

Sameer Ullah Khan
Fayaz Malik *Editors*

Drug Resistance in Cancer: Mechanisms and Strategies

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To all those who have faced the persistent challenge of cancer with unwavering courage, whose resilience and strength continue to inspire our pursuit of innovative solutions.

In memory of those who fought courageously, and in honor of those who endure, may our collective efforts lead to a future where cancer drug resistance is conquered, and hope prevails.

This book is dedicated to you—The Warriors in the Battle Against Cancer.

Dr. Sameer Ullah Khan and Dr. Fayaz Malik.

Preface

Cancer, with its complex combination of genetic mutations and cellular complexities, continues to challenge the boundaries of medical science. Despite remarkable progress in understanding the molecular underpinnings of cancer and the development of diverse therapeutic approaches, the phenomenon of drug resistance persists as a challenging hurdle in the pursuit of effective cancer treatment. The book you hold in your hands, *Drug Resistance in Cancer: Mechanisms and Strategies*, is a concerted effort to elucidate the perplexity of drug resistance in cancer and present innovative strategies to confront this challenge. The journey through these pages is a comprehensive exploration of the mechanisms that cancer cells employ to resist the treatments designed to eradicate them. From the introductory chapters laying the groundwork for the understanding of drug resistance to the specialized discussions on cancer stem cells, immune cell dynamics, and the intricate interplay within the tumor microenvironment, each section is crafted to offer profound insights into the diverse aspects of this intricate problem.

The chapters committed to elucidating the roles of epigenetic alterations, metabolic reprogramming, and intracellular compartments underscore the intricacies of cancer biology and the adaptability of malignant cells to therapeutic pressures. The exploration of autophagy's dual role and the unexpected influence of gut microbes expand the horizons of our comprehension, challenging us to consider novel dimensions in our battle against drug resistance. As the narrative unfolds, the book takes you on a journey through the current state of research on drug resistance, providing a platform for the latest discoveries, methodologies, and innovative strategies. The chapters on novel approaches and future directions illuminate the path forward, offering a beacon of hope for researchers, clinicians, and students engaged in the quest for effective cancer therapies.

We hope this compilation serves as a valuable resource, fostering not only a deeper comprehension of drug resistance mechanisms but also inspiring the development of novel therapeutic paradigms. As we navigate the complicated landscape of cancer research, it is our collective hope that the insights contained herein contribute to the ongoing discussion and collaboration essential for conquering drug resistance and improving outcomes for individuals facing the challenges of cancer. Thank you for embarking on this intellectual journey with us. May the knowledge

shared within these pages propel us closer to the day when drug resistance in cancer becomes a conquerable adversary, paving the way for more effective and personalized treatment strategies.

Srinagar, Jammu and Kashmir, India
Srinagar, Jammu and Kashmir, India

Sameer Ullah Khan
Fayaz Malik

Acknowledgments

The creation of this book, *Drug Resistance in Cancer: Mechanisms and Strategies*, has been a collective effort, and we express our heartfelt gratitude to all those who have contributed to its realization.

First and foremost, we express our deepest appreciation to our parents, whose unwavering support and belief in our endeavors have been a constant source of inspiration. Your encouragement has been a guiding light, propelling us forward in the face of challenges. To our families, whose understanding and patience during the long hours of research and writing are deeply appreciated, we extend our heartfelt thanks. Your love and support have been the foundation upon which this project stands. We acknowledge the mentors and advisors who have shaped our academic journeys, providing guidance and wisdom that has enriched the content of this book. Your commitment to nurturing the next generation of researchers is invaluable. We extend our deepest appreciation to the esteemed authors whose scholarly insights and expertise have enriched the content of this volume. Your dedication to advancing the field of cancer research is evident in the depth and breadth of the chapters you have contributed. Our special acknowledgment goes to Shariqa Jan and Kaneez Fatima who were the major writing contributors to this book, their immense efforts make this book eminently more readable.

Our sincere thanks go to the reviewers whose constructive feedback and meticulous evaluations have played a crucial role in refining the quality and coherence of the book. Your commitment to maintaining the highest standards in scientific literature is invaluable.

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Swiss/48/FM/2018-19 whose financial support has played a pivotal role in conducting the studies, acquiring essential resources, and facilitating the dissemination of knowledge.

Lastly, we thank the broader scientific community for its collaborative spirit. This book is a reflection of the collaborative efforts of many, and for that, we are truly grateful.

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About the Editors

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Abbreviations

3-MA	3-Methyladenine
4-1BB	4-1BB ligand receptor (TNFRSF9)
4-OH-tamoxifen (4HT)	4-Hydroxytamoxifen
5-Aza-2'-deoxycytidine (DAC)	Decitabine
5-azadC	5-aza-2'-deoxycytidine
5-FU	5-Fluorouracil
ABC	ATP-binding cassette
ABCC6	ATP-binding cassette subfamily C member 6
ABL	Abelson murine leukemia viral onco- gene homolog
ACT	Adoptive cell transfer
ADCC	Antibody-dependent cellular cytotoxicity
ADMA, SDMA	Asymmetric and symmetric dimethylarginine
AFP	Alpha-fetoprotein
AI	Artificial intelligence
AKT	Protein kinase B (Akt)
ALD	Alcoholic liver disease
ALDH	Aldehyde dehydrogenase
ALDH1A1	Aldehyde dehydrogenase 1A1
ALDH1A2	Aldehyde dehydrogenase 1 family member A2
ALK	Anaplastic lymphoma kinase
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AMPK	AMP-activated protein kinase
APAF1	Apoptotic peptidase activating factor 1
APC	Adenomatous polyposis coli
APCs	Antigen-presenting cells
AR	Androgen receptor
AraC	Cytarabine
ATF4	Activating transcription factor 4
ATF6	Activating transcription factor 6
ATG14 L	Autophagy-related gene 14-like
ATG5-ATG12	Autophagy-related proteins 5 and 12
ATG6	Autophagy-related gene 6

ATGs	Autophagy-related genes
ATM	Ataxia telangiectasia mutated
ATP	Adenosine triphosphate
AURKB	Aurora kinase B
B cells	B lymphocytes
B7-H3	B7 homolog 3
BafA	Bafilomycin A1
Bak	BCL2 antagonist/killer
BATF3	Basic leucine zipper ATF-like transcription factor 3
Bax	Bcl-2-associated X protein
BC	Breast cancer
Bcl2	B-cell lymphoma 2
Bcl-2l10	BCL2-like 10
BCLAF1	Bcl2-associated transcription factor 1
Bcl-xl	B-cell lymphoma-extra-large
BCRP	Breast cancer resistance protein
BET	Bromodomain and extra-terminal domain
BFT	Bacteroides fragilis toxin
BH	Bcl-2-homology
BH3 mimetics	Bcl-2 homology 3 mimetics
BH3	Bcl-2 Homology 3
Bid	BH3 interacting domain death agonist
Bim	Bcl-2-like protein 11
BM	Bone marrow
BMDCs	Bone marrow-derived cells
Bmi-1	B-lymphoma Mo-MLV insertion region 1 homolog
BMI1	B-lymphoma Mo-MLV insertion region 1 homolog
BPTES	Bis-2-(5-phenyl acetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide
BRAF	B-raf proto-oncogene
BRCA	Breast cancer gene
BRCA1/BRCA2	Breast cancer gene 1/Breast cancer gene 2
BTZ	Bortezomib
C/EBP	CCAAT/Enhancer-binding protein
CA125	Cancer antigen 125
CAFs	Cancer-associated fibroblasts
CaM-Ks	Calmodulin-dependent kinases
CAR	Chimeric antigen receptor
Caspase	Cysteine-aspartic protease
CBP	CREB-binding protein
CCL1	C-C motif chemokine ligand 1
CCL20	CC Chemokine ligand 20

CCR2	Chemokine receptor 2
CD	Cluster of differentiation
CD4+	Cluster of differentiation 4 (a glycoprotein found on the surface of immune cells)
CD4+CD25+	Cluster of differentiation 4 and 25
CD44	Cluster of differentiation 44
CD73	Cluster of differentiation 73
CD8+	Cluster of differentiation 8 (a glycoprotein found on the surface of immune cells)
CD80	Cluster of differentiation 80
CD86	Cluster of differentiation 86
CDDP	Cisplatin
CDKN1C	Cyclin-dependent kinase inhibitor 1C
CDKs	Cyclin-dependent kinases
CDSS	Clinical decision support system
cFLIPL	Cellular FLICE-like inhibitory protein long form
CI	Complex I
CK1	Checkpoint kinase 1
CKDs	Chronic kidney diseases
CLIA	Clinical Laboratory Improvement Amendments
CML	Chronic myeloid leukemia
c-Myc	Myc proto-oncogene
CO ₂	Carbon dioxide
COL11A1	Collagen type XI alpha 1
COL4A2	Collagen type IV alpha 2 chain
COX-2	Cyclooxygenase-2
COX4I1, COX4I2	Cytochrome C oxidase subunit 4 isoforms 1 and 2
CPA	Cyclophosphamide
CpG	Cytosine-phosphate-guanine
CQ	Chloroquine
CRC	Colorectal carcinoma
CRISPR/Cas9	Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9
CRKL	Crk-like protein
CRPC	Castration-resistant prostate cancer
CRS	Cytokine release syndrome
CSAs	Cancer-specific antigens
CSCs	Cancer stem cells
CSL	CBF-1, suppressor of hairless, lag-1 (transcription factor complex)
CT	Computed tomography
CTCs	Circulating tumor cells
CTF2	CCAAT-binding transcription factor 2

CTLA	Cytotoxic T-lymphocyte-associated protein
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CTLs	Cytotoxic T lymphocytes
CVDs	Cardiovascular diseases
CXCL12	C-X-C motif chemokine ligand 12
CXCR	Chemokine (C-X-C motif) receptor
CYP	Cytochrome P450
CYP1A1	Cytochrome P450 family 1 subfamily A member 1
CYP1B1	Cytochrome P450 family 1 subfamily B member 1
CYP24	Cytochrome P450 family 24 subfamily A member 1
CYP7B1	Cytochrome P450 family 7 subfamily B member 1
DAMP	Damage-associated molecular pattern
DCE MRI	Dynamic contrast-enhanced MRI
DCs	Dendritic cells
DDR	DNA damage response
DLL4	Notch ligand delta-like ligand 4
DNA	Deoxyribonucleic acid
DNAm	DNA methylation
DNAM-1	DNAX accessory molecule-1
DNA-PKcs	DNA-dependent protein kinase catalytic subunit
DNMTs	DNA methyltransferases
DOT1L	Disruptor of telomeric silencing 1-like
DOX	Doxorubicin
DR	Doxorubicin-resistant
DR	Drug resistance
DS	Doxorubicin-sensitive
DSBs	Double-strand breaks
DTP	Drug-tolerant persister
DUSP4	Dual specificity phosphatase 4
DWI MRI	Diffusion-weighted imaging MRI
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
EGFR-TKIs	Epidermal growth factor receptor tyrosine kinase inhibitors
eIF2 α	Eukaryotic initiation factor 2 α
eIF2 α	Eukaryotic translation initiation factor 2 α
ELOVL2	Elongation of very long chain fatty acids protein 2
EMT	Epithelial-mesenchymal transition
ENO1	Enolase 1
ENT1	Equilibrative nucleoside transporter 1

ENT2	Equilibrative nucleoside transporter 2
EpCAM	Epithelial cell adhesion molecule
EPI	Epirubicin
EPO	Erythropoietin
ER	Endoplasmic reticulum
ER	Estrogen receptor
ERBB2 (HER2)	Receptor tyrosine-protein kinase erbB-2 (Human epidermal growth factor receptor 2)
ERK/MAPK	Extracellular signal-regulated kinase/Mitogen- activated protein kinase
ERK1/2	Extracellular signal-regulated kinase 1/2
ERS	Endoplasmic reticulum stress
ER α	Estrogen receptor alpha
ESC	Embryonic stem cells
ESCC	Esophageal squamous cell carcinoma
ETC	Electron transport chain
EVs	Extracellular vesicles
EZH2	Enhancer of zeste homolog 2
FACS	Fluorescence-activated cell sorting
FAK	Focal adhesion kinase
FAO	Fatty acid oxidation
FAS	TNF receptor superfamily member 6
Fc	Fragment crystallizable
FDA	Food and Drug Administration
FDG-PET	18 F-fluorodeoxyglucose positron emission tomography
FIP200	Focal adhesion kinase family interacting protein of 200 kDa
FLT3	FMS-like tyrosine kinase 3
FMT	Fecal microbiota transplantation
FOXO1	Forkhead box O1
Foxp3	Forkhead box P3
FZD6	Frizzled class receptor 6
Fzd8	Frizzled 8
G0	Resting phase in the cell cycle
G1	Initial growth phase in the cell cycle
G2	Growth phase in the cell cycle
G9a	Histone methyltransferase G9a
Gal-9	Galectin-9
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GAS-STING	cGAS-STING pathway
GBM	Glioblastoma multiforme
GBM	Glioblastoma multiforme
GCLC	Glutamate-cysteine ligase catalytic subunit
GCLC	Glutamate-cysteine ligase catalytic subunit

GCLP	Good Clinical Laboratory Practice
GDF15	Growth differentiation factor 15
GEMM	Genetically engineered mouse models
GITR	Glucocorticoid-induced TNFR-related protein
GLP	G9a-like protein
GLS	Glutaminase
GLUT	Glucose transporter
GPOR	G protein-coupled estrogen receptor 1
GPR81	G-protein-coupled receptor 81
GREB1	Growth regulation by estrogen in breast cancer 1
GRP	Gefitinib-resistant cells
GRP78	Glucose-regulated protein 78
GSH	Glutathione
GSIs	Gamma-secretase inhibitors
GST	Glutathione S-transferase
GSTp	Glutathione S-transferase pi
H ⁺ -ATPase	Proton-ATPase
H2A.X	Histone 2A.X
H2AX	Histone 2AX
H2AX	Histone H2AX
H2AZ	Histone variant H2A.Z
H3K27	Histone 3 lysine 27
H3K27me3	Histone H3 lysine 27 trimethylation
H3K27me3	Trimethylation of histone 3 lysine 27
H3K36me3	Trimethylation of histone 3 lysine 36
H3K4Me3	Histone 3 lysine 4 trimethylation
H3K9	Histone 3 lysine 9
H3K9me1/2	Histone 3 lysine 9 mono/di-methylation
HATs	Histone acetyltransferases
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCQ	Hydroxychloroquine
HDAC1	Histone deacetylase 1
HDAC2	Histone deacetylase 2
HDACs	Histone deacetylases
HDR	Homology-directed repair
HDSCs	High-grade human glioma stem cells
HER2	Human epidermal growth factor receptor 2
HER3	Human epidermal growth factor receptor 3
HGF	Hepatocyte growth factor
HIAR	Hypoxia-induced angiogenesis regulator
HIC1	Hypermethylated in cancer 1
HIF-1 α	Hypoxia-inducible factor 1-alpha
HIF-1 α	Hypoxia-inducible factor 1-alpha

HK2	Hexokinase 2
HLA class Ia	Human leukocyte antigens class Ia
HLA	Human leukocyte antigen
HLA	Human leukocyte antigen
HMGB1	High-mobility group B1
HMGB1	High-mobility group box 1
hMLH1	Human MutL homolog 1
hMSCs	Human mesenchymal stem cells
HMTs	Histone methyltransferases
HMTs	Histone methyltransferases
HNF4	Hepatocyte nuclear factor 4
hnRNP	Heterogeneous nuclear ribonucleoprotein
HNSCC	Head and neck squamous cell carcinoma
HPMA	N-(2-hydroxypropyl) methacrylamide
HPV	Human papillomavirus
HR	Homologous recombination
hRFC	Human reduced folate carrier
HSP90	Heat shock protein 90
IAPs	Inhibitors of apoptosis proteins
IBD	Inflammatory bowel disease, hepatocellular carcinoma
IC50	Half-maximal inhibitory concentration
ICOS	Inducible T-cell CO-stimulator
ICs	Intracellular compartments
IDH	Isocitrate dehydrogenase
IDO1	Indoleamine 2,3-dioxygenase 1
IFN- γ	Interferon-gamma
IFN- γ	Interferon-gamma
IFP	Interstitial fluid pressure
IGF1	Insulin-like growth factor 1
IGF1R	Insulin-like growth factor 1 receptor
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-13	Interleukin-13
IL-17	Interleukin-17
IL-23	Interleukin-23
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-6	Interleukin-6
ILK	Integrin-linked kinase
IMRT	Intensity-modulated radiation therapy
iPS	Induced pluripotent stem
IRE1 α	Inositol-requiring enzyme 1 alpha
ISRIB	Integrated stress response inhibitor

ITIMs	Immunoreceptor tyrosine-based inhibitory motifs
JAK2/STAT5	Janus kinase 2/Signal transducer and activator of transcription 5
JNK	c-Jun N terminal kinase
KAT6A	Lysine acetyltransferase 6A
KDR	Kinase insert domain receptor
KIR	Killer cell immunoglobulin-like receptor
Kme1, Kme2, Kme3	Mono-, di-, and tri-methylation of lysine residues
KMTs	Lysine methyltransferases
KMTs	Lysine methyltransferases
LAG-3	Lymphocyte-activation gene 3
LAG-3	Lymphocyte-activation gene 3
LC3A/B	Microtubule-associated proteins 1A/1B light chain 3A/B
LDHA	Lactate dehydrogenase A
Lgr5	Leucine-rich repeat containing G protein-coupled receptor 5
LIR	Leukocyte immunoglobulin-like receptor
LMP	Lysosomal membrane permeabilization
LMP	Lysosomal membrane permeabilization
LMPs	Lysosomal membrane proteins
LncRNA	Long non-coding RNA
LPS	Lipopolysaccharide
LRP	Lung resistance-related protein
LRP6	Low-density lipoprotein receptor-related protein 6
M	Mitosis phase in the cell cycle
M1	Macrophage type 1
M1	Pro-inflammatory macrophages
M2	Anti-inflammatory macrophages
M2	Macrophage type 2
m6A	N6-methyladenosine
mAbs	Monoclonal antibodies
MACS	Magnetic-activated cell sorting
MAML	Mastermind-like (coactivator)
mBRCA1	Mutant BRCA1
MCAK	Mitotic centromere-associated kinesin
Mcl-1	Myeloid cell leukemia 1
MCT1	Monocarboxylate transporter 1
MDM2	Mouse double minute 2 homolog
MDR1	Multidrug resistance protein 1
MDSCs	Myeloid-derived suppressive cells
MEK	Mitogen-activated protein kinase kinase

MET	Mesenchymal-epithelial transition
MET	MET proto-oncogene
MGMT	O-6-methylguanine-DNA methyltransferase
MHC	Major histocompatibility complex
MHC-I	Major histocompatibility complex class I
MIM	Mitochondrial inner membrane
miR	MicroRNA
miR-129-5p	microRNA-129-5p
miR-137	microRNA-137
MK-0752	Gamma-secretase inhibitor used in targeting notch signaling
MKP2	MAPK phosphatase 2
MLH1	MutL homolog 1
MLL	Mixed-lineage leukemia
MM	Multiple myeloma
MMP9	Matrix metalloproteinase 9
MMPs	Matrix metalloproteinases
MOMP	Mitochondrial outer membrane permeabilization
MRI	Magnetic resonance imaging
MRP1	Multidrug resistance-associated protein 1
MRS	Magnetic resonance spectroscopy
MSC	Mesenchymal stem cell
MT	Metallothionein
MTD	Maximum tolerated dosage
mTOR	Mammalian target of rapamycin
mTORC1	Mechanistic target of rapamycin complex 1
mTORC2	Mechanistic target of rapamycin complex 2
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
mtTFA	Mitochondrial transcription factor A
Musashi-1	Musashi RNA binding protein 1
MVBs	Multivesicular bodies
NADPH	Nicotinamide adenine dinucleotide phosphate
NAFLDs	Non-alcoholic fatty liver diseases
NAT1	N-acetyl transferase 1
NCR	Natural cytotoxicity receptor
NCT	ClinicalTrials.gov Identifier
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NF-κB	Nuclear factor-kappa B
NHEJ	Non-homologous end joining
NICD	Notch intracellular domain
NK	Natural killer
NKG2A	Natural killer group 2A

NKG2D	Natural killer group 2D
NMR	Nuclear magnetic resonance
NOD/SCID	Non-obese diabetic/Severe combined immunodeficiency
Noxa	Damage-regulated apoptosis modulator
Noxa	Phorbol-12-myristate-13-acetate-induced protein 1
NPC	Nuclear pore complex
NPs	Nanoparticles
NRF2	NFE2-related factor 2
NSCL	Non-small cell lung
NSCLC	Non-small cell lung cancer
OC	Ovarian cancers
OCCs	Ovarian carcinoma cell lines
Oct	Octamer-binding transcription factor
OCT1	Organic cation transporter 1
ODD	Oxygen-dependent degradation
OS	Overall survival
OXPHOS	Oxidative phosphorylation
p16INK4a	Cyclin-dependent kinase inhibitor 2A
p300	E1A binding protein P300
p38	p38 Mitogen-activated protein kinase
p53	Tumor protein 53
p62/SQSTM1	Sequestosome 1
PAI-1	Plasminogen activator inhibitor-1
PARP-1	Poly (ADP-ribose) polymerase-1
PD	Pharmacodynamic
PD-1/PD-L1	Programmed cell death protein 1/Programmed death-ligand 1
PDAC	Pancreatic ductal adenocarcinoma
PDGF beta	Platelet-derived growth factor beta
PDH	Pyruvate dehydrogenase
PDHK1	Pyruvate dehydrogenase kinase 1
PDIA1	Protein disulfide isomerase a1
PDK1	Phosphoinositide-dependent kinase-1
PDTC	Pyrrolidine dithiocarbamate
PDX	Patient-derived tumor xenografts
PEG-E2	Prostaglandin E2
PERK	Protein kinase RNA-like endoplasmic reticulum kinase
PFK1	Phosphofructokinase 1
PFKP	Phosphofructokinase 1 platelet
PFN1	Profilin1
PFS	Progression-free survival
PGM	Phosphoglucomutase

P-gp	P-glycoprotein
pH	Potential of hydrogen
PI3K/Akt	Phosphoinositide 3-kinase/Protein kinase B
PI3KC3	Class III phosphoinositide 3-kinase
PIK3CA	Phosphatidylinositol -4,5-bisphosphate 3-kinase catalytic subunit alpha
PK	Pharmacokinetic
PKM2	Pyruvate kinase M2
PK-PD	Pharmacokinetic-pharmacodynamic
Pks	Polyketide synthases
PLGA	Poly(lactic-co-glycolic acid)
PMNs	Polymorphonuclear neutrophils
PORCN	Porcupine O-acyltransferase
PPO	Poly (propylene oxide)
PPP	Pentose phosphate pathway
PRI-724	Beta-catenin inhibitor
PRMT1-9	Protein arginine methyltransferases 1-9
PRMTs	Protein arginine methyltransferases
PRODH	Proline dehydrogenase
PSA	Prostate-specific antigen
PSCs	Pancreatic stellate cells
PSTI	Pancreatic secreted trypsin inhibitor
PTEN	Phosphatase and tensin homolog
PTGS2	Prostaglandin-endoperoxide synthase 2
PTK2	Protein tyrosine kinase 2
PTP	Protein tyrosine phosphatase
PTX	Paclitaxel
Puma	p53 upregulated modulator of apoptosis
RAF	Rapidly accelerated fibrosarcoma
RAS	Rat sarcoma
RECQL4	RecQ-like helicase 4
Rme1/MMA, Rme2	Mono- and di-methylation of arginine residues
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RP2D	Recommended phase 2 dose
RT	Radiation therapy
RTKs	Receptor tyrosine kinases
RT-PCR	Reverse transcription polymerase chain reaction
S	DNA replication phase in the cell cycle
SALL4	Sal-like protein 4
SAM	S-adenosyl-L-methionine
SASP	Senescence-associated secretory phenotype
SBRT	Stereotactic body radiation therapy
SCD1	Stearoyl-CoA desaturase 1
SCFAs	Short-chain fatty acids

SCLC	Small cell lung cancer
SCs	Stem cells
SDF-1 alpha	Stromal cell-derived factor 1 alpha
SDF-1	Stromal cell-derived factor-1
Sec62	Translocation protein SEC62
SETD2	SET domain containing 2
sgRNA	Single guide RNA
Shh	Sonic hedgehog
SILAC	Stable isotope labeling by amino acids in cell culture
siRNA	Small interfering RNA
siRNAs	Small interfering RNAs
SIRPα	Signal regulatory protein alpha
SLC19A1	Solute carrier family 19 member 1
SLC22A3	Solute carrier family 22 member 3
SMAR1	Scaffold/matrix-associated region 1
SMO	Smoothened
SMOC-2	SPARC-related modular calcium-binding protein 2
SMYD2	SET and MYND domain containing 2
SORE6	SOX2/Oct4 reporter element 6
SOX	SRY-related HMG-box
Sox2	Sex-determining region Y-box 2
SPT5	Transcription elongation factor SPT5
SRIB	Integrated stress response inhibitor
SSBs	Single-strand breaks
SSCs	Somatic stem cells
SSEA-1	Stage-specific embryonic antigen 1
SSP	Serine synthesis pathway
STAT3	Signal transducer and activator of transcription 3
STC1	Stanniocalcin 1
STF-31	A specific inhibitor of glucose transporter 1 (GLUT1)
SULT1A1	Sulfotransferase family 1A member 1
Suv39H1, Suv39H2	Suppressor of variegation 3-9 homolog 1 and 2
T cells	T lymphocytes
TAAAs	Tumor-associated antigens
TAMs	Tumor-associated macrophages
TANs	Tumor-associated neutrophils
TATI	Tumor-associated trypsin inhibitor
TCA	Tricarboxylic acid
TFs	Transcription factors
TGF-β1	Transforming growth factor beta 1
Th17	T-helper 17 cells

Th2	T-helper 2
Th2	T-helper cell type 2
Thy-1	Thymocyte differentiation antigen-1
TIGAR	Tumor protein 53-induced glycolysis and apoptosis regulator
TIGIT	T cell immunoreceptor with Ig and ITIM domains
TILs	Tumor-infiltrating lymphocytes
TIM-3	T-cell immunoglobulin and mucin-domain containing-3
TIMPs	Tissue inhibitors of metalloproteinases
TKIs	Tyrosine kinase inhibitors
TME	Tumor microenvironment
TMZ	Temozolomide
TNBC	Triple-negative breast cancer
TNFRSF16	Tumor necrosis factor receptor superfamily member 16
TPP-Pluronic F127-hyaluronic acid nano micelles	Triphenylphosphonium-pluronic F127-hyaluronic acid nano micelles
TRA-1-60	Tumor-related antigen 1-60
TRAIL	TNF-related apoptosis-inducing ligand
Tregs	Regulatory T cells
TSACP	TruSeq amplicon—cancer panel
UbQ	Ubiquinone
ULBP	UL16-binding protein
ULK1	Unc-51 like autophagy activating kinase 1
uPAR	Urokinase plasminogen activator receptor
UPR	Unfolded protein response
USP22	Ubiquitin-specific peptidase 22
USP7	Ubiquitin-specific peptidase 7
Uvrag	UV radiation resistance-associated gene
VEGF	Vascular endothelial growth factor
VHL	von Hippel-Lindau
VPS	Vacuolar protein sorting
Vps38	Vacuolar protein sorting 38
Wnt	Wingless/integrated
XRCC1	X-ray repair cross-complementing protein 1
YB-1	Y-box binding protein-1
ZBP-89	Zinc binding protein 89
ZNF143	Zinc-finger factor 143
β -catenin	Beta-catenin



Introduction to Drug Resistance in Cancer

1

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Abstract

Treating cancer has so many hurdles, and drug resistance is one of them. Treatment strategies are evolving for cancer due to innate and acquired resistance capacity in them. The mechanism behind the resistance is constantly evolving in response to new drug treatment strategies and is an outcome of the acquired or adaptive mutation expression in cancer cells. In a broader perspective, cancer drug resistance can be governed by genetic, epigenetic, proteomic, metabolic, or microenvironment cues that ultimately enable selected resistant cancer cells to survive and progress under unfavorable conditions. Although the mechanism of drug resistance has been widely studied in cancer that progressively leads to the generation of new targets for novel anticancer drugs having better efficacy than previous ones. However, due to the high variability in resistance acquired by cancer cells toward existing drugs, novel strategic options with better efficacy

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need to be explored that overcome resistance. Combination therapy is a widely used alternative with a better success rate though the risk of amplified side effects is commonplace. However, recent groundbreaking immune therapy combination with the drugs is one of the ways to overcome drug resistance and has revolutionized anticancer therapy to a greater extent. However, more study is needed to be done at genetic, epigenetic, proteomic, and metabolic levels to identify targets of different cancers that can help to develop new therapies that are more effective to the existing challenge of cancer drug resistance. This chapter will focus on the recent challenges and strategies opted by cancer cells to withstand the current therapies at the molecular level.

Keywords

Drug resistance · Apoptosis · Cancer stem cells · Multi-drug resistance · Immune cells

1.1 Introduction

Presently, one of the challenging aspects of cancer biology is drug resistance in which cancer becomes forbearing to drug treatment, thus worsening the conditions of patients (Nikolaou et al. 2018; Saha and Sarkar 2021). Although initially various cancer types are sensitive to pharmaceutical therapy, over time, they acquire resistance and become more aggressive (Lu and Chao 2012; Michaelis et al. 2019; Mir et al. 2022a). Progress in discovering targeted therapy is advancing in recent years, which has led to the approval of various impactful anticancer drugs; nonetheless, resistance still shows a big hindrance to their success besides their life-threatening side effects (Oun et al. 2018; Tao et al. 2015). Cancer cells show evolving behavior of recurrence, dormancy, and drug resistance even after using conventional treatments like surgery, radiotherapy, and static chemotherapy, which in turn give birth to cancer stem cells (CSCs), thus producing a vicious cycle of resistivity and aggressiveness (Rajesh et al. 2017; Donnenberg and Donnenberg 2005). Advanced and more potent chemotherapeutic drugs have been able to succeed the previously available anticancer drugs individually, or they have been used chronologically or co-treated with prevailing treatments already available (Citron et al. 2003; Khan et al. 2022a). Moreover, altered chemotherapeutic dose intensity tactics like intermittent administration or higher doses along with supplements and growth factors to suppress the side effects on bone marrow have proved to be effective in preventing the regrowth of tumors (Citron et al. 2003; Hryniuk and Bush 1984; Sternberg et al. 2001). But clinically, cancer drug resistance remains a major hurdle in medical oncology, therefore understanding the acquired resistance mechanism and developing next-generation targeted therapies against these hallmarks are crucial to be taken at the earliest (Hanahan and Weinberg 2000, 2011; Fouad and Aanei 2017). With the advancement in the study of drug resistance massive efforts on the development of successful therapies against RTKs, nuclear, androgen, and Her2

receptors that potentially target oncogenes or other factors that lead to the transformation of cells (Wu and Fu 2018; Roviello et al. 2016; Martin et al. 2015; Fujita and Nonomura 2019; Asano et al. 2016; Barton et al. 2017; Niikura et al. 2016; Tolaney et al. 2020). Moreover, recent approaches of using immunological therapies were proven to be more successful in the recognition and destruction of cancer cells, for example, anti-CTLA and anti-PD-1/PD-L1 therapy remarkably show antitumor activity by dysfunctioning the negative regulators of the anticancer adaptive immune system, though the low chance of resistance remains the concern as that of conventional therapy (Leach et al. 1996; Iwai et al. 2002; Noguchi et al. 2017; Juneja et al. 2017; Ribas and Wolchok 2018). In cancer patients, drug resistance can be inherent due to evolving selection pressure or arise mostly under cancer therapy pressure (Zahreddine and Borden 2013). These resistant traits in cancer cells are achieved at genomic, epigenomic, and proteomic levels by following the Darwinian selection pressure rule (Álvarez-Arenas et al. 2019; Gerlinger and Swanton 2010; Theile and Wizgall 2021). With the advent of high-throughput assays, the idea of tumor heterogeneity came into existence, though there were already some hypothetical theories that supported this emerging idea. Tumor heterogeneity contributes to attaining resistance in which a few tumor cells divide and form a subpopulation of cells that may achieve features that enable them to become irresponsive to the particular drug over time (Jamal-Hanjani et al. 2015; Alizadeh et al. 2015; Roesch 2015). This process may sometimes emerge or initiate under selective drug pressure and play a vital role in resistance (Dexter and Leith 1986; Wu et al. 2017). Some contrasting features of sensitive cancer cells and drug-resistant cells are represented in Fig. 1.1.

In this chapter, we tried to discuss the spectrum of selective mechanisms displayed by cancerous cells to resist treatment including changes affecting drug chemistry, transporters, epigenetic changes, and amplification or modification of drug targets by accumulating protective mutations, which ultimately lead to impaired apoptosis (Fig. 1.2). Gaining these changes and genetic rewiring collectively leads to the clinically more difficult problem of multidrug resistance. Here,

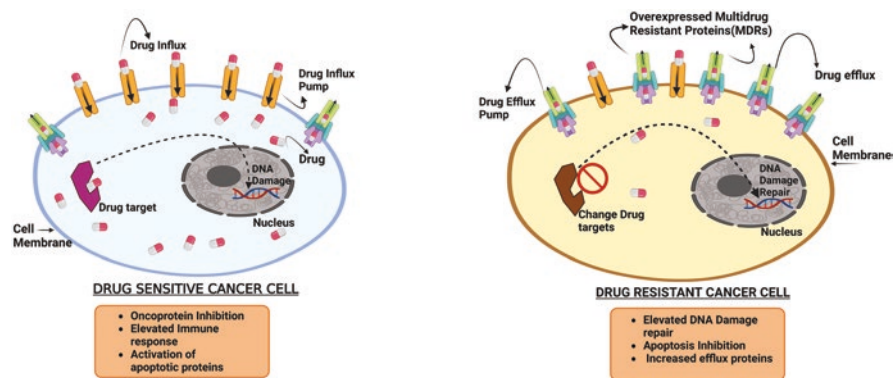


Fig. 1.1 The figure illustrates the key differences between drug-sensitive and drug-resistant cancer cells

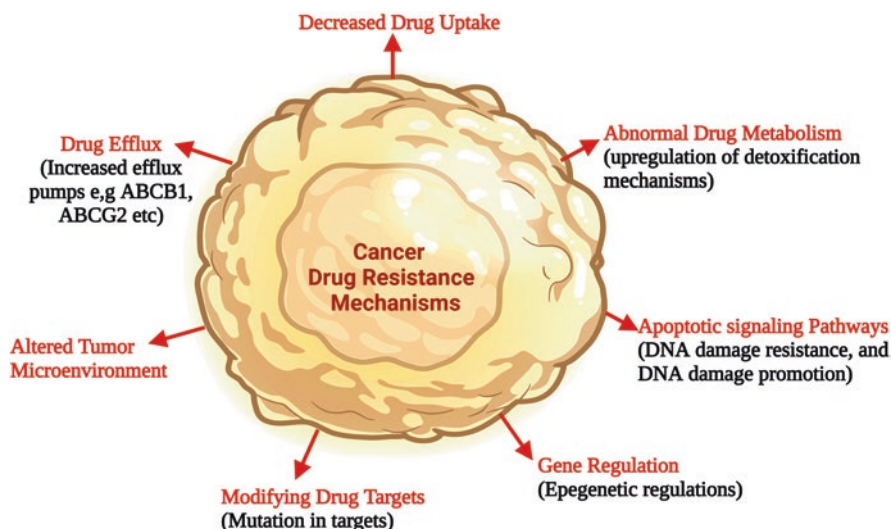


Fig. 1.2 This figure provides an overview of the diverse mechanisms employed by cancer cells to develop resistance to anticancer drugs

we tried to interrelate the multiresistant pathways with different cancer types by providing examples and some insights into these challenging aspects that can encourage the next generation of cancer therapy or the implication of immune therapy. This chapter further highlights the current challenges, prospects, and progress in cancer drug resistance that can prove to be useful in discovering more potent drugs and immune therapies.

1.2 Cancer Drug Resistance Mechanisms

Modifying Drug Targets

The molecular target of the drugs and changes to this target, such as mutations or altered expression levels, have an impact on the drug's effectiveness (Housman et al. 2014). These kinds of target modifications in malignancies may ultimately result in treatment resistance. For instance, specific anticancer medications are designed to selectively bind to topoisomerase II, a crucial enzyme that plays a pivotal role in preventing the over- or under-coiling of DNA. The interaction between DNA and topoisomerase II is typically short; however, the administration of these drugs results in the stabilization of the complex. This stabilization leads to DNA damage, obstruction of DNA synthesis, and end of mitotic processes. In these conditions, cancer cells have the ability to develop resistance via diverse mechanisms. For example, through topoisomerase II gene mutations, some cell lines have developed resistance to topoisomerase II inhibitors (Stavrovskaya 2000; Hinds et al. 1991).

Resistance to tamoxifen in breast cancer is another instance of modifications in signaling pathways. Tamoxifen functions as an ER antagonist. However, in breast cancer cells, ER signaling interacts complexly with additional growth signaling cascades, facilitating drug resistance via a number of mechanisms. Tamoxifen, for instance, could lose its estrogen blocker effect and gain a greater agonist-like effect in cancers with prominent growth factor receptor signaling, such as those with HER2 amplification, which will stimulate tumor growth (Shou et al. 2004). Furthermore, it was discovered that tamoxifen slightly increased HER2 and EGFR expression and that this increase was significantly increased when tumors developed resistance to the same drug (Massarweh et al. 2008).

Suppression of Cell Death (Inhibiting the Apoptosis Pathway)

Apoptosis, necrosis, and autophagy are three crucial processes that control the death of cells. Nevertheless, these processes' biological features set them apart from one another. Each one of them promotes cell death (D'arcy 2019; Khan et al. 2022b, 2023a, b). Apoptosis is a biological process that can be initiated by either intrinsic or extrinsic mechanisms. The external pathway involves the participation of various components including ligands, linker proteins, cell death receptors (like FAS and TNF-R), and caspases 3, 6, 7, and 8 (Jin and El-Deiry 2005). Actin as well as nuclear lamin proteins is consequently proteolyzed in the external pathway, which finally causes cell death (Lothstein et al. 1989). Pro-apoptotic proteins including Bak, Bax, and caspase-9, while antiapoptotic proteins including AKT, Bcl2, etc. are all involved in the internal route carried out by the mitochondria. Cancer cells are more resistant to chemotherapy when antiapoptotic genes (AKT, Bcl2, etc.) are upregulated and pro-apoptotic genes (Bclxl, Bax, etc.) are downregulated (Wang et al. 2018a; Mintoo et al. 2021). In addition, p53 gene alterations that result in resistance to drugs can damage DNA and stress cells by inducing apoptosis. These alterations may impede the association between DNA damage (induced by chemotherapy) and the initiation of apoptosis (Saleem et al. 2020).

Decrease in Drug Absorption

The cellular uptake of anticancer agents can take place through passive diffusion, activated transport, and facilitated diffusion mechanisms. For instance, doxorubicin and vinblastine can be absorbed passively, while nucleoside analogs may require active transport (Singh et al. 2017a). The ABC transporters usually allow the cytotoxic chemicals to pass through the cells in the direction of a concentration gradient. However, active transport is the only way for a drug to enter cells under the influence of an elevated concentration gradient (Hee Choi and Yu 2014). The majority of membrane transporters are SLC solute carriers, which transfer vitamins, minerals, and other solutes. Drug absorption can be decreased in two ways: (1) by lowering the propensity for drug binding and (2) by lowering the number of transporter

proteins. To enter cells, some substances employ particular transporters (Martinez and Amidon 2002). These transporters become inhibited by mutations, which decreases the uptake of the drugs. Patients having acute lymphoblastic leukemia (ALL) frequently develop resistance to methotrexate due to an alteration in the human folate carrier gene (hRFC). The replacement of lysine for glutamic acid in the first transmembrane domain of the hRFC protein, resulting from the mutation of the G point at nucleotide 133, has been observed to decrease the affinity of the transporter for drugs (Mansoori et al. 2017; Matherly 2001).

Efflux of Drugs

Reducing the accumulation of drugs by boosting efflux constitutes one of the more well-studied processes of cancer treatment resistance. This efflux is made possible through members of the ATP-binding cassette (ABC) transporters group of essential protein molecules, regulators at the cell membranes of normal cells (Wang et al. 2021). ABC transporters are a class of transmembrane proteins that are ubiquitously expressed across various phyla, including humans. These proteins play a crucial role in facilitating the transportation of diverse molecules across the membranes of cells. Transporters exhibit structural diversity across different proteins, with the human species containing 49 distinct members of the ABC family (Thomas and Tampé 2020). However, all transporters share a common feature of possessing two different domains, namely, a nucleotide-binding domain that is highly conserved and a transmembrane domain that is more variable (Chang and Roth 2001). The process of substrate transportation across the cell membrane involves the binding of the substrate to the transmembrane domain. This binding event triggers ATP hydrolysis at the nucleotide-binding site, leading to a conformational change that facilitates the extrusion of the substrates outside of the cell. The mechanism of efflux plays an essential part in preventing the excessive buildup of toxins in the cell (Sauna and Ambudkar 2001). The expression of ABC transporters is notably high in the epithelial cells of the liver along with the intestine. These proteins serve a protective function by facilitating the efflux of drugs and other noxious compounds into the bile duct as well as the intestinal lumen. The blood-brain barrier is also significantly sustained by their involvement (Borst and Elferink 2002; Schinkel et al. 1994).

Efflux through ABC transporters is a biological process that is commonly observed. However, it is additionally a recognized mechanism that contributes to drug resistance in cancerous cells. The involvement of three transporters, namely, multidrug resistance protein 1 (MDR1), multidrug resistance-associated protein 1 (MRP1), and breast cancer resistance protein (BCRP), has been reported in numerous instances of drug-resistant malignancies (Lubelski et al. 2007). The MDR1 gene, responsible for the production of P-glycoprotein, was the initial gene of its kind to be recognized (Gottesman et al. 2002; Szakács et al. 2004; Hilgendorf et al. 2007). The upregulation of MDR1 gene expression is observed in the liver, colon, and kidney tissues upon their malignant transformation. A study revealed that treatment with a drug called doxorubicin resulted in a substantial rise in MDR1

expression in lung carcinoma cells, whereas no noteworthy alteration in expression was detected in normal lung cells. Likewise, the expression of BCRP was found to be indicative of both drug response and survival rates among patients with small-cell lung cancer (Li et al. 2010). The utilization of a BCRP-inhibiting drug, like Gefitinib, may lead to a reduction in drug efflux in certain cases. This specific drug is an inhibitor of tyrosine kinase that works to reduce BCRP's transporter activity and reverse drug resistance (Yanase et al. 2004).

Inactivation of Drugs

The process of drug activation within an organism involves intricate mechanisms that involve the interaction of various substances with diverse proteins. The interactions between drugs and other molecules as well as proteins may result in modifications, partial degradation, or complexation of the drug, eventually resulting in its activation (Fodale et al. 2011). In order to achieve clinical efficacy, numerous anti-cancer drugs necessitate metabolic activation. Nevertheless, cancer cells have the potential to acquire resistance to these therapies by reducing drug activation. This is shown, for instance, in the therapy of acute myelogenous leukemia using cytarabine (AraC), a type of nucleoside medication that becomes active following many phosphorylation processes that change it into AraC-triphosphate (Zahreddine and Borden 2013; Sampath et al. 2006). A reduction in AraC activation caused by down-regulation or mutations in this system may result in AraC drug resistance.

The cytochrome P450 (CYP) system is another significant example of how drugs can be activated or inactivated. Typically, there are two classes in the CYP system. Class I (CYP1A1, CYP1A2, CYP3A4, and CYP2E1) is made up of the well-conserved, devoid of significant functional polymorphisms and actively involved in the metabolism of pharmaceuticals and procarcinogens. Class II (CYP2B6, CYP2C19, CYP2C9, and CYP2D6) includes the widely polymorphic and are involved in the metabolism of drugs but not procarcinogens (Plastaras et al. 2000). Due to the higher variability of class II gene sequences in comparison to class I, CYP enzymes belonging to the former class are more adept during drug metabolism and may potentially contribute to the emergence of drug resistance in cancers.

1.3 Genetic and Epigenetic Alteration as an Adaptive Response to Chemotherapy That Majorly Influences Drug Resistance

The study of hereditary phenotypic variations without change in the DNA sequence is referred to as “epigenetics” (Berger et al. 2009). Such variations are mostly caused by modifications in gene expression and function such as histone modifications and DNA methylation (Ghasemi et al. 2017). Numerous investigations have demonstrated the importance of epigenetic modifications in cancer drug-tolerant persister (DTP) cells that persist in the diverse population and become more

tolerable to higher drug pressure (Sharma et al. 2010). Additionally, it has also been suggested that altering the epigenetic landscapes aids in the maintenance and survival of CSCs (Toh et al. 2017). Moreover, to acquire various drug resistance strategies to evade anticancer therapy, cancerous cells rearrange their epigenomic landscapes via DNA methylation and changes in histone as well as non-histone proteins.

The Function of DNA Methylation in Cancer Treatment Resistance

Numerous cancer hallmarks, including cellular proliferation, infiltration, migration, and metastases, as well as clinical characteristics, like the stage of disease, survival, prognosis, and therapy response, have been linked to specific patterns of DNA methylation (DNAm) (Fouad et al. 2018). Oncogenes and tumor suppressor genes' regulatory domains frequently have a methylation signature that is different from the normal (Irizarry et al. 2009). Abnormal DNAm caused by DNMTs upregulation impacts tumor suppressive genes by hyper-methylating them, which results in transcriptional silence. Proto-oncogenes, in contrast, exhibit hypomethylation-induced transcriptional activation (Irizarry et al. 2009). It has been shown that hyper-methylation of the miR-129-5p CpG island induces miR-129-5p suppression, promoting chemoresistance in gastric carcinoma cells. The 5-azacytidine treatment significantly decreased the chemoresistance to cisplatin, 5-FU, and vincristine in gastric carcinoma MDR cell line SGC7901/VCR via restoring the activity of miR-129-5p by reducing the methylation status (Wu et al. 2014). Hypo-methylation at promoter regions may likewise be used by tumors to develop resistance to chemotherapy. For example, in the breast cancer MCF-7 cell line, the promoter region of GSTp, MDR1, uPA, and MGMT was significantly methylated, but in drug-resistant MCF-7/R, the regions were hypo-methylated. MDR1 gene's hypo-methylation was linked to P-glycoprotein overexpression, which in turn is responsible for resistance to doxorubicin (Chekhun et al. 2006).

Furthermore, the level of DNA methylation is also crucial in regulating the fate of apoptotic cells in many malignancies. At the transcriptional and posttranslational levels in cancerous cells, various anti- and pro-apoptotic signaling components are epigenetically controlled. Apoptosis resistance, for example, develops when anti-apoptotic proteins like Bcl-2, Bcl-xl, IAPs, etc. are expressed or activated more frequently (Williams and Cook 2015). Similar to this, suppressing pro-apoptotic molecules like Bid, Bax, Bim, Puma, and Noxa increases resistance to apoptosis. For example, Baharudin and coworkers conducted DNAm sequencing on 5 recurrent and 43 nonrecurrent colorectal carcinomas (CRC) patients who were receiving 5-fluorouracil (5-FU) chemotherapy (Baharudin et al. 2017). In the CRC group with recurrence compared to the nonrecurrence group, the study discovered 4787 substantially differently methylated genes; 3112 of these were hypermethylated, while 1675 were hypomethylated. Notably, numerous hypermethylated genes were linked to the MAPK signal transduction pathway that is involved in the regulation of apoptosis, which in turn leads to therapeutic resistance toward 5-FU (Baharudin et al.

2017). The study also found that 5-azadC administration improved 5-FU responsiveness, resulting in considerable growth suppression in comparison to 5-FU monotherapy in hypermethylated cell lines SW48 (Baharudin et al. 2017). In general, DNA methylation plays an important role in the development of therapeutic resistance, and targeting it in carcinoma patients would be a good therapeutic approach.

Role of Histone Modifications in Therapeutic Resistance

Modifications in histones are another epigenetic mechanism that is responsible for therapeutic resistance in cancer. A group of enzymes known as histone methyltransferases (HMTs) facilitates the methylation of histone proteins. HMTs are classified into two groups: histone lysine methyltransferases (KMTs) and protein arginine methyltransferases (PRMTs), both of which are involved in therapeutic resistance (Yang et al. 2020a). For example, the most frequent KMT is G9a, which catalyzes the synthesis of H3K9me1 and H3K9me2. Immunohistochemical testing was done by Liu and coworkers on surgically removed tumor samples from head and neck squamous cell carcinoma patients undergoing chemotherapy. They discovered that patients with higher levels of G9a were less sensitive to the first-line antitumor drug cisplatin than patients who expressed lower levels of G9a (Liu et al. 2017). The glutamate-cysteine ligase catalytic subunit (GCLC) was transcriptionally activated by G9a increases cellular glutathione (GSH), which reduces the DNA damage brought by cisplatin and increases therapeutic resistance (Liu et al. 2017). Likewise, PRMTs are also responsible for drug resistance. Cells from ovarian carcinoma that were resistant to cisplatin were shown to have elevated PRMT1 modifications (Musiani et al. 2020). Chromatin and associated proteins were most affected by the PRMT1 modification, which resulted in genotoxic stress. It has been established that PRMT1 catalyzes H4R3 and, by controlling the NF- κ B cascade, contributes to the stimulation of genes involved in the senescence-associated secretory phenotype (SASP), which in turn increases tumor cells' resistance to cisplatin by shielding them from DNA damage (Musiani et al. 2020).

1.4 Immune Cells and Tumor Microenvironment Protect Cancer Cells from Anticancer Drugs

The tumor microenvironment (TME) is a complicated and evolving “rogue organ” made up of stroma, extracellular matrix, immune cells, nervous, and lymphatic systems, as well as its blood supply. Cellular cross talk and communication between cells and the ECM are significant components of the TME. This interplay triggers the release of soluble molecules involved in immune evasion as well as ECM remodeling, which promotes tumor initiation, progression of the disease, metastasis, and most importantly treatment resistance (Balkwill et al. 2012; Hanahan and Coussens 2012; Khan et al. 2022a). The TME first mediates innate resistance just

before anticancer treatment events via regular mutual interplay between cancerous cells and adjacent TME components. Second, acquired resistance imparted by the TME typically arises as a host's adaptive mechanism to therapeutical insults. In particular, under various treatment stresses in clinical settings, the TME-induced resistance produces major effects on local disease foci as well as shapes the cancer transformation path (Sun 2016). Numerous cell types, including CAFs, immune cells, ECM components, and exosomes as well as hypoxia and acidic environment, present in the TME contribute to the therapeutic resistance (Fig. 1.3).

CAFs

CAFs are activated fibroblasts that are associated with cancerous cells and are the most prevalent population of cells in the TME (Räsänen and Vaheri 2010). The transition of fibroblasts from dormancy to activation generates a variety of oncogenic cues that allow cancer cells to evade therapies (Kalluri and Zeisberg 2006). CAFs play an important role in therapeutic resistance by secreting growth factors, chemokines, exosomes, and metabolites resulting in resistance as well as relapse (Räsänen and Vaheri 2010; Straussman et al. 2012). When chemotherapeutic drugs are used to treat esophageal squamous cell carcinoma (ESCC), CAF-secreted PAI-1 in a paracrine mode stimulates the AKT and MAPK pathways to lessen the chemotherapeutic drug-induced damage to DNA, ROS production, and cell death (Che et al. 2018; Khan et al. 2023b, c). Moreover, CAF-secreted HGF can bind to the MET receptor and stimulate the PI3K-AKT and MAPK pathways, leading to resistance to EGFR or BRAF inhibitors in colon cancer, glioblastoma, and melanoma (Luraghi et al. 2014; Fiori et al. 2019; Wadhwa et al. 2020). By stimulating FOXO 1, a forkhead transcription factor in the O-box subfamily, CAFs significantly increased the chemoresistance of ESCC cells by causing the release of TGF- β 1.

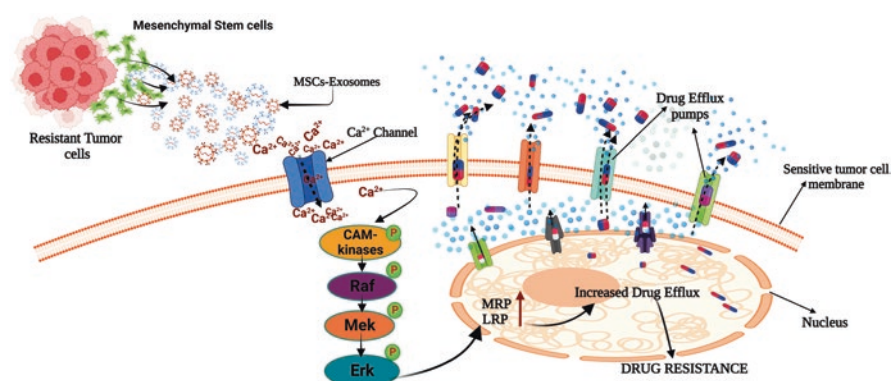


Fig. 1.3 Chemoresistance between cancer cells that are sensitive and resistant is mediated by exosomes. Through the integration of MDR transporters on the cell membrane, exosomes promote the efflux of drugs by transferring multidrug resistance (MDR) transporters from drug-resistant cells to drug-sensitive cells

Increased expression of TGF- β 1 in turn increases the expression of AKT, ERK, and STAT pathways, which shield the cancer cells from the various chemotherapeutic treatments (Zhang et al. 2017).

ECM

The ECM is another factor that is made up of fibrous proteins like collagen, elastin, and laminin as well as proteoglycans, microelements, and water, which is also responsible for therapeutic resistance (Martino et al. 2014; Walker et al. 2018). As compared to normal tissue, the ECM in tumor cells is substantially different in structure and composition. Many tumors exhibit fibroblastic/myofibroblastic infiltration, followed by the considerable buildup of collagenous ECM, which results in a phenomenon called desmoplasia. Desmoplastic stroma has been discovered to be the hindrance that causes drug resistance by obstructing anticancer drug delivery (Olive et al. 2009; Schober et al. 2014). Pancreatic cancerous cells when cultured in three-dimensional collagen demonstrated reduced responsiveness to gemcitabine treatment as well as enhanced proliferation (Sahai et al. 2013). Moreover, cells from ovarian malignancy that are resistant to cisplatin have been found to express COL11A1 (collagen type XI α 1) at elevated levels. Additionally, COL11A1 increases the chemoresistance of ovarian tumor cells by activating signal transduction pathways like the AKT and PDK1 pathways (Teng et al. 2014; Wu et al. 2015). Furthermore, the stromal cells promote drug resistance in the adjacent cells via a cell-cell interaction process known as “trogocytosis” that makes use of the integrin receptors found on cancerous cells and their communication with the ligands produced by the stromal cells. Eventually, the receptor-ligand communication stimulates intracellular signaling cascades like AKT, mTOR, NF- κ B, and STAT3 to maintain the modalities of drug resistance (Khan et al. 2022b; Jo et al. 2018).

Cell adhesion to the ECM has also been regarded as a significant determinant among the numerous TME components that affect cancer cell therapeutic resistance (Eke and Cordes 2015). Drug resistance caused by cell adhesion is dependent on the interplay between integrins and components of ECM, including laminin, collagen, and fibronectin. It has been shown that cisplatin treatment of ECMs that lack collagen or fibronectin makes cancerous cells about 40% more sensitive (Senthebane et al. 2018). Oxaliplatin’s effectiveness against colon cancer cells is also increased by suppressing the α v subunit of integrin (He et al. 2009). In esophageal cancer cells and AML, the PI3K/Akt pathway-driven upregulation of α 4 integrin is also the cause of therapeutic resistance (Cruz da Silva et al. 2019).

Exosomes

Exosomes, also known as extracellular vesicles (EVs), which are produced by big multivesicular bodies (MVBs), facilitate cell-to-cell interaction by transporting bio-active cargo, such as RNA, DNA, and proteins, as well as metabolites (Mashouri

et al. 2019). They are important signaling intermediaries in the TME, playing roles in tumor growth, TME remodeling, metastasis, angiogenesis, and treatment resistance (Yang et al. 2020b; Khan et al. 2023d). It has been discovered that exosomes can scavenge undesirable molecules in healthy cells and also regulate metabolic reprogramming. Cancerous cells hijack EVs for anticancer drug efflux, which in turn results in drug resistance. MSC-exosomes (mesenchymal stem cell-derived exosomes) integrate into gastric tumor cells, enhancing CaM-Ks (primarily CaM-KII as well as CaM-KIV) stimulation. This causes activation of downstream Raf/MEK/ERK signaling cascades, which then increases the expression of MDR-related proteins like multidrug resistance protein (MRP), multidrug resistance (MDR), and lung resistance-related protein (LRP), resulting in chemoresistance in gastric carcinoma (Fig. 1.4) (Ji et al. 2015). Ovarian cancer drug-resistant cells showed increased cisplatin exosomal export along with efflux transporters ATP7A, ATP7B, and MRP2 (ABCB2), which are the key proteins involved in drug efflux (Safaei et al. 2005).

Hypoxia and an Acidic Environment

Tumor's aberrant vasculature and high oxygen demand result in an acidic milieu with prolonged or diffusion-limited hypoxia, which reduces the availability of nutrients like glucose and vital amino acids (Zaidi et al. 2019). Additionally, tumor cells favor glycolysis over oxidative respiration, which usually converts glucose into lactic acid for the generation of ATP, resulting in successive acidic microenvironments, which are also known as the Warburg effect (Singh et al. 2017b). Oxygen deprivation stimulates the transcription factor known as a hypoxia-inducible factor (HIF)-1 α , which in turn regulates many genes implicated in several processes like angiogenesis, inhibition of apoptosis, and activation of drug efflux (Warfel and El-Deiry 2014; Xia et al. 2018). HIF-1 α has also been reported to both suppress pro-apoptotic proteins, such as TRAIL, and stimulate antiapoptotic proteins, like

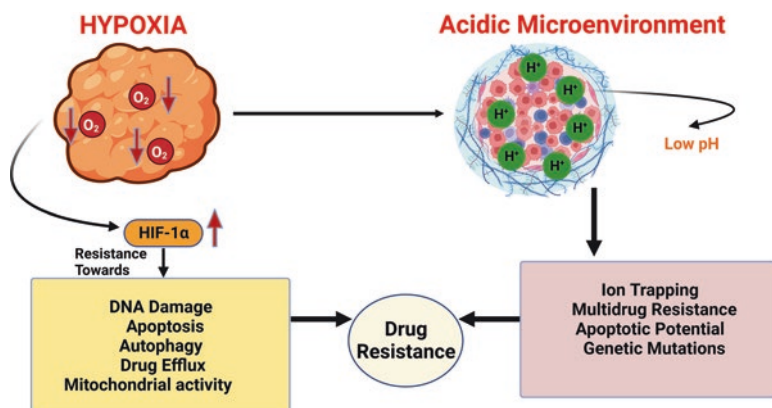


Fig. 1.4 An overview of the processes and pathways behind the HIF-mediated drug resistance

c-myc, survivin, TCF4, and STAT3, to improve tumor cells' survival in the presence of chemotherapies (Fig. 1.4) (Nishimoto et al. 2014; Zhao et al. 2016). Moreover, through the stimulation of HIF-1 α and NF- κ B as well as subsequent upregulation of Bcl-xL, hypoxia had also been shown to increase TMZ (temozolomide) resistance in GBM (glioblastoma multiforme) (Hassan et al. 2020; Chen et al. 2015).

The effectiveness of different treatments has also been shown to be affected by the TME's acidity. Due to the acidic external pH and nearly neutral or alkaline internal pH in tumors, the transportation of chemical agents across the membranes is affected (Khan et al. 2024). Therefore, drugs like doxorubicin, vincristine, mitoxantrone, and vinblastine that have acidic dissociation constants of 7.5–9.5 may have a markedly decreased rate of absorption (Khan et al. 2024; Zhou et al. 2019). As a result, these drugs' cytotoxic effect is reduced, which leads to resistant phenotypes (Nalli et al. 2019; Stepka et al. 2021). Additionally, an acidic TME modifies the metabolism, cellular proteome, and signaling cascades, promoting cancer cell stemness and treatment resistance (Khan et al. 2023e; Ahmad et al. 2022). Moreover, the acidic milieu stimulates p-glycoproteins (P-gp) activity by turning on p38 signaling, which results in multidrug resistance in rat prostate carcinoma cells (AT1) (Sauvant et al. 2008).

1.5 Immune Cells

The most prevalent immune cell types in TME are tumor-associated macrophages (TAM) (Balkwill et al. 2012). TAM invasion into TME has been linked to poor prognosis and inadequate response to chemotherapeutic agents in cancer patients (Halama et al. 2011; Lei et al. 2020; Mir et al. 2022b). TAMs are derived from circulatory Ly6C+CCR2+ monocytes. TAMs have a high degree of variability in TME and can be classified into two subgroups: classically stimulated pro-inflammatory M1 macrophages exhibiting anticancer characteristics and alternatively stimulated anti-inflammatory M2 macrophages having tumor-supporting capabilities (Liu and Cao 2015). The M2 phenotype generally promotes tumor development and treatment resistance, produces anti-inflammatory cytokines, and induces Th2 responses. In cancer conditions influenced by the microenvironment, polarization of macrophages from M1 to M2 is prevalent (Moradi-Chaleshtori et al. 2021). Under the influence of treatment, cancer cells stimulate TAMs to develop into immunosuppressive M2-polarized macrophages. M2 TAMs, in return, confer chemoresistance in cancerous cells via a variety of processes. It has been shown that ROS builds up in the gastric carcinoma cells after exposure to 5-FU causing HIF-1 α signaling to be activated and driving the production of HMGB1, which increases the migration of macrophages into the GC tumor. The recruited TAMs, in return, have a tumor-protected M2-type morphology and increase the chemoresistance of GC cells by generating growth differentiation factor 15 (GDF15), which enhances fatty acid β -oxidation in cancer cells (Yu et al. 2020).

To shield cancer cells from therapeutic attack, TAMs also secrete a variety of soluble factors into TME, such as interleukins, chemokines, etc. It has been shown

that treatment with cyclopamine, an inhibitor of the hedgehog pathway, enhanced the invasion of M2-type TAMs in breast carcinoma, reducing the effectiveness of chemotherapy because of the buildup of IL-6, which is responsible for the activation of various signaling cascades, which in turn alters the expression of many genes essential for cell cycle control, proliferation, and survival (Xu et al. 2019; Mir et al. 2023a, b). Furthermore, M2-polarized TAMs have been discovered to generate nitric oxide (NO), which shields cancer cells from cisplatin-induced death. The protective action of NO is dependent on the suppression of acid sphingomyelinase (A-SMase). NO acts by generating cyclic GMP and activating protein kinase G (PKG), which causes phosphorylation of syntaxin 4 (synt4), a protein involved in A-SMase transport and stimulation. PKG phosphorylation of synt4 causes proteasome-dependent destruction of synt4, limiting cisplatin-induced translocation of A-SMase to the cell membrane of cancer cells. This reduces cisplatin's cytotoxic action by lowering A-SMase-induced apoptosis (Perrotta et al. 2018). High infiltrations of tumor-associated neutrophils (TANs) within the TME have also been responsible for tumor growth as well as drug resistance (Incio et al. 2016). The polarization of neutrophils may influence their function in the TME. Like M1/M2 macrophage classification, TANs are also classified into anti-tumorigenic N1 and pro-tumorigenic N2 phenotypes based on functional variations (Zhang et al. 2020). Type I IFN- γ and TGF- β were thought to be crucial in the polarization of TANs changing from N1 to N2 (Pylaeva et al. 2016).

Tregs are a type of immunosuppressive T cells that are CD4+CD25+ and are distinguished by the Foxp3 expression that is required for Treg formation and differentiation. Elevated Treg invasion in the TME had been linked to worse prognosis as well as chemoresistance in glioblastoma, melanoma, colorectal, and renal cancer (Khan et al. 2023a; Liu et al. 2015; Wang et al. 2019; Long et al. 2020). It has been shown that 5-FU therapy increased the expression of CCL20 in colorectal carcinoma cells (CRC) in vivo by triggering FOXO1/CEBPB/NF- κ B signaling, which aided in the migration of Tregs into TME. Foxp3+ expression on Tregs is in turn linked to resistance-related genes such as ATP8A2, BCL2, VIM, and WNT1, which promote chemoresistance to 5-FU in CRC (Wang et al. 2019).

The platelet, a major regulator of thrombosis and hemostasis, is formed from common hematopoietic precursor cells (Contursi et al. 2017). Besides these processes, it has been shown to play an important role in the progression of cancer and therapeutic resistance (Contursi et al. 2017; D'Alessandro et al. 2015). Platelets lead to treatment resistance by secreting a variety of substances, changing chemotherapeutic pharmacokinetics, and activating a variety of signaling cascades. For example, TGF- β produced by platelets reduced cisplatin sensitivity in pancreatic carcinoma via stimulating PI3K/Akt and MEK/ERK pathways (Chen et al. 2013; Wani et al. 2021). By modifying the cancer cell cycle, DNA repair mechanisms, and MAPK levels, thrombospondin-1, clusterin, and RANTES, which are released by platelets, are shown to shield adenocarcinoma cells against anticancer agents including paclitaxel and 5-FU (Radziwon-Balicka et al. 2012). Furthermore, it has been observed that the effectiveness of platinum-based chemotherapy for NSCLC is diminished when platelet counts are high (Wang et al. 2018b). Additionally, women

with breast carcinoma having lower platelet-to-lymphocyte ratios that are administered with neoadjuvant chemotherapeutic treatment can obtain a better complete pathological response, regardless of the underlying tumor genetic subtype (Cuello-López et al. 2018).

1.6 Chemoresistance Mechanisms of Different Antineoplastic Drugs

Doxorubicin (DOX) is an anthracycline drug that is being used to treat several malignancies successfully (Thorn et al. 2011). Although DOX has demonstrated excellent efficacy in eliminating rapidly proliferating cells and slowing tumor progression in both solid and liquid tumors, drug resistance eventually develops during the DOX treatment, severely limiting its effectiveness as a cancer therapy (Table 1.1) (Thorn et al. 2011; Yang et al. 2014). It has been demonstrated that signaling cascades including PI3K and ERK/MAPK are mainly responsible for DOX resistance by stimulating replication and promoting cell cycle progression while inhibiting apoptotic and autophagic death of cells (Abrams et al. 2010; Christowitz et al. 2019). Autophagic and apoptotic death of cells is not initiated because specific proteins like Bcl-2, caspase-3/7/8/9, p62, and LC3-I/II, which are crucial for activating these processes are downregulated (Khan et al. 2023b; Christowitz et al. 2019). By increasing Akt phosphorylation, the PI3K axis has also been shown to support the development of tumors as well as DOX resistance (Abrams et al. 2010; Christowitz et al. 2019). There are three AKT isoforms (1, 2, and 3), and both phosphorylated and unphosphorylated versions of these isoforms are implicated in DOX resistance in endometrial carcinoma (Girouard et al. 2013). Additionally, constitutively activated Akt-Igene (CA)-expressing MCF-7 cells showed increased resistance to doxorubicin, 4-OH-tamoxifen (4HT), and etoposide compared to Akt-1 (CA)-deficient cells (Sokolosky et al. 2011). Drug resistance in numerous malignancies has also been linked to dual specificity phosphatase 4 (DUSP4), commonly called MAPK phosphatase 2 (MKP2). For example, in gastric cancer (GC) cells, upregulation of DUSP4 made them highly resistant to DOX. The cells became more sensitive to DOX when DUSP4 was knocked down (Kang et al. 2017). In addition, upregulation of DUSP4 induced the epithelial-mesenchymal transition (EMT) in GC cells; however, inhibiting the EMT with a Twist siRNA enhanced the DOX sensitivity of the GC cells and demonstrated that the EMT was responsible for DUSP4-induced DOX resistance (Kang et al. 2017). In addition to the signaling

Table 1.1 Different anticancerous drugs with their mechanism of action

	Drug	Drug class	Mechanism of action
1.	Doxorubicin	Anthracycline	DNA strand breakage and inhibition of topoisomerase II
2.	Paclitaxel	Taxol	Stabilizes microtubules by preventing depolymerization
3.	Cisplatin	Alkylating agent	Stop tumor growth by cross-linking guanine bases in DNA double-helix strands

pathways, multidrug resistance transporters also contribute to DOX chemotherapeutic resistance by increasing their expression under the exposure of treatment. When DOX is being used for the treatment of cancers including bladder cancer, esophageal squamous cell carcinoma, or breast cancer, MRP2 (cMOAT or ABCC2) mediates its efflux, which has been demonstrated to lead to DOX resistance (Tada et al. 2002; AbuHammad and Zihlif 2013; Yamasaki et al. 2011). Recent research has demonstrated that DOX administration to TNBC cells after primary tumor excision upregulates complement component expression in lung fibroblasts, attracting myeloid-derived suppressive cells (MDSCs), which support T cell dysfunction. However, therapeutic blockage of complement signaling in association with chemotherapy reversed chemotherapy-induced immunosuppressive activation and lessens metastatic recurrence (Monteran et al. 2022; Mir et al. 2022c) (Fig. 1.5).

Likewise, resistance to cisplatin is also a significant barrier in treatment. It has been shown that upon entry of CDDP into the cells, its chloride ions are substituted by water creating aquated entities that interact with nucleophilic sites within intracellular macromolecules (Table 1.1) (Kartalou and Essigmann 2001). While within the cell, it reacts with a range of molecules apart from DNA, such as sulfur-containing molecules like glutathione/metallothionein, which trap cisplatin and then eliminate it from the cell. The detox of ions of heavy metals is most probably facilitated by metallothionein (MT), which may also increase cisplatin resistance. Detoxification also involves glutathione (GSH) (Kartalou and Essigmann 2001). GSH produces a GSH-platinum complex in the presence of CDDP, which is then removed from the cells (Kartalou and Essigmann 2001). It is also well established that the oncoproteins like c-Myc, as well as tumor suppressor proteins including p73 and p53 that serve as transcription factors, alter cellular responsiveness toward CDDP. Zinc-finger factor 143 (ZNF143), Y-box binding protein-1 (YB-1), activating transcription factor 4 (ATF4), CCAAT-binding transcription factor 2 (CTF2), and mitochondrial transcription factor A (mtTFA) are a few transcription factors

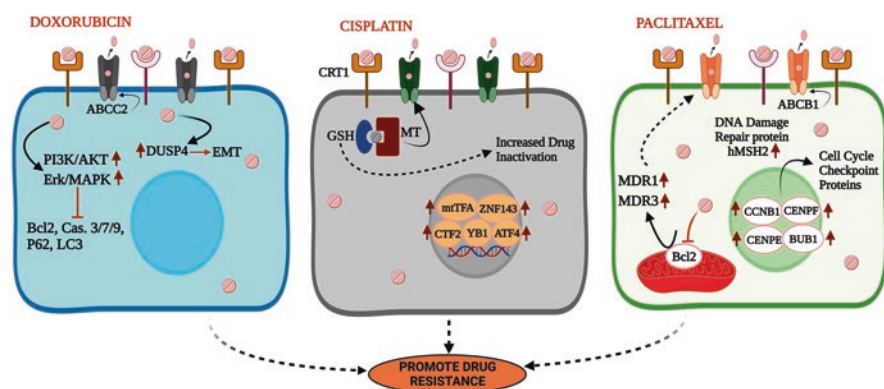


Fig. 1.5 The figure elucidates the intricate interplay between different classes of anticancer drugs and the mechanisms through which they contribute to the development of drug resistance in cancer cells

that have been linked to CDDP resistance (Torigoe et al. 2005). The removal of cisplatin-DNA adducts and the repair of cisplatin-induced DNA damages are alternate mechanisms through which cancerous cells may develop resistance to cisplatin. Some DNA repair proteins (e.g., the product of the XRCC1 gene, YB1, etc.) are responsible for such processes (Torigoe et al. 2005; Wang et al. 2004). Moreover, NF- κ B activation also contributes to cisplatin resistance in pancreatic cancer, and it has been proved that pretreatment of pancreatic cancer cells with genistein reduces NF- κ B action and helps to enhance the apoptosis-inducing impact of cisplatin, resulting in a greater antineoplastic activity *in vivo* (Florea and Büsselberg 2011).

Another drug is paclitaxel (PTX), which belongs to the taxane class of anticancer agents and develops resistance (Table 1.1). Although tubulin is the primary target for PTX, it has additionally been discovered to attack the mitochondria and block the activity of the apoptotic inhibitor protein Bcl-2 (B-cell leukemia 2) (Barbuti and Chen 2015). Like other antineoplastic drugs, PTX also contributes to chemoresistance. Changes in membrane lipids, modified cell cycle checkpoint proteins, metabolism (bio-inactivation), a rise or modification in drug targets, hindered apoptotic response proteins (Bcl-2), enhanced DNA damage repair, elevated efflux, and reduced uptake (via dysregulation of a receptor) are molecular and cellular processes that contribute to PTX chemoresistance (Barbuti and Chen 2015). The upregulation of ABCB1, also known as P-gp or MDR1, is a key factor in paclitaxel resistance. For example, it has been shown that PTX-resistant ovarian carcinoma cell lines overexpress ABCB1 (Duan et al. 2004). In these PTX-resistant OCCs (i.e., OVCAR8TR, and SKOV-3TR), ABCB1 RNA was targeted using siRNAs to assess the function of this transporter in such cells. The cell lines were treated with chemically created siRNAs or transfected with certain vectors that express specific siRNAs, and the results showed that ABCB1 protein and mRNA levels were reduced. The resistance to PTX was reduced by 7–12.4 fold in the treated cell lines (Duan et al. 2004). The alteration of microtubule dynamics is another key paclitaxel resistance factor. In PTX-resistant cells, it has been discovered that the protein expression of microtubule-associated proteins has changed. A class of motor proteins called kinesins in general supports intracellular movement and mitosis by moving along microtubules (Oliver 2012). Cancer cells that are resistant to paclitaxel have been found to have an overexpressed form of the motor protein MCAK, which is crucial for accelerating the depolymerization of microtubules (Ganguly et al. 2011). The anticancer effect of paclitaxel is hindered by the upregulation of such depolymerization motor proteins, which interfere with its ability to stabilize and polymerize microtubules.

1.7 Conclusion

In conclusion, this chapter has provided a comprehensive overview of the intricate mechanisms of drug resistance in cancer. We have explored the multifaceted mechanisms that cancer cells employ to evade the destructive power of chemotherapy, highlighting the complexity of this biological phenomenon. From genetic

mutations to microenvironmental adaptations, the resilience of cancer cells continues to challenge therapeutic strategies. However, among these challenges lies a beacon of hope. The ever-evolving landscape of cancer research has yielded promising insights into novel approaches to combat drug resistance. Targeted therapies, immunotherapies, and precision medicine are revolutionizing cancer treatment by addressing the specific vulnerabilities of cancer cells and harnessing the power of the immune system.

MCQs

1. **What is the impact of modifications to the molecular target of drugs on their effectiveness in malignancies?**
 - A. Enhancement of drug efficacy
 - B. No impact on drug effectiveness
 - C. Increased drug resistance
 - D. Rapid drug elimination

Answer C: Increased drug resistance

2. **Which enzyme is targeted by specific anticancer medications to prevent over- or under-coiling of DNA?**
 - A. DNA polymerase
 - B. RNA polymerase
 - C. Topoisomerase II
 - D. Helicase

Answer C: Topoisomerase II

3. **How do topoisomerase II inhibitors induce DNA damage in cancer cells?**
 - A. Inducing DNA over-coiling
 - B. Stabilizing the DNA-topoisomerase II complex
 - C. Inhibiting DNA synthesis
 - D. Promoting mitotic processes

Answer B: Stabilizing the DNA-topoisomerase II complex

4. **In breast cancer, what is the primary role of tamoxifen?**
 - A. DNA synthesis inhibition
 - B. Growth factor receptor activation
 - C. Estrogen receptor antagonism
 - D. Topoisomerase II inhibition

Answer C: Estrogen receptor antagonism

5. **How can breast cancer cells develop tamoxifen resistance?**
 - A. Enhanced estrogen-blocking effect
 - B. Inhibition of growth factor receptors
 - C. Reduction in HER2 expression
 - D. Interaction with additional growth signaling cascades

Answer D: Interaction with additional growth signaling cascades

6. **What effect can occur in breast cancers with HER2 amplification when treated with tamoxifen?**
 - A. Increased estrogen-blocking effect
 - B. Inhibition of tumor growth

- C. Agonist-like effect leading to tumor stimulation
- D. DNA damage induction

Answer C: Agonist-like effect leading to tumor stimulation

7. What is a well-studied process in cancer treatment resistance that involves reducing drug accumulation?

- A. Drug activation
- B. Drug efflux through ABC transporters
- C. Inhibition of tyrosine kinase
- D. Metabolic degradation

Answer B: Drug efflux through ABC transporters

8. What is the common feature shared by all members of the ABC transporter family?

- A. Identical transmembrane domains
- B. Variable nucleotide-binding domains
- C. Conserved nucleotide-binding domain and variable transmembrane domain
- D. Absence of nucleotide-binding domains

Answer C: Conserved nucleotide-binding domain and variable transmembrane domain

9. How is substrate transportation across the cell membrane facilitated by ABC transporters?

- A. Substrate binding to the nucleotide-binding domain
- B. Substrate binding to the transmembrane domain
- C. ATP hydrolysis at the transmembrane domain
- D. ATP hydrolysis at the nucleotide-binding domain

Answer B: Substrate binding to the transmembrane domain

10. How does the expression of MDR1 change in response to doxorubicin treatment in lung carcinoma cells?

- A. Decreased expression
- B. No significant alteration
- C. Substantial rise in expression
- D. Inhibition of expression

Answer C: Substantial rise in expression

11. How do cancer cells acquire resistance to therapies that require drug activation?

- A. Upregulation of drug activation pathways
- B. Downregulation or mutations in drug activation pathways
- C. Increased drug accumulation
- D. Enhanced drug efflux

Answer B: Downregulation or mutations in drug activation pathways

12. Which system involves the activation or inactivation of drugs, and its polymorphic nature contributes to drug resistance in cancers?

- A. ABC transporters
- B. Tyrosine kinase system
- C. Cytochrome P450 (CYP) system
- D. Nucleotide-binding domain system

Answer C: Cytochrome P450 (CYP) system

13. How does the tumor microenvironment (TME) contribute to treatment resistance?

- A. By inhibiting drug delivery
- B. By promoting drug responsiveness
- C. By reducing cellular communication
- D. By increasing drug delivery

Answer A: By inhibiting drug delivery

14. What is the role of cancer-associated fibroblasts (CAFs) in therapeutic resistance?

- A. They inhibit drug-induced damage to DNA
- B. They enhance drug efflux in cancer cells
- C. They increase drug sensitivity in cancer cells
- D. They inhibit Akt and MAPK pathways

Answer B: They enhance drug efflux in cancer cells

15. What role do exosomes play in treatment resistance in gastric carcinoma?

- A. They enhance CaM-Ks stimulation
- B. They inhibit Raf/MEK/ERK signaling cascades
- C. They decrease the expression of multidrug resistance proteins
- D. They scavenge undesirable molecules in healthy cells

Answer A: They enhance CaM-Ks stimulation

16. Which pathway is upregulated by cancer cells in esophageal cancer and AML, contributing to therapeutic resistance?

- A. PI3K/Akt pathway
- B. Raf/MEK/ERK pathway
- C. NF- κ B pathway
- D. JAK/STAT pathway

Answer A: PI3K/Akt pathway

17. How do cancer cells utilize extracellular vesicles (exosomes) for drug resistance?

- A. By inhibiting CaM-Ks stimulation
- B. By promoting drug efflux
- C. By suppressing MDR-related proteins
- D. By decreasing exosomal export

Answer B: By promoting drug efflux

18. What is the Warburg effect in the context of tumors?

- A. Increased oxidative respiration
- B. Conversion of lactic acid to glucose
- C. Preference for glycolysis over oxidative respiration
- D. Activation of HIF-1 α

Answer C: Preference for glycolysis over oxidative respiration

19. What transcription factor is stimulated by oxygen deprivation in tumors, regulating genes involved in angiogenesis and drug efflux?

- A. NF- κ B
- B. c-myc

C. HIF-1 α

D. STAT3

Answer C: HIF-1 α

20. **How does hypoxia contribute to drug resistance in glioblastoma multiforme (GBM)?**

A. Activation of MAPK pathways

B. Upregulation of Bcl-xL

C. Inhibition of HIF-1 α

D. Reduction of TMZ resistance

Answer B: Upregulation of Bcl-XI

21. **How does the acidity of the tumor microenvironment affect drug absorption?**

A. It increases drug absorption

B. It does not affect drug absorption

C. It decreases drug absorption

D. It stabilizes drug molecules

Answer C: It decreases drug absorption

22. **What is the role of tumor-associated macrophages (TAMs) in chemoresistance?**

A. They enhance drug absorption

B. They induce apoptosis in cancer cells

C. They produce soluble factors that confer chemoresistance

D. They inhibit HIF-1 α signaling

Answer C: They produce soluble factors that confer chemoresistance

23. **What is the role of tumor-associated neutrophils (TANs) in the tumor microenvironment?**

A. They induce apoptosis in cancer cells

B. They enhance drug sensitivity

C. They promote tumor growth and drug resistance

D. They suppress immune responses

Answer C: They promote tumor growth and drug resistance

24. **What is the significance of Tregs (regulatory T cells) in chemoresistance?**

A. They enhance chemosensitivity

B. They lead to immune activation

C. They are associated with better prognosis

D. They increase chemoresistance by promoting Treg migration

Answer D: They increase chemoresistance by promoting Treg migration

25. **Besides targeting tubulin, what additional cellular component does paclitaxel attack to inhibit apoptotic responses?**

A. Mitochondria

B. Endoplasmic reticulum

C. Nucleus

D. Golgi apparatus

Answer A: Mitochondria

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Mechanisms of Cancer Resistance to Various Therapies

2

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Abstract

Cancer therapy has witnessed remarkable advancements in eradicating cancer cells within the body, leading to improved patient outcomes and prolonged survival. This chapter provides an overview of current cancer therapies, encompassing radiation therapy, chemotherapy, targeted therapy, immunotherapy, and emerging therapeutic modalities such as viral and gene therapy. This chapter explores the intricate molecular mechanisms underpinning resistance in cancer and discusses contemporary therapeutic approaches designed to combat resistance against chemotherapy, radiation therapy, and immunotherapy. In conclusion, cancer therapy has evolved significantly, offering diverse treatment options. Continued research, clinical trials, and multidisciplinary collaboration are essential for further refining and optimizing cancer therapies, leading to improved outcomes and patient quality of life. Understanding resistance mechanisms and developing innovative therapeutic strategies are crucial for enhancing patient outcomes, making ongoing research and clinical trials indispensable.

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2.1 Introduction

Cancer therapy encompasses a broad range of treatment modalities aimed at eradicating or controlling cancer cells in the body. Over the years, significant improvements have been made in the field of cancer therapy, leading to improved outcomes and prolonged survival for many patients (Debela et al. 2021; Khan et al. 2022a). This chapter provides an overview of current cancer therapies, including radiation therapy, chemotherapy, targeted therapy, immunotherapy, and emerging therapies such as viral and gene therapy. This chapter also explores the molecular mechanisms underlying resistance in cancer and discusses the current therapeutic approaches targeting chemo, radio, and immune resistance.

Surgery remains a cornerstone of cancer treatment, offering curative potential for localized tumors. It involves the removal of cancerous tissues, lymph nodes, and sometimes adjacent structures. The development of minimally invasive techniques and robotic-assisted surgery has enhanced surgical precision and reduced patient morbidity (Deboever et al. 2022; Khan et al. 2023a).

Chemotherapy involves the administration of drugs that target rapidly dividing cancer cells. Traditional chemotherapy drugs, as well as newer agents, have been developed to improve treatment efficacy and reduce side effects (Sameer et al. 2024; Khan et al. 2023b). Chemotherapy resistance can result from alterations in drug uptake, efflux pumps, drug metabolism, and DNA repair mechanisms. Additionally, dysregulation of apoptosis pathways, stimulation of survival signaling pathways such as PI3K/Akt and MAPK, and epithelial-to-mesenchymal transition (EMT) contribute to chemotherapy resistance. Current strategies to combat chemotherapy resistance involve combination therapies, targeted agents, and novel drug delivery systems (Xu et al. 2022; Mintoo et al. 2021).

Immunotherapy harnesses the power of the immune system to recognize and attack cancer cells (Yang 2015a). Immune checkpoint inhibitors, chimeric antigen receptor (CAR) T-cell therapy, and cancer vaccines are revolutionizing cancer treatment by enhancing immune responses against tumors (Al-Haideri et al. 2022). Immunotherapy resistance can arise from immune checkpoint signaling pathways, tumor immune evasion mechanisms, and immunosuppressive tumor microenvironment. Dysregulation of PD-1/PD-L1, CTLA-4, and additional immune checkpoint molecules can hinder antitumor immune responses. Strategies to overcome immunotherapy resistance include combination immunotherapies, the development of novel checkpoint inhibitors, and modulation of the tumor microenvironment (Haibe et al. 2020).

Targeted therapy specifically targets molecular alterations in cancer cells, such as gene mutations or overexpressed proteins. These therapies can inhibit specific signaling cascades essential for tumor growth and survival. Monoclonal antibodies,

small molecule inhibitors, and immune checkpoint inhibitors are examples of targeted therapies that have shown remarkable efficacy in specific cancer types (Khan et al. 2023b; Wilkes 2018).

Radiation therapy uses high-energy radiation to kill cancer cells or inhibit their growth. It can be delivered externally (external beam radiation) or internally (brachytherapy). Advances in radiotherapy techniques, including intensity-modulated radiation therapy (IMRT) and stereotactic body radiation therapy (SBRT), have enabled precise targeting of tumors while sparing surrounding healthy tissues (Baskar et al. 2012). Resistance to radiation therapy can be attributed to DNA damage repair mechanisms, altered cell cycle checkpoints, and increased anti-oxidant defense systems. Stimulation of pro-survival signaling cascades, such as NF- κ B and PI3K/Akt, and the presence of cancer stem cells also contribute to radio-resistance (Liu et al. 2021; Hassan et al. 2020). Overcoming radiation resistance involves approaches such as combining radiation with targeted therapies, radio sensitizers, and immunomodulatory agents. Combination therapies, including the use of different treatment modalities or combining multiple drugs, are being extensively explored to maximize treatment efficacy and overcome resistance mechanisms. Additionally, supportive care measures and integrative therapies play a vital role in managing treatment side effects and improving patients' quality of life (Elbanna et al. 2021).

Emerging therapies like precision medicine and gene therapy hold promise for personalized cancer treatment. Precision medicine utilizes genomic profiling and molecular diagnostics to tailor treatment strategies based on individual patient characteristics. Gene therapy includes the introduction of genetic material into cancer cells to modify their behavior, enhance immune responses, or sensitize them to other treatments (Khan et al. 2023a; Berger and Mardis 2018).

In conclusion, cancer therapy has evolved significantly, offering a diverse range of treatment options for patients. Advances in surgery, radiation therapy, chemotherapy, targeted therapy, immunotherapy, and emerging therapies have revolutionized cancer treatment approaches. Continued research, clinical trials, and multidisciplinary collaboration are essential to further refine and optimize cancer therapies, leading to enhanced outcomes and better quality of life for cancer patients. Understanding the molecular mechanisms underlying resistance and developing innovative therapeutic strategies are essential for improving patient outcomes. Continued research and clinical trials are needed to identify predictive biomarkers, optimize treatment regimens, and develop novel therapies to overcome resistance to cancer.

2.2 Chemotherapy-Molecular Mechanisms Underlying Resistance in Cancer

Chemotherapy plays a vital role in the management of various cancer types, either as a primary treatment option or in combination with other modalities. It includes the utilization of cytotoxic drugs to target and kill cancerous cells, impeding their

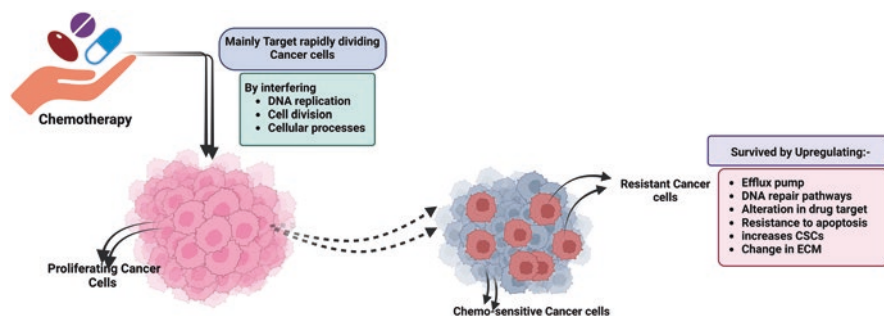


Fig. 2.1 The figure depicts the molecular mechanisms underlying resistance in cancer

growth and spread (Sameer et al. 2024; Khan et al. 2022b). The mechanisms of action of chemotherapy involve targeting rapidly dividing cancer cells by interfering with DNA replication, cell division, and cellular processes essential for tumor growth. Various classes of chemotherapy drugs, such as antimetabolites, alkylating agents, anthracyclines, taxanes, and platinum-based compounds, are employed based on the specific characteristics of the cancer being treated (Fig. 2.1) (Tilsed et al. 2022).

Chemotherapy is a common treatment option for many types of cancer, but one of the major challenges in cancer treatment is the development of resistance to chemotherapy drugs. Resistance to chemotherapy can occur via several mechanisms, which are complex and multifactorial. Chemotherapy resistance is a major hurdle in cancer treatment (Zhang et al. 2023; Wadhwa et al. 2020). There are several mechanisms by which cancer cells develop resistance to chemotherapy. Here are some of the most common mechanisms:

Efflux Pumps and Chemotherapy

Efflux pumps are one of the common mechanisms by which cancer cells develop resistance to chemotherapy drugs. These pumps actively pump out chemotherapy drugs out of cancer cells, reducing the intracellular concentration of the drugs and limiting their effectiveness (Ughachukwu et al. 2012; Khan et al. 2023c). One of the most well-known efflux pumps is P-glycoprotein, which is encoded by the MDR1 gene. P-glycoprotein is expressed in many types of cancer cells and is responsible for pumping out a wide range of chemotherapy drugs, including anthracyclines, taxanes, and vinca alkaloids. The overexpression of P-glycoprotein in cancer cells is often associated with a poor response to chemotherapy and a worse prognosis (Mintoo et al. 2021; Waghray and Zhang 2017). Other efflux pumps that have been implicated in chemotherapy resistance include the multidrug resistance-associated protein (MRP) and the breast cancer resistance protein (BCRP). These pumps can also pump out a range of chemotherapy drugs and contribute to the development of resistance (Deng et al. 2020; Zhang et al. 2020; Khan et al. 2023d). Efflux pumps

are regulated by a complex network of signaling cascades, including the PI3K/AKT, MAPK, and NF- κ B pathway. These pathways can be activated by various stimuli, such as growth factors, cytokines, and stress signals, leading to the upregulation of efflux pumps and the development of resistance (Katayama et al. 2014; Lampada et al. 2017; Tazzari et al. 2007). To overcome efflux pump-mediated resistance, several strategies have been developed. One approach is to use efflux pump inhibitors, which can block the activity of the pumps and increase the intracellular concentration of chemotherapy drugs. Examples of efflux pump inhibitors include verapamil, cyclosporin A, and tariquidar (Engle and Kumar 2022). However, clinical trials of efflux pump inhibitors have shown mixed results, and further research is needed to determine their efficacy. Another approach is to use alternative chemotherapy drugs that are not substrates for efflux pumps. For example, liposomal formulations of chemotherapy drugs can bypass efflux pumps and deliver the drugs directly to the cancer cells. Nanoparticle-based drug delivery systems, which can selectively target cancer cells and release drugs at the site of the tumor, are also being developed as a way to overcome efflux pump-mediated resistance (Waghray and Zhang 2017; Mir et al. 2022; Nalli et al. 2019).

DNA Repair and Chemotherapy

Chemotherapy drugs work by damaging the DNA of cancer cells, which leads to cell death. However, some cancer cells can develop resistance to chemotherapy by activating DNA repair pathways that can fix the DNA damage caused by the drugs (Li et al. 2021a). One of the most well-known DNA repair pathways is the homologous recombination (HR) pathway. This pathway repairs double-strand breaks in DNA, which can be caused by chemotherapy drugs such as platinum-based agents and topoisomerase inhibitors. Cancer cells can activate the HR pathway by upregulating the expression of genes implicated in the pathway, such as BRCA1 and BRCA2. Mutations in such genes are also linked with an elevated risk of breast and ovarian cancer and can confer resistance to chemotherapy (Wadhwa et al. 2020; Li et al. 2021a). Another DNA repair pathway that can contribute to chemotherapy resistance is the nucleotide excision repair (NER) pathway. This pathway repairs DNA damage caused by chemotherapy drugs such as alkylating agents and platinum-based agents. Cancer cells can upregulate the expression of genes involved in the NER pathway, such as XPA and XPG, which can increase their ability to repair the DNA damage caused by the drugs (Duan et al. 2020).

To overcome DNA repair-mediated resistance to chemotherapy, several strategies have been developed. One approach is to combine chemotherapy drugs with inhibitors of DNA repair pathways. For example, inhibitors of the PARP enzyme, which is involved in the repair of single-strand DNA breaks, are effective in treating breast and ovarian cancers with BRCA mutations. These inhibitors can prevent the cancerous cells from repairing the DNA damage caused by chemotherapy drugs, leading to cell death (Lodovichi et al. 2020; Brown et al. 2017).

Alteration in Drug Targets and Chemotherapy

Chemotherapy drugs target specific proteins or pathways that are essential for the survival and growth of cancerous cells. However, cancer cells can develop resistance to chemotherapy by altering the drug targets, either by mutations in the target proteins or by changing the expression of the proteins (Cree and Charlton 2017; Turajlic et al. 2014). One example of alteration in drug targets is EGFR gene mutation in non-small cell lung cancer (NSCLC). EGFR is a target for several chemotherapy drugs, such as tyrosine kinase inhibitors and monoclonal antibodies. However, mutations in the EGFR gene can result in constitutive stimulation of the EGFR pathway, making the cancer cells resistant to these drugs (Harrison et al. 2020). In such cases, alternative chemotherapy drugs or combination therapy with EGFR inhibitors may be used to overcome resistance.

Another example of alteration in drug targets is the increased expression of the antiapoptotic protein like Bcl-2 in some types of cancer, such as lymphoma and leukemia. Chemotherapy drugs such as venetoclax target Bcl-2 to induce cancer cell death. However, cancer cells can develop resistance to these drugs by upregulating the expression of Bcl-2 or by overexpressing other antiapoptotic proteins, such as Mcl-1. Combination therapy with venetoclax and Mcl-1 inhibitors has shown favorable outcomes in preclinical trials (Liu et al. 2022). In addition to genetic alterations in drug targets, cancer cells can also develop resistance by changing the expression of the target proteins. For example, HER2 overexpression in breast cancer is a target for chemotherapy drugs such as trastuzumab and pertuzumab. However, cancer cells can develop resistance to these drugs by downregulating the expression of HER2 (Irie et al. 2020). In such cases, combination therapy with chemotherapy drugs and HER2 inhibitors may be used to overcome resistance.

To overcome resistance due to alteration in drug targets, several strategies have been developed. One approach is to use combination therapy with multiple chemotherapy drugs that target different pathways or proteins. This can prevent the cancer cells from developing resistance to a single drug target (Hu et al. 2016). Another approach is to use alternative chemotherapy drugs or targeted therapies that are effective against the altered drug targets. Finally, the development of personalized medicine approaches, such as genomic profiling and biomarker analysis, can help to identify the specific alterations in drug targets that are responsible for resistance and guide the selection of the most effective treatment options for individual patients.

Resistance to Apoptosis and Chemotherapy

Apoptosis, or programmed cell death, is a natural process that eliminates damaged or abnormal cells from the body. Chemotherapy drugs work by inducing apoptosis in cancer cells, but some cancer cells can develop resistance to apoptosis, which can contribute to chemotherapy resistance (Mohammad et al. 2015). One mechanism by which cancer cells can develop resistance toward apoptosis is by upregulating the expression of antiapoptotic proteins. These proteins, such as Bcl-xL, Bcl-2, and

Mcl-1, inhibit apoptosis by blocking the action of pro-apoptotic proteins such as Bax and Bak (Lee et al. 2019). Cancer cells can also downregulate the expression of pro-apoptotic proteins, such as p53, which is a crucial regulator of apoptosis. This results in a decreased sensitivity of the cancer cells to chemotherapy-induced apoptosis (Hientz et al. 2017).

Another mechanism by which cancer cells become resistant to apoptosis is by activating survival pathways including the PI3K/AKT and MAPK/ERK pathways. These pathways regulate cell survival and proliferation and can promote resistance to chemotherapy-induced apoptosis by impeding pro-apoptotic proteins and stimulating antiapoptotic proteins (Rascio et al. 2021; Lee et al. 2020).

To overcome resistance to apoptosis, several strategies have been developed. One approach is to use combination therapy with chemotherapy drugs and agents that can overcome resistance to apoptosis. For example, inhibitors of antiapoptotic proteins, such as navitoclax, have been developed to increase the effectiveness of chemotherapy drugs (Tan et al. 2011). In addition, agents that activate pro-apoptotic proteins, such as BH3 mimetics, have been developed to induce apoptosis in cancer cells (Townsend et al. 2021).

Another approach is to use alternative chemotherapy drugs or targeted therapies that are effective against cancer cells with altered apoptotic pathways. For example, immune checkpoint inhibitors can induce apoptosis in cancer cells by activating the immune system to recognize and attack the cancerous cells (Lao et al. 2022). In addition, targeted therapies that inhibit the PI3K/AKT or MAPK/ERK pathways can sensitize cancer cells to chemotherapy-induced apoptosis (Lee et al. 2020; He et al. 2021).

Tumor Microenvironment and Chemotherapy

The tumor microenvironment (TME) is a multifaceted and dynamic ecosystem that comprises cancer cells, stromal cells, extracellular matrix, and immune cells. The TME plays a critical role in cancer progression and response to chemotherapy (Xiao and Yu 2021; Henke et al. 2020). Several factors in the TME can contribute to chemotherapy resistance, including hypoxia, acidic pH, and the presence of immune cells and cancer-associated fibroblasts (CAFs) (Jing et al. 2019; Khalaf et al. 2021; Ward et al. 2020; Mao et al. 2021). Solid tumors frequently exhibit hypoxia or low oxygen levels. Hypoxic cancer cells become resistant to chemotherapy by activating survival pathways, like PI3K/AKT and HIF-1 α pathways. These pathways promote cell proliferation and survival and can inhibit apoptosis and drug-induced cell death (Sebestyén et al. 2021; Muz et al. 2015). The acidic pH of the TME is another factor that can contribute to chemotherapy resistance. Acidic pH can decrease the influx of chemotherapy drugs by cancer cells and can also inhibit the activity of chemotherapy drugs. In addition, acidic pH can promote the proliferation and survival of cancer cells, making them more resistant to chemotherapy (Ward et al. 2020).

Immune cells in the TME, such as tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs), can also contribute to chemotherapy

resistance. TAMs can promote cancer cell survival and proliferation and can inhibit the activity of chemotherapy drugs. MDSCs can suppress the immune response and promote cancer cell survival and proliferation (De Cicco et al. 2020; Chen et al. 2019). CAFs are another stromal cell type in the TME that can contribute to chemotherapy resistance. CAFs can promote cancer cell survival and proliferation and can also remodel the extracellular matrix, making it more difficult for chemotherapy drugs to penetrate the tumor (Asif et al. 2021).

To overcome resistance in the TME, several strategies have been developed. One approach is to use combination therapy with chemotherapy drugs and agents that target the TME, such as hypoxia-activated prodrugs and agents that normalize the acidic pH of the TME (Feng et al. 2019; Li et al. 2021b). In addition, immune checkpoint inhibitors can be used to activate the immune response and enhance the efficacy of chemotherapy (Liu et al. 2020). Another approach is to use targeted therapies that specifically target cells in the TME, such as CAFs. For example, agents that target the CAFs in the TME, such as halofuginone and pifrenidone, have shown promising results in preclinical studies (Wang et al. 2022).

Cancer Stem Cells and Chemotherapy

Cancer stem cells (CSCs) are a small subpopulation of cancer cells with self-renewal capacity and the ability to differentiate into multiple cell types within a tumor. CSCs are believed to play a critical role in cancer progression and recurrence and have been implicated in chemotherapy resistance (Khan et al. 2023b; Yu et al. 2012). One mechanism by which CSCs can develop resistance to chemotherapy is by upregulating drug efflux pumps, such as ABC transporters. These transporters can pump chemotherapy drugs out of the CSCs, making them less sensitive to the drugs (Begicevic and Falasca 2017). In addition, CSCs can activate survival pathways, such as the PI3K/AKT pathway, which can promote cell survival and inhibit apoptosis (Yoon et al. 2021).

Another mechanism by which CSCs can develop resistance to chemotherapy is by undergoing a quiescent or dormant state. This state can protect the CSCs from the cytotoxic effects of chemotherapy drugs, as the drugs are most effective against rapidly dividing cells (Khan et al. 2023d; Basu et al. 2022). To overcome resistance in CSCs, several strategies have been developed. One approach is to use combination therapy with chemotherapy drugs and agents that specifically target CSCs. For example, agents that target CSC-specific markers, such as CD44 or CD133, have shown potential results in preclinical trials (Desai et al. 2019). In addition, agents that inhibit survival pathways, such as the PI3K/AKT pathway, can sensitize CSCs to chemotherapy-induced apoptosis (Wadhwa et al. 2020; Yang et al. 2020a; Budillon et al. 2019). Another approach is to use alternative chemotherapy drugs or targeted therapies that are effective against CSCs. For example, PARP inhibitors are effective against CSCs by inhibiting DNA repair pathways, which are critical for CSC survival (Gillespie et al. 2023).

2.3 Immunotherapy in Cancer Treatment

Immunotherapy has emerged as a breakthrough approach to cancer treatment, utilizing the immune system’s intrinsic ability to recognize and remove cancer cells (Sahu and Suryawanshi 2021). This treatment modality aims to stimulate or restore the immune system’s capability to identify and attack cancer cells, or employs immune system components, such as antibodies, to specifically target cancer cells (Mukherjee et al. 2022). By enhancing immune recognition and activation, immunotherapy holds promise for a wide range of cancer types. Several types of immunotherapy are used for the treatment of cancer such as immune checkpoint inhibitors, adoptive cell therapy, cancer vaccines, etc. (Fig. 2.2).

Immune Checkpoint Inhibitors

Immune checkpoint inhibitors are a type of immunotherapy that has shown remarkable success in the treatment of various cancer types such as bladder cancer, lung cancer, melanoma, and renal cell carcinoma (Shiravand et al. 2022). Immune checkpoint inhibitors work by blocking certain proteins on cancer cells or immune cells, known as immune checkpoint proteins, which can suppress the immune system’s capacity to identify and kill cancer cells. Immune checkpoint inhibitors work by

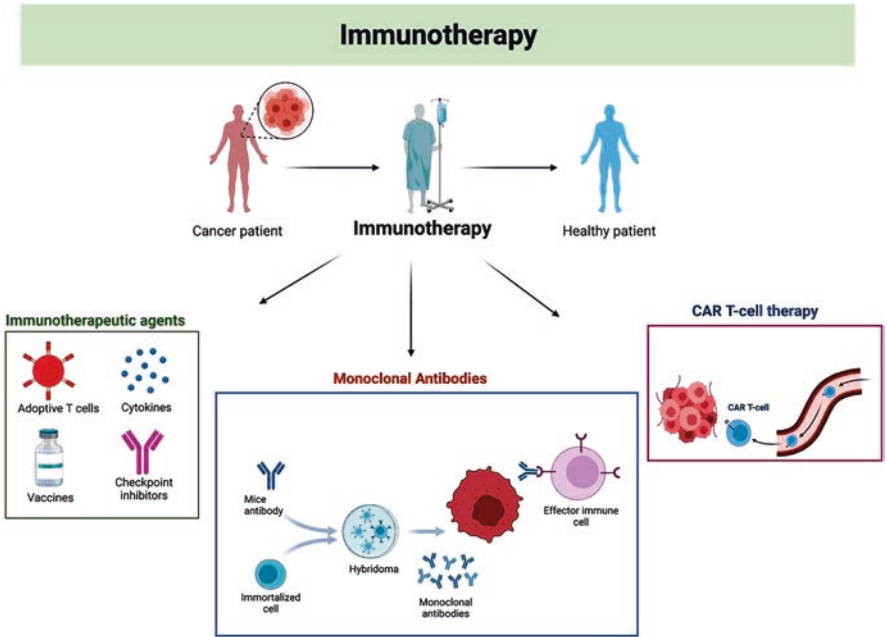


Fig. 2.2 The figure illustrates how immunotherapy enhances the inherent power of the immune system to identify and eliminate cancer cells

preventing these proteins from communicating with the immune system, which makes it easier for the immune system to identify and combat cancer cells (Shiravand et al. 2022).

The most well-known immune checkpoint proteins are PD-1 and CTLA-4. Immune checkpoint inhibitors that target PD-1 or its ligand, PD-L1, have been authorized for the treatment of various types of cancers, such as bladder cancer, melanoma, and lung cancer (Wojtkowiak et al. 2011; Jiang et al. 2019).

Immune checkpoint inhibitors have shown promising results in clinical trials, with some patients attaining long-term remission and improved survival. However, not all patients respond to immune checkpoint inhibitors, and some may experience adverse effects, like fatigue, rash, and immune-related adverse events (Naimi et al. 2022).

CAR T-Cell Therapy

This is a type of cellular therapy in which T cells are taken from the blood of the patient, genetically engineered to identify and attack cancer cells, and then infused back into the patient's body. CAR T-cell therapy has shown noteworthy success in the treatment of certain types of blood cancers, such as B-cell lymphomas and leukemias (Fig. 2.2) (Hasegawa and Hosen 2019). There are various processes involved in the CAR T-cell treatment process. Initially, the patient's blood is used to extract T cells, which are then genetically modified to express chimeric antigen receptors (CARs) on their surface in a lab. These CARs are made to identify particular antigens present on the outside of cancerous cells. After being created, the CAR T cells are multiplied in a lab to create a substantial amount of cells and subsequently reinfused into the patient. Once inside the patient's body, the CAR T cells can recognize and bind to cancer cells that express the targeted antigen. This triggers the CAR T cells to become activated and attack the cancer cells, ultimately leading to their destruction (Amini et al. 2022; Beauvais et al. 2020). CAR T-cell therapy has shown remarkable success in clinical trials, with some patients attaining long-term remission and improved survival. However, not all patients respond to CAR T-cell therapy, and some may experience adverse effects, such as cytokine release syndrome (CRS) or neurotoxicity (Smith and Venella 2017; Tallantyre et al. 2021; Velasco et al. 2023; Huang et al. 2023). Cytokine release syndrome occurs when the CAR T cells become activated and release cytokines in large amounts, which can cause fever, low blood pressure, and other flu-like symptoms (Smith and Venella 2017). Neurotoxicity can occur when the CAR T cells cross the blood-brain barrier and cause inflammation in the brain, leading to confusion, seizures, and other neurological symptoms (Tallantyre et al. 2021; Velasco et al. 2023). The identification of biomarkers that can predict response to CAR T-cell therapy and guide the selection of patients for treatment is an active area of research. Moreover, new approaches are being developed to enhance the safety and effectiveness of CAR T-cell therapy, such as the use of "off-the-shelf" CAR T-cells and the development of CAR T-cells that can recognize multiple antigens.

Cancer Vaccines

Cancer vaccines represent a promising frontier in cancer immunotherapy, aiming to train the immune system to recognize and target cancer cells. Unlike traditional vaccines, which are used to prevent infectious diseases, cancer vaccines are designed to treat existing cancers (Schiller et al. 2022). There are two main types of cancer vaccines: prophylactic vaccines and therapeutic vaccines. Prophylactic vaccines are intended to inhibit the growth of cancer by targeting infectious agents that can cause cancer, such as the human papillomavirus (HPV) and hepatitis B virus (HBV) (Tsai 2015; Crews et al. 2021). Therapeutic vaccines, on the other hand, are intended to stimulate an immune response against existing cancer cells (Saxena et al. 2021). Therapeutic cancer vaccines can be divided into several categories based on the type of antigen they target. These include tumor-associated antigens (TAAs), cancer-specific antigens (CSAs), and neoantigens (Buonaguro and Tagliamonte 2020). Tumor-associated antigens are proteins that are present on the surface of cancer cells and are also expressed on normal cells, albeit at lower levels. Cancer-specific antigens, on the other hand, are proteins that are only expressed in cancer cells. Neoantigens are antigens that are generated by mutations in the cancer cell's DNA and are unique to that particular cancer cell (Leko and Rosenberg 2020; Okarvi and AlJammaz 2019; Xie et al. 2023). Therapeutic cancer vaccines have shown promise in clinical trials, with some patients achieving improved survival and long-term remission. However, their efficacy is highly dependent on the patient's immune system and the specific characteristics of the cancer being targeted (Saxena et al. 2021; Buonaguro and Tagliamonte 2020).

Moreover, cancer vaccines are often used in combination with other immunotherapies, such as immune checkpoint inhibitors and CAR T-cell therapy, to enhance their efficacy. This combination approach has shown promise in clinical trials, with some patients achieving improved outcomes compared to either therapy alone (Alard et al. 2020; Kleponis et al. 2015).

Adoptive Cell Transfer

Adoptive cell transfer (ACT) is a type of immunotherapy that involves the transfer of immune cells, such as T cells, into a patient to treat cancer. ACT can be used to treat cancer in several ways, including the transfer of tumor-infiltrating lymphocytes (TILs), genetically modified T cells, and natural killer (NK) cells (Rosenberg et al. 2008). TILs are immune cells with the ability to identify and destroy cancerous cells. TILs can be isolated from a patient's tumor and grown into a big cell population in a lab. Once expanded, the TILs are infused back into the patient's body, where they can recognize and attack the cancerous cells (Zhao et al. 2022).

Genetically modified T cells, such as CAR T-cells, can also be used in ACT. These T cells are engineered to express a chimeric antigen receptor (CAR) on their surface, which allows them to identify and attack cancerous cells that express a specific antigen (Marofi et al. 2021). NK cells are another type of immune cell that can be

used in ACT. NK cells can recognize and kill cancer cells without prior exposure to the antigen, making them a potentially powerful tool in cancer immunotherapy (Paul and Lal 2017). ACT has shown potential in the treatment of certain types of cancer, particularly hematological malignancies including leukemia and lymphoma. In some cases, patients have achieved long-term remission and improved survival. However, the efficacy of ACT is highly dependent on the patient's immune system and the specific characteristics of the cancer being targeted (Ferrer et al. 2022). ACT can also be associated with adverse effects, such as cytokine release syndrome (CRS) and neurotoxicity, similar to CAR T-cell therapy. Careful patient selection and management of side effects are necessary to optimize its efficacy and minimize toxicity (Yang 2015b; Morris et al. 2022; Siegler and Kenderian 2020).

Immunomodulatory Agents

Immunomodulatory agents are drugs that can augment or suppress the immune system's response to cancer cells (Fig. 2.2) (Matsushita and Kawaguchi 2018). They work by interacting with the immune system's signaling pathways, activating or inhibiting immune cells, and altering the tumor microenvironment to promote an anti-tumor response (Locy et al. 2018; Quach et al. 2010). Examples of immunomodulatory agents include checkpoint inhibitors, such as pembrolizumab and nivolumab, which target the PD-1 and CTLA-4 receptors on T-cells, and CAR T-cell therapy, which genetically modifies T cells to recognize and attack cancer cells (Hussaini et al. 2021). Some examples of immunomodulatory agents used in cancer therapy include the following:

Interferons: These are naturally occurring proteins that can enhance the immune system's response to cancerous cells. They work by activating immune cells such as NK cells and T cells and by inhibiting the growth of cancer cells. Interferons are used to treat several cancer types, including melanoma and leukemia (Yu et al. 2022).

Interleukin-2: This is a protein that can stimulate the growth and stimulation of immune cells, including T cells and NK cells. It is employed in the treatment of metastatic melanoma and renal cell carcinoma (MacDonald et al. 2021).

Immune checkpoint inhibitors: As discussed earlier, these are drugs that block inhibitory signaling pathways in the immune system, allowing immune cells to attack cancer cells more efficiently. Examples of checkpoint inhibitors include pembrolizumab, nivolumab, and ipilimumab. They are utilized to treat several cancer types, including bladder cancer, melanoma, and lung cancer (Hussaini et al. 2021).

CAR-T cell therapy: This is a type of immunotherapy that includes engineering a patient's T cells to express chimeric antigen receptors (CARs) that can identify and attack cancerous cells. CAR-T cell therapy has shown promising results in treating several types of blood cancers, such as non-Hodgkin's lymphoma and acute lymphoblastic leukemia (Hasegawa and Hosen 2019).

Cytokine inhibitors: These are drugs that can block the activity of cytokines, which are proteins that can promote inflammation and contribute to the growth of cancer cells. Examples of cytokine inhibitors include tocilizumab, which targets interleukin-6, and ustekinumab, which targets interleukin-12 and interleukin-23. These drugs are used to treat inflammatory conditions and certain types of cancer, such as multiple myeloma (Rider et al. 2016; Nielsen et al. 2020).

Immunomodulatory agents can have side effects, such as flu-like symptoms, fatigue, and immune-related adverse events such as skin rash and diarrhea (Bascones-Martinez et al. 2014). However, they are generally well tolerated and have shown promising results in improving cancer treatment outcomes. Ongoing research is needed to identify new targets and develop new immunomodulatory agents to improve the effectiveness of cancer therapy.

2.4 The Mechanisms Underlying Cancer Resistance to Immune Therapy

Immunotherapy has shown promise in treating a variety of cancers, including bladder cancer, lung cancer, melanoma, kidney cancer, and lymphoma. However, not all patients respond to immunotherapy, and some may experience side effects, such as fatigue, rash, and immune-related adverse events (Gupta et al. 2022; Tan et al. 2020).

Furthermore, immunotherapy can be expensive, and its cost-effectiveness is an active area of research. In addition, the identification of biomarkers that can predict response to immunotherapy and guide the selection of patients for treatment is also an active area of research (Tan et al. 2020). Although immune therapy has demonstrated promising outcomes in treating cancer, all patients didn't respond to these treatments. Resistance to immune therapy can arise through various mechanisms, including the following:

Tumor Microenvironment

Tumor cells can create an immunosuppressive microenvironment that prevents immune cells from attacking cancer cells. This can occur through the secretion of immunosuppressive cytokines, such as TGF-beta and IL-10, or the recruitment of immune-suppressive cells, such as regulatory T-cells and MDSCs (Lorenzo-Sanz and Muñoz 2019).

Lack of Tumor Antigens

Immune therapy works by recognizing and attacking specific antigens expressed by cancer cells. However, some tumors may lack these antigens or may have mutations that prevent recognition by the immune system. This can lead to a lack of response to immune therapy (Leko and Rosenberg 2020).

Immune Checkpoint Activation

Immune checkpoint proteins, such as PD-1, CTLA-4, and LAG-3, act as “brakes” on the immune system, preventing excessive immune activation and tissue damage. Tumor cells can upregulate these checkpoint proteins, leading to immune suppression and resistance to immune therapy (He and Xu [2020](#)).

Dysfunction of Effector T Cells

T cells play a critical role in the immune response against cancer cells. However, cancer cells can disrupt the function of effector T cells by inhibiting their activation, impairing their migration to the tumor site, or inducing their exhaustion or anergy (Xia et al. [2019](#)).

Alterations in Antigen Presentation Machinery

Cancer cells can alter the expression of molecules involved in antigen presentation, such as MHC molecules and TAP proteins, which are necessary for T-cell recognition and activation (Dhatchinamoorthy et al. [2021](#)).

Resistance to Apoptosis

Cancer cells can become resistant to apoptosis, the process of programmed cell death that eliminates damaged or abnormal cells. This can prevent immune cells from effectively eliminating cancer cells (Zhu et al. [2017](#)).

Epigenetic Changes

Epigenetic changes, such as DNA methylation and histone modification, can alter gene expression and cellular behavior, including resistance to immune therapy (Cheng et al. [2019](#)).

2.5 Radiotherapy-Molecular Mechanism Underlying Resistance in Cancer

Radiation therapy is currently one of the main methods used to treat cancer. Resistance to radiotherapy, on the other hand, might lead to treatment failure or even cancer recurrence. The usefulness of radiotherapy as a curative and palliative treatment prompted technological breakthroughs in this area, allowing larger doses to be delivered to the tumor more accurately, sparing healthy tissues and lowering

adverse effects (Bernier et al. 2004). As a result, an estimated 50% of patients now receive an external beam of radiotherapy as part of their treatment (Delaney et al. 2005). The response of cancer cells to radiation is associated with radioresistance and radiosensitivity, which is the most important component in regulating therapeutic effects (Wu et al. 2015). Radiotherapy-induced damage to DNA regulates the proliferation of cells and alters the cell cycle, eventually resulting in apoptosis or other sorts of programmed death pathways (Wang et al. 2018; Mir et al. 2023a). Radioresistance in cancer is typically caused by the repair response to radiation-induced DNA damage, cell cycle dysfunction, cancer stem cell (CSC) resiliency, and the transition from EMT. Understanding the molecular variations that cause radioresistance may lead to novel diagnostic indicators and treatment targets to increase radiation efficacy. Furthermore, the activation of antioxidant enzymes by NRF2, NF- κ B, and HIF-1 reduces the ROS-dependent harmful effects of radiation therapy (Khan et al. 2023a; Galeaz et al. 2021). This defensive activity lowers the treatment's effectiveness, as its mode of action is mostly based on the generation of free oxygen radicals (Galeaz et al. 2021).

Repair of Radiation-Induced DNA Damage

Research has demonstrated that radiation therapy can either directly harm DNA by IR (ionizing radiation) or indirectly by increasing the absorption of high-energy wavelengths by molecules surrounding DNA, which can produce extremely reactive free radicals that can damage DNA (Azzam et al. 2012). Such radicals cause the creation of reactive oxygen species (ROS) and consequent oxidative stress, causing cancer cells to be damaged (Aprioku 2013). To kill tumor cells, radiation generates intracellular DNA damage in the form of single-strand breaks (SSBs), abasic sites, and double-strand breaks (DSBs), and DSBs are the most harmful (Borrego-Soto et al. 2015). DSBs involve breakdowns in the phosphodiester backbone of the two DNA strands that are disconnected by ten bases or less (Hanai et al. 1998). DSB formation upregulated linearly with increasing radiation dosage (Rothkamm and Löbrich 2003). Eukaryotes have evolved the capability to restore DNA damage on their own. The major DSB repair processes are HR and NHEJ (Grabarz et al. 2012). To repair DSBs, homologous DNA sequences are required by HR from sister chromatids, thus constrained to cell cycle stages where sister chromatids are present (late S to G2) (Zhao et al. 2017). NHEJ, on the other hand, is unrestrained repair machinery that joins two damaged ends directly and does not need sequence similarities. As a result, NHEJ takes place throughout the cell cycle (Emerson and Bertuch 2016; Mir et al. 2023b). NHEJ is triggered by Ku proteins, which are heterodimers composed of Ku80 and Ku70 components. It has a great attraction for the endpoints of DNA, and once attached, it develops a holoenzyme with the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) (Reynolds et al. 2012). Radiation sensitivity is determined by the extent of DNA damage generated within the cell and the capability of cells to activate repair mechanisms via DNA damage response (DDR) pathways (Jackson and Bartek 2009). Radioresistant cancer cells

are more likely to raise the DDR rate. For instance, *in vitro*, the expression of Ku80 is associated with radiation resistance, and the knocking down of Ku80 regains sensitivity (Chang et al. 2006). In fact, after radiotherapy, the expression of the Ku80 protein is linked to locoregional failure and death (Moeller et al. 2011). Additionally, ATM plays a vital role in NHEJ repair by phosphorylation at Th-2609 of DNA-PKcs in response to radiation (Chen et al. 2007).

Radiation-Induced Damage to DNA Leads to Proliferation

The cell cycle is a tightly controlled series of measures that permits cells to multiply. The cell cycle passes through the G0 (resting) phase, G1 (initial growth phase), S (DNA replication), G2 (growth), and M (mitosis) phases. Cell cycle advancement is organized by cyclin-dependent kinases (CDKs) and cyclins (Mir et al. 2023b; Singh et al. 2017). Checkpoints detect errors during the synthesis of DNA and the segregation of chromosomes to maintain the integrity of the cell cycle. When the structure of DNA gets impaired by radiation, the cell cycle of the concerned tissue might be deferred or paused to allow for DNA repair (Lu et al. 2022). As a result, tumor cells can boost radiation resistance by modulating cell-cycle arrest. Cyclin K, for example, controls the G2/M checkpoint by modulating the β -catenin/cyclin D1 axis (Yao et al. 2020). TopBP1 (Topoisomerase II-binding protein 1) or Claspin is a key arbitrator in the checkpoint that enhances the CHK1 phosphorylation in lung cancer cell resistance to radiotherapy (Choi et al. 2014). Checkpoint pathways keep CDKs dormant until a DNA lesion is repaired. The activation of ataxia telangiectasia mutated (ATM) following the identification of DSBs induced by radiation is a critical event in the activation of checkpoints. When damage in DNA is detected in the G1 period after irradiation, ATM primarily phosphorylates H2AX near the DSB (Stucki and Jackson 2006). ATM and Chk2 alleviate p53 by separating it from MDM2, which activates effector targets like p21. In G1, the buildup of p21 prevents cyclin/CDK complexes and halts cell-cycle advancement. According to Deng et al., full deletion of p21 or p53 leads to complete loss of the G1 checkpoint (Hutchinson et al. 2020). Moreover, cancer cells may proceed to the S phase despite receiving significant dosages of radiation during the late G1 phase (Deckbar et al. 2010). ATM is upregulated in numerous radiation-resistant tumors, and the ATM pathway controls cell cycle checkpoints to make cancerous cells radiotherapy-resistant following IR-induced DNA damage (Bian et al. 2020). High levels of ATM expression have been reported to improve the repair of DNA damage as well as stimulate radiation resistance, and in human cancer, its expression is correlated with resistance to radiotherapy (Bian et al. 2020; Tribius et al. 2001). ATM expression inhibition can increase cancer cell sensitivity to IR by deferring DNA DSB repair, blocking translocation of NF- κ B, suppressing p38 phosphorylation, and stimulating c-Jun N terminal kinase (JNK) activity (Zhou et al. 2013; Carruthers et al. 2015; He et al. 2017). Damage to DNA during the S phase of the cell cycle may disrupt the advancement replication fork, and base impairment may result in point mutations due to base mispairings. If DSBs aren't repaired, more DSBs and chromosomal breaks

may arise. As a result, cell-cycle advancement is halted following DSB formation to allow for repair (Hutchinson et al. 2020). Repair of radiation-induced DNA destruction is insufficient in cancer cells with mutant p53, but it is capable enough to produce clones with an accretion of genetic mutations that result in resistance. Additionally, loss-of-function mutations of p53 prevent apoptosis and cell-cycle arrest, leading to cell survival and radiation failure (Valente et al. 2013). In another study, the zinc finger E-box binding homeobox 1 (ZEB1) is phosphorylated and stabilized by the kinase ATM. Then, ZEB1 binds to ubiquitin-specific peptidase 7 (USP7), which improves USP7's capacity to deubiquitylate and stabilize the checkpoint kinase 1 (CK1) (Zhang et al. 2018).

Radiation-Induced EMT

Epithelial-mesenchymal transition (EMT) is a kind of process by which epithelial cells convert into mesenchymal cells and gain migrant capacities (Brabletz et al. 2018). Loss of intercellular adhesion and the reduced manifestation of E-cadherin is one of the features of EMT, and this steady reduction is mediated by Snail and Slug (Lamouille et al. 2014). Various studies also demonstrate that radiation causes cancer cells of esophageal squamous cell carcinoma (ESCC) and CRC cells to undergo EMT and activate the Wnt/ β -catenin signaling (dos Reis Bastos et al. 2014; Liu et al. 2019). Previous studies on the esophageal radiation-resistant KYSE-150RR cancer cell line have shown that radiation exposure mainly affects EMT by down-regulating the expression of the PTEN gene and activating the PKB (Akt)/Snail signaling (He et al. 2015). Additionally, PTEN, a potential gene of tumor suppressor, can increase the radiosensitivity of tumor cells by inhibiting tumor growth and metastasis by acting on phosphatidylinositol-3-kinase (PI3K) (Khan et al. 2023c; McKenna and Muschel 2003). Moreover, cancer cells of NSCLC that endured IR therapy also displayed EMT-like features (Gomez-Casal et al. 2013).

Immunomodulatory Response Against Radiation Therapy

In addition to cytotoxicity, radiation influences the regulation of immune cells in both the tumor and its microenvironment. Radiation stimulates antigen-specific antitumor immune reactions via a variety of pathways. These pathways comprise: (1) boosting tumor-infiltrating lymphocyte activation and proliferation, (2) altering chemokines that attract cytotoxic T cells preferentially and result in MHC I upregulation, (3) promoting tumor neoantigen release through inflammatory cell death, and (4) dendritic cell activation and migration. Following radiation, many inhibitory immune cells are attracted to the microenvironment of the tumor. As a result, the tumor cells counteract the therapeutic action of radiation therapy at the site of the tumor. Tumor-infiltrating myeloid cells as well as Tregs are among the attracted inhibitory immune cells, and they play a critical function in redesigning the immunosuppressive tumor microenvironment. For example, CCL2 (C-C motif

chemokine ligand 2) is increased in cancer cells after radiation therapy and thus promotes the recruitment of TAMs and Tregs via the CCL2/chemokine receptor 2 (CCR2) axis (Mondini et al. 2019; Kalbasi et al. 2017). Similarly, CCL5 (C-C motif chemokine ligand 2) can enhance the influx of immunosuppressive CCR5+ monocytes into the cancer location (Connolly et al. 2016). In irradiated breast cancer cells, radiation therapy enhances the expression of CD73, which is an adenosine-generating enzyme. This, in turn, may inhibit the intrusion of typical dendritic cell type 1 as well as T cells into tumors (Wennerberg et al. 2020). Surprisingly, the radiation therapy-activated cancer cell-intrinsic GAS-STING signaling enhances immune avoidance by enhancing the expression of PD-L1 (Du et al. 2022). A previous study discovered that lactate produced from tumor cells stimulates MDSCs through the GPR81/mTOR/HIF-1 α /STAT3 pathway and is obligatory for the radio-resistance of pancreatic cancer (Yang et al. 2020b).

Signaling Pathways in Radiation Resistance

DNA damage caused by irradiation and the oxidative emergency reaction both activate separate signaling pathways in cells. Subject to the degree of DNA damage, either apoptosis or survival pathways may be triggered. The EGFR, Akt/cyclin D1/CDK4 survival signaling system, Wnt/ β -catenin, NF- κ B, and autophagy have all been linked to radiological resistance in cancer (Singh et al. 2017; Su et al. 2015; Woodward et al. 2007; Chen et al. 2015; Shimura et al. 2012; Bai et al. 2012; Khan et al. 2023e).

The EGFR receptor is a transmembrane tyrosine kinase receptor that is stimulated by ligands like EGF and TGF. Ligand-mediated EGFR activation results in intracellular domain autophosphorylation, with downstream activation of the PI3K and Ras pathways to promote persistence and proliferation. Patients with elevated EGFR have a greater rate of post-radiation locoregional failure, implying that EGFR plays a role in radiation resistance (Ang et al. 2002). Radiation mimics ligand-receptor interactions by promoting autophosphorylation of EGFR, which results in hyperactivation of the Ras and PI3K pathways, which promotes tumor cell proliferation and survival, and ultimately radiation failure (Schmidt-Ullrich et al. 1997).

Stimulated NF- κ B signaling controls its downstream directed genes, like c-Myc and cyclin D1, which prevent tumor cells from death and promote their growth, invasion, and metastasis as well as their resistance to chemotherapy and radiotherapy (Deorukhkar and Krishnan 2010). For instance, in gliomas, the radioresistance of cells is increased when the prostaglandin-endoperoxide synthase 2 (PTGS2)/NF- κ B signaling is activated (Hassan et al. 2020; Tan et al. 2019). In human HCC, Aurora-A boosts NF- κ B activity and also encourages the production of its downstream targets, including Bcl-2, PARP, Mcl-1, and caspase-3, which lessens the radiation-induced death of parental cells (Shen et al. 2019). Parallel results were seen in melanoma and breast tumors, pointing to the possibility of targeting NF- κ B to overcome radiation resistance (Hassan et al. 2020; Ahmed et al. 2013; Munshi

et al. 2004). Blocking the NF- κ B pathway can make tumors more sensitive to radiation. For instance, In NSCLC, ginsenoside Rg3 suppresses NF- κ B stimulation, which lowers the production of NF- κ B gene products and renders lung cancer cells susceptible to gamma radiation (Fig. 2.3) (Nalli et al. 2019; Wang et al. 2015; Ahmad et al. 2022; Wani et al. 2021).

Autophagy, a highly conserved mechanism, facilitates the destruction of malfunctioning organelles and proteins inside the cells. The initiation of the autophagy system functions as an adaptive and survival mechanism that offers metabolic sustenance in the existence of cellular stresses (such as radiation exposure), thus reducing the effectiveness of radiation (Khan et al. 2022a, b; Nam et al. 2013). It has been observed that radiation-induced autophagy shields breast tumor cells against damage caused by radiation (Chaachouay et al. 2011). According to clinical investigations, hypoxia prompts autophagy and enhances radiation resistance in tumor cells (Chaachouay et al. 2015). Hypoxia-induced autophagy leads to radioresistance in lung cancer cells via c-Jun-mediated Beclin1 expression, and Beclin1 induces autophagy primarily via suppressing osteopontin (Zou et al. 2014; Chang et al. 2012). Several studies demonstrated that inhibition of autophagy increases death in radioresistant cells (Wani et al. 2021). For example, the suppression of autophagy by 3-MA in an ESCC cell line increased apoptosis and decreased tumor cell proliferation, resulting in radiosensitivity of ESCC cells in both in vitro and in vivo (Chen et al. 2015).

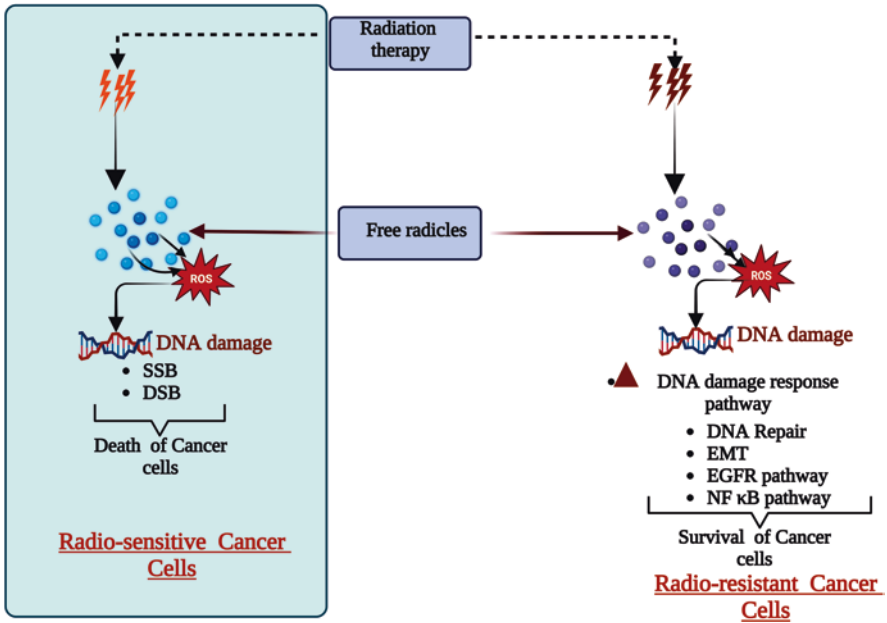


Fig. 2.3 Radiation therapy and DNA damage intricate interplay and the emergence of drug resistance in cancer

2.6 Targeted Therapies and Cancer

Targeted therapies are a type of cancer treatment that specifically targets the molecular or genetic alterations that drive the growth and survival of cancerous cells. These therapies are designed to be more selective and less toxic than traditional chemotherapy, which can also damage healthy cells (Nalli et al. 2019; Pottier et al. 2020). Targeted therapies can be classified into several categories, including the following:

Small Molecule Inhibitors

Small molecules are drugs that bind to specific proteins or enzymes that are overactive in cancer cells, thereby blocking their activity and preventing cancer growth. These drugs are small enough to pass through the cell membrane and target intracellular proteins or enzymes that are critical for cancer cell survival. Examples of small molecule inhibitors include vemurafenib, which targets the BRAF mutation in melanoma (Zhong et al. 2021). Small molecule inhibitors can target a variety of molecular pathways that are deregulated in cancer cells, including the following:

Tyrosine Kinase Inhibitors

These drugs target enzymes called tyrosine kinases, which are associated with signaling cascades that regulate cell growth and survival. Examples of tyrosine kinase inhibitors include imatinib, which targets the BCR-ABL fusion protein in chronic myeloid leukemia, and sunitinib, which targets multiple tyrosine kinases in renal cell carcinoma (Pottier et al. 2020).

Proteasome Inhibitors

These drugs inhibit the proteasome, a cellular complex that degrades proteins and is necessary for cancer cell survival. Examples of proteasome inhibitors include bortezomib, which is used to treat mantle cell lymphoma and multiple myeloma (Manasanch and Orłowski 2017).

PI3K/AKT/mTOR Pathway Inhibitors

These drugs target components of the PI3K/AKT/mTOR pathway, which is implicated in regulating cell growth and survival. Examples of these inhibitors include everolimus, which targets mTOR in breast and renal cell cancer, and idelalisib, which targets PI3K in chronic lymphocytic leukemia (Du et al. 2018; Bhatti et al. 2018).

Epigenetic Modifiers

These drugs target enzymes that modify the structure of DNA or histones, which can affect gene expression and cancer cell growth. Examples of epigenetic modifiers include azacitidine and decitabine, which are used to treat myelodysplastic syndrome (Pan et al. 2016). Small molecule inhibitors can have side effects, including

diarrhea, skin rash, and fatigue, and can also be associated with the development of resistance over time (Guilhot 2004). Therefore, combination therapy with other drugs or immunotherapies is often used to improve their efficacy and overcome resistance.

Monoclonal Antibodies

Monoclonal antibodies (mAbs) are a type of targeted therapy that uses laboratory-produced antibodies to specifically target cancer cells. These antibodies are designed to bind to specific proteins that are highly expressed on the surface of cancer cells, which can prevent the cancer cells from growing and dividing or trigger the immune system to attack and destroy them (Zahavi and Weiner 2020). Examples of mAbs include trastuzumab, which targets the HER2 receptor in breast carcinoma, and rituximab, which targets the CD20 protein in non-Hodgkin's lymphoma (Cortés et al. 2022; Papageorgiou et al. 2022). The use of mAbs as cancer therapy has revolutionized cancer treatment. Some examples of mAbs used in cancer therapy include the following:

Rituximab: This antibody targets the CD20 antigen on the surface of B-cell lymphoma cells. It is utilized in the treatment of non-Hodgkin's lymphoma and chronic lymphocytic leukemia (Papageorgiou et al. 2022).

Trastuzumab: This antibody targets the HER2 protein on the surface of breast cancer cells that overexpress this protein. It is used to treat HER2-positive breast cancer (Cortés et al. 2022).

Bevacizumab: This antibody targets the vascular endothelial growth factor (VEGF) protein, which is implicated in the formation of new blood vessels that supply nutrients to tumors. It is used to treat several cancer types, including colorectal, lung, and kidney cancer (Itatani et al. 2018).

Pembrolizumab: This antibody targets the programmed cell death protein 1 (PD-1) receptor on immune cells, which can prevent the immune system from attacking cancerous cells. It is used to treat several cancer types, including melanoma and lung cancer (Prasad and Kaestner 2017).

Monoclonal antibodies can be used alone or in combination with other cancer therapies, such as chemotherapy or radiation therapy. They are generally well tolerated but can have side effects such as infusion reactions, fever, and fatigue (Baldo 2013).

2.7 Resistance Mechanisms to Current Targeted Treatments

Targeted medication therapy has recently produced positive healing results. With their great selectivity, minimal toxicity, and excellent therapy index, molecularly targeted medicines are now the main focus of research and development for novel

antitumor medications. Drug resistance, nevertheless, significantly restricts its therapeutic application perspective (Attwood et al. 2021; Bedard et al. 2020). Drug development for cancer with new forms of targets, like miRNAs, has produced positive results. At the moment, many approaches are being utilized clinically to combat drug resistance, including new-generation anticancer drugs for drug-resistant alterations, multi-targeted medications, and combination treatments, like as targeted drugs in combination with immunotherapy. Though overall survival has increased due to these treatment options, medication resistance has not been overcome (Zhong et al. 2021).

Resistance to EGFR Inhibitors

The human epidermal growth factor receptor family includes the extracellular protein ligands called EGFR, often referred to as HER1 or ERBB-1 (Herbst 2004). ERBB2 (HER2), ERBB3 (HER3), and ERBB4 (HER4) are members of the HER family in addition to HER1. The distribution of EGFR is mainly present on the mammalian surfaces of epithelial cells, glial cells, fibroblasts, and other cells and its associated signaling cascades are crucial to physiological activities like proliferation, cell growth, and differentiation. Mutations in the EGFR are the most frequent type of mutations in many cancer types (Ye et al. 2021). The acquired mechanism of EGFR drug resistance is mainly categorized into two types: the EGFR-dependent mode of action (on-target), which includes the EGFR T790M mutation, and the EGFR-independent mode of action (off-target), like as the utilization of bypasses to stimulate the signaling cascade or alternative pathways to spread tumor cells to the EGFR signaling route (Majidpoor and Mortezaee 2021). The stimulation of downstream signaling cascades, including the RAS or PI3K signaling cascades, is one of the off-target resistance mechanisms (Fig. 2.4). The following describes the mechanism of EGFR inhibitor resistance:

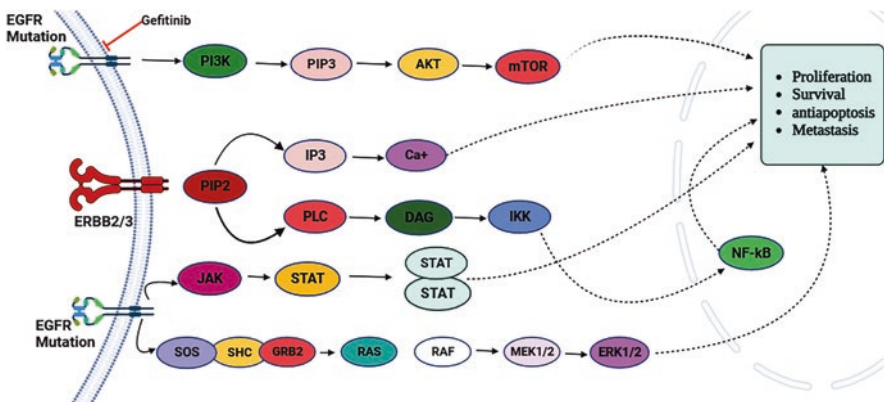


Fig. 2.4 The figure highlights EGFR-mediated pathways leading to enhanced cell survival, heightened proliferation, promotion of metastatic potential, and the inhibition of apoptosis, which in turn leads to drug resistance

Mutations in T790M

The primary factor for resistance to first- and second-generation inhibitors of the EGFR is the T790M mutations. The vast majority of patients with treatment resistance had the T790M mutation, according to numerous studies (Kobayashi et al. 2005). The ATP binding site in the kinase region of the cell undergoes structural modifications, which in turn results in increasing EGFR's affinity for ATP and decreasing its affinity for inhibitors. As a result, the first/second generations of EGFR-TKIs are unable to continue blocking the associated signal transduction pathways, allowing EGFR to be activated permanently (Pao et al. 2005; Yun et al. 2008).

MET Mutation and Amplification

One of the main causes of many malignancies is the proto-oncogene known as mesenchymal-epithelial transition factor (MET), which is found on the seventh chromosome. The MET gene produces the C-met protein, which is mostly expressed by epithelial cells and functions as the hepatocyte growth factor (HGF) receptor. HGF controls cell proliferation and development by triggering a series of tyrosine kinase signaling upon interacting with the C-MET receptor (Bottaro et al. 1991). Regional or local MET gene duplication, often referred to as MET gene amplification, can result in aberrant stimulation of HGF/MET signaling cascades like RAS-MAPK, PI3K-AKT, Wnt/ β -catenin, and STAT. Due to its ability to stimulate the ERBB3 phosphorylation, which was initially dependent upon EGFR, as well as the downstream PI3K-Akt signaling cascade, MET gene amplification was discovered by Engelman et al. to be a key process causing EGFR-TKI acquired resistance (Engelman et al. 2007). Research has indicated that mutations in D1228 and Y1230 of MET result in the development of acquired resistance toward MET inhibitors by impacting their stability and binding with the MET kinase domain. Moreover, according to the case reports, the D1228N mutation in the kinase domain of MET resulted in persistent phosphorylation of the MET protein, thereby inducing aberrant stimulation of the MET signaling cascade and ultimately conferring resistance for crizotinib (Qi et al. 2011; Tiedt et al. 2011; Heist et al. 2016). The coexistence of MET amplification, as well as T790M mutations of EGFR, has been observed in numerous cancer patients, and the effectiveness of either T790M inhibitor alone or EGFR-TKI in combination with MET inhibitor is limited, as reported in the literature (Gou et al. 2016). The study conducted by Cao and colleagues revealed that drug resistance can be attributed to mutations within the MET gene. Exon 14 is the most frequently occurring mutation. The occurrence of MET 14 exon jump alterations leads to the deletion of binding regions for Y1003 and C-CBL E3 ubiquitin ligase. This deletion results in a decrease in receptor ubiquitination, hindering the degradation of MET protein and leading to the prolonged stimulation of the proto-oncogene MET, which in turn results in resistance (Cao et al. 2018).

Mutation in KRAS Gene

The KRAS gene is classified as a proto-oncogene and is responsible for encoding the K-RAS protein, a constituent of the RAS-RAF/MEK-ERK signaling cascade

(Tsuchida et al. 1982). In cases where KRAS undergoes mutation, the downstream signals remain activated, specifically the PI3K-AKT-mTOR signaling pathway, which is responsible for regulating cell generation as well as for controlling cell division. This ultimately leads to the development of tumors due to the disorganized proliferation and development of cells. The study conducted by Moll and colleagues revealed the presence of activated ERBB signals in individuals with lung cancer, which were attributed to the KRAS gene. They discovered that KRAS plays a crucial role in driving the expression of various ERBB receptor tyrosine kinases (RTKs) in lung cancers. The stimulation of ERBB can enhance the RAS signal core signaling routes, stimulate additional members of the ERBB family, and facilitate the proliferation of tumor cells carrying KRAS alterations, thereby promoting the growth of tumors in the body. This process has led to the emergence of resistance to drugs to first-generation TKIs (Moll et al. 2018).

Expression of SPINK1 Protein

The Serine Protease Inhibitor Kazal-Type 1 (SPINK1), which is also referred to as tumor-associated trypsin inhibitor (TATI) or pancreatic secreted trypsin inhibitor (PSTI) was initially isolated from the urine of individuals with ovarian cancer (Huhtala et al. 1982). The level of expression in cancerous cells was later shown to be inversely linked with the clinical outcome (Gouyer et al. 2008; Gao et al. 2021). SPINK1 is considered to be a member of the senescence-associated secretory phenotypes (SASP) that are commonly linked to the process of aging. According to Sun et al.'s research, radiation therapy, and chemotherapy induce apoptosis of cancerous cells as well as stromal cells in the microenvironment of tumors (TME), leading to the production of SASP (Sun et al. 2012). Chen et al. observed increased levels of SPINK1 protein among individuals with prostate cancer who underwent chemotherapy in a clinical cohort. The administration of SPINK1 monoclonal antibody plus Cetuximab resulted in a reduction of resistance to drugs in cancer cells. Hence, it has been observed that stromal cells present in the TME undergo accelerated aging during chemotherapy, leading to the production and secretion of a significant quantity of SASP factors. One of the key factors among them is SPINK1, which has the potential to activate the residual cancerous cells in the post-treatment phase, thereby conferring resistance to conventional therapeutic agents (Chen et al. 2018; Nemec et al. 2021).

Resistance to BCR-ABL Inhibitors

Abnormalities in the chromosomes are responsible for more than 90% of instances of CML. In 1960, Peter Noel discovered an abnormal chromosome that results in the fusion of the BCR-ABL1 gene via (9; 22)(q34; q11) translocation. The BCR-ABL1 inhibitor is commonly used as the primary therapeutic approach for patients with chronic myeloid leukemia (CML); however, it is not entirely effective in

preventing the development of drug resistance (Nowell 1962). The primary cause of drug resistance is attributed to the mutation of the BCR-ABL1 gene, with the T315I mutation being the most prevalent form (Cortes et al. 2013). The mutation results in the removal of crucial oxygen molecules that are essential for the formation of hydrogen bonds between imatinib and ABL kinases. Additionally, it generates a steric hindrance that impedes the binding of most TKIs, thereby decreasing the affinity for the inhibitor and ultimately culminating in drug resistance. Bixby et al. discovered that drug-resistant individuals often have elevated p-glycoprotein efflux pump expression and drugs that block p-glycoprotein function can improve imatinib sensitivity (Bixby and Talpaz 2009). In clinical specimens of patients with medication resistance, it has been discovered that BCR-ABL tyrosine kinase function is frequently reinstated by phosphorylation of the adaptor protein CRKL, suggesting that imatinib is no longer able to block its target, resulting in drug resistance (Gorre et al. 2001). Additionally, according to An et al. study, certain TKI-resistant individuals have reduced organic cation transporter 1 (OCT1) protein expression, which is necessary for imatinib entrance into cells (An et al. 2010).

Resistance to ERK Inhibitors

Extracellular regulated protein kinases (ERK) are situated downstream of the RAS/RAF/MEK/ERK signaling process. Research has indicated that the BRAF V600E alteration can result in the sustained stimulation of the BRAF protein. This triggered BRAF protein functions as a monomer, irrespective of the upstream RAS kinase, thereby resulting in persistent activation of the ERK pathway. Consequently, this can result in the development of resistance to ERK inhibitors (Yao et al. 2015; Wan et al. 2004). By using mutagenesis, several researchers discovered that ERK1 (G186D) and ERK2 (C65Y, Y36N, and G37S) mutants increase A11 melanoma cell's resistance to ERK1/2 inhibitor medications (Goetz et al. 2014; Jha et al. 2016). According to reports, exome sequencing conducted on the ERK1-R system revealed that drug-resistant individuals exhibited on-target alterations in both ERK1 as well as ERK2. Such mutants comprise ERK2 mutations like C65F, Y36H, G37C, and G37A, and ERK1 mutations included G186D and A191V (Jaiswal et al. 2018).

Resistance to Androgen Receptor (AR) Inhibitors

Prostate cancer has emerged as a major concern to the well-being of elderly men (Liang et al. 2020). AR inhibitors are a key medication for prostate cancer treatment because they bind to androgen receptors in cancerous cells and prevent testosterone and dihydrotestosterone from activating those receptors, which is crucial in combating cancer. The antiandrogen medications flutamide and nilutamide have reportedly been shown to stimulate the AR signaling system and increase the production of AR target genes in addition to having no inhibitory effects on the T878A and H875Y mutant AR. The reason for this, according to Zhao et al., could be that additional

hormonal ligands, like estradiol and progesterone, induce these alterations and result in treatment resistance (Zhao et al. 1999). Mu et al. discovered that some cancers have the ability to transform AR-dependent luminal epithelial cells to AR-independent basal-like cells, which are resistant to androgen-blocking medications. Cancerous cells develop cell lineage plasticity and lose the activities of TP53 and RB1, which results in the overexpression of SOX2 protein that eventually results in resistance toward AR inhibitors (Mintoo et al. 2021; Mu et al. 2017).

Resistance to BRAF Inhibitors

B-RAF is a protein that plays a role in cell proliferation and is encoded by the human proto-oncogene BRAF. Inhibitors of B-RAF kinase include sorafenib and vemurafenib. The FDA has approved Sorafenib for the treatment of primary liver cancer and Vemurafenib for metastatic melanoma, both with promising outcomes. In clinical studies, several individuals developed resistance to treatment despite the drug's great efficacy (Das Thakur et al. 2013). Studies have shown that B-RAF (V600E), MAPK activation, and stimulation of alternate survival pathways are among the mechanisms behind acquired resistance to BRAF inhibitors (Montagut et al. 2008; Jänne et al. 2009; Poulikakos et al. 2010). In contrast to secondary mutations of B-RAF (V600E), Nazarian et al. discovered that cancer tissue resistance toward vemurafenib is brought on by overexpression of PDGFR or mutations of N-RAS (Nazarian et al. 2010). Individuals with BRAF V600E NSCLC who had developed resistance to BRAF/MEK inhibitors were enrolled in the institutional randomized match-R study by Facchinetti et al., who also carried out a thorough molecular investigation. They discovered that reactivation of the RAS-RAF-MEK and PI3K-AKT-mTOR networks in NSCLC BRAF V600E mutations following BRAF/MEK inhibitor therapy led to drug resistance (Facchinetti et al. 2020).

2.8 Immune Evasion

Some cancerous cells can develop mechanisms to evade the immune system, which can limit the efficacy of targeted immunotherapies. For example, cancer cells can downregulate the expression of the target antigen, making them less visible to the immune system, or develop mutations that prevent recognition by immune cells (Khan et al. 2023c; Ahmad et al. 2022; Kim and Cho 2022).

To overcome resistance to targeted therapies, several strategies are being explored, including the development of combination therapies that target multiple signaling pathways, the identification of predictive biomarkers to identify patients who are likely to respond to therapy, and the use of immunotherapy to enhance the immune system's ability to identify and eliminate cancer cells. Additionally, continued research into the underlying mechanisms of resistance to targeted therapies is crucial for the development of more effective treatments for cancer.

2.9 Combination Therapies for Cancer

Combination therapies for cancer involve the use of multiple treatment modalities, such as chemotherapy, radiation therapy, targeted therapy, and immunotherapy, to improve the efficacy of treatment and overcome resistance. In recent years, combination therapy has emerged as a promising approach to overcome resistance and enhance treatment efficacy in various types of cancer. By targeting multiple pathways and leveraging synergistic effects, combination therapy offers a comprehensive strategy to combat cancer resistance (Bayat Mokhtari et al. 2017).

The rationale behind combination therapy lies in the understanding that cancerous cells utilize a multitude of mechanisms to evade the cytotoxic effects of single-agent treatments. These mechanisms include genetic alterations, activation of survival pathways, immune evasion, and tumor microenvironment-mediated resistance. By targeting multiple vulnerabilities simultaneously, combination therapy can disrupt various resistance mechanisms and improve treatment outcomes (Singh et al. 2017; Bayat Mokhtari et al. 2017).

Types of Combination Therapy

Chemotherapy with targeted therapies: Combining conventional chemotherapy agents with targeted therapies that inhibit specific molecular targets has shown promise in overcoming resistance. The cytotoxic effects of chemotherapy can be enhanced by targeting signaling pathways involved in tumor growth and survival. Examples include combining cytotoxic agents with tyrosine kinase inhibitors or mABs against growth factor receptors (Rodriguez et al. 2007; Jin et al. 2023).

Immunotherapy with targeted therapies or chemotherapy: The immune system plays a critical role in cancer surveillance and elimination. Combining immunotherapy, such as immune checkpoint inhibitors or adoptive cell therapies, with targeted therapies or chemotherapy can enhance immune responses against cancer cells while simultaneously targeting cancer-specific vulnerabilities (Shahid et al. 2019; Bailly et al. 2020).

Integration of different targeted therapies: Tumors often exhibit heterogeneity and harbor multiple driver mutations. Combining different targeted therapies that address distinct molecular targets can lead to additive or synergistic effects. This approach aims to maximize tumor control by simultaneously targeting various pathways crucial for tumor growth and survival (Gumusay et al. 2020).

Mechanisms of Synergistic Effects

Combination therapy can exert synergistic effects through various mechanisms. These include:

Enhanced tumor cell killing: Different treatment modalities can act synergistically to induce greater tumor cell death. For example, targeted therapies can sensitize cancer cells to the cytotoxic effects of chemotherapy, leading to enhanced tumor cell killing (Rodriguez et al. 2007; Jin et al. 2023).

Modulation of signaling pathways: Combination therapy can disrupt multiple signaling pathways simultaneously, leading to more profound inhibition of tumor growth and survival. By targeting redundant or compensatory pathways, combination therapy can overcome resistance mechanisms that arise from single-agent treatment (Dent et al. 2009).

Immune system activation: Combining immunotherapy with other treatment modalities can potentiate immune responses against cancer cells. Immunotherapeutic agents can enhance the recognition of cancer cells by the immune system and overcome immune evasion mechanisms, leading to improved antitumor immune responses (Shahid et al. 2019; Bailly et al. 2020).

Clinical Applications and Challenges

Combination therapy has demonstrated clinical benefits in various cancer types, including melanoma, lung cancer, breast cancer, and hematological malignancies. However, designing effective combination regimens poses several challenges. These include identifying the optimal drug combinations, determining the most effective sequencing or scheduling of treatments, managing potential overlapping toxicities, and understanding the potential emergence of resistance to combination therapies (Zhou et al. 2023).

2.10 Other Emerging Therapies

Oncolytic Virus Therapy

Oncolytic virus therapy is a type of cancer therapy that uses viruses to selectively target and destroy cancer cells. The viruses used in oncolytic virus therapy are engineered to infect and replicate within cancerous cells while sparing healthy cells (Engeland and Bell 2020). Oncolytic viruses can be designed to target specific types of cancer cells by modifying the virus to express proteins or receptors that interact with proteins or receptors found on the surface of cancer cells (Engeland and Bell 2020). Additionally, the virus can be engineered to express therapeutic genes that enhance the immune response against the cancer cells, or that sensitize the cancer cells to other cancer treatments, such as chemotherapy or radiation therapy (Zeng et al. 2021; Lan et al. 2020). Once the oncolytic virus has infected the cancer cell, it replicates and causes the cancer cell to burst, releasing viral particles and antigens (Chiocca and Rabkin 2014). These antigens stimulate an immune response against the cancer, further enhancing the efficacy of the treatment (Zeng et al. 2021). Oncolytic virus therapy has shown promise in clinical studies, particularly in the treatment of melanoma, head and neck cancer, and

pancreatic cancer. In some cases, patients have achieved complete responses and long-term remission (Fukuhara et al. 2016). However, oncolytic virus therapy can also be associated with side effects, such as flu-like symptoms, inflammation at the site of injection, and immune-related adverse events. Careful patient selection and management of side effects are necessary to optimize its efficacy and minimize toxicity (Buijs et al. 2015).

Tumor Suppressor Gene Therapy

This approach involves introducing genes that can suppress cancerous cell growth. Tumor suppressor genes such as TP53, which regulates cell division and prevents the formation of tumors, can be introduced into cancer cells to slow or stop their growth (Aubrey et al. 2016; Morris and Chan 2015). Gene therapy for cancer is still in the early stages of development, and several challenges need to be addressed, including improving the delivery of genetic material to cancer cells, minimizing off-target effects, and ensuring the long-term safety of the treatment. However, several promising preclinical and clinical studies have shown that gene therapy has the potential to improve cancer treatment outcomes and may be a promising approach for the development of new cancer therapies (Das et al. 2015).

Gene Therapy

Gene therapy is a type of treatment that involves introducing genetic material into cells to modify or correct abnormal genes that contribute to the development of diseases, including cancer. Gene therapy for cancer aims to improve the body's ability to recognize and destroy cancer cells or to enhance the effects of conventional cancer therapies (Das et al. 2015). Examples of gene therapy include the delivery of tumor suppressor genes or the use of viral vectors to selectively target cancer cells (Montaño-Samaniego et al. 2020). There are several approaches to gene therapy for cancer, including the following:

Gene Replacement

In this approach, a normal gene is introduced into the body to replace a defective or missing gene that contributes to cancer development. For example, in some types of inherited cancers, such as retinoblastoma, a faulty copy of the RB1 gene can cause tumor formation (Aubry et al. 2020). Gene replacement therapy can be used to replace the faulty gene with a functional copy, which may help prevent or treat cancer.

Gene Editing

This approach involves modifying the DNA of cancer cells to correct or eliminate mutations that contribute to cancer development. One technique for gene editing is CRISPR/Cas9, which can be used to introduce targeted changes to the DNA sequence (Zhang et al. 2021). This approach is still in the early stages of development and requires more research to determine its safety and efficacy.

2.11 Conclusion

This chapter has delved into the multifaceted landscape of cancer therapy, exploring the intricate molecular mechanisms underlying resistance and the evolving strategies to overcome it across various treatment modalities (Fig. 2.5). In conclusion, cancer therapy is a complex and ever-evolving field. As we gain a deeper understanding of the molecular mechanisms driving resistance, we can develop more effective treatment strategies. Collaboration between researchers, clinicians, and multidisciplinary teams is essential to advance our knowledge, refine therapeutic approaches, and ultimately improve patient outcomes. The quest to unravel resistance mechanisms and develop innovative therapies continues, offering hope for better outcomes and quality of life for individuals battling cancer.

MCQs

1. Which DNA repair pathway repairs double-strand breaks in DNA, often caused by platinum-based agents and topoisomerase inhibitors?

- A. Nucleotide excision repair (NER)
- B. Base excision repair (BER)
- C. Homologous recombination (HR)
- D. Mismatch repair (MMR)

Answer C: Homologous recombination (HR)

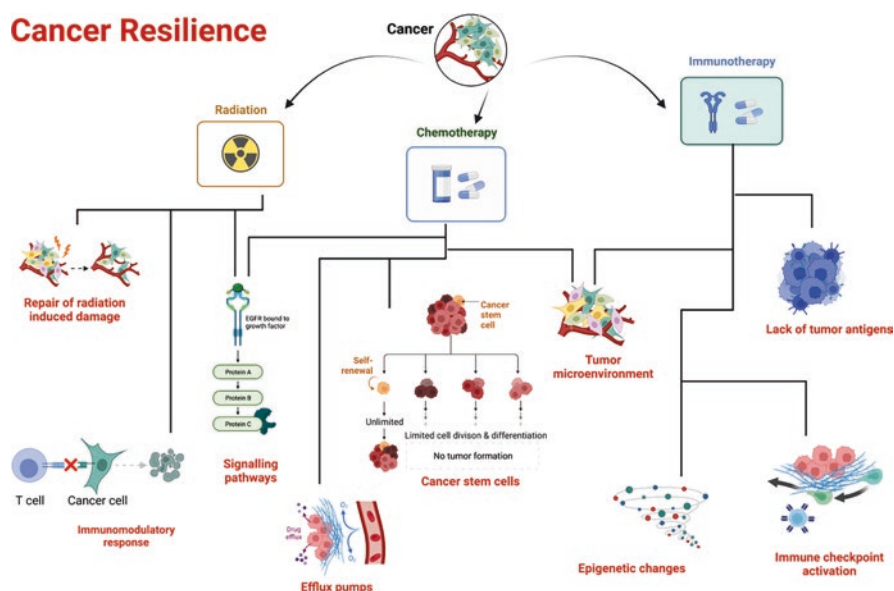


Fig. 2.5 Different mechanisms of cancer resistance

2. **Mutations in which genes are linked with an elevated risk of breast and ovarian cancer can confer resistance to chemotherapy by activating the HR pathway?**

A. XPA and XPG
B. BRCA1 and BRCA2
C. PARP
D. EGFR

Answer B: BRCA1 and BRCA2

3. **What is one strategy to overcome DNA repair-mediated resistance to chemotherapy?**

A. Using alternative chemotherapy drugs
B. Combining chemotherapy drugs with DNA repair inhibitors
C. Downregulating drug targets
D. Enhancing gene mutations

Answer B: Combining chemotherapy drugs with DNA repair inhibitors

4. **In non-small cell lung cancer (NSCLC), resistance to chemotherapy drugs targeting EGFR can occur due to:**

A. Overexpression of Bcl-2
B. Mutations in the HER2 gene
C. EGFR gene mutation
D. Downregulation of BRCA1

Answer C: EGFR gene mutation

5. **How do cancer cells often develop resistance to apoptosis during chemotherapy?**

A. Upregulating expression of pro-apoptotic proteins
B. Downregulating survival pathways
C. Activating immune checkpoint inhibitors
D. Upregulating expression of antiapoptotic proteins

Answer D: Upregulating expression of anti-apoptotic proteins

6. **Which of the following proteins inhibits apoptosis by blocking the action of pro-apoptotic proteins such as Bax and Bak?**

A. p53
B. Bcl-2
C. Mcl-1
D. XPA

Answer B: Bcl-2

7. **Resistance to chemotherapy-induced apoptosis can occur by activating survival pathways such as:**

A. PI3K/AKT and HIF-1 α
B. NER and MAPK/ERK
C. HR and PARP
D. ABC transporters and PI3K/AKT

Answer A: PI3K/AKT and HIF-1 α

8. **The acidic pH in the tumor microenvironment (TME) contributes to chemotherapy resistance by:**

- A. Enhancing drug uptake by cancer cells
- B. Inhibiting chemotherapy drug activity
- C. Promoting apoptosis in cancer cells
- D. Activating pro-apoptotic proteins

Answer B: Inhibiting chemotherapy drug activity

9. **PARP inhibitors are effective against cancer stem cells (CSCs) by:**

- A. Activating DNA repair pathways
- B. Inhibiting survival pathways
- C. Promoting quiescence in CSCs
- D. Enhancing drug efflux pumps

Answer A: Activating DNA repair pathways

10. **What is the main objective of immunotherapy in cancer treatment?**

- A. Inhibit DNA replication in cancer cells
- B. Stimulate or restore the immune system's ability to recognize and remove cancer cells
- C. Induce mutations in cancerous DNA
- D. Inhibit cell division in cancer cells

Answer B: Stimulate or restore the immune system's ability to recognize and remove cancer cells

11. **Which type of immunotherapy works by blocking certain proteins on cancer or immune cells known as immune checkpoint proteins?**

- A. CAR T-cell therapy
- B. Cancer vaccines
- C. Immunomodulatory agents
- D. Immune checkpoint inhibitors

Answer D: Immune checkpoint inhibitors

12. **What are the most well-known immune checkpoint proteins targeted by immune checkpoint inhibitors?**

- A. p53 and Mcl-1
- B. PD-1 and CTLA-4
- C. HER2 and EGFR
- D. BRCA1 and BRCA2

Answer B: PD-1 and CTLA-4

13. **Cytokine release syndrome (CRS) can occur as a side effect in which type of immunotherapy?**

- A. Cancer vaccines
- B. Immunomodulatory agents
- C. CAR T-cell therapy
- D. Adoptive cell transfer

Answer C: CAR T-cell therapy

14. **What is the purpose of cancer vaccines in immunotherapy?**

- A. To prevent infectious diseases
- B. To stimulate the growth of cancer cells

- C. To inhibit the growth of cancer by targeting infectious agents
- D. To induce mutations in cancer cells

Answer C: To inhibit the growth of cancer by targeting infectious agents

15. **What is the primary goal of adoptive cell transfer (ACT) in cancer treatment?**

- A. Inhibit immune response
- B. Suppress T-cell activation
- C. Transfer immune cells into a patient to treat cancer
- D. Block immune system signaling pathways

Answer C: Transfer immune cells into a patient to treat cancer

16. **What is the role of cytokine inhibitors in cancer therapy?**

- A. Block the activity of cytokines, promoting inflammation
- B. Enhance the immune system's response to cancerous cells
- C. Inhibit DNA repair pathways in cancer cells
- D. Stimulate the growth of cancer cells

Answer A: Block the activity of cytokines, promoting inflammation

17. **What is the primary consequence of radiation-induced DNA damage in cancer cells?**

- A. Enhanced cell proliferation
- B. Activation of antioxidant enzymes
- C. Apoptosis or programmed death pathways
- D. EMT induction

Answer C: Apoptosis or programmed death pathways

18. **What is the most harmful form of DNA damage induced by radiation therapy?**

- A. Single-strand breaks (SSBs)
- B. Abasic sites
- C. Double-strand breaks (DSBs)
- D. Homologous recombination

Answer C: Double-strand breaks (DSBs)

19. **What is the role of cyclin K in the cell cycle during radiation therapy?**

- A. Initiates apoptosis
- B. Modulates G2/M checkpoint
- C. Induces DNA repair
- D. Suppresses DNA damage

Answer B: Modulates G2/M checkpoint

20. **Which signaling pathway is associated with radiological resistance in cancer cells?**

- A. Epidermal growth factor receptor (EGFR)
- B. PI3K/Akt pathway
- C. Wnt/ β -catenin pathway
- D. All of the above

Answer D: All of the above

21. How does radiation therapy influence the regulation of immune cells in the tumor microenvironment?

- A. Suppresses tumor-infiltrating lymphocytes
- B. Attracts inhibitory immune cells
- C. Inhibits MHC I upregulation
- D. Decreases dendritic cell activation

Answer B: Attracts inhibitory immune cells

22. What is the primary focus of research and development for novel anti-tumor medications?

- A. Chemotherapy
- B. Immunotherapy
- C. Molecularly targeted medicines
- D. Radiotherapy

Answer C: Molecularly targeted medicines

23. What is the primary factor for resistance to first- and second-generation EGFR inhibitors?

- A. MET mutation
- B. SPINK1 expression
- C. T790M mutation
- D. KRAS mutation

Answer C: T790M mutation

24. Which protein, when overexpressed, can lead to resistance in ERK inhibitors?

- A. MET
- B. SPINK1
- C. p-glycoprotein
- D. ERK1/2

Answer D: ERK1/2

25. What is a potential cause of acquired resistance to BRAF inhibitors in melanoma?

- A. Secondary mutations of B-RAF (V600E)
- B. Overexpression of PDGFR
- C. Mutations of N-RAS
- D. All of the above

Answer D: All of the above

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Role of Cancer Stem Cells in Drug Resistance

3

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Abstract

The chapter on the “Role of Cancer Stem Cells in Drug Resistance” explores the intricate mechanisms underlying the resistance of cancer stem cells (CSCs) to therapeutic interventions. CSCs, a subpopulation within tumors endowed with self-renewal and differentiation capabilities, have been implicated in the persistence and recurrence of cancers. Moreover, the role of CSCs in promoting tumor heterogeneity and driving metastasis further complicates treatment strategies. The chapter explores the multifaceted ways in which CSCs contribute to drug resistance, including enhanced DNA repair mechanisms, activation of antiapoptotic pathways, and the ability to evade immune surveillance. It also explains the unique features of CSCs that differentiate them from typical cancer cells. The chapter examines recent advancements in targeting CSCs to overcome drug resistance, emphasizing the importance of developing novel therapeutic approaches that specifically address the unique biology of CSCs.

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Keywords

Cancer stem cells · Cancer stem cell markers · Immune cells · Drug resistance · Signaling pathways

3.1 Introduction

Surgery, radiation, and chemotherapy are the three basic cancer treatments that are frequently utilized to treat patients. While radiation therapy and chemotherapy can be combined to treat many different types of cancer, surgery can be used to effectively eradicate malignancy from the human body (Lakhani et al. 2007). Modern chemotherapeutic drugs are effective against initial tumors and their residuals following treatment with surgery or radiation therapy (Pützer et al. 2017; Nalli et al. 2019). The heterogeneity within malignancies, however, reduces the effects of chemotherapy, which contributes to the failure of treatment and the progression of the disease (Ahmad et al. 2022; Reid et al. 2017). Chemoresistance is a significant issue in the therapy of cancer patients because it reduces the effectiveness of chemo agents since cancer cells develop a resistance to the chemicals employed in the therapy (Trédan et al. 2007). It is frequently linked to cancers developing into metastatic or more aggressive forms (Senthebane et al. 2017; Fesler et al. 2017; Di Fiore et al. 2014; Chen et al. 2017). The available evidence indicates that the chemoresistance and recurrence of cancer can be attributed to a specific subset of cancer cells known as cancer stem cells (CSCs). These cells possess the capacity for self-renewal and differentiation into diverse cancer cell lineages upon exposure to chemotherapeutic drugs. Additionally, CSCs are capable of inducing cell cycle arrest, which facilitates their resistance to both chemotherapies as well as radiotherapy (Singh and Settleman 2010; Kiyohara et al. 2017; Fang and Kitamura 2018; Cojoc et al. 2015; Mir et al. 2023a).

The CSC theory of tumor growth has been described as a hierarchy, with cells at the highest rank possessing an extreme potential for self-renewal and differentiation in a substantial population of cells that have a restricted capacity for proliferating (Singh et al. 2015; Prasetyanti and Medema 2017). While CSCs and normal stem cells have several characteristics in common, such as limitless proliferative potential and the capacity for self-renewal, their respective molecular signatures are still poorly understood (O’Flaherty et al. 2012; Rossi et al. 2020). The molecular profile of CSCs is now being characterized to more effectively target them. It is thought that approaches to target CSCs are crucial for understanding the progress of cancer treatments and the potential of treatment modalities (Sun et al. 2019; Vassalli 2019). Finding and comprehending this cell subpopulation, which promotes the growth and spread of tumors, is still a challenge. To subject them to targeted treatment, the first obstacle is to define their unique markers. Aldehyde dehydrogenase (ALDH) constitutes one of the recently proposed markers for CSCs. It is used to detect CSCs since it exhibits greater metabolic activity compared to naive cancerous cells (Yang et al. 2020a; Khan et al. 2024).

Through the use of an ALDH-specific cell-sorting approach, Sullivan and colleagues in 2010 were the first to locate and describe lung CSCs in basic human cell cultures (Sullivan et al. 2010). Based on these findings, it was found that ALDH high+ cells expressed higher levels of Notch pathway transcripts. Numerous investigations in vitro as well as in vivo demonstrate that ALDH high+ cells contributed significantly to the pool of self-regenerating NSCLC stem-like cells with elevated tumorigenic potential. Masciale and colleagues recently found and isolated pulmonary CSCs from initial tumors using ALDH expression, indicating their capability to generate in vitro tumor spheres (Masciale et al. 2019, 2020; You et al. 2018; Aponte and Caicedo 2017).

A distinctive property of CSCs is their capability for self-renewal paired with a significant differentiation capability. This enables the preservation of a stem cell pool and the production of a diverse lineage of differentiated tumor cells to continuously renew the tumor (Wang et al. 2013a; Tan et al. 2006; Gómez-López et al. 2014; Tassan and Kubiak 2017; Vinogradova et al. 2015; Saga et al. 2019; Dzobo et al. 2016; Plaks et al. 2015; Jopling et al. 2011). Three distinct cell subpopulations were identified in 2019 by Olmeda et al. These were differentiated cells, CSCs, and additional cell populations (Hass et al. 2020; Melzer et al. 2017; Zheng et al. 2021). Each cell type is formed by an arrangement built on molecular cascades as well as interactions (Hass et al. 2020; Wani et al. 2021). Although CSCs have been identified as a driver of the plasticity, heterogeneity, and growth of the CSC subpopulation as well as promoting niche development that is necessary for the maintenance of CSC (Hass et al. 2020; Melzer et al. 2017; Zheng et al. 2021), they appear to be an essential component in the survival of tumor and its propagation because they persist inside the tumor microenvironment (TME). Through the development of “stressor foci,” which are fueled by immune distress brought on by cellular collisions and cause the spread of malignant cells and CSCs from the vasculature to the bloodstream, such combinations of variables may aid in the metastatic progression (Sullivan et al. 2010; Masciale et al. 2020; Khan et al. 2023a). The emergence of resistance to standard cancer therapies, as well as the growth and relapse of tumors, is caused by such biological events.

Cancer Stem Cells (CSCs): Their Origin and Surface Markers

CSCs, additionally referred to as tumor-initiating cells (TICs), have been extensively investigated over the last decade, with particular emphasis on their putative origin, cellular markers, mechanistic study, and the creation of therapeutic strategies targeting their pathways (Valent et al. 2012; Peitzsch et al. 2013). Bonnet and Dick published the first clear proof of CSCs in 1997 when they found a subset of cells from leukemia expressing the surface marker CD34 but not CD38 (Mintoo et al. 2021). After transplantation, the CD34+/CD38– subset had the ability to begin tumor formation in NOD/SCID recipient mice (Valent et al. 2012). CSCs have also been seen in a variety of solid tumor types other than blood cancer (Joosse and Pantel 2013). Although it had been suggested in vitro in 2002 by the isolation of

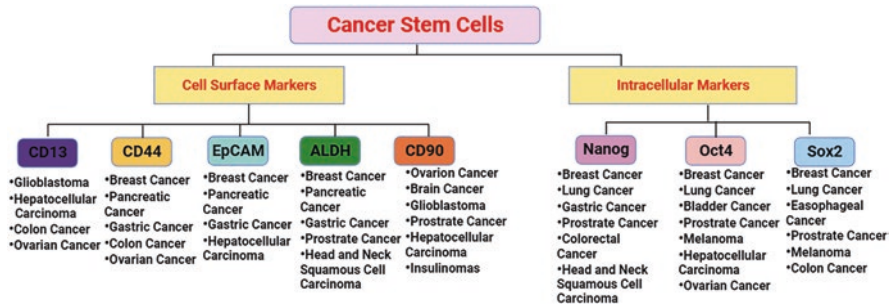


Fig. 3.1 The figure depicts key cell surface and intracellular markers associated with cancer stem cells (CSCs)

clonogenic (sphere-forming) cells obtained from human brain tumors (Ignatova et al. 2002), the very first proof of the existence of CSCs in solid tumors in vivo was discovered and characterized as CD44+CD24–/low lineage cells in 2003 in immunodeficient mice following transplanting human breast cancer cells (Al-Hajj et al. 2003). CSC populations have also been discovered throughout time from several different solid tumors, including brain, colon, liver, lung, breast, pancreatic, and ovarian cancer as well as melanoma (Fig. 3.1) (Schatton et al. 2008; Eramo et al. 2008; O’Brien et al. 2007; Klonisch et al. 2008; Singh et al. 2003).

Though the hierarchical framework and way of evolution of malignancies are explained by the CSC concept, the origins of CSCs remain uncertain and debatable (Brunner et al. 2012; Morrison et al. 1997). Depending on the type of tumor, accumulating hypotheses propose that CSCs may be produced from adult stem cells, adult precursor cells that have undergone mutations, or from differentiated cells or cancer cells that have gained stem-like traits by dedifferentiation (Hilgendorf et al. 2007; López-Lázaro 2015; Barker et al. 2009; Burns et al. 2005; Baccelli and Trumpp 2012; Friedmann-Morvinski and Verma 2014). Adult human mesenchymal stem cells (hMSCs) with telomerase transduction have been demonstrated in studies to spontaneously convert when cultured for an extended period, demonstrating that these cells are also the source of CSCs (Burns et al. 2005; Rubio et al. 2005). It’s interesting to note that CSCs develop from a combination of different tissue stem cells as well as their tissue-specific stem cells. As an example, bone marrow-derived cells (BMDCs) could serve as a crucial source for several tumor forms, including teratoma, brain tumors, and even stomach cancer (Liu et al. 2006). Additionally, it has been shown that progenitor cells or cells with differentiation that have gained “stemness” features as a consequence of the buildup of additional genetic or epigenetic defects can give rise to CSCs (Baccelli and Trumpp 2012).

Cell Surface Markers of CSCs

Fluorescence-activated cell sorting (FACS) and magnetic-activated cell sorting (MACS) may identify CSCs using various markers on the cell surfaces. The use of traditional surface markers including CD133, epithelial cell adhesion molecule

(EpCAM), CD44, and CD90 is widespread. Some CSC surface markers, though, are also present in conventional stem cells. As a result, numerous markers must be used for precise CSC targeting.

CD133

A penta-span membrane-bound glycoprotein called CD133 was first discovered in mouse neuroepithelial SCs and later discovered in human tissues (Shmelkov et al. 2005). In certain malignancies, CD133 was employed as a CSC marker. Singh et al. in 2004 (Singh et al. 2004) discovered that CD133 serves as a marker for CSCs in brain tumor-initiating cells (TICs). Their findings indicated that the injection of a minimal quantity of 100 CD133⁺ cells resulted in the development of a tumor that might have been sequentially transplanted and was a phenocopy of the individual's original tumors. Conversely, the injection of 10⁵ CD133⁻ cells engrafted yet failed to lead to tumor formation. Subsequently, CD133 was recognized as a marker for CSCs in various types of malignancies such as glioblastoma (Beier et al. 2007), hepatocellular carcinoma (HCC) (Ma et al. 2007), ovarian cancers (OCs) (Baba et al. 2009), and colon tumors (Ricci-Vitiani et al. 2007). Nevertheless, the expression of CD133 alone may not invariably signify the cancer stem cell phenotype. Researchers have therefore directed their attention toward examining joint signatures. The combination of CD133 and Nestin has been proposed as a potentially ideal marker specific to CSCs among individuals with glioma (Zhang et al. 2008; Colman et al. 2010). A unique HCC subgroup was identified using the combination of CD44 and CD133 (Khan et al. 2022a). The xenografts expressing CD133⁺CD44^{high} were found to be capable of producing metastasis in the lungs or liver of nude mice, whereas the xenografts expressing CD133⁺CD44^{-/low}, CD133⁻CD44^{high}, or CD133⁻CD44^{-/low} did not exhibit such metastatic behavior (Hou et al. 2012). Naotsugu Haraguchi and his colleagues demonstrated that the CD133⁺CD44⁺ subset has the potential to serve as a marker for TICs in colon cancer in humans (Haraguchi et al. 2008). Aldehyde dehydrogenase (ALDH) was found to be involved in colon cancers' stemness within the CD133⁺CD44⁺ subpopulation in the year 2009 (Huang et al. 2009). Ines A. Silva analyzed ovarian cancer cell lines utilizing ALDH and CD133. The results indicated a higher rate of proliferation in ALDH⁺CD133⁺ cells compared to ALDH⁺CD133⁻ cells, which suggests an increased concentration of ovarian cancer stem cells within the ALDH⁺CD133⁺ cell population (Silva et al. 2011). Recent studies have demonstrated that, in ovarian cancer, cells expressing both L1 cell adhesion molecule (L1CAM) and CD133 (double-positive L1CAM⁺CD133⁺ cells) exhibit greater spherogenic as well as clonogenic capabilities compared to cells lacking L1CAM and CD133 expression (L1CAM⁻CD133⁻ cells). Additionally, these double-positive cells have been linked to resistance to radiotherapy (Terraneo et al. 2020).

CD44

CD44, a transmembrane glycoprotein lacking kinase activity, is purported to have a function in CSCs (Chen et al. 2018). As previously noted, CD44 was initially employed as a marker for CSCs in breast cancer (Al-Hajj et al. 2003) and then subsequently in other types of cancer such as colorectal cancer (CRC) (Dalerba et al.

2007; Du et al. 2008), ovarian cancer (OC) (Alvero et al. 2009), pancreatic cancer (Lee et al. 2008), and gastric cancer (Takaishi et al. 2009). A meta-analysis of CRC studies showed that a combination of CD44 as well as CD133 markers was associated with a sevenfold rise in tumor-causing potential. In contrast, CD133 alone was associated with a 1.45-fold change, and CD44 alone was associated with a twofold rise (Abbasian et al. 2019). In addition, it was observed that the capacity to implant in vivo in mice with immunodeficiency was limited to a minority subset of epithelial cells with high expression of EpCAM and CD44 in colorectal cancer (Dalerba et al. 2007). Moreover, the tumor-initiating potential could be enhanced by the combination of ALDH with either CD44 or CD133 (Huang et al. 2009; Dolgin 2019). The research conducted by the Team, led by Chenwei Li (Li et al. 2011), revealed that cellular populations expressing CD44 (0.5–5%) and c-Met exhibited a notable ability for self-renewal as well as displayed the greatest tumor-forming potential among all the cell populations investigated in the context of pancreatic cancer.

The study revealed that CD44+ gastric cancer cells within the population of gastric CSCs exhibited self-renewal properties as well as the capacity to generate differentiated descendants, ultimately leading to the emergence of CD44– cells (Takaishi et al. 2009). The research conducted by Phu Hung Nguyen (Nguyen et al. 2017) demonstrated that CD44 and ALDH are highly specific biomarkers for the identification and isolation of tumor-causing and chemoresistant gastric CSCs in non-cardia gastric carcinomas, regardless of the tumor's histologic categorization.

EpCAM

A transmembrane glycoprotein called EpCAM is present on the outer surface of normal epithelial cells (Boesch et al. 2018). EpCAM is becoming more widely identified as a distinct CSC marker for several malignancies, including breast cancer (Al-Hajj et al. 2003), pancreatic cancer (Li et al. 2007), colon cancer (Shmelkov et al. 2008), and HCC (Yamashita et al. 2009). EpCAM high/CD44+ cells gave rise to CRC, which replicated the whole morphologic as well as phenotypic variety of its parental lesions as well as maintained a distinct phenotype (Dalerba et al. 2007). Additionally, CD166 may be another differently expressed biomarker for CSC isolation in CRC (Dalerba et al. 2007). In contrast to EpCAM–/CD133– HCC cells, EpCAM+/CD133+ cells seem to represent a CSC subset in HCC (Chen et al. 2011).

ALDH

By the process of oxidation of retinoic acid, ALDH detoxifies intracellular aldehydes and may be involved in the differentiation of SCs (Chute et al. 2006). ALDH can be employed as a sole marker of CSCs in head and neck squamous cell carcinoma (HNSC) (Clay et al. 2010). ALDH is frequently coupled to additional CSC markers, and cells that are ALDH high CD44+CD24– and ALDH high CD44+CD133+ may play a significant role in mediating breast CSCs (Crocker et al. 2009). Further investigation was done, and the findings showed that HCC cells with tumor-forming capability had the following hierarchical organization: CD133+ALDH+ > CD133+ALDH– > CD133–ALDH– (Ma et al. 2008).

CD90

A cell adhesion protein and the immunoglobulin superfamily's smallest member, CD90, is a glycoprotein additionally referred to as thymocyte differentiation antigen-1 (Thy-1) (Kisselbach et al. 2009). In prostate cancer (True et al. 2010), HCC (Yang et al. 2008), insulinomas (Buishand et al. 2016), OC (Chen et al. 2016), and other cancers, CD90+ cells were discovered to represent CSCs that were able to predict how well patients would respond to sorafenib (Kim et al. 2019). Co-expression with another surface marker, CD44, resulted in a much more aggressive phenotype, exhibiting a greater propensity for metastasis as well as self-renewal, compared to the CD90+CD44− ones (Wang et al. 2013b).

Intracellular Markers

The most crucial characteristics of CSCs are self-renewal along with the capacity to distinguish into a number of additional distinct cell types (Zhang and Wang 2008). Nanog, Oct4, and Sox2 are transcription factors (TFs) that are crucial for the development of early embryonic stages (Zhang and Wang 2008). Based on this, Shinya Yamanaka's team discovered that multiple transcription regulators (Sox2, Oct4, c-Myc, and Klf4) may reprogram differentiated cells into induced pluripotent stem (iPS) cells by causing them to take on a pluripotent phenotype over a period of a few weeks (Papp and Plath 2011). When the reprogramming factors are expressed, certain cells begin to proliferate quickly and lose their differentiated cell properties with substantial somatic gene downregulation. With the capacity to self-renew as well as differentiate, CSCs may reactivate or reexpress these transcription variables (Zhang and Wang 2008). The optimal CSC markers, in theory, correspond to those that are necessary to preserve their stemness properties. As a result, Nanog, Oct4, and Sox2 can be thought of as intracellular CSC markers.

Nanog

A key part of the second embryonic cell-fate specification is played by Nanog, a homeobox transcriptional factor (Cavaleri and Schöler 2003). However, differentiated cells lack this molecule, which is necessary for maintaining pluripotency (Mitsui et al. 2003; Chambers et al. 2003). By using genome-wide expression assessment, Kristian Almstrup, and coworkers (2004) discovered in 2004 that testicular carcinoma in situ had characteristics similar to embryonic SC: Nanog was increased throughout the progression to embryonic cancer. Nanog expression was found to be directly associated with CSCs (CD133high/CD44high) in 2010 (Botchkina et al. 2010), and Nanog+ NSCLC cells were found to have CSC characteristics (Ghanei et al. 2020). In addition, Nanog status regulates the transition from cancer cells to CSCs (Liu et al. 2020). Furthermore, Nanog expression has been linked to increased ALDH activity, as well as cellular radioresistance (Dehghan Harati et al. 2019) and chemoresistance (Lai et al. 2019).

Oct4

By interacting with octamers, which control the expression of several genes, Oct4 performs its homeodomain transcription factor activity (Schöler et al. 1990). Early expression of Oct4 in the preimplantation embryo controlled early developmental processes in mice (Schöler 1991; Nichols et al. 1998). Monk's research in 2001 was the first to link Oct4 to cancer (Monk and Holding 2001). Ponti and colleagues discovered in 2005 (Ponti et al. 2005) that the CD44+CD24− subpopulation of cells expressed Oct4 and generated new tumors following CSCs in breast cancer. Oct4 high cells exhibit greater SC-like characteristics as compared to Oct4 low cells, including chemoresistance, self-renewal, and increased xenograft tumor formation (Koo et al. 2015).

Sox2

Sox2 is a member of the high-mobility class transcription factor family and is essential for the upkeep and early growth of undifferentiated ESCs. In CSC research, Sox2 is frequently utilized as a stemness-related marker. Sox2 expression was found to be higher in ALDH and CD133+ NSCLC cells (Masciale et al. 2019; Hsu et al. 2011). Sox2 has been demonstrated to be a marker for CSCs in bladder carcinoma by Zhu and colleagues (2017). It was discovered that SORE6+ cells are much more tumorigenic than SORE6− cells in a reporter system (SORE6) that permits monitoring of live cells expressing Sox2 and/or Oct4 (Pádua et al. 2020; Menendez et al. 2020).

3.2 Properties of CSCs That Distinguished Them from Typical Cancer Cells

Cancer is triggered by the buildup of alterations in genes, resulting in the loss of signaling pathway regulation, which leads to the development of self-sufficient growth signals, avoidance of programmed cell death “apoptosis,” sustained angiogenesis, infinite capacity for replication, and the ability to invade neighboring tissues (Sadikovic et al. 2008). CSCs are derived from mutations of normal stem cells triggered by environmental changes, and chronic inflammation, as well as by EMT (Tang 2012). CSCs are also known as tumor-initiating cells because they share numerous critical traits with somatic stem cells (SSCs), including self-renewal, infinite proliferation potential, delayed replication, resistance to drugs, and the potential to differentiate, dividing into cancer cells making the bulk of the tumor (Hayat 2012). CSCs share the same molecular and cellular pathways as SSCs; however, CSCs lack the regulatory system required to avoid uncontrolled multiplication (Spillane and Henderson 2007). According to CSC theory, CSCs can develop into cancer cells and are responsible for tumor growth and metastasis. For instance, Dick and colleagues found a CD34+/CD38− subset in the samples of acute myeloid leukemia patients and in in-vivo experiments, and this subset of cells can develop into

leukemia cells (Bonnet and Dick 1997). Moreover, the differentiation of CSC into cancer cells has also been observed in a variety of solid cancers. Furthermore, both in vitro and in vivo studies show that CD133+ tumor-initiating cells in the brain tumor give rise to multi-lineage CD133– cells (astrocytes, oligodendrocytes, or neurons) (Singh et al. 2004). The rapid growth of metastatic cancer and its resistance to chemotherapy and radiotherapy are still common in many patients, despite the advancements in treatments. Evidence suggests that CSCs, a cancer cell subpopulation with many similarities to SSCs, play a vital role in treatment failure (Bao et al. 2013). Although CSCs account for a small proportion of the entire tumor mass, these cells can re-establish the tumor bulk. For example, the existence of CSCs in the tumor mass is accountable for tumor regrowth as shown by findings from a xenotransplantation experiment using brain tumors in NOD/SCID mice (Singh et al. 2004). CSCs were first discovered in leukemia and have since been reported in malignancies of the brain, pancreas, colon, and breast (Fig. 3.2) (Lenz and Kahn 2014; Ren et al. 2018).

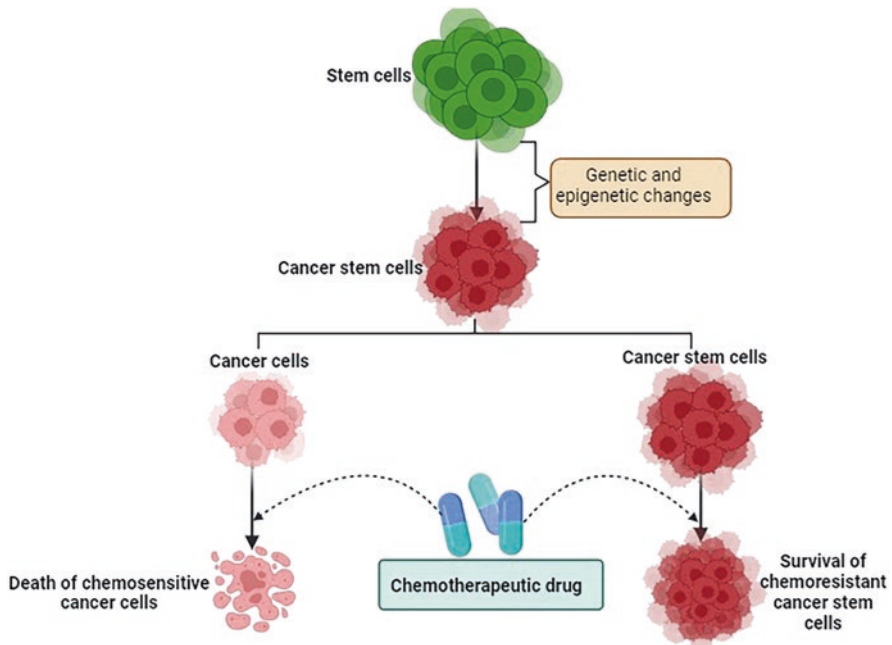


Fig. 3.2 The figure illustrates the unique characteristics that set cancer stem cells (CSCs) apart from typical cancer cells. These unique features include self-renewal capacity, which enables CSCs to generate identical copies of them, and heightened resistance to conventional cancer treatments, such as chemotherapy contributing to their role in tumor initiation, progression, and recurrence

Expression of Stemness-Related Markers

The manifestation of stemness-related markers has been discovered in adult stem cells and embryonic stem cells (ESCs), the two primary forms of human stem cells, as well as in CSCs. These molecular markers and associated pathways are involved not only in normal stem cell maintenance and self-renewal but also in regulating the stemness of CSCs (Pattabiraman and Weinberg 2014; Medema 2013). Investigating these characteristics could assist in elucidating the process of CSC-driven carcinogenesis and may lead to new approaches for CSC-targeted cancer therapy.

Transcriptional Factors Related to Stemness in Cancer

Yamanaka et al. demonstrated that pluripotent stem cells may be generated from embryonic fibroblasts of mice by combining the manifestation of four TFs currently known as the Yamanaka factors (c-Myc, Oct4, Klf4, and Sox2) (Takahashi and Yamanaka 2006). Presently, around 25 TFs have been described as being expressed in stem cells. OCT4, KLF4, SOX2, Nanog, and SALL4 form a fundamental governing linkage for the maintenance and self-renewal of ESCs (Zhao et al. 2017). These TFs are strongly expressed in ESCs but are frequently reduced in normal somatic cells, except for some adult stem cell types. A growing body of evidence suggests that TFs, which are specific to embryos, are aberrantly expressed in human tumor samples, demonstrating the presence of CSCs (Monk and Holding 2001; Zhao et al. 2012). Retroactive investigations on a cohort of patients have also shown the linkage of TF expression to survival consequences in particular tumor types, implying that expression of TF levels may be beneficial in predicting patient prognosis (Schoenhals et al. 2009). Detecting the degree of expression of these TFs, for example, using immunohistochemical staining might thus aid in cancer diagnosis, classification, and therapy approaches.

OCT4

OCT4 expression has been found in tumors of the bladder, ovary, lung, brain, bladder, ovary, prostate, renal, testicular, and leukemia using RT-PCR and immunohistochemistry (Schoenhals et al. 2009). In addition, increased OCT4 expression has been linked to a poor prognosis in bladder cancer (Hatefi et al. 2012), prostate cancer (De Resende et al. 2013), medulloblastoma (Rodini et al. 2012), and esophageal squamous cell carcinoma (ESCC) (Li et al. 2012).

SOX2

SOX2 has been found in the lung, prostate, brain, breast, liver, and testicular tumors, and its expression has been related to a poor prognosis in stage I lung adenocarcinoma, gastric carcinoma, squamous cell carcinoma, ovarian cancer, and small cell lung cancer (Zhao et al. 2017).

KLF4

KLF4 has been discovered to be expressed in tumors of the breast, brain, head and neck, oral, prostate, and testis, as well as leukemia and myeloma (Schoenhals et al. 2009). KLF4 expression can also be used to predict the prognosis of colon cancer (Patel et al. 2010) and HNSC (Tai et al. 2011). Moreover, nuclear KLF4 localization has been linked to the aggressive phenotype of early-stage breast cancer, as well as a poor prognosis in nasopharyngeal and oral malignancies (Zhao et al. 2017).

Nanog

Nanog is expressed in the tumors of the breast, brain, prostate, colon, liver, and ovary (Hart et al. 2005). High Nanog expression encourages the EMT, a critical developmental phase for cancer cells to acquire stem cell features (Mani et al. 2008). Nanog has also been linked to a detrimental prognosis in malignancies such as breast, colorectal, gastric, lung, ovarian, and liver (Zhao et al. 2017).

c-Myc

c-Myc is a transcriptional factor that is involved in both stem cells and cancer cells. Overexpression of c-Myc, one of the most researched oncogenes, has been found to promote cancer in animal models. Furthermore, overexpression of c-Myc has been found in up to 70% of human malignancies, including the brain, breast, colon, pancreas, head and neck, prostate, renal, salivary-gland, and testis tumors, as well as leukemia and lymphoma (Schoenhals et al. 2009; Kim and Orkin 2011; Dang 2012). The expression of c-Myc has also been associated with the worst prognosis in hepatocellular carcinoma and early uterine cervix carcinoma (Zhao et al. 2017).

SALL4

SALL4 expression has been found in malignancies of the breast, liver, colon, ovary, and testis, as well as leukemia (Chen et al. 2013; Wang et al. 2014a). SALL4 expression has been evaluated as a poor prognostic marker in liver carcinoma (Oikawa et al. 2013; Yong et al. 2013) gliomas (Zhang et al. 2015), and myelodysplastic syndromes (Wang et al. 2013c).

Surface Markers Related to Stemness in Cancer

Cell surface proteins make it possible to isolate and investigate distinct cell types using magnetic sorting or flow cytometry. Furthermore, they allow for precise targeting, which is useful for disease monitoring and treatment delivery. In a similar way to a stemness-related transcription factor, many surface markers that are overexpressed in stem cells, such as TRA-1-60, EpCam, SSEA-1, ALDH1A1, Lgr5, CD47, CD49f, CD133, CD44, CD13, CD19, CD20, CD24, CD26, CD27, CD34, CD38, CD45, CD66c, CD90, CD166, TNFRSF16, CD105, CD117/c-kit, and CD138, are also expressed in human cancers (Walcher et al. 2020). Among these, CD44 and CD133 are the most often employed markers in CSC research and are also therapeutic targets in malignancies (Zhao et al. 2017).

CD44 is a transmembrane glycoprotein involved in cell division, motility, adhesion, and signaling (Basakran 2015). It is consistently produced in both fetal and adult hematopoietic stem cells, and when it binds to its principal ligand, hyaluronic acid, it mediates cell-cell contact and signal transduction. CD44 is found in a variety of malignancies, including breast, bladder, colon, glioma, gastric, osteosarcoma, head and neck, ovarian, leukemia, pancreatic, and prostate cancers, as well as osteosarcoma. According to reports, CD133 has a role in cell growth and development and is expressed in hematopoietic stem cells, endothelial progenitor cells, glioma, and neuronal and glial stem cells (Li 2013; Muraro et al. 2012). Almost all tumor types can be identified by CD133 expression, and in the NOD-SCID mouse model, CD133+ tumor cells exhibit stem cell-specific traits like self-renewal, differentiation, and tumor development (Singh et al. 2004). CD133+ cells exhibit chemoresistance and radioresistance after being injected into mice with impaired immune systems. Studies have been conducted on the utilization of CD133 as a potential therapeutic target for metastatic melanoma (Rappa et al. 2008), colon cancer (Catalano et al. 2012), and ovarian cancer (Skubitz et al. 2013). Drug delivery has also employed CD133 as a potential target (Smith et al. 2008).

A variety of different CSC surface markers appear to function in particular kinds of tumors. SSEA-1, for example, has been demonstrated to be expressed in human colonic cancer and glioblastoma (Mao et al. 2009; Lin et al. 2017). TRA-1-60 has been linked to prostate cancer (Giwerzman et al. 1993). Lgr5 is expressed in head and neck, colon, and stomach tumors (Barker et al. 2007; Simon et al. 2012). CD90 has been seen in high-grade human glioma (He et al. 2012), as well as liver and lung tumors (Zhao et al. 2017), whereas CD117 has been employed as a CSC marker in leukemia (Eren et al. 2016), gastrointestinal stromal tumors (Sarlomo-Rikala et al. 1998), oral squamous cell carcinomas (Mărgăritescu et al. 2011), and ovarian tumors (Luo et al. 2011). CD24 has been implemented together with CD44 in breast cancer cell lines to demonstrate that CD44+/CD24– cancer cells exhibit treatment resistance and invasive characteristics (Muraro et al. 2012; Sheridan et al. 2006; Adamczyk et al. 2014). CD24 has also been found to be an independent predictive marker for non-small cell lung cancer (Lee et al. 2010; Karimi-Busheri et al. 2013) and ovarian cancer (Kristiansen et al. 2002).

Other Important Markers of Stemness

Many stemness-related markers such as ALDH, Bmi-1, Musashi-1, Nestin, TIM-3, and CXCR are neither TFs nor cell surface proteins. ALDH enzymes, which are found everywhere in the cells, cause the irreversible oxidation of cellular aldehydes in the cytoplasm. ALDH enzyme activity has been discovered to be high in ESCs, adult hematopoietic and brain stem cells, and CSCs. ALDH activity in CSCs has been linked to the expression of ALDH1A1, which has been shown to govern stem cell self-protection, differentiation, and population expansion. ALDH has been linked to a poor prognosis in HDSCs (Qian et al. 2014; Yang et al. 2014). It is also being considered as a potential treatment target in ovarian (Brigham et al. 2012) and

non-small cell lung malignancies (Liu et al. 2016a). BMI1 is a protein that is necessary for hematopoietic stem cell self-renewal (Gong et al. 2006) as well as neural stem cell self-renewal (Molofsky et al. 2003). In head and neck cancer models, drug-induced BMI1 expression has been demonstrated to improve stem cell populations (Nör et al. 2014). BMI1 has been identified as a prognostic marker in oligodendroglial tumors and breast cancer (Zhao et al. 2017). Nestin and Musashi-1 have both been found in neural stem cells (Strojnink et al. 2007), where they both play critical roles in stem cell maintenance and self-renewal. Nestin's expression has been found in altered cells from many human cancers, and it has been linked to the clinical course of a few diseases (Neradil and Veselska 2015). Furthermore, Nestin co-expression with other stem cell markers has been identified as a CSC characteristic (Wang et al. 2009).

3.3 CSCs as Biomarkers

The use of biomarkers in therapy, diagnosis, and prognosis has grown in popularity during the last few decades. Specifically, biomarker analysis in cancer patients during the pre- and post-therapeutic periods is essential to identify many types of cells that are associated with cancer cell progression and subsequent post-therapeutic reversion. CSCs are a type of tumor cell that can originate tumors and generate relapses. Because of their significance, various biomarkers that distinguish CSCs have been found and associated with therapy, diagnosis, and prognosis. The expression of specific CSC surface markers has allowed for the identification of CSCs in several solid tumors. The surface markers CD29, CD24, CD90, CD44, CD133, ALDH1, and ESA (epithelial-specific antigen) have been used to identify CSCs up to this point (Xia 2014; Ajani et al. 2015; Ding et al. 2015). They can express several markers specific to each type of CSC, depending on the tissue type from which they are derived. Most significantly, the expression of these markers can be exploited to target CSCs specifically for therapeutic purposes (Burgess and Huang 2016).

3.4 Cross Talk Between Immune Cells and CSCs in Therapeutic Resistance in Cancer

CSCs have been proven to be more tumorigenic than conventional cancer cells present in solid tumors, and they may be able to escape the immune response of the host because of their phenotypic and functional features, which allow them to survive and propagate all over the body (Codony-Servat and Rosell 2015). Tumor cells may evade the host immune response by a variety of methods, including the generation of immunosuppressive factors, poor tumor antigen expression, absence of manifestation of human leukocyte antigens (HLAs), and co-stimulatory molecules (Chikamatsu et al. 2011). Several studies show that CSCs are capable of regulating the function of immune cells, including macrophages, T cells, B cells, and NK cells (Shi et al. 2011).

3.5 Immunomodulatory Properties of CSCs and Tumor Microenvironment

The tumor microenvironment, which comprises the ECM and non-tumor cells such as cancer-associated fibroblasts (CAFs), endothelial cells, and immune cells, can govern the expansion of CSCs (Albini et al. 2015; Korkaya et al. 2012). Stromal cells can influence CSC activity through paracrine signaling. For example, hepatocyte growth factor (HGF) released by myofibroblasts promotes signaling pathways such as Wnt and causes non-CSCs to de-differentiate into CSCs (Vermeulen et al. 2010). Similarly, the TME vasculature promotes carcinogenesis and offers a dedicated environmental niche for CSCs. In colon cancer, endothelial cells have been demonstrated to induce the CSC phenotype by releasing the DLL4 (Notch ligand Delta-like ligand 4) (Hoey et al. 2009) and also produce growth factors that stimulate stem cells like phenotype in glioblastoma. The CSC niche maintains cancer cells functioning and protects them from the attack of immune cells. The ongoing communication of CSCs with the microenvironment of tumors provides the ability to resist surveillance and abolition by immune cells, allowing their existence and growth (Sistigu et al. 2020; Hinshaw and Shevde 2019). Thus, understanding the ability of cancer cells to evade immune recognition is an essential requirement for establishing more effective therapeutic options.

MDSCs release chemokine and cytokine to lessen the effectiveness of immunotherapy (Welte et al. 2016). In CSCs, mTOR signaling increases the intrusion and accumulation of MDSCs at the tumor sites (Welte et al. 2016). CD133+ CSCs in melanoma promote TGF- β 1 manifestation and attract immunosuppressive MDSCs to the tumor location (Shidal et al. 2019). Furthermore, leukemia stem cells generate TIM-3/Galectin 9 (Gal-9) on the surface and increase the amount of invading TAMs and MDSCs, resulting in decreased immune responses against tumors (Gao et al. 2014). Furthermore, MDSCs produce exosome S100A9, which increases the action of the STAT3/NF- κ B signaling and the synthesis of prostaglandin E2 (PGE2), increasing the stemness and survival of cancer cells (Hassan et al. 2020; Wang et al. 2019a). These outcomes imply that CSC-MDSC communications influence CSC stemness, resulting in tumor development and progression (Kuroda et al. 2018).

Regulatory T cells (Tregs) are a subset of CD4+ T cells that often include Foxp3+CD25+CD4+ T cells and have tumor-promoting properties (Sakaguchi et al. 2010). Tregs suppress the host immune system by secreting immunosuppressive cytokines like IL-10, IL-35, and TGF- β , thus decreasing the action of the effector T cells as well as other immune cells. Moreover, CSCs cause Treg cell infiltration in glioblastoma through the costimulatory molecule PD-L1, soluble Galectin-3, and TGF- β release, whereas ABCB5+ melanoma cells induce Treg cell intrusion through a B7-2-dependent process (Beck et al. 2011). Similarly, CSCs release CCL1 to attract Tregs, which then secrete TGF- β 1 and IL-17 to promote CSC self-renewal. CSCs may influence the Th17/Treg equilibrium by modulating the formation of cytokines like IL-8 and IL-6, as well as the chemokine CCL5 in the TME (Yu et al. 2012). It has been suggested that Th17/Treg homeostasis has a dual role in promoting or suppressing cancer (Knochelmann et al. 2018). Moreover, under hypoxic

conditions, Tregs produce VEGF to support the existence, stemness, and self-renewal of CSCs.

Natural killer (NK) cells are cytotoxic lymphocytes that are accountable for eliminating tumor cells. Higher cytotoxic action of NK cells has been linked with a lower threat of malignancy (Levy et al. 2011). Apart from antibody-dependent cellular cytotoxicity (ADCC), NK cells identify target cells in cell-cell interactions via a range of stimulating and repressing receptors. Stimulating receptors such as NKG2D, NCR, and NKG2C as well as repressing receptors like Ly49 attach to MHC or HLA class I molecules and cellular stress ligands, resulting in the response of NK cells (Zhang et al. 2020). In many cancers like human colon cancer, oral squamous carcinoma, glioblastoma, melanoma, and preferential NK cell killing of CSCs have been seen in response to cancer cell exposure (Lei and Lee 2021). CSCs are specifically targeted using the SC markers CD24+, CD133+, and ALDH+, confirming the involvement of NK cells in successfully targeting and destroying CSCs. This specific identification of CSCs is thought to be arbitrated by NKG2D, Nkp30, and DNAM-1 stimulating receptor (Tallerico et al. 2016). CSCs may be able to avoid recognition by natural killer cells by going into a dormant state and decreasing ULBP ligands (ligands of NKG2D) that bind to and activate NK cells (Malladi et al. 2016). Furthermore, CSC may suppress cytotoxic T cells by decreasing the expression of MHC-I and increasing PD-L1 manifestation, subsequent in the host immunosuppression (Alhabbab 2020). A study examined the immunological features of CSC-like cells (CD44+) to tumor cells (CD44-), revealing that CD44+ cancer cells exhibit an immunosuppressive phenotype. This result was investigated by inhibiting activated T cell growth, inducing Treg polarization, and suppressing Th1 cells, which results in the malfunction of the effector and cytotoxic T cells (Chikamatsu et al. 2011). An alternative study found that CSCs that are isolated from therapy-resistant tumors exhibit pro-inflammatory tumorigenic features, such as promoting the macrophage colony-stimulating factor-making by stimulating the interferon regulatory factor-5 (IRF-5) pathway (Yamashina et al. 2014). Moreover, ALDH+ CSCs in breast cancer avoid NK cells by lowering the manifestation of NKG2D ligands MICB and MICA via miR20a regulation (Wang et al. 2014b). In conclusion, NK-mediated death plays a crucial part in the immune response by reducing CSCs with the help of numerous stimulating and repressing receptors. However, CSCs may avoid particular targeting by changing the manifestation of these receptors. A new study additionally revealed that, depending on the cancer type, NK cells may conflict with their conventional cytotoxic action by encouraging EMT in CSCs (Huergo-Zapico et al. 2018).

Macrophages are divided into two types: pro-inflammatory (M1) macrophages and anti-inflammatory (M2) macrophages (Chen et al. 2019). TAMs typically have an M2 type, which performs immunosuppressive and pro-cancer behaviors and is associated with the progression and recurrence of cancer (Malfitano et al. 2020). According to emerging research, CSCs influence the immune TME by attracting macrophages and encouraging their pro-cancer characteristics, while in turn, TAMs are essential for CSC maintenance and self-renewal at the primary site of tumors due to the coupling of STAT3 and NF- κ B signaling cascades (Sainz et al. 2016). It

has been suggested that CSCs have an innate immunosuppressive program that involves attracting macrophages to the tumor spot and directing them toward M2 polarisation (Brissette et al. 2012). This capability of CSCs is frequently observed in malignancies of the ovary, glioma, liver, breast, and lung via activating the NF- κ B and STAT3 cascades as well as cytokines including interleukin (IL-10 and IL-8) (Mitchem et al. 2013; Fang et al. 2014). For instance, CD133+ cells secrete IL-8 to cause M2 polarization of TAMs in hepatocellular carcinoma (Xiao et al. 2018). CSCs produce more of the chemoattractants C-C motif chemokine ligands (CCL2, CCL5), VEGF-A, and neurotensin in glioblastoma than the majority of the tumor produces (Yi et al. 2011). After being recruited into the TME, TAMs are then used as a “niche” for supporting CSC expansion. These penetrating TAMs secrete inflammatory cytokines such as IL-6, IL-10, IL-1 β , TGF- β , and MFG-E8 by triggering the NF- κ B signaling pathway (Fang et al. 2014; Jinushi et al. 2011; Wan et al. 2014). These cytokines that are tumor-promoting interact with their receptors, causing STAT3 activation in neighboring CSCs. This causes a vicious loop of NF- κ B activation and maintenance of cancer cell stemness. In a mouse model, for example, breast cancer cells treated with TAM-conditioned media result in increases in the Sox-2, Oct3/4, Nanog, and ALDH1 expression (Nnv and Kundu 2018). Surprisingly, it has been postulated that CSCs in leukemia ensure their persistence in the niche of leukemic stem cancer by enhancing the expression of TIM-3 (mucin domain-containing protein 3), which stimulates MDSCs and following differentiation into TAMs (Raggi et al. 2016).

Dendritic cells (DCs) are basically APCs that trigger inherent (innate) or acquired (adaptive) immune responses (Ma et al. 2013). Immature DCs acquire antigens that are derived from tumors and display them to immune cells on their cell surface using appropriate costimulatory molecules, subsequently activating an antigen-specific immune response and the establishment of memory B and T cells (Ravindran et al. 2019). An increasing body of research has shown that CSCs play an important role in immune avoidance by altering the phenotypes of DC and inhibiting their recruitment to TME. Moreover, CSCs are accountable for altering the functional differentiation and stimulation of DCs, converting DCs to tolerogenic or reducing their ability to stimulate T cells (Jachetti et al. 2015; Zhong et al. 2019). In colorectal cancer, CD133+ CSCs weaken DC function by lowering the number of activated DCs (Szaryńska et al. 2018). The EpCAM+ HCC subtype possesses stemness characteristics and stimulates the production of alpha-fetoprotein (AFP), which prevents DC maturation, differentiation, and T cell proliferation (Yamashita et al. 2009). Additionally, CSCs produce the immunosuppressive cytokines IL-4, IL-10, IL-13, and TGF- β as well as demonstrate higher coinhibitory molecules like PD-L1, B7-H3, and IDO1 (Lei and Lee 2021). These molecules are essential for the buildup of immunosuppressive DCs, which limit the immune system's ability to fight tumors by promoting T cell tolerance and the recruitment of T regulatory cells (Pardee et al. 2014). TGF- β is known for having a detrimental impact on immune response, concerning DCs, TGF- β limits DC stimulation by repressing the expression of its costimulatory markers CD80 and CD86 as well as MHC class II (Lei and Lee 2021). Furthermore, TGF- β stimulates Wnt/ β -catenin stimulation and thus weakens the

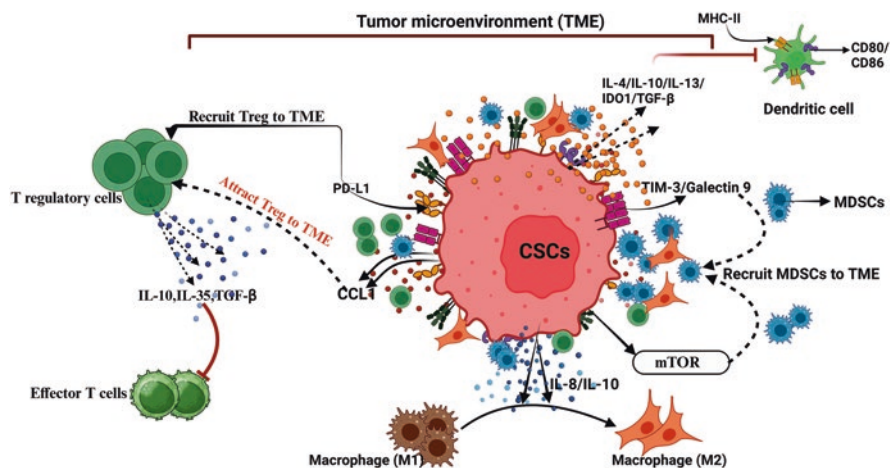


Fig. 3.3 The figure depicts the dynamic cross talk between immune cells and cancer stem cells (CSCs) within the tumor microenvironment, elucidating their pivotal role in therapeutic resistance. The bidirectional signaling pathways between immune cells and CSCs contribute to the development of resistance mechanisms against therapeutic interventions, including chemotherapy and immunotherapy

recruitment of BATF3+ DCs, which is associated with CD8+ T cell penetration (Spranger et al. 2017). TGF-β via β-catenin pathway weakens T cell-mediated immune investigation and diminishes DC recruitment to the tumor site, rendering immune escape of HCC and resistance to anti-PD1 treatment (De Galarreta et al. 2019). Furthermore, in a metastatic ovarian carcinoma, TGF-β promotes the growth of immunosuppressive DCs that express PD-L1, which reduces CD8+ T cell activation (Krempski et al. 2011). Cancer and stromal cells in the TME express CXCR4 and its ligand CXCL12 to withstand CSC self-renewal and attract regulatory DCs. These DCs build CXCL12 in an autocrine way and use a feed-forward process to sustain CSC stemness (Fig. 3.3) (Sultan et al. 2017).

3.6 Development of CSC-Specific Immunotherapy

In the past 10 years, CTLA-4 and PD-1 inhibitors have transformed cancer investigation and re-emphasized the importance of immunology in the creation of new therapeutics. Effective CSC targeting may necessitate extremely precise identification of the population of CSC. Immunological targeting of CSCs in therapeutic advance is currently concentrated on three key approaches: niches, phenotypes, and antigens related to CSCs. NK and DC cells have intrinsic effector and antigen-presenting characteristics that make them suitable immunotherapy candidates. Additionally, numerous studies showed that chemotherapy or radiation results in CSC proliferation as well as an increase in the expression of MICA and MICB on CSC (Ames et al. 2015). This emphasizes the potential for NK cell treatment to be

used in combination with conventional drugs to eradicate CSCs. Numerous current clinical trials are treating advanced solid tumors with NK cell infusion (NCT03592706 and NCT04162158) or in combination therapy (NCT03319459). The goal of adaptive NK cell therapy is to improve and enhance the anticancer properties of NK cells obtained from both allogeneic and autologous sources (Lei and Lee 2021). According to multiple preclinical investigations, DC immunization has strong immune effects. It is speculated that triggering DCs with CSC-designated antigens will help to eradicate CSC with higher precision and successfully inhibit CSC-caused tumor recurrence and metastasis (Pang et al. 2019). Sipuleucel-T is the first FDA-authorized DC vaccination for advanced prostate carcinoma, proving DCs as a successful treatment method in immunotherapy (Cheever and Higano 2011). Clinical investigations, however, indicated only a 10–15% response rate to DC vaccinations for different tumor types (Anguille et al. 2014). DC vaccines targeted to CSC have recently been shown to improve the eradication of melanoma CSCs in a murine tumor model using a permutation of PD-L1 and CTLA-4 obstructions, with an improved CD8+ T cell response, elevated IFN- γ , and suppressed TGF- β manifestation (Zheng et al. 2018). The findings highlight the utility of DC vaccines based on CSC in combinational therapy.

Since 2017, the FDA has permitted the utilization of CAR-T cell treatments targeting CD19 in the treatment of lymphoma (US Food and Drug Administration 2017). A growing number of preclinical investigations have shown that immunological regulation of CAR-T cells is beneficial in limiting tumor progression and improving the survival of the host (Chong et al. 2017; Kloss et al. 2018). CAR-T treatment, which demonstrates good results in leukemia and lymphoma treatment, has been suggested for targeting CSCs. EpCAM, CD133, and CD90 of CSCs have been recognized as CAR-T cell antigens (Guo et al. 2018). An EpCAM-targeted CAR-T cell clinical study is underway to treat EpCAM+ tumors. To reduce harmfulness to healthy cells, these CSC indicators can be combined with an inhibitory receptor specific for healthy tissues.

Apart from directly affecting CSC phenotypes and markers, scientists have also focused on targeting the CSC niche, which subsidizes CSC self-renewal and immune evasion. CSCs retain self-renewal by forming a positive feedback loop with immunosuppressive cells like TAMs via Wnt/ β -catenin, JAK/STAT3, and NF- κ B cross talk stimulation, as well as the release of inhibitory cytokines like TGF- β , IL-6, and IL-8 (Nengroo et al. 2022). Wnt/ β -catenin-targeted drugs are now in clinical trials, including anti-FZD receptor mAb (monoclonal antibody) (Vantictumab), β -catenin inhibitor PRI-724, and WNT974, which is a small-molecule porcupine inhibitor (Katoh 2017, 2018). To address the immune evasive capability of CSCs in Wnt-directed malignancies, Wnt-targeted actions are recommended to be adopted as combination therapy with ICIs like ipilimumab and nivolumab or tyrosine kinase inhibitors (Katoh 2017). IL-6 inhibition has previously been shown to inhibit NSCL carcinoma and the growth of H460 lung CSCs (Yi et al. 2012). Tocilizumab, an IL-6 receptor mAb, has been shown to decrease the pre-metastatic capacity of breast

CSCs and increase the efficacy of cisplatin contrary to TNBC (Alraouji et al. 2020). The FDA has approved the medicine for the treatment of rheumatoid arthritis, and it is presently in phase II testing for the treatment of metastatic melanoma in conjunction with ICIs like ipilimumab and nivolumab (NCT03999749). Angiogenesis' functions in favoring immunosuppressive TME and CSC self-renewal have been thoroughly investigated; therefore, a conjunction use with a VEGF inhibitor offers an innovative approach to immunotherapy. In a phase III clinical investigation (NCT03434379), bevacizumab and the PD-L1 inhibitor atezolizumab outperform than sorafenib in terms of OS and PFS in patients with advanced HCC (Finn et al. 2020). In recognition of this good consequence, the FDA authorized the combination of Atezolizumab and bevacizumab as first-line therapy for patients with unresectable HCC (Yang et al. 2020b).

The direct effect of immunosuppressive cells like TAMs with zoledronic acid effectively suppressed the proliferation of CSCs derived from cervical cancer by lowering stemness and triggering death (Wang et al. 2019b). The medication is now being evaluated in phase III clinical trials (NCT02622607) to determine if it can prevent bone metastases in individuals with metastatic lung cancer. Antibodies targeting CD47 also evade a critical immune evasion process, the "Don't eat me" signal mediated by CD47/SIRP α (Russ et al. 2018). ALX148, a CD47-inhibiting protein, was well tolerated in patients with advanced malignancies when combined with anticancer antibodies and standard treatment (NCT03013218). Hu5F9-G4, an anti-CD47 antibody, is also well tolerated and has encouraging effects in leukemia SCs when used in conjunction with Azacitidine (NCT03248479). These results indicate that targeting CD47 as part of predictable cancer treatment could be an effective strategy for combating CSC-derived immune evasion. While CSC-targeted therapies such as NK cells, CAR-T therapies, and DC vaccines are still being investigated, targeting the CSC niche may be a viable immunological treatment approach in cancer elimination, based on multiple promising preclinical outcomes. Similarly, significant work will be needed further to overcome the negative effects of most immunotherapies, such as resistance.

3.7 Dysregulated Signaling Cascades That Direct CSCs in Regulating Treatment Resistance

A complex signaling network controls the homeostasis of regular stem cells and the improper activation or suppression of it promotes neoplastic transformation. Because of these abnormalities, CSCs can self-renew and differentiate, giving malignancies a stem-like quality. Such signaling cascades are essential for the maintenance and survival of CSCs' stemness, just like they are for their regular counterparts. CSCs' resistance to therapy is mediated by several signaling mechanisms. Hedgehog (Shh), Wnt/ β -catenin, Notch, and NF- κ B pathways are some of the most significant and well-studied signaling pathways.

Wnt/ β -Catenin Signaling

The widely conserved Wnt pathway is well known to play a crucial role in tissue homeostasis as well as embryonic growth (Sedgwick and D'Souza-Schorey 2016). A wide variety of malignancies commonly exhibit aberrant stimulation of Wnt signaling components, such as Axin, adenomatous polyposis coli (APC), β -catenin, and Wnt1, which is linked to the development and spread of cancer (Yang et al. 2016; Yu et al. 2021). The noncanonical Wnt route doesn't depend on β -catenin, whereas the canonical Wnt system does.

The canonical Wnt signaling seems to have a significant role in sustaining cancer cells' stem cell-like characteristics in the establishment of CSC regulation (De Sousa E Melo and Vermeulen 2016). Canonical Wnt signaling, possibly by controlling the expression of the CSC indicator OCT-4, is essential for sustaining lung CSC properties (Prabavathy et al. 2018). Additional CSC markers like CD24, CD44, Prom1, and ALDH1 are increased by the Wnt/ β -catenin route, increasing tumor stemness (De Sousa E Melo and Vermeulen 2016). These CSC markers were likewise overexpressed in Wnt+ glioma cells, demonstrating the contribution of Wnt/ β -catenin signaling to the maintenance of glioblastoma stemness (Liu et al. 2018a). The dedifferentiation of colorectal carcinoma (CRC) cells, a unique indicator of stemness, was also facilitated by the nuclear translocation of β -catenin (Liu et al. 2016b; Malta et al. 2018). On the other hand, squamous cell cancer completely regressed after β -catenin gene ablation caused the loss of CSC populations (Malanchi et al. 2008).

Although the first-line therapy for CRC, 5-fluorouracil (5-FU), can slow the development of tumors, recent research found that 5-FU may also activate CSCs through the Wnt/ β -catenin signaling route, leading to chemotherapy resistance in patients with CRC (Cho et al. 2020). The Wnt/ β -catenin pathway is activated by the m6A modification-Sec62- β -catenin, which increases stemness as well as chemotherapy resistance in CRC (Liu et al. 2021).

Over 50% of breast tumors have an elevated level of the Wnt/ β -catenin pathway, which is an indicator of a poor prognosis (Khramtsov et al. 2010). Additionally, breast CSCs have increased expression of the activated β -catenin protein. In breast cancer cells that overexpressed the protein HER2, inhibiting β -catenin signaling greatly reduced tumor growth and metastasis (Schade et al. 2013; Khan et al. 2023b).

Resistance to CSC-mediated treatment is regulated by the Wnt/ β -catenin pathway. The CSC phenotype as well as resistance to sorafenib in HCC is caused by PTK2 promoter hypomethylation, which also increases PTK2 overexpression and stimulates Wnt signaling (Fan et al. 2019). Additionally, SMOC-2 can bind to FZD6 and LRP6, activating Wnt and causing resistance to paclitaxel as well as cisplatin (Lu et al. 2019). In addition, by directly interacting with a promoter and offering castration resistance, Wnt signaling is a crucial mechanism that controls the widely recognized SC marker CD44 (Yun et al. 2016). Additionally, it has been noted that the transcription factor Sox8 promotes the Wnt/ β -catenin cascade by attaching to the FZD7 promoter, ultimately resulting in resistance to cisplatin (Xie et al. 2018).

Notch Signaling

A biologically regulated process called notch signaling plays a role in the early growth of the heart, the central nervous system, and numerous other organs (Clara et al. 2020; Dandawate et al. 2016). The Notch signal plays a crucial function in the development and dissemination of cancer. Four Notch transmembrane receptor types (Notch 1–4) and five membrane-bound cellular surface ligands (JAG 1–2 and DLL 1–3) have been identified in mammalian cells (Wang et al. 2011). The Notch intracellular domain (NICD), the functional component of Notch receptors, is generated by the proteolysis process after the binding of the ligand with the receptor. When NICD is activated, the NICD-CSL complex is produced, which subsequently attracts MAML and p300 coactivators, triggering the subsequent genes of Notch signaling, that is, c-Myc, Hes-1, NF- κ B, HER2, p21, and cyclin-D1 (Fortini 2009; Mir et al. 2023b; Singh et al. 2017).

Radiation and chemotherapeutic resistance are promoted by CSCs via activating the Notch cascade (Capodanno et al. 2018; Panaccione et al. 2016). The inhibition of Notch signaling may make CSCs more susceptible to radiation treatment and drugs like cisplatin or carboplatin (Panaccione et al. 2016; Muñoz-Galván et al. 2019). In the case of breast cancer, Nanog controlled Notch signaling as well as ALDH activity and radiation resistance (Dehghan Harati et al. 2019). Additionally, the interaction between NF- κ B and Notch in triple-negative breast cancer (TNBC) contributes to therapeutic resistance; Jagged1 causes the antiapoptotic gene cIAP-2 to be transcribed in the nucleus in an NF- κ B-dependent manner. Additionally, Notch can be controlled by extracellular signals (Hossain et al. 2018). ILK is essential for controlling IL-6-induced Notch1 stimulation and CSC growth via γ -secretase assembly at the caveolae, according to En-Chi Hsu (Hsu et al. 2015). Additionally, inhibition of ADAM-17, a crucial element of Notch cascades by Nectin-4's, may partially revert resistance toward 5-FU (Nayak et al. 2019).

NF- κ B Pathway

Through the link between inflammation and stemness, the NF- κ B pathway promotes both chronic as well as acute inflammation in tumors. It is essential to tumor biology and controls important events throughout the onset and advancement of various cancers (Naugler and Karin 2008; Biswas et al. 2004). The p50–p65 dimer is NF- κ B's primary physiological constituent. When combined with additional transcription factors, the active p50–p65 dimer is subsequently stimulated by posttranslational modifications and delivered to the cell's nucleus, where it induces the expression of the target genes.

Recently, it was discovered that CSCs selectively trigger NF- κ B signaling. Salinomycin, an NF- κ B inhibitor, has the ability to cause apoptosis in OC that is resistant to cisplatin (Parajuli et al. 2013). Additionally, Twist2 transcriptionally promotes NF- κ B stimulation, and NF- κ B promotes Twist2 expression, producing a

positive feedback loop that drives EMT and increases CSC-like features (Wu et al. 2017a). Furthermore, the activation of NF- κ B has been shown to stimulate hypoxia-related signaling pathways associated with stemness. Additionally, NF- κ B has been observed to reverse apoptotic cell death triggered by reactive oxygen species (ROS) in CSCs (Zhu et al. 2013; Khan et al. 2023c). Furthermore, the hypothesis proposing a sequential stimulation of the IL-1 α and IL-6 signaling cascades caused by HER2 was substantiated by the subsequent pieces of evidence: (1) the upregulation of IL-1 α expression is facilitated by HER2 via the stimulation of the PU.1 transcription factor through the MAPK pathway. (2) The secreted IL-1 α molecule binds to its receptor, leading to the stimulation of NF- κ B. This stimulation, in turn, triggers a feedback system that results in the binding and stimulation of the IL-1 α and IL6 promoters. (3) The secreted IL-6 molecule binds to its receptor, initiating the stimulation of the downstream STAT3 transcription factor (Liu et al. 2018b). The HER2/NF- κ B model has the potential to facilitate tumorigenesis and confer resistance to chemotherapy. Bhushan Thakur reported the existence of an additional autocrine loop, wherein cisplatin specifically induces NF- κ B activation in CSCs (Thakur and Ray 2017). This activation subsequently triggers a bimodal feedback loop involving NF- κ B-TNF α and NF- κ B-PIK3CA. This process serves to establish an autocrine loop through the activation of TNF α -NF- κ B in CSCs. Additionally, it enhances PIK3CA and PI3K/AKT signaling, ultimately resulting in the stabilization of NF- κ B (Thakur and Ray 2017). The stimulation of the PI3K/AKT pathway has been found to confer resistance toward cisplatin by modulating the expression of antiapoptotic genes, leading to an increase in cFLIP, as well as the downregulation of proapoptotic genes, resulting in a reduction in Bax and PUMA. A steady supply of NF- κ B via the TNF-NF- κ B autocrine loop, along with improved NF- κ B stabilization by stimulated AKT, sustains an antiapoptotic, quiescent CSC phase that provides resistance to chemotherapies in resistant cells (Thakur and Ray 2017; Wadhwa et al. 2020).

Sonic Hedgehog Cascade

The Shh (Sonic Hedgehog) pathway was first discovered in *Drosophila melanogaster*, commonly known as the fruit fly, and plays a crucial role in the process of embryonic development. The interaction between the Hedgehog (Hh) ligand and its receptor Patched (PTCH) facilitates the transfer of glioma-associated protein 1/2 (Gli1/2) to the cell's nucleus through the involvement of smoothened (SMO). This translocation process is responsible for initiating the Shh target genes transcription (Hui et al. 2013). The protein Shh plays an important role in controlling the growth, specialization, and movement of specific cells in a manner that is dependent on their location, timing, and concentration of Shh (Merchant and Matsui 2010).

The stimulation of the Hedgehog signaling cascade has been shown to have a stimulatory effect on the expression of genes that regulate cell proliferation, namely, cyclin D1, cyclin D2, N-Myc, Hes1, and Igf-2 (Merchant and Matsui 2010). The

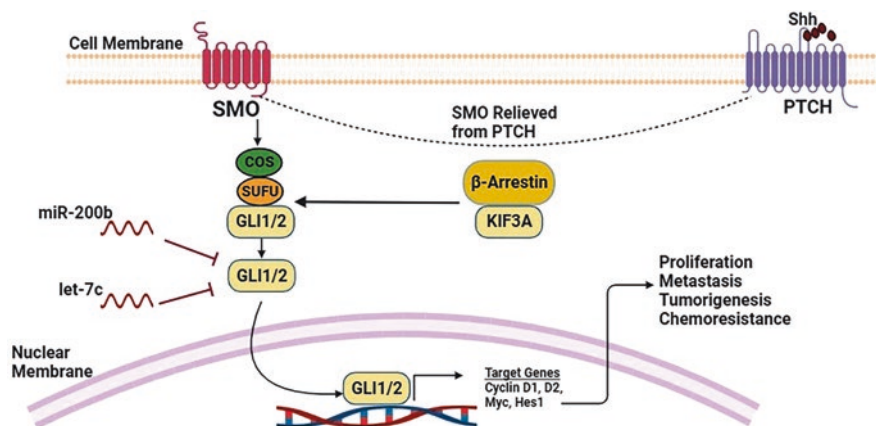


Fig. 3.4 The figure elucidates the role of the Hedgehog (Hh) signaling pathway in contributing to therapy resistance within cancer stem cells (CSCs)

Shh has been found to be associated with the development of chemoresistance (Chen et al. 2011). The Shh signaling pathway plays a regulatory role in the ABCG2 efflux pumps (196), as well as in ALDH activity (Li et al. 2019; Zhang et al. 2012). Additionally, it has been observed to reverse resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs). Moreover, the Shh signaling pathway was found to sustain the phenotypes associated with CSCs and malignant transformation in CD44+ gastric carcinoma cells. Additionally, inhibiting the Shh pathway demonstrated the potential to counteract chemotherapy resistance in CD44+ cells (Yoon et al. 2014). The LncRNA-cCSC1 has been found to have a role in activating the Shh signaling pathway and exerting regulatory control over the expression of CD44 as well as CD133 (Zhou et al. 2020). On the other hand, the expression of miR-200b and let-7c resulted in a notable reduction of Shh-induced erlotinib resistance in CSCs (Ahmad et al. 2013). Furthermore, Shh actively engages in intracellular signaling that effectively coordinates the regulation of CAFs and cancer stem cells CSCs, ultimately leading to the development of therapy resistance (Fig. 3.4) (Valenti et al. 2017).

3.8 Treatment Strategies Targeting Dysregulated Signaling in CSCs

Agents That Inhibit the Wnt/β-Catenin Signaling Pathway

OMP-54F28 refers to a fusion protein that merges the cysteine-rich domain of Fzd8 with the immunoglobulin Fc domain. This fusion protein functions by competing with the native Fzd8 receptors for its ligands, thereby inhibiting Wnt signaling (Le et al. 2015). A total of 26 individuals participated in a phase 1 clinical trial

investigating the efficacy of the anticancer agent OMP-54F28249 (Jimeno et al. 2017). Additionally, another phase 1b study was conducted to assess the dosage escalation of OMP-54F28 when used with carboplatin and paclitaxel among patients with persistent platinum-sensitive ovarian carcinoma. The scope of further inquiry is constrained due to the presence of bone toxicity (Moore et al. 2019) (Fig. 3.5).

PRI-724 functions as a β -catenin inhibitor. The administration of PRI-724 resulted in a decrease in drug resistance and CSC characteristics in TNBC (Ring et al. 2018), as well as a downregulation of Sox2 and CD44 gene expression (Chan et al. 2015). Additionally, the co-administration of PRI-724 and cisplatin exhibited a synergistic effect in inhibiting cellular proliferation (Chan et al. 2015). Although preclinical studies have demonstrated the potential of this treatment to address treatment resistance by specifically targeting CSCs (Zhang et al. 2018; Kartha et al. 2018), its clinical efficacy in cancer treatment remains undetermined. Furthermore, it is important to note that liver injury could potentially manifest as a serious adverse event (Kimura et al. 2017). Furthermore, it is important to note that the occurrence of liver injury is a potentially serious adverse event associated with this treatment (Kimura et al. 2017).

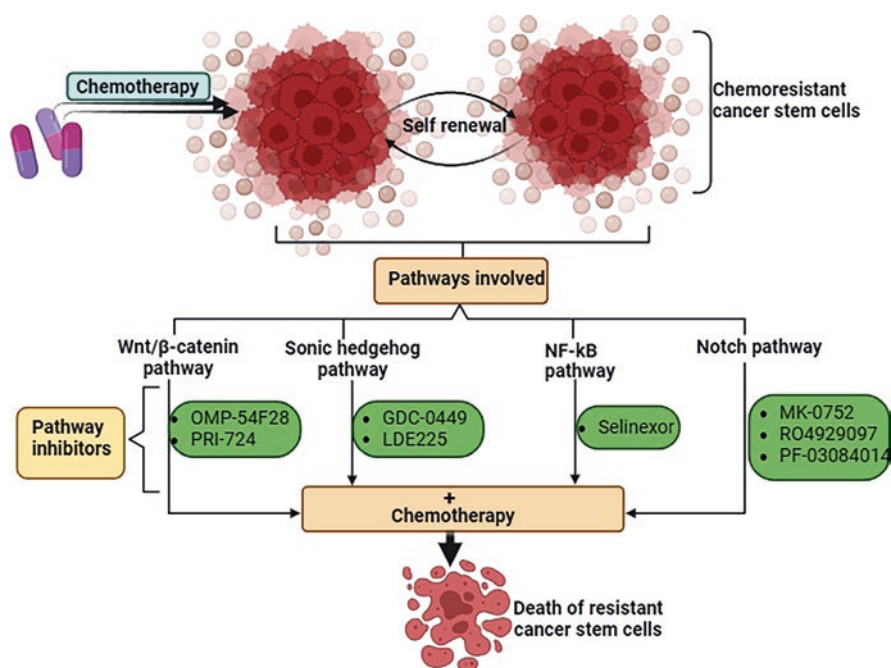


Fig. 3.5 The figure outlines therapeutic approaches aimed at mitigating dysregulated signaling pathways within cancer stem cells (CSCs). Various molecular targets and signaling cascades implicated in CSC maintenance, self-renewal, and resistance to treatment are depicted. Treatment strategies include small molecule inhibitors, and other targeted agents designed to modulate specific signaling components critical for CSC survival

Notch Pathway-Targeting Agents

The primary clinical strategies employed to target Notch signaling involve the utilization of γ -Secretase inhibitors (GSIs), specifically MK-0752 and RO4929097, as well as the application of antibodies towards the Notch receptor or ligand. A multi-center study employing a nonrandom Bayesian adaptive design was conducted to assess the safety of a combination therapy involving MK-0752 and gemcitabine to establish the recommended phase 2 dose (RP2D) (Cook et al. 2018). Tumor response evaluation was conducted on a cohort of 19 patients, revealing that 13 individuals exhibited stable disease, while one patient attained a confirmed partial response. Furthermore, a combination of MK-0752 and docetaxel has the potential to reduce the proportions of CD44+CD24[−] and ALDH⁺ cells (Fig. 3.5) (Schott et al. 2013).

RO4929097, a novel gamma-secretase inhibitor (GSI), has demonstrated the potential to enhance the sensitivity of prostate cancer cells to antiandrogen therapy (Rice et al. 2019). Furthermore, it has been observed that the administration of RO4929097 leads to a decrease in the expression of IDO1 in cervical CSCs. This reduction is accompanied by a decrease in the binding of the Notch intracellular domain (NICD) to the IDO1 promoter. Additionally, the use of RO4929097 has been found to enhance the sensitivity of xenograft tumors to radiation therapy (Low et al. 2020). Moreover, it has been observed that RO4929097 has the potential to effectively combat the development of tamoxifen resistance in CSCs within the context of human breast cancer (Simoës et al. 2015). In a phase 0/1 clinical trial, a cohort of 21 patients diagnosed with either anaplastic astrocytoma or glioblastoma were administered a combination treatment of RO4929097, temozolomide, and radiotherapy (Xu et al. 2016). As a result, a notable reduction in the population of CD133 CSCs was observed. A total of 13 participants were included in a phase 1 clinical trial that investigated the effects of RO4929097 in combination with bevacizumab on patients suffering from recurrent cancerous glioma (Pan et al. 2016). The study reported an average overall survival of 10.9 months, along with an average progression-free survival of 3.7 months.

Moreover, it is worth noting that PF-03084014 is classified as a gamma-secretase inhibitor (GSI). The growth of HCC was effectively inhibited by PF-03084014 through the inhibition of cancer stemness (Wu et al. 2017b). Additionally, it has been reported that the compound PF-03084014 exhibits the ability to augment the antitumor efficacy of docetaxel specifically in prostate cancer stem-like cells (Wang et al. 2020). The administration of PF-03084014 in conjunction with gemcitabine demonstrated a decrease in putative CSCs in pancreatic ductal adenocarcinoma (PDA). Significantly, in an orthotopic model characterized by high aggressiveness, the combined administration of PF-03084014 and gemcitabine demonstrated efficacy in triggering apoptosis, impeding tumor cell proliferation, and suppressing angiogenesis. As a result, this combination therapy led to a reduction in primary tumor growth and effectively controlled the spread of metastatic cells, surpassing the effects observed with gemcitabine treatment alone (Yabuuchi et al. 2013). Additionally, an observed phenomenon in breast cancer involved the synergistic impact of PF-03084014 and docetaxel, which was attributed to their combined

targeting of CSCs. A series of studies (Zhang et al. 2013; Locatelli et al. 2017; Villalobos et al. 2018; Kummar et al. 2017) have investigated the effects of PF-03084014 on advanced TNBC, desmoid fibromatosis, and aggressive fibromatosis. These studies have shown potential results in the treatment of desmoid tumors during phase 1 trials.

NF- κ B Pathway-Specific Agents

Selinexor is an orally administered inhibitor of exportin 1, a nuclear export protein, which effectively suppresses NF- κ B signaling. The Selinexor molecules exhibit a synergistic effect when combined with gemcitabine as well as nanoparticle albumin-bound (nab)-paclitaxel, resulting in the inhibition of pancreatic ductal adenocarcinoma (PDAC) growth and disintegration of cancer stem cell (CSC) spheroids (Azmi et al. 2020; Khan et al. 2022b). The Food and Drug Administration (FDA) recently gave accelerated permission to the combination of Selinexor and low-dose dexamethasone for the therapy of triple-class refractory multiple myeloma (MM) (Dolgin 2019). This decision was made due to the observed objective response to treatment in patients who exhibited resistance to multiple inhibitors of the proteasome when treated with Selinexor-dexamethasone (Chari et al. 2019). Selinexor may potentially contribute to the reversal of resistance to therapy in relapsed patients. The combination of Selinexor and gemcitabine has demonstrated the ability to effectively inhibit CSC spheroids in the phase 1b trial for PDAC (Azmi et al. 2020) (Fig. 3.5). This study has identified Selinexor as a potentially promising therapeutic agent specifically designed to target CSCs.

Agents That Target the Shh Pathway

Vismodegib (GDC-0449) and Sonidegib (LDE225) are oral SMO antagonists that have been permitted by the FDA (Dummer et al. 2016; Axelson et al. 2013). Ahmad and colleagues (Ahmad et al. 2013) demonstrated that the development of resistance to erlotinib was facilitated by CSCs. Moreover, the researchers found that the suppression of the Shh signaling cascade using GDC-0449 led to a reduction in the expression of CSC markers. Consequently, this intervention sensitized cells undergoing EMT to the effects of drug treatment (Ahmad et al. 2013). Furthermore, it has been observed that the administration of GDC-0449 has the potential to minimize stemness as well as reduce resistance to radiation as well as carboplatin (Wang et al. 2019c). Additionally, the administration of GDC-0449 in vivo halted the intracellular signaling pathway regulated by Shh, resulting in a decrease in the expansion of CAFs and CSCs (Valenti et al. 2017).

A phase 2 clinical trial was carried out to evaluate the efficacy of Vismodegib for individuals with multiple basal-cell carcinomas (Dréno et al. 2017). The trial included two groups, namely, Group A and Group B, which received intermittent

dosage schedules of Vismodegib. The results indicated that both groups demonstrated favorable long-term activity in the treatment of multiple basal-cell carcinomas. In the EDALINE phase 1 clinical study, a total of 12 patients diagnosed with advanced triple-negative breast cancer (TNBC) were enrolled. Among these patients, it was observed that three individuals obtained clinical benefits from the combination treatment involving an SMO inhibitor as well as docetaxel chemotherapy. Notably, one patient achieved a complete response to the treatment (Cazet et al. 2018).

Sonidegib, also known as LDE225, is a highly effective and selective inhibitor of the SMO protein. In murine models of TNBC, neoplastic cells secrete Hedgehog ligand, which leads to the reprogramming of cancer-associated fibroblasts (CAFs). This reprogramming process creates a favorable microenvironment that facilitates the development of a chemoresistant, CSC phenotype (Cazet et al. 2018; Khan et al. 2023d). This is achieved through the upregulation of FGF5 expression and the generation of fibrillar collagen by the CAFs. The application of SMO inhibitors in the stromal therapy of patient-derived xenografts resulted in the downregulation of CSC marker expression. This downregulation, in turn, enhanced the sensitivity of tumors to docetaxel, a chemotherapy drug. Consequently, this treatment approach significantly improved overall survival rates and reduced the burden of metastatic spread (Cazet et al. 2018; Khan et al. 2023e).

3.9 Conclusion

In conclusion, understanding the pivotal role of cancer stem cells in drug resistance has profound implications for improving cancer treatment outcomes. The unique characteristics of CSCs, such as their quiescence and ability to reconstitute tumors, pose significant challenges to conventional therapies. The chapter highlights the need for innovative therapeutic strategies that selectively target CSCs, aiming to disrupt their contributions to treatment resistance and tumor progression. As research progresses, unraveling the complex interplay between CSCs and the tumor microenvironment will be essential for designing tailored and effective treatments. The insights gained from this exploration provide a foundation for developing precision medicine approaches that can enhance the success of anticancer therapies and pave the way for more durable responses in the clinical setting.

MCQs

1. **What is a significant issue associated with chemoresistance in cancer therapy?**
 - A. Increased effectiveness of chemo agents
 - B. Reduction in cancer aggressiveness
 - C. Decreased metastatic potential
 - D. Progression of the disease

Answer D: Progression of the disease

2. **What specific subset of cancer cells is associated with chemoresistance and recurrence?**

- A. Macrophages
- B. Cancer stem cells (CSCs)
- C. Fibroblasts
- D. T lymphocytes

Answer B: Cancer stem cells (CSCs)

3. **Which marker is proposed for detecting CSCs based on their higher metabolic activity?**

- A. CD34
- B. CD38
- C. Aldehyde dehydrogenase (ALDH)
- D. Notch

Answer C: Aldehyde dehydrogenase (ALDH)

4. **What characteristic of CSCs enables the preservation of a stem cell pool and continuous renewal of the tumor?**

- A. Rapid apoptosis
- B. Limited differentiation capability
- C. Self-renewal paired with significant differentiation capability
- D. High susceptibility to radiation

Answer C: Self-renewal paired with significant differentiation capability

5. **Which of the following is a characteristic surface marker for identifying CSCs in breast cancer?**

- A. CD34+/CD38
- B. CD44+CD24–/low
- C. ALDH high+
- D. Notch pathway transcripts

Answer B: CD44+CD24–/low

6. **Which technique can be used to identify cancer stem cells (CSCs) using various surface markers?**

- A. X-ray imaging
- B. Fluorescence-activated cell sorting (FACS)
- C. CT scans
- D. PET scans

Answer B: Fluorescence-activated cell sorting (FACS)

7. **What is CD133 recognized as in various types of malignancies?**

- A. A tumor suppressor
- B. A cancer stem cell marker
- C. An oncogenic protein
- D. A chemotherapy drug

Answer B: A cancer stem cell marker

8. **Which combination of markers has been proposed as a potentially ideal marker specific to CSCs in individuals with glioma?**

- A. CD44 and CD90
- B. CD133 and Nestin

- C. EpCAM and CD133
- D. CD44 and epidermal growth factor receptor (EGFR)

Answer B: CD133 and Nestin

9. Which transmembrane glycoprotein is purported to have a function in CSCs and was initially employed as a marker for CSCs in breast cancer?

- A. CD90
- B. CD133
- C. CD44
- D. EpCAM

Answer C: CD44

10. In pancreatic cancer, which cellular populations exhibited a notable ability for self-renewal and the greatest tumor-forming potential?

- A. CD133+ and Nestin+
- B. CD44+ and c-Met+
- C. CD44– and EpCAM+
- D. CD133– and ALDH+

Answer B: CD44+ and c-Met+

11. Which combination of markers may play a significant role in mediating breast CSCs?

- A. ALDH high CD44+CD24–
- B. CD90+CD44–
- C. ALDH high CD44+CD133+
- D. CD133+ALDH+

Answer C: ALDH high CD44+CD133+

12. What is CD90 also known as?

- A. Epidermal growth factor receptor (EGFR)
- B. Thy-1 (thymocyte differentiation antigen-1)
- C. CD133
- D. ALDH

Answer B: Thy-1 (thymocyte differentiation antigen-1)

13. What surface marker, when co-expressed with CD90, results in a more aggressive phenotype with a greater propensity for metastasis and self-renewal?

- A. CD133
- B. ALDH
- C. CD44
- D. CD24

Answer C: CD44

14. What role does Nanog play in embryonic cell-fate specification?

- A. Promotes differentiation
- B. Maintains pluripotency
- C. Enhances cellular radioresistance
- D. Inhibits stemness properties

Answer B: Maintains pluripotency

15. **What characteristics are associated with Nanog+ NSCLC cells?**

- A. Increased chemoresistance
- B. Decreased ALDH activity
- C. Lower stemness properties
- D. Reduced radioresistance

Answer A: Increased chemoresistance

16. **Which transcription factor is a member of the high-mobility class and is frequently utilized as a stemness-related marker in CSC research?**

- A. Nanog
- B. Oct4
- C. Sox2
- D. CD44

Answer C: Sox2

17. **What has significantly transformed cancer investigation in the past decade, re-emphasizing the importance of immunology in the creation of new therapeutics?**

- A. Antibiotics
- B. Chemotherapy
- C. CTLA-4 and PD-1 inhibitors
- D. Radiation therapy

Answer C: CTLA-4 and PD-1 inhibitors

18. **What is the purpose of combining Wnt-targeted actions with immune checkpoint inhibitors (ICIs) in cancer treatment?**

- A. To increase the cost of treatment
- B. To reduce side effects
- C. To enhance the effectiveness of ICIs
- D. To avoid drug interactions

Answer C: To enhance the effectiveness of ICIs

19. **Which cytokine has been shown to inhibit the growth of lung CSCs, and its inhibition is being explored as a potential treatment strategy?**

- A. TGF- β
- B. IL-6
- C. IL-8
- D. IFN- γ

Answer B: IL-6

20. **What is targeted by antibodies like ALX148 and Hu5F9-G4 in the context of immune evasion in cancer cells?**

- A. CSC phenotypes
- B. CD19
- C. CD47
- D. PD-L1

Answer C: CD47

21. **Which pathway is widely conserved and plays a crucial role in tissue homeostasis and embryonic growth?**

- A. Notch pathway
- B. NF- κ B pathway

- C. Sonic Hedgehog pathway
- D. Wnt pathway

Answer D: Wnt pathway

22. What positive effect is observed when inhibiting β -catenin signaling in breast cancer cells that overexpress the protein HER2?

- A. Increased tumor growth
- B. Enhanced metastasis
- C. Reduced tumor growth and metastasis
- D. No effect

Answer C: Reduced tumor growth and metastasis

23. How does the Shh (Sonic Hedgehog) pathway contribute to chemotherapy resistance in CD44+ gastric carcinoma cells?

- A. It inhibits ABCG2 efflux pumps
- B. It reverses ALDH activity
- C. It sustains phenotypes associated with CSCs
- D. It promotes the expression of miR-200b

Answer C: It sustains phenotypes associated with CSCs

24. In which type of breast cancer does the interaction between NF- κ B and Notch contribute to therapeutic resistance?

- A. HER2-positive
- B. Triple-negative
- C. Hormone receptor-positive
- D. Inflammatory

Answer B: Triple-negative

25. How does the Shh pathway affect the expression of CD44 and CD133 in cancer stem cells (CSCs)?

- A. Reduces CD44 expression
- B. Activates CD133 expression
- C. Inhibits CD44 expression
- D. Activates CD44 and CD133 expression

Answer D: Activates CD44 and CD133 expression

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Immune Cells: Critical Players in Drug Resistance

4

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and Fayaz Malik

Abstract

The chapter explores the complicated relationship between immune cells and cancer drug resistance. The immune cell composition in the tumor microenvironment (TME), encompassing B cells, effector and regulatory T cells, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and tumor-associated neutrophils (TANs), plays a decisive role in tumorigenesis. Various studies showed that MDSCs can create immunosuppressive microenvironment, allowing cancer cells to evade drug-induced cytotoxicity. The dynamics of the tumor microenvironment, influenced by immune cells, play a crucial role in modulating drug responsiveness. The chapter navigates through the signaling pathways, molecular cross talk, and adaptive mechanisms that define the immune landscape in the context of cancer drug resistance. It explores the potential of immunotherapeutic interventions, such as immune checkpoint inhibitors, to recalibrate immune responses and overcome resistance mechanisms. In essence, this exploration underscores the pivotal role of immune cells in shaping the destiny of cancer drug resistance. By deciphering the intricacies of these

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interactions, the chapter aims to provide insights that pave the way for innovative therapeutic strategies, pushing the boundaries of cancer treatment toward greater efficacy and resilience against resistance.

Keywords

Immune cells · Drug resistance · TME · Tregs · TAMs · Platelets · Cytokines

4.1 Introduction

The interplay between the immune system and tumor cells presents a complex dynamic, as the immune system possesses the ability to identify and eliminate tumor cells. However, owing to the remarkable adaptability of tumor cells, they can employ various mechanisms to evade immune surveillance or actively hinder the function of immune effector cells (Gonzalez et al. 2018). As the progression of malignancies occurs, the cells that have undergone transformation have the ability to modify the surrounding microenvironment in a way that benefits their growth. This generally involves promoting inflammation and facilitating communication within the milieu that supports tumor development (Gonzalez et al. 2018). The dys-regulated immunological and stromal milieu can contribute to the stimulation of stem cell signals, which have been frequently linked to increased resistance against cytotoxic and targeted treatments (Clara et al. 2020; Khalaf et al. 2021; Fracchiolla et al. 2017). Cancers are often linked to an inflammatory reaction, which is initiated by inflammatory signals generated by stimulated stromal and cancerous cells in reaction to necrosis occurring at the core of the tumor (Vendramini-Costa and Carvalho 2012; Yoshimura 2006). Nevertheless, cancer cells have the ability to induce invading immune cells to adopt immunosuppressive and protumorigenic phenotypes (Sameer et al. 2024). The process of activating or polarizing cells, including TAMs, B cells, MDSCs, TANs, and specific subsets of T lymphocytes like regulatory T cells, has the ability to both suppress adaptive immune responses and directly communicate with cancer cells, thereby influencing their fate and sustainability (Fig. 4.1). The stimulation of downstream stem cell processes can be facilitated by cytokines and growth factors that are generated by immune cells. This activation leads to an enhancement of self-renewal capabilities and contributes to the development of therapeutic resistance (Huang et al. 2017; Jahandideh et al. 2020). Therefore the manipulation of immunological signaling holds potential as a viable approach to impede self-renewal and enhance the efficacy of therapeutic interventions. The investigation of protumorigenic immune cell activity has primarily focused on comprehending the involvement of tumor-associated myeloid cells (Porta et al. 2018). Recent research has indicated that the reduction of myeloid populations can enhance the responsiveness of tumors to established treatment modalities, such as chemotherapy in cases of pancreatic cancer (Leinwand and Miller 2020) and androgen-deprived therapy in cases of prostate cancer (Basaria et al. 2002). The role of TAMs in pancreatic cancer has been observed to facilitate

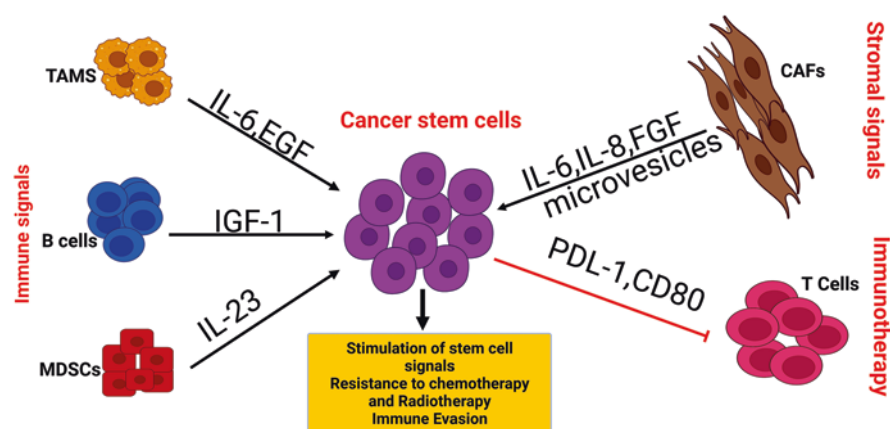


Fig. 4.1 The figure depicts the intricate interplay between stromal and immune signals, which leads to the activation of stem cell signals and the subsequent enhancement of therapeutic resistance in cancer

the activity of cancer stem cells (CSCs) by activating the STAT3 pathway, which is a crucial signaling mechanism involved in promoting cell survival (Takao et al. 2012). The fraction of CSCs was substantially reduced through the depletion of macrophages, resulting in higher sensitivity to chemotherapy in an in vivo setting (Takao et al. 2012). Furthermore, in preclinical models, the combined targeting of TANs and TAMs significantly enhanced chemosensitivity (Mantovani et al. 2021). In the context of prostate cancer, it has been observed that interleukin (IL)-23 derived from MDSCs activates STAT3 in cancerous cells. Furthermore, it has been demonstrated that depleting MDSCs or administering an anti-IL-23 blocking antibody effectively prevents or delays the development of resistance toward androgen deprivation therapy in autochthonous models (Calcinotto et al. 2018). In addition to myeloid cells, current evidence suggests that B lymphocytes may also play a role in the development of resistance to targeted treatments. Therefore, the inhibition of immune signaling that promotes tumor growth is a promising approach to enhance treatment efficacy. This can be achieved by directly interfering with stem cell signals or targeting specific stem cell populations.

4.2 Involvement of Immunosuppressive Immune Network in Therapeutic Resistance

Regulatory T Cells

Regulatory T cells, often known as Tregs, exhibit a diverse range of phenotypic and functional characteristics (Sakaguchi et al. 2020). Tregs exist in several subtypes, such as CD4⁺ and CD8⁺ T cells, and are primarily distinguished by the presence of distinct markers. One example of Tregs, specifically CD4⁺CD25⁺ Tregs, is their

active role in preserving immune homeostasis and promoting immunologic self-tolerance (Sakaguchi et al. 2020). The Forkhead box protein P3 (FoxP3) is of significant importance in both the developmental process and the suppressive capabilities of Tregs. The differentiation between Tregs and activated CD4+CD25–T cells is primarily governed by a crucial regulator (Fontenot et al. 2003). Tregs exhibit a wide array of co-signaling receptors that are of utmost importance in the regulation of the immune response. Several receptors, such as CTLA-4, LAG-3, GITR, ICOS, OX40, PD-1, TIGIT, TIM-3, and 4-1BB, are observed to be expressed on Tregs. The upregulation of certain receptors by Tregs and cancer cells plays a significant role in facilitating immune evasion by tumors (Fig. 4.2) (Fontenot et al. 2003; Shiravand et al. 2022; Kumar et al. 2018).

Tregs play a significant and complex role in cancer drug resistance. A persistent decrease in Tregs was found following the delivery of docetaxel in non-small cell lung cancer (NSCLC) and after the use of Folinic acid/5-FU/oxaliplatin in gastric carcinoma (Li et al. 2014; Wang et al. 2018a). A significant dosage of cyclophosphamide (CPA) results in the reduction of the Tregs population, aligning with its potent lymphopenic properties. According to research conducted on mice suffering from fibrosarcoma, it was observed that Tregs that managed to survive CPA treatment exhibited a quick increase in their proliferation rate after chemotherapy. Consequently, this hindered the progression of the immune response against the

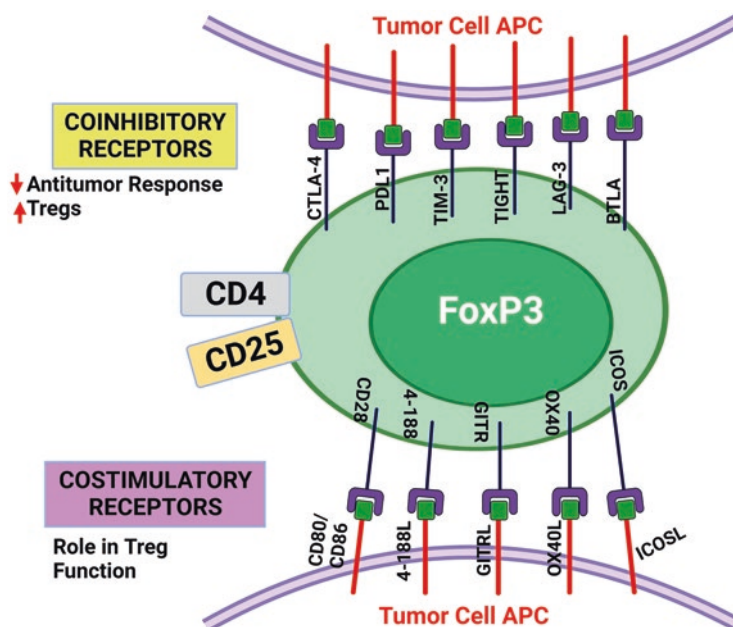


Fig. 4.2 The figure illustrates the pivotal role of Forkhead box protein P3 (FoxP3) and co-signaling receptors in governing the function of regulatory T cells (Tregs) and their impact on immune evasion by tumors

tumor following lymphodepletion (Saida et al. 2015). When administered in minimal quantities over an extended duration, CPA has the capacity to specifically diminish the proliferation of Tregs, including those present in the TME. This finding indicates that the administration of low-dose CPA has the potential to strengthen immune responses against tumors without causing significant lymphopenia (Tanaka and Sakaguchi 2017). The discovery of the potential application of CPA in conjunction with other drugs could serve as a valuable tool in enhancing antitumor effects during chemotherapy, as well as in strengthening adoptive T-cell therapy through lymphodepletion processes. The studies have indicated that patients exhibiting a larger proportion of Tregs within the CD4+ T cell subset before undergoing chemotherapy experienced more unfavorable long-term results. Nevertheless, the presence of Treg cells has been suggested as a potential biomarker with predictive capabilities in several types of cancer, such as TNBC and ovarian cancer (Saleh and Elkord 2020). Radiotherapy is a crucial component of cancer therapy; however, its efficacy may be hindered by the upregulation of certain markers such as CTLA-4, PD-L1, STAT3, and TIM-3. These signals have the potential to stimulate the proliferation of Tregs, hence resulting in resistance to treatment (Knitz et al. 2021; Cao et al. 2009).

Myeloid-Derived Suppressor Cells

MDSCs are a diverse group of immature myeloid cells that include precursors for dendritic cells (DCs), granulocytes, and macrophages. These cells accumulate in case of chronic inflammation and tumor growth (Gabrilovich et al. 2012; Kumar et al. 2016; Parker et al. 2015; Meirou et al. 2015). MDSCs are generated from hematopoietic precursor cells in the bone marrow as a result of dysregulated myelopoiesis caused by persistent production of inflammatory mediators (Gabrilovich et al. 2012; Meirou et al. 2015; Solito et al. 2014). These cells possess notable immunosuppressive and tumorigenic properties (Gabrilovich et al. 2012; Marvel and Gabrilovich 2015). There is a frequent association between elevated levels of MDSCs and resistance to chemotherapy, radiotherapy, and immunotherapy (Fig. 4.3). MDSCs have been found to enhance the recruitment of T-regulatory cells with suppressive properties to the tumor site. This phenomenon has been associated with the development of resistance to both radiation and chemotherapy (Wainwright et al. 2013). Chemotherapy in numerous clinical trials has been found to result in heightened immunosuppression as an adverse effect. Temozolomide is a frequently employed chemotherapeutic drug in the treatment of glioblastoma. Although it exhibits antitumor properties and enhances patient survival, it can also elicit elevated expression of hypoxia-related genes, specifically HIF-1 α and VEGF (Fisher et al. 2007). These substances facilitate a series of pro-tumor processes, including angiogenesis and cellular repair. The injection of temozolomide systemically is also associated with immuno-depletion, which might be advantageous when administered along with immunotherapies. However, this poses an additional challenge in establishing the antitumor action in lymphocytes. Carboplatin has been observed to enhance an MDSC mediated pathway in mouse models of lung cancer, resulting in

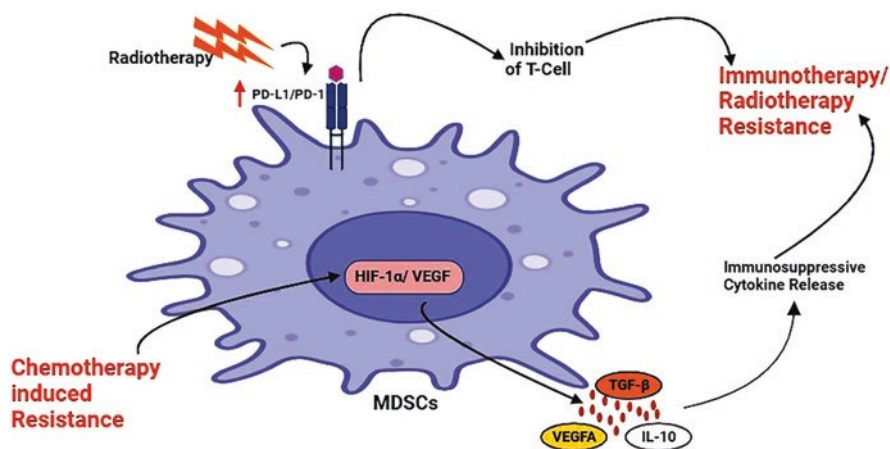


Fig. 4.3 The figure illustrates the multifaceted mechanisms employed by myeloid-derived suppressor cells (MDSCs) in conferring resistance to therapeutic interventions in cancer

the development of resistance. This resistance is facilitated by the upregulation of VCAM/RANTES and activation of IL13/33 pathways (Anestakis et al. 2020). The immune suppressive function of chemo-induced MDSCs is facilitated by the stimulation of IL-10 production in CD4+/Foxp3+ regulatory T-cells (Tregs) (Anestakis et al. 2020).

Radiation therapy (RT) has the potential to decrease MDSC levels inside the tumor microenvironment (TME). However, it is important to note that MDSCs have a very short lifespan and are often replenished by circulating MDSCs. These circulating MDSCs are generally not impacted by RT and tend to be elevated in individuals with glioma (Raychaudhuri et al. 2011). MDSCs provide a significant challenge to the effectiveness of immune checkpoint blockade therapy. This is primarily attributed to their ability to directly inhibit the immune response and indirectly promote the development of immunosuppressive characteristics in other immune cells within the TME. In the case of glioblastoma, tumor cells, including CSCs, have an influence on MDSCs, leading to the development of an immunosuppressive phenotype in both local and systemic environments (Raychaudhuri et al. 2011; Feng et al. 2018).

Tumor-Associated Macrophages

Macrophages are the predominant population of immune cells inside the tumor microenvironment (Tie et al. 2020; Hill et al. 2020). Specifically, macrophages that are localized within the tumor microenvironment are commonly referred to as TAMs (Sedighzadeh et al. 2021). Traditionally, macrophage phenotypes have been categorized as M1 and M2 macrophages. The phenotype of macrophages is

influenced by the Tumor Immunity in the Microenvironment (TIME) (Stout et al. 2005; Biswas and Mantovani 2010; Weizman et al. 2014; Amit and Gil 2013). The M1 phenotype demonstrates pro-inflammatory reactions and tumoricidal properties, while the M2 phenotype is characterized by anti-inflammatory attributes and pro-tumor characteristics (Noy and Pollard 2014; Poh and Ernst 2018; Zheng et al. 2013).

The activation of M1 macrophages is induced by Th1 cytokines, such as IFN- γ or lipopolysaccharide (LPS) stimuli. This activation then leads to the secretion of pro-inflammatory cytokines by M1 macrophages, including IL-6, IL-12, IL-23, and TNF- α . The M2 macrophage is facilitated by Th2 cytokines, including IL-4 and IL-13, as well as glucocorticoids and immunoglobulin complexes. M2 macrophages play a crucial role in the regulation of angiogenesis, wound healing, and tissue remodeling, as evidenced by previous studies, and they promote the proliferation, progression, neovascularization, and dissemination of diverse malignancies (Huang et al. 2017; Chanmee et al. 2014; Zhu et al. 2014; Chen et al. 2017). The mechanisms underlying the tumor-promoting role of TAMs following chemotherapy encompass several pathways. These pathways involve enhanced recruitment of immunosuppressive TAMs, polarization toward a pro-tumor phenotype, induction of a tumor-promoting Th17 immune response, and stimulation of antiapoptotic processes in cancerous cells (Zurrida and Veronesi 2015).

The study conducted by Baghdadi et al. aimed to examine the impact of tumor supernatants derived from doxorubicin-resistant (DR) and doxorubicin-sensitive (DS) lung cancer cell lines on the polarization of monocytes. The researchers discovered that monocytes, when exposed to tumor supernatants derived from the DR cell line A549-DR, exhibited notably elevated expression levels of CD68 and CD163 (M2 marker), in contrast to the DS group A549-DS (Baghdadi et al. 2016). Furthermore, it was observed that monocytes, when exposed to chemoresistant tumor supernatants derived from A549-DR cells, underwent differentiation into M2 macrophages and exhibited an immunosuppressive phenotype characterized by increased PD-L1 expression and decreased expression of the MHC class I and II costimulatory markers CD80 and CD86. Furthermore, TAMs expressing CD68 and CD163 markers have been found to secrete a range of molecules that play a role in tumor growth and resistance to chemotherapy. These factors include immunosuppressive cytokines such as TGF β and IL10, the pro-angiogenic factor VEGF, and factors that facilitate invasion, such as MMP9 and MMP13 (Baghdadi et al. 2016).

Tumor-Associated Neutrophils (TANs)

TANs have the ability to induce resistance to a particular therapeutic treatment through many pathways (Shaul and Fridlender 2019). As an illustration, TANs have the ability to directly impact cancer cells by either intensifying or diminishing their reaction to a specific therapy through the generation of reactive oxygen species (ROS) or modifications in the pathways responsible for repairing DNA damage

(Butin-Israeli et al. 2019; Bui et al. 2021; Bui and Sumagin 2019; Khan et al. 2022a). TANs have the potential to modify the effectiveness of drugs by restricting the accessibility or durability of delivered therapeutic agents (Glicksman et al. 2017). TANs have the potential to influence the development of angiogenic vessels, hence potentially affecting the effectiveness of therapeutic interventions. The research examined tumors in mice and humans and observed that VEGFR1⁺ CXCR4⁺ PMNs (polymorphonuclear neutrophils) located in hypoxic areas exhibited a significant affinity for vascular repair through angiotropism. Additionally, these PMNs were found to be predisposed to releasing elevated quantities of matrix metalloproteinase 9 (MMP9) upon stimulation by vascular endothelial growth factor-A (VEGF-A) (Massena et al. 2015). The cleavage of VEGF-A by MMP9 results in the production of an activated product that exhibits a higher propensity for angiogenesis compared to the unprocessed protein (Massena et al. 2015). Moreover, in a mouse model of pancreatic dysplasia, TANs expressing MMP9 were shown to boost tumor angiogenesis. However, TAMs expressing MMP9 did not have the same effect. This promotion of angiogenesis by MMP9-expressing TANs was found to be reliant on the association of MMP9-induced ligand/receptor interaction between VEGF-A and VEGFR1 (Nozawa et al. 2006). In addition to MMP9, TANs also release significant amounts of BV8/prokineticin, a known contributor to vascular development (Bui and Sumagin 2019; Shojaei et al. 2007). The presence of BV8 (Shojaei et al. 2007), MMP9 (Nozawa et al. 2006), and VEGF-A (Scapini et al. 2004) suggests that TANs located in hypoxic scars play a crucial role in the development of obstructed blood flow and the maintenance of high interstitial fluid pressure (IFP) microenvironment, which in turn hampers the effective delivery of therapeutic drugs (Itatani et al. 2020). In a genetically engineered mouse model, the targeted inhibition of angiogenic TAN subsets expressing VEGFR1 has demonstrated a significant reduction in IFP and an enhanced therapeutic effectiveness of VEGF inhibitors such as bevacizumab (Avastin), sorafenib, or sunitinib (Heldin et al. 2004). TANs have the capacity to stimulate the formation of new blood vessels (angiogenesis) and diminish the effectiveness of therapies that target angiogenesis by either directly releasing interleukin-17 (IL-17) (Chung et al. 2013) or indirectly triggering the activation of cathepsin B/Nlrp3 inflammasome, resulting in excessive production of interleukin-1 β (IL-1 β) (Bruchard et al. 2013). Therefore, it can be observed that IL-17⁺ PMNs make up a significant proportion of IL-17-producing cells in gastric malignancies in humans (Li et al. 2017) has a role in promoting tumor angiogenesis in this context (Pan et al. 2015; Liu et al. 2019). In various murine cancer models, the administration of gemcitabine in combination with 5-fluorouracil treatment induced the release of cathepsin B by TANs as well as MDSCs. This release, facilitated by the Nlrp3 inflammasome, subsequently led to an IL-1 β -dependent upregulation of IL-17 production and the development of angiogenic vessels (Bruchard et al. 2013). Collectively, these data suggest that TANs have the potential to hinder the therapeutic benefits of antiangiogenic therapy by indirectly facilitating tumor angiogenesis via the IL-17/IL-1 β paracrine system (Fig. 4.4).

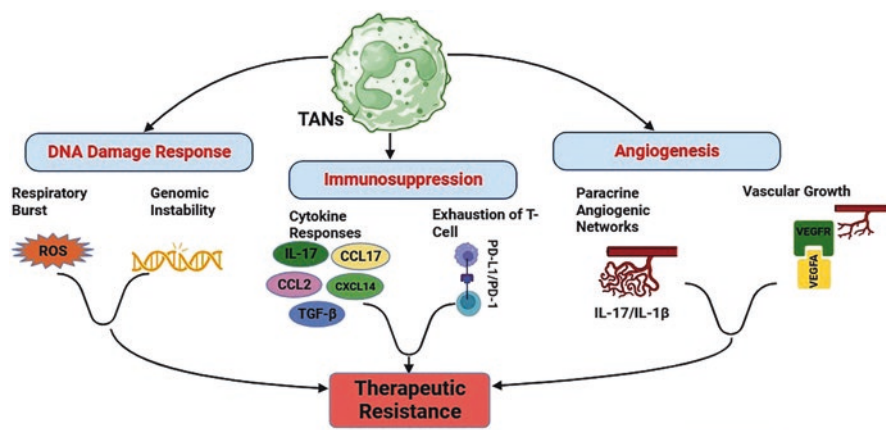


Fig. 4.4 The figure illustrates the diverse mechanisms and signaling pathways through which tumor-associated neutrophils (TANs) may confer therapeutic resistance

4.3 Role of Platelets in Drug Resistance

Platelets are recognized for their involvement in the processes of hemostasis and thrombosis (Ruggeri 2002; van der Meijden and Heemskerk 2019; Khan et al. 2023a). These anucleated cells are derived from the process of cytoplasmic disintegration of megakaryocytes, primarily occurring in the bone marrow. These cells are characterized by the presence of multiple types of granules, namely, α -granules, dense granules, and lysosomes, which store numerous mediators (Khan et al. 2022b). Platelets play a crucial role in connecting tissue destruction or dysfunction with an inflammatory reaction, serving to facilitate the repair of the injury. However, when platelet activation becomes unregulated, it can result in persistent inflammation that is implicated in various pathological conditions such as atherothrombosis as well as cancer (Gawaz et al. 2005; Gay and Felding-Habermann 2011; Contursi et al. 2017; Hassan et al. 2020). Extensive research spanning over five decades has been dedicated to examining the pivotal role of platelets in the growth of tumors (Khan et al. 2023b). Thus far, numerous processes pertaining to the relationship between cancer cells and platelets have been investigated and elucidated. The majority of these mechanisms revolve around the utilization of active platelets to promote tumor growth, angiogenesis, and dissemination (Huong et al. 2019). Upon receiving signals that activate tumors, there is a significant alteration in the expression of specific proteins generated from platelets (Han et al. 2014; Labelle et al. 2011). Furthermore, platelets have the ability to sequester and transport tumor-associated substances that contribute to the processes of angiogenesis and metastases. Consequently, the composition of the platelet proteome in individuals with cancer differs from that of individuals without illness, or those who are considered to be in a healthy state. Several recent investigations have identified potential

platelet protein biomarkers that could be utilized for the early detection of different forms of cancer (Lomnytska et al. 2018; Sabrkhany et al. 2018; Zhu et al. 2018). In addition to the process of diagnosing cancer, the assessment of platelet amount is also employed for the purposes of cancer prognosis and the monitoring of treatment efficacy.

Multiple investigations have demonstrated that platelets derived from cancer patients undergoing chemotherapy can potentially impact the effectiveness of medication therapy across a wide range of cancer types (Haemmerle et al. 2018; Khan et al. 2023c). For example, it has been shown that elevated platelet levels can diminish the effectiveness of platinum-based therapy in non-small cell lung cancer (NSCLC) (Wang et al. 2018b). The combination of paclitaxel and carboplatin treatment has also been proposed as a medication regimen that may help preserve platelet levels (Pertusini et al. 2001). The study reported a substantial increase in blood thrombopoietin levels at 5 h following infusion and a sustained elevation on the seventh day in patients who received carboplatin plus paclitaxel when compared to normal controls (Pertusini et al. 2001). Additionally, it has been observed that patients with breast cancer who have a low platelet-to-lymphocyte ratio and undergo neoadjuvant chemotherapy have a greater likelihood of achieving a complete clinical response. This effect is observed regardless of the molecular subtype of the main tumor (Cuello-López et al. 2018). Moreover, the estimation of the ratio of platelets to lymphocytes and its fluctuations throughout the course of chemotherapy has proven to be valuable in forecasting a more precise prognosis for patients with metastatic biliary tract cancer (Cho et al. 2017). For example, a negative association between the level of the ratio of platelets to lymphocytes and survival prognosis has been observed in gastric carcinoma patients (Gong et al. 2018) and BC patients (Xu et al. 2017) following neoadjuvant treatment. In patients with acute myeloid leukemia, the restoration of hematopoiesis, particularly the excessive recovery of platelets following induction therapy, is regarded as a noteworthy indicator of relapse-free survival (Yamazaki et al. 2017). When considering the impact of chemotherapeutics on megakaryocytic progenitor cells, it is important to note that their sensitivity may differ. Consequently, the degree of platelet dysfunction can be attributed to both the specific medications used and their respective dosages, as outlined in Table 4.1.

The primary determinant governing the development of resistance to chemotherapy is the increased rate of proliferation exhibited by cancer cells, which is facilitated by the stimulation of antiapoptotic proteins or the genetic transformation of cancer cells via epithelial-mesenchymal transition (EMT), and it is believed that platelets may have an impact on these processes (Elaskalani et al. 2017; Ahmad et al. 2022). Platelets demonstrate a pro-proliferative role by releasing several growth factors, enabling them to counteract the antiproliferative impacts of chemotherapeutic drugs (Elaskalani et al. 2017). The administration of ABT-263 to patients is restricted by its dose-dependent effect on platelet count, as it suppresses BCL-xL in platelets and leads to thrombocytopenia (Polier et al. 2015). However, the absence of caspase-9 results in resistance to ABT-737, which in turn inhibits the exposure of phosphatidylserine and delays the onset of thrombocytopenia induced by APT-737 in vivo (White et al. 2012).

Table 4.1 The impact of platelet-mediated chemotherapy on cancerous cells

Drug	Impact of platelets	Reference
Aspirin	The administration of aspirin has been shown to reduce the capacity of platelets to promote the proliferation of colorectal cancer cells	Mitrugno et al. (2017)
Chemotherapy	Following chemotherapy, platelets increase procoagulant action and may have an impact on NSCLC's hypercoagulative state	Ma et al. (2017)
Resveratrol	The inhibitory effects of resveratrol on lung tumor metastasis are achieved through the disruption of the platelet-tumor cell amplification loop	He et al. (2017)
Platinum	Elevated platelet levels have been found to diminish the effectiveness of platinum-based therapy in patients with NSCLC	Wang et al. (2018b)
Rituximab	The absence of detectable platelet autoantibodies is correlated with a lack of response to rituximab therapy in individuals with immune thrombocytopenia	Porcelijn et al. (2017)
Neoadjuvant chemotherapy	The pretreatment platelet/lymphocyte ratio has been identified as a potentially significant prognostic factor for predicting the response to neoadjuvant chemotherapy in patients with breast cancer	Xu et al. (2017)

Moreover, the intricate relationship between gemcitabine/cisplatin and platelets is rooted in the process of epithelialization. The release of TGF- β 1 has been observed to have two effects: (1) the activation of EMT in tumor tissues and (2) the stimulation of activated platelets by direct contact (Sharma et al. 2014; Naugler and Karin 2008; Khan et al. 2023d). The promotion of MAPK and PI3K/Akt signaling in pancreatic cancer cells, leading to reduced responsiveness to cisplatin, is mostly facilitated by stimulated platelet-derived TGF- β 1, instead of by direct interactions between platelets and tumor cells (Chen et al. 2013; Wadhwa et al. 2020). Emerging research consistently shows the significant role of platelets in the resistance to chemotherapy (Elaskalani et al. 2017). For example, it has been observed that platelets have the ability to augment the survival of adenocarcinoma cells and provide resistance to conventional anticancer agents (Radziwon-Balicka et al. 2012; Khan et al. 2023e). Differential levels of platelets have been observed to have a major impact on the responsiveness to taxane-based treatment in clinical settings. Additionally, it has been found that transfusions of platelets can effectively neutralize the antitumor impact of chemotherapy in cases of ovarian cancer (Bottsford-Miller et al. 2015). In a recent study conducted by Takeuchi and colleagues, the researchers investigated the interaction between the platelet-enhancing agent rosuvastatin and eltrombopag. They specifically focused on the breast cancer resistance protein (BCRP) and proposed that the BCRP located in the small intestine could be the primary site of interaction among rosuvastatin and eltrombopag in humans (Takeuchi et al. 2014).

In addition, there exist other conventional resistance mechanisms that can potentially play a substantial role in drug resistance, including the upregulation of drug efflux pumps situated on the cellular membrane or enhanced drug

metabolism (Broxterman et al. 2003). Previous studies have proposed that platelets have a substantial expression of multidrug resistance-associated protein 4 (MRP4), both in the plasma membrane and in the membrane of dense granules (Köck et al. 2007). MRP4 has been found to provide resistance against nucleoside-based cytotoxic agents (Ritter et al. 2005). The upregulation of MRP4, along with its distinct localization throughout the process of differentiation into megakaryocytes, lends support to the notion of platelet-specific functions. Conversely, the decreased expression of this transporter during leukocyte differentiation may have effects on chemotherapy (Oevermann et al. 2009; Minto et al. 2021). Additionally, another efflux protein that has been examined in platelets is MRP1. Its physiological function is documented as the active transportation of leukotriene C₄, which is known to be the substrate with the greatest binding capacity for MRP1, in human platelets. The association between the number of platelets and MRP1 expression has been observed to be correlated with an unfavorable outcome in diverse cancer types (Fang et al. 2018; Jedlitschky et al. 2012). Furthermore, it has been observed that in cases of hepatocellular cancer, the p0SK-Hep1 cells exhibit resistance to apoptosis induced through both cisplatin and doxorubicin (Ling et al. 2012).

Another potential method through which platelets may contribute to resistance against anti-angiogenic treatment is by scavenging anti-angiogenic substances. Both sunitinib and bevacizumab have the potential to be absorbed by platelets, thereby influencing the absorption and pharmacodynamics of those anti-angiogenic drugs (Huijbers et al. 2016). In contrast, it was observed that the administration of G3139, an antisense oligodeoxynucleotide targeting Bcl-2, resulted in a decrease in the total count of platelets and alleviation of fatigue before Adriamycin, vincristine, and dexamethasone (AVD) chemotherapy. This finding implies that G3139 has the potential to counteract conventional resistance mechanisms and reinstate the responsiveness of myeloma tumor cells to AVD therapy (Van de Donk et al. 2004). The efficacy of imatinib has been observed to be notably high in individuals with chronic-phase chronic myeloid leukemia (CML) who have demonstrated resistance or intolerance to interferon, particularly in cases where platelet counts are within the normal range (Cervantes et al. 2003).

The potential of the platelet stimulation index as a cancer biomarker to predict cancer resistance to chemotherapy can also be inferred in Table 4.2. One recent study has indicated that platelets could potentially serve as cancer biomarkers for the identification of cisplatin/gemcitabine resistance in the treatment of NSCLC (Shi et al. 2018). Platelets in patients with castration-resistant prostate carcinoma can serve as carriers of prostate cancer biomarkers, thereby facilitating the prediction of treatment response to abiraterone (Tjon-Kon-Fat et al. 2018). One possible explanation for this phenomenon is that larger platelets have the capacity to release a greater quantity of cytokines when stimulated compared to smaller platelets in cancer cells. Consequently, certain cytokines may activate tumor drivers such as EMT or TGF- β , resulting in the development of resistance to chemotherapy (Shi et al. 2018).

Table 4.2 Platelets' roles in cancer cells resistance to drugs

Cancer type/patients	Drug	Role of platelet	References
Castration cancer patients	Abiraterone	Platelets carry prostate tumor biomarkers and can predict therapy response	Tjon-Kon-Fat et al. (2018)
Immune thrombocytopenia patients	Rituximab	The absence of detectable platelet autoantibodies has been found to be correlated with a lack of response to rituximab therapy	Porcelijn et al. (2017)
Non-small cell lung cancer	Gemcitabine/cisplatin	The platelet activation index has the potential to serve as a predictive marker for resistance to gemcitabine/cisplatin treatment	Shi et al. (2018)
Serum in patients	Paclitaxel/carboplatin	The occurrence of platelet-sparing phenomena has been found in individuals undergoing chemotherapy with carboplatin and paclitaxel	Pertusini et al. (2001)

4.4 Regulation of Therapeutic Resistance by Cytokines and Chemokines

Several investigations have indicated that dysregulated cytokine expression plays a pivotal role in several mechanisms of drug resistance (Zahreddine and Borden 2013; Holohan et al. 2013; Ho and Piquette-Miller 2006). Cytokines, a term used in this context to encompass a wide range of secreted molecules such as chemokines, growth factors, pro- and anti-angiogenic factors, adipokines, soluble receptors, and extracellular proteases, are known to have significant involvement in several cellular processes, both in normal physiological conditions and in pathological states. Abnormal cytokine signaling directs the growth of tumor cells and the development of stromal blood vessel systems, both of which play a crucial role in facilitating progressive tumor growth (Singh et al. 2017). Cytokines have a significant role in various physiological processes that exhibit a strong association with carcinogenesis, tumor progression, and metastasis (Wani et al. 2021). These processes encompass inflammation (Hansson 2005), migration of cells (Moser et al. 2004), angiogenesis (Belperio et al. 2000), and apoptosis (Janes et al. 2005; Nalli et al. 2019). Furthermore, there is a growing body of evidence indicating that the cytokines released by cancerous cells and their surrounding stroma are significantly involved in several processes of treatment resistance (de Visser and Jonkers 2009; McMillin et al. 2013). Despite the limited existing knowledge on the impact of cytokines on drug resistance, it has become evident that cytokines hold significant potential as treatment targets and biomarkers. Therefore, there is an urgent demand for a deeper investigation into their role in these specific circumstances.

The TME is recognized as a crucial and progressively valued factor in the development of tumors and the advancement of cancer (Turley et al. 2015; Quail and Joyce 2013; Hanahan and Weinberg 2011). The cellular composition of the TME is diverse, encompassing a range of cell types originating from the bone marrow, such

as neutrophils, mast cells, macrophages, and others. Additionally, the TME consists of endothelial cells derived from blood or lymphatic sources, pericytes, CAFs, and MSCs. The nonmalignant cells and the soluble substances they release have the potential to impact the growth and spread of cancer cells through various mechanisms. These include promoting cell survival, facilitating invasiveness, and altering the response to drugs. Immune cells have the potential to facilitate the development of cancer by the secretion of cytokines and growth factors. These substances can induce the proliferation of epithelial cells and produce reactive oxygen species, ultimately leading to damage to DNA (Bhowmick et al. 2004). The proliferation of endothelial cells and subsequent formation of diseased vasculatures occur as a result of an excessive production of vascular endothelial growth factor (VEGF). Pericytes, which are attracted to the tumor through the presence of platelet-derived growth factor-beta (PDGF beta) gradients (Abramsson et al. 2003), localize themselves on the outer surface of blood arteries, where they are believed to contribute to immunosuppressive mechanisms. Fibroblasts, which are the primary nonimmune stromal cells in the TME, play a crucial role in both the synthesis and remodeling of the ECM within the tumor stroma. Additionally, they facilitate cancer cell proliferation through the release of growth factors in a paracrine manner (Fig. 4.5).

The presence of stroma-mediated therapeutic resistance processes poses a significant obstacle to the efficacy of cancer treatment, mostly attributed to the limited availability of specific target molecules that can be effectively targeted by therapeutic interventions. The significance of the TME and its various stromal cell types in the development of targeted drug resistance is gradually gaining recognition. In fact, a particular study utilized antibody arrays to investigate the secretomes of

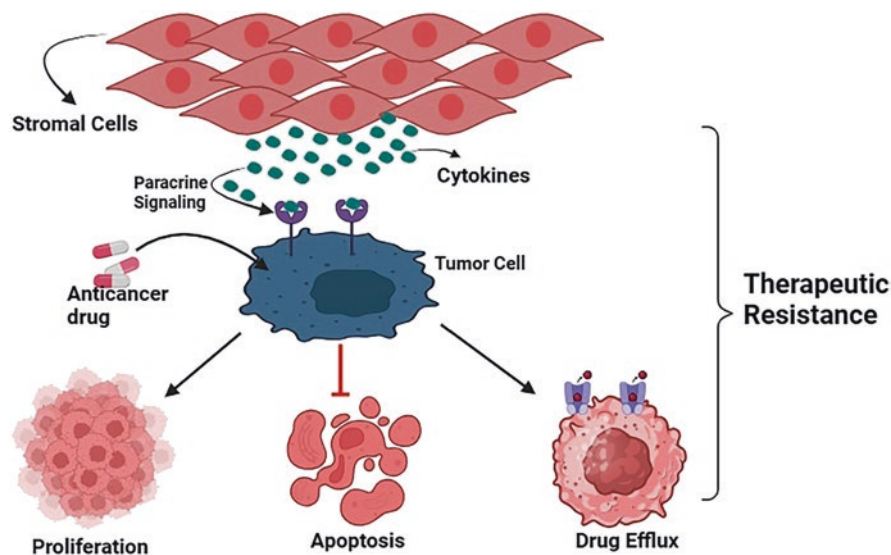


Fig. 4.5 The figure depicts the paracrine model of cancer drug resistance, highlighting the pivotal role of the tumor microenvironment in influencing resistance mechanisms

stromal cells, aiming to initiate the exploration of this inquiry. It was subsequently found that the tumor stroma possesses the ability to directly induce innate resistance to RAF inhibitors employing hepatocyte growth factor (HGF) production (Straussman et al. 2012). In this study, a co-culture system was employed to investigate the potential of 23 distinct stromal cell types in inducing innate resistance against 35 diverse anticancer medicines, encompassing both cytotoxic and oncoprotein-targeted treatments, across 45 cancer cell lines. Based on the extensive dataset analyzed, it was shown that stroma-mediated resistance was a prevalent phenomenon, particularly concerning the administration of targeted medications. Moreover, the observed resistance was replicated when only stromal cell media was used, suggesting that a soluble secreted component was accountable for this phenomenon. The examination of the medium using antibody arrays indicated a significant expression of HGF, leading to the stimulation of downstream MAPK and PI3-kinase/Akt signaling pathways via MET. This stimulation conferred rapid resistance to the RAF inhibitor PLX4720 (Straussman et al. 2012). Similar to other investigations that utilize a dual inhibition approach, the simultaneous disruption of HGF and MET activity led to the reversal of drug resistance. This finding further supports the notion that combination targeted therapy holds promise as a treatment option for melanoma. Moreover, this study serves as an illustrative example of how this approach can effectively elucidate the pathways behind drug resistance.

Pancreatic cancer is characterized by having the most unfavorable prognosis among all types of tumors and demonstrates a significant level of resistance toward currently accessible chemotherapeutic treatments. The significance of pancreatic stellate cells (PSCs) in the advancement of pancreatic cancer (Apte et al. 2004) and their possible role in chemoresistance (Huanwen et al. 2009; Guan et al. 2014) has been demonstrated. In a recent investigation, the mechanism of chemoresistance in pancreatic cancer mediated by PSCs was examined. The study focused on the involvement of SDF-1 α /CXCR4 signaling, which has been found to play a crucial role in several epithelial malignancies (Bertran et al. 2013; Conley-LaComb et al. 2013; Choi et al. 2014). The study revealed that primary PSCs exhibited expression of SDF-1 α , whereas pancreatic cancer cells (PCCs) displayed a high level of expression of its receptor, CXCR4. The cytotoxicity and apoptosis generated by gemcitabine in PCCs were inhibited by the culture media derived from PSCs. This inhibitory effect was counteracted by the addition of a neutralizing antibody against SDF-1 α and could be reproduced by treating the PCCs with recombinant SDF-1 α . Furthermore, the administration of recombinant SDF-1 α resulted in an upregulation of IL-6 expression in PCCs, which was reliant on the presence of CXCR4. Additionally, this treatment led to an increase in the phosphorylation of FAK, ERK1/2, Akt, and p38. Collectively, the findings from this study indicate that PSCs have the ability to provide resistance to chemotherapy in PCCs through the paracrine signaling of SDF-1 α and its receptor CXCR4. This signaling pathway leads to the stimulation of intracellular FAK-Akt and ERK1/2, followed by the establishment of an autocrine loop involving IL-6 (Zhang et al. 2015).

The bone marrow (BM) consists of hematopoietic cells as well as non-hematopoietic cells, such as endothelial cells, BM stromal cells, osteoblasts, and

osteoclasts. The cellular components and secreted soluble substances, such as cytokines, chemokines, and growth factors in addition to the ECM, collectively form the complete bone marrow milieu (Manier et al. 2012). The bone marrow milieu is a critical factor in influencing the differentiation, proliferation, migration, survival, and medication resistance of leukemia cells. An instance of a c-KIT inhibitor, imatinib, is a remarkably efficacious therapy for chronic myeloid leukemia (CML). It functions by inhibiting the BCR-ABL kinase oncogene present in CML cells. Nevertheless, it has been established that the BM is capable of inducing resistance to imatinib in K562 cells by the release of soluble factors originating from the BM stroma. These factors have been found to enhance the phosphorylation of STAT3 at tyrosine 705, leading to an upregulation of anti-apoptotic proteins (Li et al. 2015). Moreover, the efficacy of ABL tyrosine kinase inhibitors (TKIs) in treating CML is limited to a small proportion of patients, mostly because the stimulation of survival signals in CML stem/progenitor cells (SPCs) occurs via many pathways, including JAK2/STAT5 (Traer et al. 2012). Nevertheless, there have been reports indicating that the co-administration of ruxolitinib, a JAK1/2 inhibitor, with nilotinib, a tyrosine kinase inhibitor, resulted in a decrease in activity of the JAK2/STAT5 pathway. This combined therapy approach has also shown efficacy in eliminating CML CD34+ cells in both in vitro as well as in vivo settings. Therefore, targeting the JAK2/STAT5 pathway is a significant therapeutic approach for eliminating persistent illness in individuals with CML (Gallipoli et al. 2014).

Acute myeloid leukemia (AML) is a malignancy characterized by its aggressive nature and limited response to chemotherapy. The response of AML to chemotherapy is heavily influenced by the crucial role played by the dynamic interplay between AML cells and the BM microenvironment (Tabe and Konopleva 2014). For instance, the efficacy of nilotinib and imatinib, which are inhibitors of c-KIT/ABL, has been demonstrated in inhibiting the proliferation of two c-KIT mutant AML cell lines. However, this inhibitory effect was substantially reduced when these cells were grown in a conditioned medium derived from BM stromal cells, specifically either HS-5 or the primary BM stromal cells. Following an examination of several cytokines' capacity to prevent c-KIT-induced apoptosis in AML cell lines, it was determined that treatment with G-CSF can mimic the observed effects of HS-5 conditioned media (Gordon et al. 2014). Therefore, the study provided evidence of the significant involvement of G-CSF in regulating the reaction of AML cells to c-KIT inhibition.

4.5 Addressing Treatment Resistance by Targeting Immune Cells

Tumor cells have the capacity to generate an immunosuppressive TME by the secretion of several signaling factors that possess immunosuppressive properties. This phenomenon leads to the evasion of tumor surveillance by the body's immune system and further exploits immune cells to facilitate their own proliferation. Immune checkpoints refer to molecules that are present in immune cells and possess the

ability to modulate the extent of immunological activation. These molecules play a crucial role in the prevention of autoimmunity (Li et al. 2019). Nevertheless, tumor cells adeptly exploit the inhibitory impact of immune checkpoints on many immune cells, including T cells, DCs, macrophages, and NK cells. The tumor tissue can evade cytotoxic damage produced by T-cells by upregulating the expression of ligands for checkpoint molecules. This mechanism results in immune suppression, ultimately facilitating the development, invasion, and metastasis of cancer (Postow et al. 2018). The primary approach in immunotherapy for combating tumors is the use of immune checkpoint inhibitors (ICIs), which are antagonists capable of competitively binding to checkpoint molecules, including PD-1/PD-L1 and CTLA4/B7 (Bagchi et al. 2021). ICIs are actively involved in promoting immunosuppression through direct mechanisms. Due to their remarkable anticancer properties, they have been extensively utilized in clinical contexts and have demonstrated significant therapeutic efficacy in diverse cancer therapy modalities (Ihrig et al. 2020).

ICIs for CD8+ T Cells

The most traditional T-cell-associated immunological checkpoint is programmed death 1 (PD-1), an inhibitory transmembrane protein that is mostly produced on the T-cell surface (Wei et al. 2017; Ahmadzadeh et al. 2009). Its primary physiological role is to attach to the ligands PD-L1 or PD-L2, which inhibits the immune toxic activity of T cells. It then identifies the healthy tissue cells in the body and keeps them safe from toxic damage (Keir et al. 2008). However, in a particular study, it was observed that the PD-1 receptor expression in effector T cells was substantially elevated due to the presence of various atypical inflammatory conditions within the TME, as well as the unusually elevated expression of PD-1 ligand in tumor cells. This increase in PD-1 receptor expression ultimately led to the failure of T-cell function. Furthermore, the activation of the PD-1 signaling pathway has the potential to convert effector T cells into Tregs, leading to compromised immune responses against tumors (Amarnath et al. 2011; Francisco et al. 2009). PD-1 inhibitors, which are specifically engineered to restore the immune response of T cells, exhibit potent anticancer properties. Several clinical trials have been conducted to evaluate the efficacy of PD-1 inhibitors as anticancer agents, with promising outcomes observed in terms of cancer treatment efficacy (clinical trials). PD-1 inhibitors have demonstrated efficacy in obstructing the interaction between T cells and the tumor cell immunological checkpoint PD-1/PD-L1. This is achieved by competing with newly formed PD-1 receptors on the T cell's surface, hence reactivating the immunotoxic capabilities of T cells and producing favorable antitumor outcomes. In a separate clinical trial conducted by Ansell and Ramchandren et al., respectively, it was determined that the PD-1 antibody nivolumab exhibited notable therapeutic efficacy and satisfactory safety profiles when used for the therapy of recurrent and resistant classical Hodgkin's lymphoma (Ramchandren et al. 2019; Ansell et al. 2015).

Furthermore, CTLA-4, also known as cytotoxic T lymphocyte antigen-4 or CD152, is a prominent immune checkpoint inhibitor signaling route linked with T

cells. It functions as a coinhibitory receptor located on the surface of T cells and primarily facilitates tumor immunosuppression via antigen-specific signaling pathways. CTLA-4 is a member of the immunoglobulin superfamily, and it facilitates the induction of a quiescent state in T lymphocytes by interacting with the B7 ligand found on antigen-presenting cells (APCs) (Chen and Flies 2013). CTLA-4 is also involved in the trans-endocytosis process of APCs on B7-1 and B7-2 molecules, thereby inhibiting their interaction with CD28 and subsequently decreasing the abundance of CD28 (Chen and Flies 2013; Qureshi et al. 2011). Moreover, CTLA-4 serves to restrict the duration of interaction between the T-cell receptor (TCR) and APCs (Schneider et al. 2006). Additionally, CTLA-4 has the ability to induce de-phosphorylation and subsequent deactivation of positive immune activating signals (Saito and Yamasaki 2003). It has also the capability to send signals that directly impede the activation of T cells, leading to the suppression of T-cell proliferation and stimulation, as well as the decreased secretion of pro-effector cytokines like IL-12 and cytotoxic enzymes like perforin (Greenwald et al. 2001).

ICIs for Macrophages

Macrophages play a crucial role as primary effectors of the innate immune system, continuously sensing both activating and inhibitory cues to trigger phagocytosis and cytokine release (Komohara et al. 2016). SIRP α is a receptor with inhibitory properties that possesses several intracellular tyrosine-based immunosuppressive motifs (ITIMs). These motifs are primarily found on the APCs surface, including macrophages (Veillette and Chen 2018). The ligand known as CD47, which binds to TIM-3, functions as a signal that inhibits phagocytosis and prevents macrophages from engulfing healthy cells. Hematopoietic cells that lack CD47 are rapidly engulfed by macrophages, leading to the activation of dendritic cells (Alvey and Discher 2017). Nevertheless, it is worth noting that CD47 exhibits a significant upregulation on the cellular membrane of malignant tumor cells, hence functioning as a crucial method to evade immune surveillance. Upon binding to the ligand CD47, SIRP α undergoes phosphorylation and subsequently recruits phosphatases SHP-1 and SHP-2. This recruitment leads to the inhibition of phagocytosis and the functional action of macrophages (Matlung et al. 2017; Barclay and Van den Berg 2014). In recent years, the utilization of SIRP α inhibitors to selectively target macrophages has gained prominence as a viable approach to counteract the tumor-induced suppression of macrophages. In a study conducted in 2020, Kuo et al. employed the SIRP α blocker known as hAB21 to facilitate the process of macrophage-mediated antibody-dependent phagocytosis of tumor cells in an in vitro setting. The researchers discovered that the administration of a SIRP α antibody resulted in an augmentation of the therapeutic effect of rituximab in lymphomas that express CD20. Additionally, it was observed that the SIRP α antibody considerably boosted the efficacy of PD-1/PD-L1 inhibitors in combating tumor growth (Kuo et al. 2020).

ICIs for NK Cells

The primary inhibitory receptors found in NK cells consist of killer cell immunoglobulin-like receptor (KIR), natural killer Group 2A (NKG2A), and leukocyte immunoglobulin-like receptor (LIR). The regulation of these molecules is mediated by MHC-I molecules expressed on the tumor cell's surface. The KIR is a constituent of the immunoglobulin superfamily, with the ability to identify and bind to classical human leukocyte antigens A, B, and C (HLA class Ia) molecules. Numerous studies have consistently shown that the utilization of KIR inhibitors can effectively augment the anticancer efficacy of NK cells both in vitro and in vivo settings (Benson et al. 2011; Romagné et al. 2009). In addition, there are now several active clinical trials (NCT01256073) investigating the efficacy of KIR inhibitors. These trials have demonstrated promising results in terms of inhibiting tumor growth, while also showing minimal side reactions in patients who have achieved first full remission (Vey et al. 2012). Furthermore, NKG2A belongs to the C-type lectin receptor family and has the ability to form a dimer with CD94, resulting in the formation of the NKG2A/CD94 receptor. This receptor is responsible for the recognition of nonclassical HLA-E class I molecules. NKG2A represents a significant immunological checkpoint as well. In a study conducted by Pascale Andre and colleagues in 2018, it was observed that the inhibition of NKG2A in an in vitro setting resulted in the enhancement of the immunological response exhibited by NK cells. Moreover, the concurrent administration of an anti-NKG2A antibody with an anti-PD-L1 antibody has the potential to enhance its efficacy in regulating tumor growth. Additionally, the researchers have verified the safety and effectiveness of Monalizumab, an anti-NKG2A antibody, when used in conjunction with cetuximab in a Phase II clinical trial (NCT02643550) for patients with recurrent or advanced HNSC carcinoma (Carlsten et al. 2016).

4.6 Conclusion

In conclusion, the role of immune cells in cancer drug resistance underscores the intricate interplay between the host immune system and cancer cells within the tumor microenvironment. The dynamic involvement of cytotoxic CD8+ T cells, tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs) contributes significantly to the complex landscape of therapeutic responses. While cytotoxic CD8+ T cells stand as primary effectors, TAMs and MDSCs exhibit dual roles in tumor development and immunosuppression, challenging the effectiveness of immunotherapy. The exploration of combination therapies, such as immune checkpoint inhibitors, has demonstrated promise in overcoming resistance mechanisms. However, the persistence of acquired immune resistance poses a continued challenge, urging further research to unravel the intricate mechanisms involved. As we navigate the evolving understanding of immune cell dynamics in cancer drug resistance, personalized and adaptive therapeutic strategies emerge as essential tools in enhancing treatment outcomes and addressing the heterogeneous nature of immune responses across diverse cancer types.

MCQs

1. **What is the primary challenge in the interplay between the immune system and tumor cells?**
 - A. Immune system's inability to recognize tumor cells
 - B. Tumor cells' ability to evade immune surveillance
 - C. Lack of communication between immune cells
 - D. Limited adaptability of tumor cells

Answer B: Tumor cells' ability to evade immune surveillance

2. **What can dysregulated immunological and stromal milieu contribute to cancer progression?**
 - A. Inhibition of stem cell signals
 - B. Suppression of inflammatory reactions
 - C. Enhanced sensitivity to treatments
 - D. Stimulation of stem cell signals and resistance to treatments

Answer D: Stimulation of stem cell signals and resistance to treatments

3. **Which immune cells have been primarily investigated for their role in promoting cancer stem cells (CSCs) and therapeutic resistance?**
 - A. T lymphocytes
 - B. Myeloid cells
 - C. B lymphocytes
 - D. Natural killer cells

Answer B: Myeloid cells

4. **How has the reduction of myeloid populations impacted the responsiveness of tumors to established treatment modalities?**
 - A. It has decreased sensitivity to chemotherapy
 - B. It has no impact on treatment responsiveness
 - C. It has enhanced responsiveness to chemotherapy
 - D. It has increased resistance to targeted treatments

Answer C: It has enhanced responsiveness to chemotherapy

5. **According to recent research, what role might B lymphocytes play in the development of resistance to targeted treatments?**
 - A. Inhibiting stem cell signals
 - B. Enhancing treatment efficacy
 - C. Promoting tumor growth
 - D. No significant role observed

Answer C: Promoting tumor growth

6. **What is the primary function of regulatory T cells (Tregs) in the immune system?**
 - A. Inducing inflammation
 - B. Promoting immunologic self-tolerance
 - C. Enhancing immune homeostasis
 - D. Activating immune effector cells

Answer B: Promoting immunologic self-tolerance

7. Which marker is of significant importance in both the developmental process and suppressive capabilities of Tregs?
- A. CD4
 - B. FoxP3
 - C. CTLA-4
 - D. PD-1

Answer B: FoxP3

8. Which immune cells are associated with resistance to chemotherapy, radiotherapy, and immunotherapy, and enhance the recruitment of T-regulatory cells with suppressive properties?
- A. Natural killer cells
 - B. B cells
 - C. Myeloid-derived suppressor cells (MDSCs)
 - D. Dendritic cells

Answer C: Myeloid-derived suppressor cells (MDSCs)

9. How does temozolomide, a chemotherapeutic drug for glioblastoma, impact immunosuppression?
- A. Reduces hypoxia-related gene expression
 - B. Elevates expression of HIF-1 α and VEGF
 - C. Enhances immune responses against tumors
 - D. Suppresses MDSCs activation

Answer B: Elevates expression of HIF-1 α and VEGF

10. In the context of lung cancer, what pathway is activated by carboplatin-induced MDSCs, leading to resistance development?
- A. IL-10 pathway
 - B. VCAM/RANTES pathway
 - C. PD-1 pathway
 - D. STAT3 pathway

Answer B: VCAM/RANTES pathway

11. What challenge do myeloid-derived suppressor cells (MDSCs) pose to immune checkpoint blockade therapy?
- A. They enhance the immune response
 - B. They inhibit the immune response directly
 - C. They have no impact on immune checkpoint blockade therapy
 - D. They induce inflammation

Answer B: They inhibit the immune response directly

12. What are the two main phenotypes of macrophages, and how do they differ in their characteristics?
- A. M1 and M3; pro-tumor characteristics
 - B. M2 and M3; anti-inflammatory attributes
 - C. M1 and M2; pro-inflammatory and tumoricidal (M1) vs. anti-inflammatory and pro-tumor (M2)
 - D. M1 and M2; pro-inflammatory and pro-tumor characteristics

Answer C: M1 and M2; pro-inflammatory and tumoricidal (M1) vs. anti-inflammatory and pro-tumor (M2)

13. What activates M1 macrophages, and what cytokines do they secrete?

- A. Activation by Th2 cytokines; secretion of TGF β and IL10
- B. Activation by IFN- γ or LPS stimuli; secretion of IL-6, IL-12, IL-23, and TNF- α
- C. Activation by IL-4 and IL-13; secretion of angiogenic factors
- D. Activation by PD-L1; secretion of immunosuppressive cytokines

Answer B: Activation by IFN- γ or LPS stimuli; secretion of IL-6, IL-12, IL-23, and TNF- α

14. How do TAMs (Tumor-associated macrophages) contribute to tumor progression and resistance to chemotherapy?

- A. By inducing apoptosis in cancer cells
- B. By promoting immune responses
- C. By enhancing the recruitment of immunosuppressive TAMs and adopting a pro-tumor phenotype
- D. By inhibiting angiogenesis

Answer C: By enhancing the recruitment of immunosuppressive TAMs and adopting a pro-tumor phenotype

15. In the study conducted by Baghdadi M et al., what differentiation was observed in monocytes exposed to doxorubicin-resistant (DR) tumor supernatants?

- A. Differentiation into M1 macrophages
- B. Differentiation into dendritic cells
- C. Differentiation into M2 macrophages with an immunosuppressive phenotype
- D. No differentiation observed

Answer C: Differentiation into M2 macrophages with an immunosuppressive phenotype

16. How do TANs (Tumor-associated neutrophils) impact the effectiveness of therapeutic drugs targeting angiogenesis?

- A. By inhibiting angiogenesis
- B. By directly releasing interleukin-17 (IL-17)
- C. By enhancing the delivery of therapeutic drugs
- D. By promoting tumor regression

Answer B: By directly releasing interleukin-17 (IL-17)

17. What role does cathepsin B/Nlrp3 inflammasome play in TANs' impact on angiogenesis and therapeutic drug delivery?

- A. Inhibits angiogenesis
- B. Activates cathepsin B
- C. Indirectly triggers IL-17 production and IL-1 β upregulation
- D. Promotes tumor regression

Answer C: Indirectly triggers IL-17 production and IL-1 β upregulation

18. How do platelets contribute to cancer resistance to chemotherapy?

- A. By promoting apoptosis in cancer cells
- B. By inhibiting the activation of caspase-9

- C. By releasing growth factors and counteracting anti-proliferative effects of chemotherapeutic drugs
- D. By suppressing BCL-xL in platelets

Answer C: By releasing growth factors and counteracting anti-proliferative effects of chemotherapeutic drugs

19. How might platelets potentially impact resistance against anti-angiogenic treatment?

- A. By promoting the absorption of anti-angiogenic substances
- B. By scavenging anti-angiogenic substances
- C. By releasing cytokines that enhance angiogenesis
- D. By decreasing platelet count

Answer B: By scavenging anti-angiogenic substances

20. What is the potential role of platelets as cancer biomarkers in predicting chemotherapy resistance?

- A. Predicting resistance based on platelet size
- B. Identifying resistance to platinum-based therapy
- C. Assessing platelet count fluctuations
- D. All of the above

Answer D: All of the above

21. What is the significance of the TME in the context of cancer development, and what types of cells does it encompass?

- A. Targeted Medication Environment; includes only immune cells
- B. Therapeutic Modulation Efficiency; includes endothelial cells and pericytes
- C. Tumor Microenvironment; includes diverse cell types and soluble substances
- D. Tissue Malignancy Evaluation; includes only cancer cells

Answer C: Tumor Microenvironment; includes diverse cell types and soluble substances

22. How do fibroblasts contribute to cancer cell proliferation in the TME?

- A. By promoting apoptosis in cancer cells
- B. By suppressing growth factors
- C. By releasing chemokines
- D. By releasing growth factors in a paracrine manner

Answer D: By releasing growth factors in a paracrine manner

23. How does the bone marrow microenvironment influence the resistance of leukemia cells to imatinib in chronic myeloid leukemia (CML)?

- A. By inhibiting the release of soluble factors
- B. By suppressing JAK1/2 activity
- C. By enhancing phosphorylation of STAT3
- D. By promoting apoptosis in CML cells

Answer C: By enhancing phosphorylation of STAT3

24. How does the bone marrow microenvironment affect the proliferation of AML cells in response to nilotinib and imatinib?

- A. Promotes apoptosis in AML cells
- B. Inhibits phosphorylation of STAT3

- C. Enhances the efficacy of tyrosine kinase inhibitors
- D. Reduces the inhibitory effect of tyrosine kinase inhibitors

Answer D: Reduces the inhibitory effect of tyrosine kinase inhibitors

25. What is the primary physiological role of PD-1 in T cells?

- A. Activation of immune responses
- B. Inhibition of immune toxic activity
- C. Induction of T-cell proliferation
- D. Stimulation of cytokine secretion

Answer B: Inhibition of immune toxic activity

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Tumor Microenvironment: Multiway Role in Drug Resistance

5

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Abstract

The tumor microenvironment (TME) is an ecosystem that surrounds a tumor inside the body. TME contains a complex and dynamic system that consists of various cellular and noncellular components that cross talk with each other and with the tumor cells, thus supporting tumor progression. The intricate interplay

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between tumor cells and their surrounding microenvironment has emerged as a pivotal factor influencing the development of drug resistance in cancer treatment. This book chapter explores the multifaceted roles that the tumor microenvironment (TME) plays in mediating resistance to therapeutic interventions by elucidating the dynamic interactions between tumor cells, immune cells, stromal cells, extracellular matrix components, blood vessels, and soluble factors within the TME. It unravels the complexity of resistance mechanisms through a comprehensive exploration of key signaling pathways, cellular cross talk, and microenvironmental factors, and the chapter highlights the contribution of TME-driven adaptive strategies such as immune evasion, epithelial-mesenchymal transition, and angiogenesis to therapeutic resistance. It also highlights specific facets including the role of cancer-associated fibroblasts (CAFs) and mesenchymal stem cells in cancer drug resistance. The influence of the extracellular matrix (ECM) in fostering drug resistance and the active participation of the vascular system in sustaining resistance mechanisms are dissected in this chapter. Further digging into physiochemical exchanges, homotypic and heterotypic interactions, exosomes, cytokines, and chemokines as critical mediators of resistance. The chapter culminates by showcasing innovative strategies targeting the TME to overcome drug resistance, potentially revolutionizing cancer treatment paradigms. In essence, this chapter provides a comprehensive understanding of the TME's intricate role in drug resistance, spanning diverse elements and interactions while illuminating groundbreaking approaches to mitigate resistance challenges in oncology.

Keywords

Cancer · EMT · CAF · Drug resistance · Hypoxia · Drug efflux

5.1 Introduction

Cancer, a labyrinth of genetic anomalies and cellular disturbances, has captivated the scientific community for generations. Cancer, ranked among the five gravest global maladies, exhibits a persistent escalation in its prevalence with each passing year. Cancer, an intricate interplay of genetic mutations, environmental factors, and disrupted cellular pathways, stands as one of the most complex and formidable adversaries to human health (Khan et al. 2022a). It embodies distinctive biological traits characterized by aberrant cellular differentiation, incessant proliferation, and uncontrolled expansion, invasion, and metastasis (Khan et al. 2023a). At its core, cancer embodies an uncontrolled proliferation of cells, often forming masses known as tumors that encroach upon healthy tissues and perturb vital physiological processes. Chemotherapy and radiotherapy are the most common cancer treatments, but they often face drug resistance and severe side effects that limit their effectiveness (Erin et al. 2020). Therefore, researchers have explored the complex factors that affect cancer progression beyond the genetic mutations within cancer cells. One

such influential factor is the tumor microenvironment (TME), a dynamic and intricate environment that surrounds and interacts with cancer cells (Erin et al. 2020). The concept of the TME has revolutionized our understanding of cancer biology, shedding light on the profound impact of the local environment in which tumors thrive. Notably, the TME was implicated in fostering several of these hallmark traits, redefining its centrality in cancer progression (Erin et al. 2020). The TME is the complex and dynamic network of cellular and noncellular components that surrounds and interacts with the tumor cells. The TME includes various types of stromal cells, such as fibroblasts, endothelial cells, immune cells, and extracellular matrix (ECM) molecules, such as collagen, fibronectin, hyaluronan, and laminin (Ma et al. 2018). The TME also encompasses different niches that reflect the spatial and temporal heterogeneity of the tumor, such as the primary site, the intermediate site (e.g., lymph nodes), and the secondary site (e.g., distant organs) of metastasis.

Metastasis, once perceived as a linear and mechanical process, is now understood to be intricately linked to the TME. The journey of cancer cells from their origin to distant sites involves a series of orchestrated events driven by intricate cellular cross talk and the manipulation of the local environment. The TME, consisting of diverse components such as stromal cells, immune cells, blood vessels, and extracellular matrix, serves as a nurturing and protective niche for cancer cells during their invasive endeavors (Khan et al. 2023b). This protective cocoon allows cancer cells to evade the immune system's surveillance and develop mechanisms of resistance against therapeutic interventions, ultimately culminating in treatment failure. The mechanisms of TME-mediated drug resistance involve multiple factors, such as altered drug delivery and efflux, enhanced survival and repair pathways, adaptive phenotypic changes, and immunosuppressive signals (Mir et al. 2023).

This chapter delves deep into the multifaceted landscape of the TME and its pivotal role in drug resistance. By deciphering the intricate cross talk between cancer cells and their surroundings, we gain insights into the mechanisms through which cancer cells manipulate the TME to their advantage. This newfound understanding has paved the way for innovative therapeutic strategies aimed at disrupting the symbiotic relationship between cancer cells and their microenvironment, thus overcoming drug resistance and improving treatment outcomes.

5.2 Role of Cancer-Associated Fibroblasts (CAFs) in Therapeutic Resistance

The intricate system known as the tumor microenvironment (TME) comprises a complex interplay of cellular and molecular elements, significantly impacting cancer progression and therapeutic response (Eble and Niland 2019). Among the diverse noncancerous cell types inhabiting this milieu are fibroblasts, which play multifaceted roles (Belhabib et al. 2021; Khan et al. 2023c). These cells, usually quiescent, become activated during wound healing, driving extracellular matrix (ECM) remodeling, and releasing growth factors and cytokines that orchestrate epithelial proliferation and immune cell infiltration (Brassart-Pasco et al. 2020; Hassan

et al. 2020). However, in the context of cancer, this activation can transform into an irreversible state due to epigenetic alterations, giving rise to cancer-associated fibroblasts (CAFs).

CAFs emerge from a diverse array of cellular origins, with notable progenitors encompassing normal fibroblasts, epithelial cells, endothelial cells, adipocytes, stellate cells, bone marrow-derived cells, and mesenchymal stem cells (MSCs) (Simon and Salhia 2022). In a specific instance, in a xenograft model of breast cancer, fibroblasts can be prompted by endogenous factors like transforming growth factor-beta 1 (TGF- β 1) and stromal cell-derived factor-1 (SDF-1) to differentiate into myofibroblasts through an autocrine mechanism (Mezawa et al. 2019).

CAFs exhibit distinct characteristics compared to their quiescent counterparts and exert a significant impact on cancer progression within the TME. Their heightened proliferation, motility, and increased secretion of factors such as growth factors, chemokines, and matrix-modifying enzymes contribute to an environment conducive to tumor growth and metastasis (Brassart-Pasco et al. 2020; Minto et al. 2021; Khan et al. 2022a). Notably, CAFs also play a role in establishing and maintaining cancer stem cell niches through ECM remodeling and morphogen secretion. Furthermore, the interplay between CAFs and cancer cells extends to metabolic symbiosis, wherein these cells dynamically reprogram their metabolic profiles, including lipid metabolism, fostering a pro-tumorigenic system (Khan et al. 2023a; Chan et al. 2019).

The realm of clinical oncology has witnessed a transformation through targeted therapies, but their efficacy is hampered by primary and acquired resistance rooted not only in cancer cells themselves but also in the TME (Zahan et al. 2020; Khan et al. 2023d). The intricate role of CAFs, which are highly adaptable cells, in mediating resistance to anticancer therapies, particularly molecular interventions, is an area deserving more attention. While progress has been made in the effectiveness of cancer chemotherapies, achieving complete cures remains a challenge, especially in advanced cases. Drug resistance, a persistent issue, has primarily been linked to intrinsic cancer cell characteristics, yet the interplay between (Hameed et al. 2018) the tumor microenvironment (TME) and cancer cells also plays a pivotal role. CAFs, as crucial TME components, significantly influence tumor progression through soluble factor secretion (Farhood et al. 2019).

How Do Cancer-Associated Fibroblasts (CAFs) Orchestrate Resistance to Anticancer Therapies?

Alongside extensively explored cell-autonomous resistance mechanisms involving oncogenic mutations and alternate signaling pathways, the past decade has revealed the emergence of “non-cell-autonomous” resistance strategies. CAFs have emerged as key players in this context, particularly in the realm of targeted therapies (Farhood et al. 2019). They utilize a diverse toolkit, including modulation of extracellular matrix components, controlled release of bioactive factors and extracellular

vesicles, and dynamic shifts in metabolic activity. Notably, CAFs' impact extends beyond direct interactions with cancer cells, as they intricately integrate into the tumor microenvironment (TME), influencing therapeutic effectiveness (Linares et al. 2021). For instance, CAFs can affect microvessel permeability, impacting drug distribution, and also shape immune responses, influencing the outcomes of both immunotherapies and targeted treatments (Knipper et al. 2023). In essence, CAFs form a complex nexus that interweaves various resistance mechanisms across diverse therapeutic modalities. In this chapter, we will explore various ways in which cancer-associated fibroblasts (CAFs) contribute to drug resistance.

Role of CAFs in Drug Resistance in Hypoxic Microenvironments

Low oxygen levels, or hypoxia, are a common feature of the TME and are one of the main reasons for tumors becoming resistant to drugs (Li et al. 2017; Khan et al. 2023e). Cancer cells often face hypoxia, which helps them grow (Bridgford et al. 2018). In fast-growing tumors, the cells are far from the blood vessels, which makes it hard for drugs to reach the tumor, especially in a low-oxygen environment (Chou et al. 2012). For instance, NSCLC cells with EGFR mutations that were exposed to high doses of gefitinib under hypoxia developed drug-resistant cells, called GRPs. These cells also had high expression of genes related to Cancer stem cells. This process was mainly driven by an increase in IGF1 expression by HIF1, which activated IGF1R on GRPs, making them resistant to gefitinib and increasing the number of CSCs (Fig. 5.1) (Murakami et al. 2014). HIF-1 α expression is also high in CAFs (Petrova et al. 2018), which suggests that these cells may make NSCLC resistant to drugs by affecting the hypoxic microenvironment.

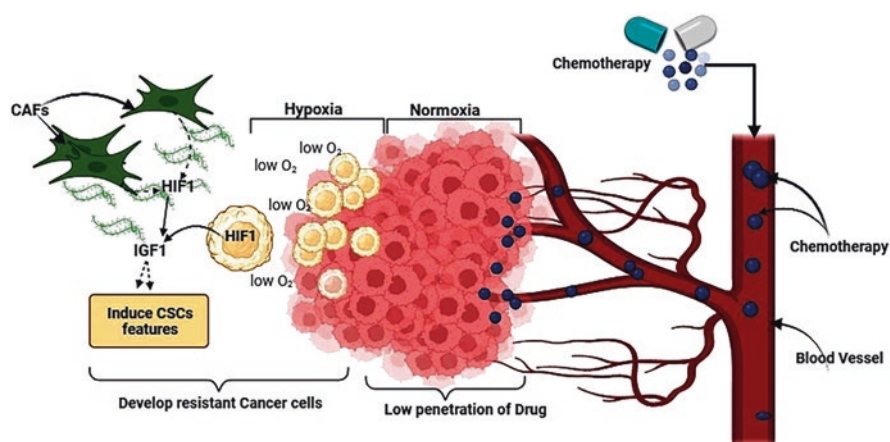


Fig. 5.1 The above figure demonstrates the formation of chemoresistant cancer cells in hypoxic conditions

Role of CAFs in Drug Resistance by Revascularization

Vascular endothelial growth factor (VEGF) stands as a pivotal HIF-regulated gene integral to angiogenesis. VEGF instigates endothelial cell activation via VEGFR, thereby fostering angiogenesis and augmenting endothelial cell proliferation (Sakurai and Kudo 2011). In the realm of breast cancer, the collaborative action of HIF-1 α and G protein-coupled estrogen receptor 1 (GPER) orchestrates the transcriptional upregulation of VEGF in hypoxic cancer-associated fibroblasts (CAFs), thereby propelling endothelial tube formation (De Francesco et al. 2013). Noteworthy observations include the ablation of fibroblast-specific HIF-1 α in a murine mammary tumor model, yielding normative vascular structures and heightened tumor proliferation—a phenomenon recapitulated upon VEGF ablation in fibroblasts (Kim et al. 2012). A quantitative proteomics analysis using stable isotope labeling by amino acids in cell culture (SILAC) revealed that hypoxic mammary CAFs secrete various angiogenic factors, influencing both intracellular and secretory proteins related to cell metabolism and angiogenesis (Kim et al. 2022). Interestingly, a significant portion of the analyzed proteins were not previously recognized as HIF-1 α target genes. Hypoxia-induced angiogenesis regulator (HIAR) in CAFs modulates the levels of pro-angiogenic factor stanniocalcin 1 (STC1) and antiangiogenic factor collagen type IV alpha 2 chain (COL4A2), stimulating VEGFA secretion and promoting endothelial cell sprouting (Kugeratski et al. 2019). Bevacizumab, an antiangiogenic monoclonal antibody, counteracts the VEGF ligand, impeding angiogenesis. However, due to limited efficacy, combination therapies with other anticancer agents are necessary (Kazazi-Hyseni et al. 2010).

Role of CAFs in Drug Resistance by Immunotherapy Resistance

Immune checkpoint inhibitors are important drugs for advanced non-small cell lung cancer (Raju et al. 2018). They target proteins such as PD-1, PD-L1, and CTLA-4 that regulate the immune response. Examples of these drugs are nivolumab, pembrolizumab, atezolizumab, and tremelimumab, which have been approved by the US FDA for lung cancer treatment (Kloten et al. 2019). However, most patients with lung cancer do not respond to these drugs and show primary resistance (Pu et al. 2018). CAFs can cause resistance by preventing immune cells from reaching and attacking the tumor cells (Fig. 5.2). They do this by changing the ECM, which is the network of molecules that surrounds the cells. A dense ECM blocks the contact and movement of immune cells (Fig. 5.2) (Salmon et al. 2012; Nicolas-Boluda et al. 2021). It also stops immune checkpoint inhibitors from binding to their targets and allows the tumor cells to escape the immune system (Fig. 5.2) (Salmon et al. 2012). The ECM is made of molecules such as collagen, laminin, and fibronectin. Lysyl oxidase is an enzyme that links collagen molecules together to form a dense ECM, which hinders the migration of immune cells and reduces the effect of PD-1 inhibitors (Nicolas-Boluda et al. 2021). CAFs that produce a lot of lysyl oxidase like-1

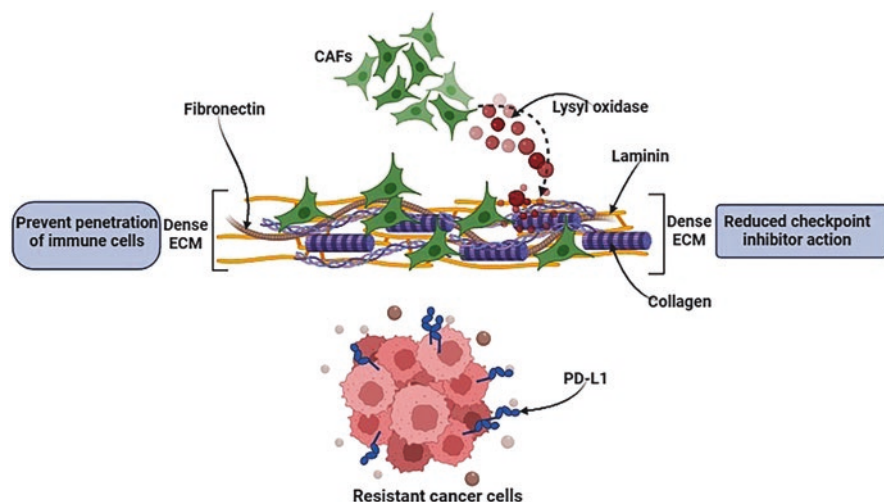


Fig. 5.2 The figure shows the role of CAFs in modulating ECM, thus promoting immunotherapy-resistant cancer cells

can change the collagen matrix *in vivo* (Zeltz et al. 2019), which suggests that CAFs make NSCLC resistant to immunotherapy drugs through the ECM. More research is needed to understand how CAFs and immune checkpoint inhibitors are related.

Role of CAFs in Drug Resistance by Fostering Cancer Stemness

CAFs are essential cells in driving the dedifferentiation of cancer cells into cancer stem cells (CSC), which have the ability to differentiate, self-renew, immortalize, expel chemotherapeutic drugs, and mediate cancer cell proliferation after drug treatment, leading to chemotherapy resistance (Aramini et al. 2022). CAFs secrete chemokines and growth factors that induce and maintain tumor cell stemness by activating the corresponding signaling pathways, resulting in chemotherapy resistance.

- Chemokines are small proteins (mostly 8–10 kDa) that belong to the interleukin family (IL-6, IL-8, IL-17A, and IL-1 α). They recruit leukocytes to the sites of infection and have a key role in the inflammatory response.
 - IL-6: CAF-derived IL-6 enhances cancer cell stemness and promotes chemotherapy resistance (Huang et al. 2019), for example, IL-6 is a multifunctional cytokine originally identified as involved in immunomodulation and immune response. In gastric cancer, lung cancer, and head and neck squamous cell carcinoma, CAF-secreted IL-6 supports the CSC phenotype by activating the STAT3 and TGF- β /SMAD signaling pathways, causing chemoresistance (Fig. 5.3) (Fang et al. 2022). IL-6 neutralizing antibody significantly increases

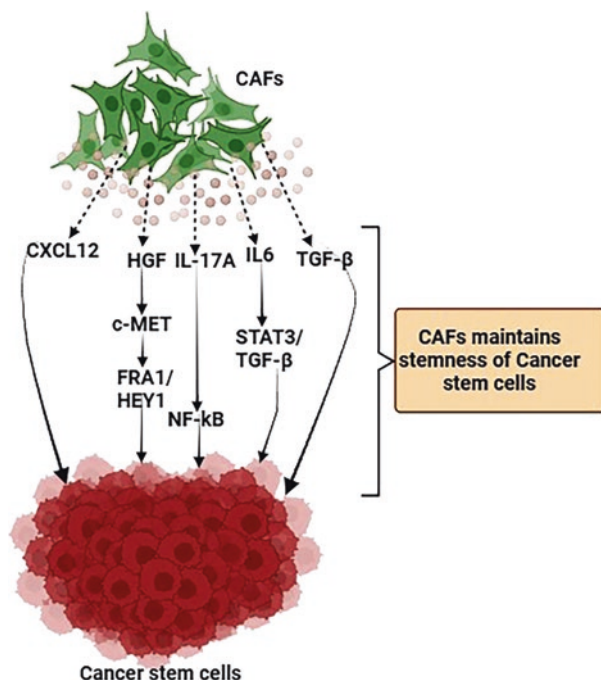


Fig. 5.3 The figure shows that CAFs release various chemokines and growth factors in maintaining CSCs

the chemosensitivity of pancreatic cancer cells by counteracting the CAF-induced drug-resistant phenotype (Zheng et al. 2022).

- IL-8: In breast cancer, thyroid cancer, and SCLC, CAF-released IL-8 may maintain cancer cell stemness by binding to their CXCR1 and CXCR2 receptors (Huang et al. 2019).
- IL-17A: The upregulation of NF-κB by IL-17A may be a possible mechanism for maintaining CSC, and targeting NF-κB may inhibit the role of IL-17A in cytotoxic therapy (Fig. 5.3) (Lotti et al. 2013; Wani et al. 2021).
- IL-1α: CAF-produced IL-1α acts on myeloid cells in the TME to induce the production of TSLP, which stimulates BCL2 expression and promotes tumor survival and lung metastasis (Brunetto et al. 2019).
- Growth factors
 - Transforming growth factor-beta (TGF-β): CAFs can induce cancer cell dedifferentiation and stemness by secreting TGF-β (Bu et al. 2020), which supports CSCs' tumor-initiating potential in response to chemotherapy (Calon et al. 2015). TGF-β activates EMT and transcription factors that promote a CSC-like state. TGF-β also stimulates CAFs to secrete CXCL12, which regulates breast cancer cell stemness via the CXCL12/CXCR4 axis (Fig. 5.3) (Huang et al. 2019). Moreover, TGF-β sustains the expression of DNA-binding regulators that are associated with stemness in various cancers (Itoh

et al. 2014). Thus, TGF- β can directly or indirectly modulate cancer cell stemness and resistance to drugs.

- Hepatocyte growth factor (HGF): HGF is a growth factor that affects many biological processes, and it binds to c-Met, which is made by a proto-oncogene. When c-MET is activated by HGF, it turns on its tyrosine kinase activity (Maulik et al. 2002). In HCC, HGF from CAFs interacts with c-MET and turns on the cMET/FRA1/HEY1 pathway, which controls HCC stemness. Also, in metastatic CRC, HGF from CAFs can stop cetuximab, a drug that blocks the EGF receptor, from working (Fig. 5.3). This makes CRC-initiating cells not respond to cetuximab (Luraghi et al. 2014).

Role of CAFs in Drug Resistance by Metabolic Modulation

CAFs can change the metabolism of cancer cells by causing Warburg and anti-Warburg effects and taking part in glutamine metabolism, which leads to resistance to drugs. CAFs can help cancer cells survive in the low-nutrient TME by releasing substances such as alanine (Sousa et al. 2016; Parker et al. 2020), deoxycytidine (Dalín et al. 2019), proline (Olivares et al. 2017), and lipids (Auciello et al. 2019), which allow tumor cells to adapt their energy production and use. This phenomenon of changing the metabolic pathways of tumor cells is called “metabolic reprogramming” and is one of the features of cancer (Yoshida 2015). Mitochondrial dysfunction alters the metabolic phenotype of cancer cells, impairing the oxidative phosphorylation of glucose to CO₂ and enhancing the aerobic glycolysis of glucose to lactate. This is known as the Warburg effect (Mitchell and Engelbrecht 2017; De Rosa et al. 2015), which is mediated by pyruvate kinase M2 (PKM2). PKM2 is overexpressed in NSCLC cell lines and confers resistance to cisplatin (Suzuki et al. 2019). Under hypoxic conditions, cisplatin-resistant cells secrete exosomes enriched with PKM2, which are internalized by cisplatin-sensitive cells. Exosomal PKM2 modulates the glycolytic flux in treatment-sensitive cells, promoting cell survival and inhibiting apoptosis. Moreover, in the TME, exosomes from cisplatin-resistant cells deliver PKM2 to CAFs, and the metabolically reprogrammed CAFs release pyruvate and lactate, contributing to chemotherapeutic resistance (Wang et al. 2021a; Wadhwa et al. 2020). In addition, CAF autophagy releases lactic acid, ketone bodies, and glutamine to create a nutrient-rich microenvironment that supports tumor growth (Mitchell and Engelbrecht 2017; Khan et al. 2022b).

5.3 Mesenchymal Stem Cells’ Potential Role in Cancer Drug Resistance

Mesenchymal stem cells (MSCs) are a type of adult stem cell that can be found in various tissues throughout the body, including bone marrow, adipose tissue (fat), umbilical cord tissue, and other connective tissues (Han et al. 2019; Pittenger et al. 2019; Lan et al. 2021). They are characterized by their ability to differentiate into

multiple cell types, including bone cells, cartilage cells, fat cells, and muscle cells. One of the key characteristics of MSCs is their self-renewal ability, which means they can divide and produce more stem cells while maintaining their undifferentiated state (Xuan et al. 2021; Gentile 2022; Nalli et al. 2019). This property makes them a valuable tool in regenerative medicine and tissue engineering, as they can potentially be used to repair or replace damaged or diseased tissues. In addition to their differentiation potential, MSCs possess immunomodulatory properties (Gomez-Salazar et al. 2020; Hmadcha et al. 2020). They can modulate the immune response by interacting with various immune cells, such as T cells, B cells, natural killer cells, and dendritic cells. MSCs can suppress immune cell activation and inhibit the production of pro-inflammatory molecules, making them attractive candidates for the treatment of immune-related disorders and conditions characterized by excessive inflammation (Jiang and Xu 2020; Song et al. 2020; Singh et al. 2017).

Mesenchymal stem cells (MSCs) gained significant attention in regenerative medicine due to their ability to differentiate into multiple cell types and their immunomodulatory properties. However, there is growing evidence suggesting that MSCs may also play a role in cancer drug resistance (Fig. 5.4) (Han et al. 2019; Song et al. 2020).

Tumor Microenvironment

MSCs have been shown to interact with cancer cells within the tumor microenvironment. They can migrate to the site of the tumor in response to various chemokines

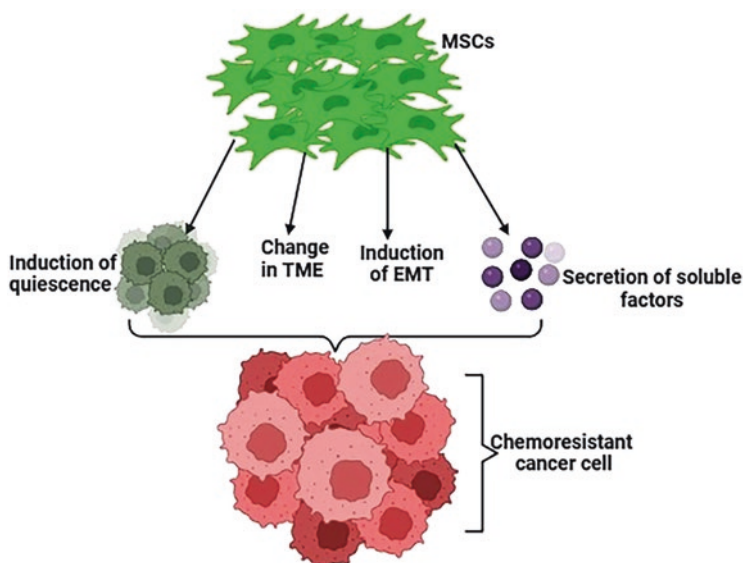


Fig. 5.4 The figure shows how mesenchymal stem cells are involved in the induction of resistance in cancer cells

and growth factors produced by cancer cells. Once in the tumor microenvironment, MSCs can secrete factors that promote tumor growth, angiogenesis (formation of new blood vessels), and metastasis. These factors can also contribute to drug resistance by providing a protective niche for cancer cells (Ahn 2020; Hass 2020; Aravindhan et al. 2021).

Induction of Quiescence

MSCs can induce a state of quiescence, or dormancy, in cancer cells. Quiescent cancer cells are less susceptible to conventional cancer treatments such as chemotherapy, which primarily target actively dividing cells. By entering a quiescent state, cancer cells can evade the effects of chemotherapy and subsequently resume proliferation once the treatment is discontinued (Aleksenko et al. 2018; Rumman et al. 2018; Vermeulen et al. 2023). In breast cancer, tumor dormancy can be generated by the involuntary fusion of MSC with breast cancer cells (Melzer et al. 2021).

Secretion of Soluble Factors

MSCs secrete a variety of soluble factors, including growth factors, cytokines, and extracellular vesicles, which can influence the behavior of cancer cells. Some of these factors can enhance the survival and growth of cancer cells while protecting them from the cytotoxic effects of anticancer drugs. Additionally, MSCs can produce factors that modulate the immune system, creating an immunosuppressive environment that hinders the efficacy of immunotherapy (Sobacchi et al. 2017; Han et al. 2024).

Epithelial-Mesenchymal Transition (EMT)

EMT is a biological process through which cancer cells acquire mesenchymal properties and become more invasive and resistant to therapy. MSCs have been shown to induce EMT in cancer cells, promoting their resistance to chemotherapy and targeted therapies. EMT also contributes to the development of cancer stem cells, which are believed to be highly resistant to anticancer treatments (Kletukhina et al. 2019; Shibata et al. 2019). For example, co-culturing of MSCs with gastric cancer or breast cancer cells decreases the expression of E-cadherin and increases the expression of EMT markers like vimentin, SNAIL, and TWIST (Xue et al. 2015; Martin et al. 2010).

Overall, the interactions between MSCs and cancer cells in the tumor microenvironment can promote drug resistance through multiple mechanisms. These include providing a supportive niche for cancer cells, inducing quiescence, secreting factors that protect cancer cells from drug-induced cell death, and promoting EMT and the

development of cancer stem cells. Understanding the role of MSCs in cancer drug resistance is important for the development of more effective therapeutic strategies against cancer.

5.4 Role of Extracellular Matrix in Drug Resistance

The extracellular matrix (ECM) is a complex three-dimensional network of proteins, glycoproteins, proteoglycans, and polysaccharides that surrounds and supports cells within tissues (Kusindarta and Wihadmadyatami 2018; Zhang et al. 2021). It provides structural integrity and mechanical support to tissues and organs while also playing essential roles in cellular functions, signaling, and tissue homeostasis (Karamanos et al. 2021). The ECM forms a scaffold that holds cells together and provides mechanical support to tissues. It contributes to the tensile strength and elasticity of tissues, enabling them to withstand physical forces (Valdoz et al. 2021; Yi et al. 2022). The ECM contains adhesive proteins, such as fibronectin and laminin, which facilitate cell adhesion and migration. Cells interact with specific ECM components through cell surface receptors, such as integrins, to anchor themselves and move within tissues during processes such as development, wound healing, and immune responses (Walker et al. 2018; Bandzerewicz and Gadomska-Gajadur 2022). The ECM is crucial for tissue organization and morphogenesis during development. It guides cell movements, shapes tissues and organs, and helps establish appropriate cellular arrangements and cell-cell interactions (Nicolas et al. 2020; Lemke and Nelson 2021).

The ECM undergoes continuous remodeling and turnover processes. Cells can synthesize, degrade, and modify ECM components through the action of enzymes such as matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) (Quintero-Fabián et al. 2019). This remodeling is essential for tissue repair, regeneration, and maintenance of tissue homeostasis. The extracellular matrix (ECM) plays a critical role in various aspects of cancer progression, including drug resistance (Fig. 5.5) (Cabral-Pacheco et al. 2020; Winkler et al. 2020). For example, ECM shields SCLC cells from cell death caused by chemotherapy by stimulating $\beta 1$ integrins, which trigger the activation of phosphoinositide-3-OH kinase (PI3-kinase). This activation prevents the activation of caspase-3 and the subsequent occurrence of apoptosis produced by etoposide (Rintoul and Sethi 2002).

Physical Barrier

The ECM can act as a physical barrier that limits the penetration and distribution of anticancer drugs within the tumor. The dense and fibrous nature of the ECM can hinder the effective delivery of chemotherapy agents, making it difficult for drugs to reach all cancer cells. This limited drug penetration can lead to incomplete eradication of cancer cells and the emergence of drug-resistant populations (Fig. 5.5) (Tahmasebi et al. 2019; Huang et al. 2021).

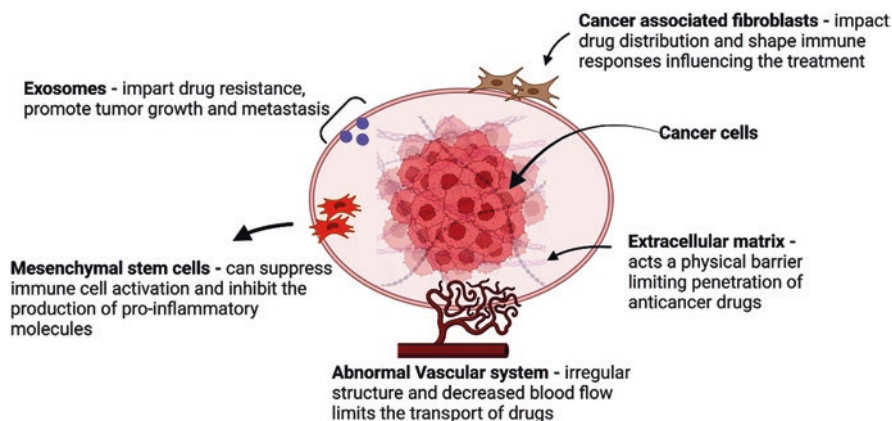


Fig. 5.5 The figure shows the role of exosomes, CAFs, MSCs, ECM, and abnormal angiogenesis in drug resistance

Drug Sequestration

Components of the ECM, such as proteoglycans and glycosaminoglycans, can bind to chemotherapeutic agents, effectively sequestering them and reducing their availability for interaction with cancer cells. This sequestration can reduce the effective concentration of drugs at the tumor site, reducing their efficacy and promoting drug resistance (Hraběta et al. 2020; Tomaszewski et al. 2021).

Activation of Survival Signaling Pathways

The ECM can activate various signaling pathways that promote cell survival and drug resistance. For example, interactions between cancer cells and the ECM can activate integrin signaling, which can lead to the activation of pro-survival pathways such as PI3K/Akt and MAPK/ERK. These pathways can enhance the survival and growth of cancer cells, making them more resistant to the cytotoxic effects of anti-cancer drugs (Hastings et al. 2019; Yuan et al. 2023).

Activation of Pro-survival Signaling in Stromal Cells

The stromal cells within the tumor microenvironment, such as cancer-associated fibroblasts and immune cells, can also interact with the ECM and become activated. These activated stromal cells can secrete factors that promote drug resistance in cancer cells. ECM can stimulate the secretion of cytokines and growth factors that enhance cell survival and confer resistance to anticancer drugs (Nallanthighal et al. 2019; Popova and Jücker 2022).

5.5 Vascular System's Contribution to Drug Resistance

The vascular system, also known as the circulatory system, is an essential network of blood vessels that transport blood, nutrients, oxygen, hormones, and other vital substances throughout the body. It consists of three main types of blood vessels: arteries, veins, and capillaries (Gnidovec et al. 2020). While the vascular system does play a role in drug resistance in certain contexts, it is more directly associated with the delivery of drugs to target tissues rather than contributing to drug resistance itself. The vascular system facilitates the transport of drugs to the target site, but it can also pose challenges that affect the effectiveness of drug therapy (Trammel and Sapra 2020; Saha and Sarkar 2021).

Limited Drug Delivery

The vascular system is responsible for delivering drugs to the target tissues and cells. However, tumor blood vessels often exhibit abnormal characteristics, such as irregular structure, increased permeability, and poor blood flow. These abnormalities can impair the delivery of anticancer drugs to the tumor site, leading to insufficient drug concentrations within the tumor and reduced therapeutic efficacy (Fig. 5.1) (Singh et al. 2019; Mitchell et al. 2021).

Increased Interstitial Fluid Pressure

In solid tumors, the abnormal tumor vasculature can contribute to increased interstitial fluid pressure (IFP) within the tumor microenvironment. Elevated IFP acts as a physical barrier that hinders the penetration and distribution of drugs in the tumor tissue. Insufficient drug penetration can limit the exposure of cancer cells to therapeutic agents, reducing their effectiveness and promoting drug resistance (Stewart 2020; Gao et al. 2020).

Development of Hypoxia

Abnormal tumor vasculature can result in regions of poor oxygen supply, known as hypoxia, within the tumor. Hypoxic conditions are associated with drug resistance, as they can lead to altered cellular metabolism and activation of survival pathways in cancer cells. Hypoxia can also promote the development of drug-resistant cancer stem cells and contribute to the upregulation of drug efflux pumps, which remove drugs from cancer cells (Fig. 5.1) (Li et al. 2021; Emami Nejad et al. 2021).

Poor Drug Penetration

Tumor blood vessels often exhibit abnormal characteristics, such as leakiness, irregular structure, and inefficient blood flow. These abnormalities can limit the distribution and penetration of anticancer drugs into the tumor tissue, leading to insufficient drug concentrations at the target site. Inadequate drug penetration can result in incomplete eradication of cancer cells, allowing resistant cell populations to persist and contribute to drug resistance (He et al. 2020; Munir 2022).

Drug Efflux Pumps

Blood vessels in various tissues, including tumors, can express drug efflux pumps, such as P-glycoprotein (P-gp). These pumps actively transport drugs out of cells, reducing their intracellular concentrations and limiting their efficacy. High expression of drug efflux pumps in tumor blood vessels can contribute to drug resistance by actively removing anticancer drugs from cancer cells, making them less susceptible to treatment (Gupta et al. 2020; Wang et al. 2022). Bronger et al. examined the levels and distribution of six members of the ATP-dependent efflux pumps known as the multidrug resistance protein family (ABCC1-ABCC6, previously known as MRP1-MRP6), as well as six organic anion uptake transporters (OATP1A2, OATP1B1, OATP1B3, OATP1C1, OATP2B1, and OATP4A1), in 61 human glioma specimens with various histologic subtypes (Bronger et al. 2005). The real-time polymerase chain reaction (PCR) analysis revealed the presence of ABCC1, ABCC3, ABCC4, and ABCC5 gene expressions. In addition, they identified the presence of OATP uptake transporter genes SLCO1A2, SLCO1C1, SLCO2B1, and SLCO4A1. Immunofluorescence microscopy revealed that only OATP1A2 and OATP2B1 proteins were present in the luminal membrane of endothelial cells that make up the blood-brain barrier and the blood-tumor barrier, but not in the glioma cells (Bronger et al. 2005).

5.6 Exosomes: Potential Drivers of Cancer Drug Resistance

Exosomes are small extracellular vesicles that play a significant role in cell-to-cell communication within the tumor microenvironment (Zhong et al. 2021). They are released by various cell types, including tumor cells, stromal cells, and immune cells, and carry bioactive molecules such as proteins, nucleic acids (RNA and DNA), lipids, and signaling molecules (Hu et al. 2019). Exosomes have been shown to have diverse functions within the tumor microenvironment, including promoting tumor growth, metastasis, and drug resistance (Santos and Almeida 2020; Paskeh et al. 2022). Some key roles of exosomes in the tumor microenvironment:

Intercellular Communication

Exosomes act as mediators of intercellular communication within the tumor micro-environment. They can transfer their cargo of proteins, RNA, and other molecules to recipient cells, influencing their behavior and phenotype (Challagundla et al. 2015). Exosomes can transmit signals between tumor cells, stromal cells, and immune cells, leading to the modulation of cellular functions and promoting tumor progression (Bian et al. 2019; Conti et al. 2020).

Promotion of Tumor Growth and Angiogenesis

Exosomes derived from tumor cells can enhance tumor growth by stimulating proliferation, migration, and invasion of neighboring cells. They can also promote angiogenesis, the formation of new blood vessels, by delivering pro-angiogenic factors to endothelial cells. This supports the supply of nutrients and oxygen to the growing tumor (Arneth 2019; Anderson and Simon 2020).

Metastasis Promotion

Exosomes have been implicated in facilitating the metastatic spread of tumor cells. They can prime the pre-metastatic niche by preparing distant sites for tumor cell colonization. Exosomes derived from primary tumor cells can promote the invasion and migration of cancer cells to distant organs, thereby contributing to metastasis (Vasilaki et al. 2021; Neophytou et al. 2021).

Immune Modulation

Exosomes play a crucial role in modulating immune responses within the tumor microenvironment. Tumor-derived exosomes can carry immunosuppressive molecules and promote the differentiation of immune cells, such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), which suppress the anti-tumor immune response. They can also induce immune cell apoptosis and impair the function of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells, leading to immune evasion by tumor cells (Miyauchi et al. 2019; Wang et al. 2021b).

Drug Resistance

Exosomes have been implicated in promoting drug resistance in tumor cells. They can transfer drug efflux pumps, such as P-glycoprotein (P-gp), to recipient cells,

leading to increased drug efflux and reduced intracellular drug concentrations. Exosomes can also transfer molecules involved in DNA repair and antiapoptotic pathways, promoting cell survival and resistance to chemotherapy (Seebacher et al. 2021; Kaemmerer et al. 2021). Ning et al. provided evidence that in MCF7/ADM cells, a type of human breast cancer cells resistant to adriamycin (ADM), ADM did not accumulate in the nuclei as anticipated. Instead, it was released from the cells via exosomes. Simultaneously, there was a notable presence of P-gp in the exosomes, along with elevated levels of its regulator, transient receptor potential channel 5 (TrpC5). The restoration of P-gp was achieved by employing a particular blocker (T5E3) that targets the TrpC5 channel. This blocker effectively restricted the efflux of drugs, leading to an enhanced sensitivity of breast cancer cells to chemotherapy (Ning et al. 2017).

Diagnostic and Therapeutic Potential

Exosomes have gained interest as potential biomarkers for cancer diagnosis and prognosis. They can be isolated from various body fluids, such as blood, urine, and saliva, and their cargo can reflect the genetic and molecular characteristics of the originating tumor cells. Furthermore, exosomes hold promise as therapeutic targets or delivery vehicles for anticancer agents, including small molecules, nucleic acids, and proteins (Gargiulo et al. 2020; Latowska et al. 2020). For example, Tian et al. demonstrated that doxorubicin is effectively loaded onto iRGD peptide functional exosomes via electroporation, resulting in a drug loading efficiency that is 20 times higher. They also find that exosomes exhibit significant tumor-targeting capability and antitumor effectiveness in both *in vitro* and *in vivo* tests, owing to the inclusion of iRGD peptide (Tian et al. 2014).

Overall, exosomes play a multifaceted role in the tumor microenvironment, influencing various aspects of tumor growth, metastasis, immune response, and drug resistance. Understanding their functions and mechanisms of action may provide insights into the development of novel therapeutic strategies for cancer treatment.

5.7 Targeting Tumor Microenvironment to Overcome Drug Resistance

Targeting the tumor microenvironment is a promising approach to overcoming drug resistance in cancer. The tumor microenvironment consists of various components, including stromal cells, immune cells, blood vessels, extracellular matrix, and signaling molecules. These components interact with tumor cells and contribute to the development of drug resistance (Erin et al. 2020; Kaemmerer et al. 2021). Some strategies for targeting the tumor microenvironment to overcome drug resistance are as follows:

Modulating Immune Responses

The immune cells present in the tumor microenvironment can either promote or inhibit tumor growth and drug resistance. Enhancing the antitumor immune response by activating immune cells, such as T cells and natural killer (NK) cells, can help overcome drug resistance. Immune checkpoint inhibitors, which block inhibitory receptors on immune cells, have shown success in some cancers by reactivating immune responses against tumor cells (Malla et al. 2020; Madeddu et al. 2022). For example, Blocking CTLA4 leads to a widespread improvement in immunological responses that rely on helper T cells. Conversely, engaging CTLA4 on TReg cells boosts their ability to suppress immune activity (Pardoll 2012).

Targeting Angiogenesis

Tumors require a sufficient blood supply for growth and metastasis. Inhibiting angiogenesis, the formation of new blood vessels, can disrupt the tumor microenvironment and reduce nutrient and oxygen supply to the tumor cells. Antiangiogenic therapies, such as drugs targeting vascular endothelial growth factor (VEGF) or its receptors, can normalize the tumor vasculature, increase drug delivery, and sensitize tumor cells to chemotherapy or other targeted agents (Ma et al. 2018; Hameed et al. 2018). Avastin (bevacizumab), a genetically engineered antibody that specifically targets the signaling pathway of VEGF/VEGFR in the tumor microenvironment, has received approval from the Food and Drug Administration (FDA) for the treatment of specific women with advanced Ovarian cancer (Arora et al. 2021).

Disrupting Stromal Support

Cancer-associated fibroblasts (CAFs) and other stromal cells within the tumor microenvironment contribute to drug resistance by providing a supportive niche for tumor growth and metastasis. Targeting these stromal cells or the signaling pathways that regulate their activity can disrupt the tumor-stromal interactions and enhance the effectiveness of anti-cancer therapies (Maacha et al. 2019; Baghban et al. 2020).

Modifying the Extracellular Matrix

The extracellular matrix (ECM) within the tumor microenvironment can create physical barriers that limit drug penetration into the tumor. ECM-targeting strategies, such as using ECM-degrading enzymes or agents that remodel the ECM, can improve drug delivery to tumor cells and overcome resistance (Kaemmerer et al. 2021; Liu et al. 2022).

Modulating Tumor Metabolism

The metabolic state of tumor cells and the surrounding microenvironment can influence drug response. Targeting metabolic pathways in tumor cells or modifying the metabolic interactions within the tumor microenvironment can sensitize resistant cells to treatment. For example, combining targeted therapies with agents that inhibit specific metabolic pathways or modulate nutrient availability can improve treatment outcomes (Xia et al. 2020; Targhazeh et al. 2022).

Dual Targeting Approaches

Combining therapies that directly target tumor cells with agents that modify the tumor microenvironment can enhance treatment efficacy. This approach addresses both intrinsic and extrinsic factors contributing to drug resistance and improves the chances of overcoming resistance mechanisms (Han et al. 2024; Taghipour et al. 2022).

It is important to note that the tumor microenvironment is complex and heterogeneous, and different strategies may be effective depending on the specific tumor type and context. Combination therapies that target both tumor cells and the microenvironment, along with personalized treatment approaches, hold promise for overcoming drug resistance and improving patient outcomes.

5.8 Conclusion

The tumor microenvironment is a dynamic and complex milieu surrounding cancer cells that significantly influences tumor development, progression, and response to therapy. Comprising a variety of cellular and noncellular components, including fibroblasts, immune cells, blood vessels, and the extracellular matrix, the tumor microenvironment plays a crucial role in shaping the behavior of cancer cells. Understanding the intricate interplay within the tumor microenvironment is crucial for developing more effective and targeted cancer therapies. It represents a dynamic area of research with the potential to improve treatment outcomes and address challenges such as drug resistance and metastasis. Below table shows the various cellular components and their role in the drug-resistance

S. no.	Aspect	Description	Reference
1.	Cellular components	Tumor cells, immune cells (T cells, B cells, macrophages), fibroblasts, endothelial cells.	Ferrari et al. (2019), Certo et al. (2021)
2.	Extracellular matrix (ECM)	Collagen, fibronectin, hyaluronic acid, proteoglycans.	Zhang et al. (2021), Karamanos et al. (2021)
3.	Cytokines and chemokines	Signalling molecules influencing cell behaviour, migration and immune response.	Nallanthighal et al. (2019), Masih et al. (2022)

(continued)

S. no.	Aspect	Description	Reference
4.	Therapeutic targets	Potential targets for cancer therapies, including immunotherapies and anti-angiogenic agents.	Singh et al. (2019), Mitchell et al. (2021)
5.	Multiway drug resistance mechanism	Increased expression of drug efflux pumps, enhanced DNA repair mechanisms, altered drug targets, epithelial-mesenchymal transition.	Gupta et al. (2020), Costa and Vale (2022), Saitoh and Oda (2021)
6.	Interleukins	T-cell growth factor, regulates immune response, anti-inflammatory cytokine.	Edwardson et al. (2019), Marques et al. (2020)
7.	Clinical implications	Impact on treatment efficacy, challenges in overcoming multiway drug resistance, and personalized treatment strategies.	Hmadcha et al. (2020), Lu et al. (2019)

MCQs

1. What is the role of immune system in cancer prevention?

- A. Suppressing the growth of normal cells
- B. Attacking and destroying cancer cells
- C. Promoting the formation of tumors
- D. Inhibiting chemotherapy effectiveness

Answer B: Attacking and destroying cancer cells

2. What are cancer-associated fibroblasts (CAFs)?

- A. Cancer cells with fibrous characteristics
- B. Fibrous proteins found in tumors
- C. Fibroblasts residing near cancer cells
- D. Normal cells unaffected by cancer

Answer C: Fibroblasts residing near cancer cells

3. In what ways do CAFs contribute to the development of a drug-resistant phenotype in cancer cells?

- A. By inhibiting angiogenesis
- B. By promoting cell differentiation
- C. By inducing cancer cell quiescence
- D. By influencing drug efflux mechanisms

Answer D: By influencing drug efflux mechanisms

4. How do mesenchymal stem cells interact with cancer cells in tumor microenvironment?

- A. The directly attack and destroy cancer cells
- B. They promote cancer cell apoptosis
- C. They provide a supportive niche for cancer cells
- D. They inhibit angiogenesis

Answer C: They provide a supportive niche for cancer cells

5. How can mesenchymal cells influence the extracellular matrix in a way that contributes to drug resistance?

- A. By reducing matrix stiffness

- B. By promoting drug penetration
- C. By increasing matrix degradation
- D. By secreting factors that enhance matrix integrity

Answer C: By increasing matrix degradation

6. **What term describes the ability of mesenchymal cells to differentiate in various cell types, potentially influencing cancer behaviour?**

- A. Dedifferentiation
- B. Plasticity
- C. Metastasis
- D. Proliferation

Answer C: Metastasis

7. **Which type of therapy focuses on modifying the tumor microenvironment, including the interactions with the mesenchymal cells to improve cancer treatment outcomes?**

- A. Targeted therapy
- B. Immunotherapy
- C. Angiogenesis inhibitors
- D. Stromal-targeted therapy

Answer D: Stromal-targeted therapy

8. **What role does the vascular system play in cancer progression?**

- A. Suppressing tumor growth
- B. Providing nutrients to cancer cells
- C. Inducing apoptosis to cancer cells
- D. Inhibiting angiogenesis

Answer B: Providing nutrients to cancer cells

9. **How do cancer cells adapt to the vascular system to resist chemotherapy?**

- A. By increasing drug uptake
- B. By promoting vessel normalisation
- C. By inducing tumor hypoxia
- D. By activating antiangiogenic factors

Answer C: By inducing tumor hypoxia

10. **How can the vascular system contribute to the development of multidrug resistance in cancer cells?**

- A. By inhibiting drug efflux mechanisms
- B. By promoting drug uptake in cancer cells
- C. By creating a protective environment
- D. By inducing cancer cell apoptosis

Answer C: By creating a protective environment

11. **What factor secreted by the tumor microenvironment can stimulate angiogenesis and contribute to drug resistance?**

- A. Tumor necrosis factor (TNF)
- B. Vascular endothelial growth factor (VEGF)
- C. Interleukin-6 (IL-6)
- D. Transforming growth factor-beta (TGF- β)

Answer B: Vascular endothelial growth factor (VEGF)

12. What is the role of pericytes in the vasculature of the tumor microenvironment in relation to drug resistance?

- A. Inhibiting angiogenesis
- B. Enhancing drug sensitivity
- C. Creating a barrier to drug penetration
- D. Promoting vessel normalization

Answer C: Creating a barrier to drug penetration

13. What role do physiochemical interactions play in drug resistance within the tumor microenvironment?

- A. Enhancing drug sensitivity in cancer cells
- B. Creating a barrier to drug penetration
- C. Inhibiting DNA repair mechanisms
- D. Inducing cancer cell apoptosis

Answer A: Enhancing drug sensitivity in cancer cells

14. What physiochemical factors contribute to the limited penetration of drugs into tumor tissues?

- A. High interstitial fluid pressure
- B. Low extracellular pH
- C. Elevated levels of oxygen
- D. Increased drug uptake by cancer cells

Answer A: High interstitial fluid pressure

15. What factors in the tumor microenvironment can modulate drug resistance by altering drug metabolism in cancer cells?

- A. Fibroblasts
- B. Immune cells
- C. Cytokines
- D. Enzymes

Answer D: Enzymes

16. What do homotypic interactions in the tumor microenvironment primarily involve?

- A. Interaction between cancer cells and immune cells
- B. Interaction between cells of same type
- C. Interaction between cancer cells and blood vessels
- D. Interactions between cancer cells and extracellular matrix

Answer B: Interaction between cells of same type

17. Which cell adhesion molecules are commonly involved in homotypic interactions between cancer cells?

- A. Integrins
- B. Cadherins
- C. Selectins
- D. Immunoglobulins

Answer B: Cadherins

18. What are cytokines in the context of tumor microenvironment?

- A. Cancer cells
- B. Signalling molecules

- C. Blood vessels
- D. Extracellular matrix components

Answer B: Signaling molecules

19. **What type of cytokines are typically associated with pro-inflammatory responses in the tumor microenvironment?**

- A. Interleukins
- B. Growth factors
- C. Transforming growth factors
- D. Chemokines

Answer A: Interleukins

20. **Which chemokine is known for its role in promoting angiogenesis in the tumor microenvironment?**

- A. CCL2
- B. CXCL8
- C. CXCL12
- D. CCL5

Answer B: CXCL8

21. **Why is targeting the tumor microenvironment considered a strategy to overcome drug resistance?**

- A. It directly kills cancer cells
- B. It induces mutation in cancer cells
- C. It creates a supportive niche for cancer cells
- D. It inhibits DNA repair mechanism

Answer A: It directly kills cancer cells

22. **Which type of immune cells are commonly found in the tumor microenvironment and play a role in antitumor immunity?**

- A. Natural killer cells
- B. B cells
- C. Mast cells
- D. Neutrophils

Answer A: Natural killer cells

23. **What is the primary role of the ECM in contributing to drug resistance?**

- A. Enhancing drug penetration
- B. Sequestering chemotherapeutic agents
- C. Activating survival signaling pathways
- D. Facilitating drug delivery

Answer B: Sequestering chemotherapeutic agents

24. **What abnormal characteristics are often observed in tumor blood vessels that affect drug delivery?**

- A. Decreased permeability
- B. Efficient blood flow
- C. Irregular structure and leakiness
- D. Normalized vascular architecture

Answer C: Irregular structure and leakiness

25. How do exosomes contribute to drug resistance in cancer cells?

- A. By inhibiting drug efflux pumps
- B. Promoting drug penetration
- C. Enhancing cellular metabolism
- D. Transferring drug resistance-related molecules

Answer D: Transferring drug resistance-related molecules

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Cancer Drug Resistance and Metabolic Reprogramming

6

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and Sameer Ullah Khan

Abstract

There is a complex and strong association between metabolic reprogramming and the phenomenon of drug resistance in cancer. Cancer cell metabolic modifications and crosstalk with cellular and noncellular components are essential to support their growth and proliferation and support anticancer therapy resistance. The chapter explores the unintended consequences of chemotherapy interventions, revealing how therapeutic measures can induce metabolic shifts in cancer cells that inadvertently contribute to the emergence of chemoresistance. The chapter navigates through the complicated interplay between key genetic players and the metabolic shifts that support drug resistance mechanisms. Finally, the chapter proposes innovative strategies to manipulate cancer cell metabolism as a promising avenue for overcoming drug resistance. This comprehensive examina-

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tion not only elucidates the complex link between metabolic reprogramming and drug resistance but also suggests potential therapeutic interventions, presenting a roadmap for future research in the evolving landscape of cancer treatment.

Keywords

Chemoresistance · Metabolic reprogramming · Oncogenes · Tumor suppressor genes

6.1 Introduction

Metabolic reprogramming, which refers to the metabolic modifications observed in tumor cells, is recognized as one of the fundamental characteristics of malignancies (Khan et al. 2023a; Lee et al. 2018a). The promotion of cellular proliferation in tumors necessitates the reprogramming of metabolic processes, resulting in alterations to metabolic pathways in order to provide sufficient ATP or intermediates for the synthesis of macromolecules under stressful conditions, hence satisfying the demands of accelerated cell division. The metabolic restructuring observed in tumor cells is thought to be a result of metabolic adaptation. This adaptation is thought to happen due to alterations in oncogenes, which subsequently disrupt the production and function of certain metabolic enzymes and alter the flow of metabolic pathways. Ultimately, these changes have an impact on the overall metabolism of the cells. Alterations in cellular metabolism not only facilitate the progression of tumors (Faubert et al. 2020; Qiao et al. 2020; Lee et al. 2018a; Vernucci et al. 2019; Boroughs and DeBerardinis 2015; Nalli et al. 2019) but also play a role in the development of resistance to chemotherapeutic agents (Cardoso et al. 2018; Maria et al. 2017; Rahman and Hasan 2015; Pan et al. 2017). The observed alterations can be attributed to the metabolic adjustments made by tumor cells in response to chemotherapeutic stress. These adaptations encompass changes in bioenergetics, oxidative stress, and lipid metabolism (Khan et al. 2023b; Hassan et al. 2020). Additionally, tumor cells exhibit an augmented reliance on glucose and glycolysis, as well as an upregulation in polyamine synthesis, among other modifications (Zhang et al. 2016a, 2017; Lee et al. 2017a; You et al. 2019). The precise mechanism underlying metabolic adaptation in drug-resistant (DR) cells remains poorly known; nevertheless, accumulating data indicates that the expression of certain metabolic genes or signal cascades is dysregulated in these cells (Cruz-Bermúdez et al. 2019; Chen et al. 2019; Ghosh et al. 2015). This phenomenon results in an elevation in the absorption of glucose and glutamine by drug-resistant (DR) cells (Montopoli et al. 2011; Catanzaro et al. 2015). Additionally, it triggers the stimulation of many pathways, including glycolysis, glutaminolysis, and fatty acid oxidation (Masamha and LaFontaine 2018; Galicia-Vázquez and Aloyz 2018). Furthermore, it stimulates the generation of antioxidants (Catanzaro et al. 2015; Lopes-Coelho et al. 2016) and induces metabolic remodeling within mitochondria (Ghosh et al. 2015; Khan et al. 2023c).

The investigation into the impact of cellular metabolism on tumor viability and proliferation demonstrated that tumor cells exhibit increased sensitivity to nutritional scarcity and augmented reliance on certain metabolites. Fast or fast-mimicking diets have been employed as an adjunctive approach to enhance the efficacy of cancer therapy (Nencioni et al. 2018). The modulation of a particular food regimen has the potential to influence the metabolic processes of tumor cells, hence impacting the efficacy of tumor treatment (Gao et al. 2019). Furthermore, alongside *in vivo* evidence, experimental findings conducted *in vitro* demonstrate that the manipulation of cell metabolism is a viable approach to enhance the efficacy of tumor therapy (Zhao et al. 2013). Previous *in vitro* investigations have demonstrated that the manipulation of metabolite levels or the selective targeting of specific genes within metabolic pathways can potentially restore the sensitivity of cancer cells to chemotherapeutic drugs (Masamha and LaFontaine 2018; Long et al. 2017; Zhou et al. 2010; Jang et al. 2017). This chapter provides an overview of the metabolic alterations observed in tumor cells as they acquire chemoresistance and explores the potential for mitigating chemoresistance through metabolic regulation.

6.2 Changes in Metabolism in Cancer Cells

Cancer cells have distinct characteristics compared to normal cells, such as heightened proliferative capacity, self-renewal capability, and resistance to apoptosis (Khan et al. 2022a, 2023d). Cells that undergo neoplastic transformation exhibit modifications in their ability to acquire nutrients, reorganize bioenergetic pathways, and undergo metabolic changes (Singh et al. 2017). Dysregulated metabolism is recognized as one of the seven hallmarks of cancer, a concept that originated from Otto Warburg's breakthrough study in 1956 (Hanahan and Weinberg 2011). The researcher made a novel observation on the metabolic behavior of cancer cells during the investigation. Specifically, it was found that tumors employ glycolysis as a means to generate energy even in the presence of oxygen (Warburg 1956). This result presents a contradiction to the typical energy production mechanism in healthy cells, where oxidative phosphorylation (OXPHOS) is favored over glycolysis in the presence of oxygen (Fig. 6.1).

The field of cancer metabolism research has witnessed steady growth, garnering significant attention from medical professionals and researchers over the past decade. This surge in interest emerges from the collective endeavor to comprehend the complicated relationship between aberrant metabolic processes and the development of cancer (Weinberg and Hanahan 2000; Ahmad et al. 2022). For instance, research investigations have revealed that throughout the process of transformation, cancer cells necessitate uninterrupted bioenergetic mechanisms and substantial quantities of ATPs to maintain a consistent supply of energy (Kodama et al. 2020; Liu et al. 2021). Consequently, cancer cells employ a diverse array of metabolic strategies to fulfill their energy demands. These strategies encompass heightened food consumption, activation of several alternate metabolic pathways, and upregulation of numerous metabolic enzymes and proteins (Khan et al. 2023a; Cosset et al.

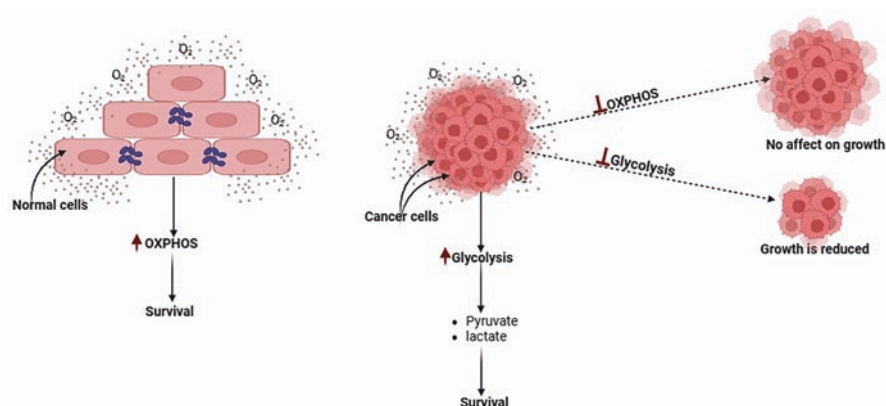


Fig. 6.1 The above figure shows that cancer cells rely on glycolysis even in the presence of oxygen; inhibition of glycolysis decreases cell growth, while no such effect was found when inhibition of OXPHOS takes place

2017; Torres-Guzmán et al. 2017; Dai et al. 2020). In more recent times, there has been a proposition suggesting that the dysregulation of metabolism in cancer is associated with the resistance of the disease to pharmaceutical interventions. According to recent research, it has been found that cancer cells that are resistant to chemotherapy may undergo metabolic reprogramming as a result of exposure to specific pharmacological or molecular inhibitors (Jones et al. 2018; Pei et al. 2020; Pellegrini et al. 2018). As an illustration, subsequent to undergoing conventional chemotherapy, a subset of cancer cells that managed to survive exhibited a shift in their metabolic processes, prioritizing the synthesis of membrane skeletons and nucleotide blocks. These cells then entered a state of dormancy, persisting for a prolonged duration (Pei et al. 2020; Joshi et al. 2021). During the phase of dormancy, cellular preparations are made to facilitate the emergence of resilient clones and may trigger subsequent relapse (Joshi et al. 2021). In the context of cancer, aberrant cells undergo a multifaceted metabolic reprogramming mechanism in order to sustain their heightened energy demands. The observed phenomenon is distinguished by an elevated absorption of essential nutrients, such as glucose and glutamine, a heightened rate of glycolysis leading to augmented extracellular acidity, and enhanced synthesis of metabolic enzymes and proteins (Liu et al. 2021; Wei et al. 2020; Bergers and Fendt 2021). Elevated glucose uptake is observed as a characteristic manifestation of dysregulated metabolism in cancer (Dai et al. 2020; Libby 2020). The investigation of breast cancer cells has revealed the presence of enhanced glucose consumption and elevated production of pyruvate and lactate in an aggressive tumor model (Fujita et al. 2020). Moreover, the suppression of glycolytic function using the glycolytic inhibitor 2-deoxyglucose (2-DG) resulted in a decrease in cell growth. Conversely, inhibiting mitochondrial function with 3-nitropropionic acid, a tricarboxylic acid (TCA) cycle inhibitor, did not have an impact on invasiveness (Fujita et al. 2020). These observations suggest that the invasive

properties of cells are dependent on glycolysis rather than the mitochondrial energy pathway. Increased expression of GLUT facilitates accelerated glucose consumption, which is associated with the oncogenic processes of the transcription factor NF- κ B (Gilmore 2021; Sommermann et al. 2011). GLUT1 and GLUT3 have been associated with several types of cancer, such as breast cancer, colorectal carcinoma, leukemia, and glioblastoma. These associations have been observed within the 14 homologous families of GLUT proteins (Cosset et al. 2017; Dai et al. 2020; Sommermann et al. 2011; Wang et al. 2019). Elevated levels of GLUT1 and GLUT3 expression have been associated with unfavorable clinical outcomes in individuals diagnosed with colorectal cancer and glioblastoma (Cosset et al. 2017; Dai et al. 2020).

The p53 protein is widely recognized as a crucial tumor suppressor, exerting substantial influence on various aspects of normal cellular processes such as growth, development, apoptosis induction, cell cycle regulation, DNA repair, and genome stability maintenance. The occurrence of mutations or the reduction in levels of this protein is linked to the development of a majority of cancer types (Vousden and Ryan 2009; Mintoo et al. 2021). The regulatory actions of p53 are mediated by an intricate network. The cellular processes that are subject to regulation by the p53 protein encompass reactive oxygen species (ROS) management, DNA damage recognition and repair, cell cycle control, autophagy, and, more recently, metabolic regulation. There is a growing body of evidence indicating that p53 has a significant influence on the regulation of glycolysis and oxidative phosphorylation. This suggests that p53 plays a coordinating role in these metabolic pathways and serves as a crucial regulator of the Warburg effect. Metabolic enzymes, such as glucose transporters, glycolytic enzymes, and TCA cycle enzymes, have been identified as downstream targets of the p53 protein. The presence of p53-responsive regions within the promoters of the PGM and hexokinase II genes indicates that p53 has the potential to modulate certain aspects of glycolysis. The p53 protein can inhibit glycolysis, hence attenuating the heightened glycolytic activity commonly observed in cancer cells (Vousden and Ryan 2009). The p53 protein has the ability to suppress the production of glucose transporters, particularly GLUT1 and GLUT4 (Fig. 6.2) (Schwartzberg-Bar-Yoseph et al. 2004), leading to a decrease in glucose absorption and an elevation in tumor protein 53-induced glycolysis and apoptosis regulator (TIGAR) (Ahmad et al. 2017).

The rate of glycolysis is elevated in several types of malignancies as a result of the overexpression of PFK1 (Sha et al. 2021; Inaishi et al. 2022; Umar et al. 2020). PFK1 encompasses three distinct isoforms that exhibit differential expression patterns across various organs and tissues. These isoforms are referred to as PFK1 platelet (PFKP), PFK1 muscle, and PFK1 liver (Lee et al. 2017b). Nevertheless, it is widely observed that PFKP experiences dysregulation in various types of cancer, such as lung, breast, liver, and kidney malignancies (Fujita et al. 2020; Sha et al. 2021; Inaishi et al. 2022). In a model of brain cancer with PTEN deficiency, the protein AKT phosphorylated and stabilized PFKP, thereby facilitating glycolysis and subsequently encouraging the growth of the tumor (Lee et al. 2020). In addition, it has been established that PFKP is associated with the process of

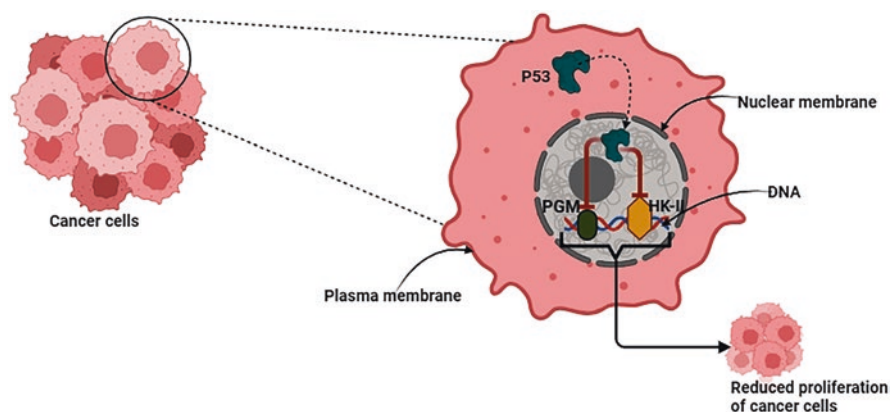


Fig. 6.2 The figure depicts the role of P53 in metabolic regulation. P53 decreases the process of glycolysis by inhibiting the activity of PGM and HK-II, thus decreasing the proliferation of cancer cells

epithelial-mesenchymal transition in cells affected by breast cancer. Moreover, the overexpression of PFKF has been identified as an unfavorable prognostic indicator in individuals who have experienced BRCA1 loss (Isa 2022; Yeerken et al. 2020). PKM2 is an additional glycolytic enzyme that operates at the terminal stage of the glycolytic pathway and exhibits elevated expression levels in numerous cancer types characterized by elevated glycolytic activity (James et al. 2020; Zahra et al. 2020). The enzyme facilitates the conversion of phosphoenolpyruvate into pyruvate and ATP, hence conferring a competitive advantage to tumor cells that exhibit its expression. This phenomenon has been observed in pancreatic cancer cells (James et al. 2020). Interestingly, PKM2 undergoes translocation to the plasma membrane and specifically supplies ATP to the ATP-dependent calcium pump, thereby preventing excessive accumulation of cytoplasmic calcium and subsequent cellular demise (James et al. 2020). The dysregulation of PKM2 has also been reported in cells that experience impaired mitochondrial activity, resulting in the inhibition of glycolytic flux into the TCA cycle (Li et al. 2019). Because of the elevated glycolytic activity, there is a consequential buildup of lactate in the extracellular space, leading to an acidic tumor microenvironment that promotes metastasis, vascularization, and resistance (Estrella et al. 2013). Both in vitro and in vivo studies have shown that an acidic microenvironment provides a growth advantage for aggressive colon and breast cancers (Estrella et al. 2013; de la Cruz-López et al. 2019). The resistance to drugs and reduced effectiveness of drugs are observed in colon cancer cells that have acclimated to an acidic environment (Pellegrini et al. 2018). In the same way, cancer cells necessitate adaptation to sustain a diminished extracellular pH. Consequently, cancer cells exhibit an increased expression of various membrane transporters, including sodium-hydrogen exchanger-1 and monocarboxylate transporters (MCTs). These transporters have been associated with tumors characterized by elevated metabolic rates, such as in cervix squamous cell carcinoma and

B-cell lymphoma (Estrella et al. 2013; Pinheiro et al. 2014; Noble et al. 2017). Cancer cells additionally depend on the pentose phosphate pathway (PPP) and serine synthesis pathway (SSP) as alternate metabolic pathways to glycolysis and OXPHOS. T-cell acute lymphoblastic leukemia (T-ALL) cells exhibit a metabolic adaptation characterized by the limitation of glycolysis and redirection of glycolytic intermediates toward the PPP and SSP (Wang et al. 2017). These metabolic alterations play a crucial role in providing essential building blocks for tumor growth and safeguarding against oxidative stress-induced damage. It is noteworthy that the process of glycolysis experiences a decrease in activity as a result of the phosphorylation of PFKP and PKM2 by cyclin D3-CDK6 kinase, which serves as a crucial element within the cellular cycle apparatus (Wang et al. 2017). The synthesis of nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione (GSH) is enhanced by the inhibition of these glycolytic enzymes (Wang et al. 2017). Moreover, enhanced uptake of glutamine has been shown in various types of malignancies (Kodama et al. 2020). In a study conducted by Yoo et al. (2020), it was observed that the augmented uptake of glutamine, facilitated by glutamine-supported OXPHOS, effectively met the ATP requirements of pancreatic cancer cells exhibiting high rates of proliferation (Yoo et al. 2020). Elevated levels of glutamine have been observed to concurrently increase cellular glutathione (GSH) levels, impeding the formation of ROS and providing cellular protection against damage induced by chemotherapy (Jones et al. 2019; Koo et al. 2023; Khan et al. 2022b). There exists also a correlation between dysregulated glutamine metabolism and augmented expression of oncogenes, such as c-Myc (Kodama et al. 2020).

6.3 Metabolic Modifications Are Essential to Support Cancer Cells' Growth and Proliferation

Numerous pharmaceutical agents designed to combat cancer specifically focus on inhibiting the metabolic process of glycolysis in actively proliferating cells (Jones et al. 2018; Pei et al. 2020). According to recent studies, it has been found that cancer cells that manage to survive treatment are able to evade therapy by redirecting their metabolic processes toward alternate pathways, such as OXPHOS, PPP, and SSP (Jones et al. 2018; Pei et al. 2020; Joshi et al. 2021). The cancer cells exhibit a latent state for a duration of time before potentially undergoing reactivation, hence resulting in the occurrence of a relapse at a later point (Pei et al. 2020; Joshi et al. 2021; Shiratori et al. 2019). In their investigation on acute myeloid leukemia (AML), Jones et al. made a significant finding about the presence of glycolytic-dependent treatment-sensitive and OXPHOS-dependent treatment-resistant leukemia cells (Jones et al. 2019). Interestingly, the cells that depend on OXPHOS did not exhibit a reliance on glucose as their primary source of nutrition. Instead, these cells relied on the metabolic processes of amino acids for their nutritional needs. It has been suggested that cells that are resistant to treatment have the potential to modify their metabolism in a manner that promotes the generation of lipids (Joshi et al. 2021; Zhang et al. 2022). A comprehensive analysis involving many omics approaches

revealed that cells treated with an EZH2 inhibitor exhibited an upregulation in fatty acid synthesis, accompanied by a downregulation in the levels of TCA and amino acids (Zhang et al. 2022). Moreover, it has been observed that the inhibition of glycolysis can potentially lead to a metabolic change in invasive breast cancer cells, causing them to rely more on the PPP and SSP (Wang et al. 2017; Kim et al. 2017). However, the mechanism by which cancer cells alter their metabolic processes to develop resistance remains unknown. Previous studies have established a correlation between dysregulated expressions of oncogenes, specifically RAS and MAPK, in resistant subclones and compensatory energy acquisition (Fig. 6.3) (Joshi et al. 2021; Raho et al. 2020; Wani et al. 2021). A transcriptomic analysis using RNA-seq was conducted on samples obtained from relapsed or resistant cases of AML. The findings revealed an overabundance of intrinsic RAS expression in the resistant subclones (Pei et al. 2020; Joshi et al. 2021). The activation of the RAS pathway has been observed to induce mitochondrial fusion, resulting in an upregulation of OXPHOS activity (Li et al. 2019). In addition, it has been observed that cancer cells exhibit a redirection of glycolytic flux into the PPP as a compensatory mechanism when the glycolysis pathway is obstructed. This redirection serves to enhance the levels of NADPH within the cells (Kim et al. 2017). NADPH is essential not only for the synthesis of macromolecules during the cellular growth phase but also for providing protection to cells against oxidative damage. Glutathione reductase (GSH reductase) enzymatically lowers oxidized GSH to maintain lower levels of GSH in the presence of nicotinamide adenine dinucleotide phosphate (NADPH). This enzymatic process plays a crucial role in safeguarding cancer cells against oxidative damage induced by ROS. It is worth mentioning that there exists a correlation between reduced levels of ROS and the stemness attributes exhibited by cancer cells that possess resistance to treatment (Jones et al. 2018; Kim et al. 2017; Khan et al. 2023e). This association provides insight into the mechanisms employed by resistant cells to safeguard their survival. Moreover, in the context of anticancer therapy,

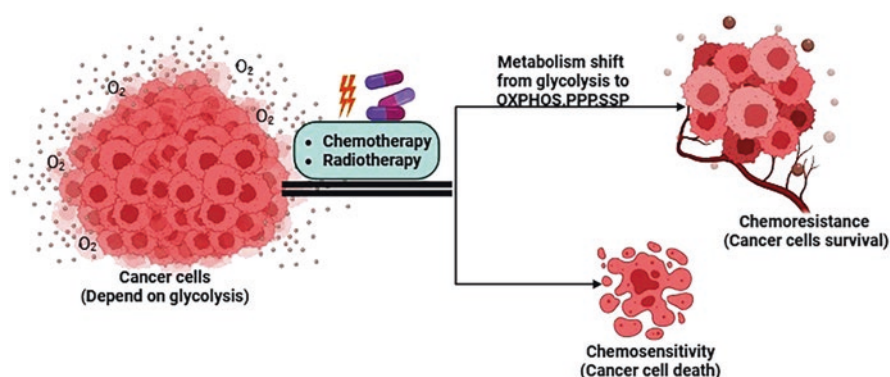


Fig. 6.3 The figure shows that the metabolism of chemotherapy-treated cancer cells shifts from glycolysis to OXPHOS, PPP, and SSP as metabolic adaptations in resistant cells. Sensitive cells are not able to adapt to such metabolism resulting in death

the transition from glycolysis to lipid metabolism is intricately linked to the reprogramming of the cell cycle and the modification of global methylation status (Joshi et al. 2021; Zhang et al. 2022; Lee et al. 2017c). AML cells harboring FLT3 mutations and exhibiting metabolic alterations have been observed to evade the early cell cycle arrest in the G0/G1 phase induced by treatment (Joshi et al. 2021). The surviving cells exhibited enrichment in the metabolism of sphingolipids or phospholipids during the evolutionary process toward resistance. This was subsequently accompanied by an increase in NRAS mutation and a more pronounced activation of AURKB signaling (Joshi et al. 2021). These molecular changes played a crucial role in driving the cells out of a dormant state and promoting their transition to a highly proliferative state. A further investigation integrated transcriptomic and metabolomic data obtained from solid tumor samples subjected to treatment with GSK126, a specific inhibitor of EZH2 methyltransferase (Zhang et al. 2022). The study revealed that treatment-resistant samples exhibited epigenetic modifications, including a decrease in the level of H3K27me3. This decrease in H3K27me3 resulted in increased expression of lipid biosynthesis target genes, including SCD1 and ELOVL2 (Zhang et al. 2022).

6.4 Chemotherapy-Induced Metabolic Reprogramming Promotes Chemoresistance

Adaptation to Oxidative Stress

Redox equilibrium and antioxidant capability are crucial for the survival of a cell and its development. Cisplatin is a platinum-based compound that is frequently employed in the field of oncology for cancer therapy (Wadhwa et al. 2020). The anti-tumor mechanism of platinum is characterized by the induction of cell apoptosis through DNA damage and the promotion of oxidative stress mediated by ROS (Poisson et al. 2015; Marullo et al. 2013; Kim et al. 2019). Ovarian cancer cells that exhibit resistance to cisplatin demonstrate the presence of an active pathway for synthesizing GSH and possess elevated levels of intracellular GSH (Table 6.1). This suggests that augmenting cellular GSH serves as a favorable mechanism for managing the oxidative stress induced by cisplatin (Poisson et al. 2015). One illustrative instance involves the phenomenon wherein lung cancer cells that have developed resistance to cisplatin exhibit an augmented production of ROS as a result of metabolic reprogramming. Consequently, these cells become more reliant on oxidative metabolism as compared to glycolysis (Cruz-Bermúdez et al. 2019; Wangpaichitr et al. 2017a, b). Elevated levels of ROS and various metabolic changes have been observed to facilitate the process of EMT (Wangpaichitr et al. 2017a). These cells are far more sensitive to glutamine deficiency and take up more glutamine. To make GSH, glutamine is changed into glutamate, which lowers the level of ROS in the cells. Selective inhibition of glutamate flux has been demonstrated to induce cell death specifically in cisplatin-resistant cells (Wangpaichitr et al. 2017a). The glutamate and glycine from glutathione (GSH) are primarily derived from glutamine,

Table 6.1 The main metabolic alterations in cancer cells that are resistant to chemotherapy

Cancer type	Approach used	Name of drug	Changes in metabolism	Reference
Breast cancer cells	NMR	Paclitaxel	Glutamate, glutamine, GSH↑, proline, lysine, valine↓	Stewart et al. (2016)
Chronic myeloid leukemia	NMR	Imatinib	Phosphocreatine, creatine↑, lactate↓	Dewar et al. (2010)
ESCC cells	NMR	5-Fluorouracil	Glutamine, lactate↑, glutamate↓	Zhang et al. (2017)
Glioblastoma multiforme cells	NMR	Temozolomide	Alanine, creatine, choline, and phosphorylcholine↓	St-Coeur et al. (2015)
Lung cancer cells	Fluorimeter	Cisplatin	ROS↑	Wangpaichitr et al. (2017a)
Mouse mammary tumor tissues	NMR	Paclitaxel	Choline↑	van Asten et al. (2015)

whereas cysteine does not originate from glutamine (Lopes-Coelho et al. 2016). Cysteine is the thiol constituent of GSH. The thiol groups derived from GSH, as well as cysteine-glycine and cysteine, can sequester platinum molecules, thereby preventing their interaction with cellular components and the formation of protein and DNA adducts (Lopes-Coelho et al. 2016). Cysteine plays a crucial role in the proliferation and survival of cancer cells, as well as in the metabolic reprogramming process of cancer (Kang et al. 2019; Nunes et al. 2018a, b; Combs and DeNicola 2019). The presence of cysteine has a significant influence on the ability of cancer cells to adapt to hypoxic conditions, thereby playing a role in the development of resistance to platinum-based chemotherapy drugs that are effective in hypoxia-induced cancer treatment. Hence, the regulation of cysteine bioavailability could potentially be employed as a strategic approach to counteract resistance to both hypoxia and carboplatin (Nunes et al. 2018a, b). Cysteine serves as a significant supplier of pyruvate for cancer cells, partially substituting glucose. The breakdown of cysteine results in the production of approximately 20% of the pyruvate found within the cells (Yu et al. 2019).

Other anticancer drugs, besides cisplatin, have also been observed to elicit alterations in the cellular levels of GSH. Paclitaxel, also known as Taxol, specifically interacts with cellular tubulin, resulting in disruptions in the assembly of the mitotic spindle, segregation of chromosomes, and the process of cell division. The levels of glutamine, glutamate, and GSH were observed to increase in triple-negative breast cancer cells following treatment with paclitaxel (Stewart et al. 2016). Sorafenib functions as a pharmacological agent that inhibits tyrosine kinases. The synthesis of GSH in leukemia cells that are resistant to sorafenib was found to be increased, suggesting that these resistant cells have undergone unique metabolic as well as redox modifications (You et al. 2019). Erlotinib is a pharmaceutical agent classified as an

inhibitor of the epidermal growth factor receptor (EGFR). It is primarily employed in the treatment of cancers characterized by mutations in the EGFR gene, which drive the progression of the disease. The development of drug resistance in tumors is induced by the secondary mutation of T790M EGFR. Nevertheless, the metabolomic profiles of cells that are responsive to erlotinib and those that are resistant to it indicate a noteworthy decrease in GSH levels in T790M EGFR cells. The presence of the T790M mutation has been observed to impede the activity of NFE2-related factor 2 (NRF2), resulting in the downregulation of the expression of enzymes involved in the synthesis of GSH (Li et al. 2016). GSH supplementation made resistant cells more responsive to erlotinib. On the other hand, lowering GSH levels caused sensitive cells to become erlotinib-resistant.

In bortezomib-resistant cells, glucose was directed toward the PPP and SSP, resulting in an augmentation of the antioxidant activity of these cells (Zaal et al. 2017). Bortezomib functions as a pharmacological agent that inhibits the activity of proteasomes. The inhibition of proteasome activity induces endoplasmic reticulum stress (ERS) and triggers the stimulation of the unfolded protein response (UPR), ultimately resulting in apoptosis via various mechanisms, such as excessive generation of ROS. The production of a reduced form of NADPH by the PPP serves to maintain intracellular levels of GSH and ensure the maintenance of redox balance within the cell. The increased level of activity observed in the SSP can be attributed to the upregulation of PHGDH (3-phosphoglycerate dehydrogenase), which serves as the enzyme responsible for regulating the rate of serine synthesis. The downregulation of PHGDH resulted in a reduction of ROS levels by upregulating the synthesis of GSH, consequently facilitating cellular proliferation and enhancing resistance to bortezomib (Wu et al. 2020).

Lipid Metabolism Adaptation

There is mounting evidence indicating that lipid metabolism undergoes reprogramming in tumor cells as a response to chemotherapy (Cao 2019). Preliminary investigations have shown that cisplatin-resistant ovarian cancer cells possess a greater baseline concentration of intracellular mobile lipids derived from the hydrolyzed acyl chains within triacylglycerides, in comparison to cisplatin-sensitive counterparts (Musacchio et al. 2009; Luciani et al. 2009). Comparative lipidomic profiling was conducted to confirm the occurrence of comparable reprogramming in the metabolism of lipids in cisplatin-resistant bladder cancer (Lee et al. 2018b). The metabolomic study conducted on lung carcinoma cells and their cisplatin-resistant derivative revealed the presence of 40 distinct metabolites. These metabolites primarily encompassed amino acids, phospholipids, fatty acids, and metabolites associated with energy metabolism (Weipeng et al. 2018). The examination of five distinct metabolite groups revealed that there was an elevation in the concentration of short-chain acylcarnitines as well as selected lysophosphatidylcholines, while there was a decrease in the levels of acyl-alkyl-phosphatidylcholines and one sphingolipid in erlotinib-resistant human pancreatic carcinoma cells when compared to

the erlotinib-responsive parental cells. The findings indicated that there were alterations in the metabolism of choline phospholipid and acetyl-CoA following the development of resistance to erlotinib in pancreatic cancers (Lee et al. 2017a). The levels of choline were found to be significantly elevated in the tissues of BRCA1-mutated mouse mammary tumors that were resistant to docetaxel, in comparison to the docetaxel-sensitive control group (van Asten et al. 2015). Temozolomide is a chemotherapeutic agent that functions as a DNA alkylating agent. The administration of temozolomide increased the levels of choline as well as phosphorylcholine in glioblastoma multiforme (GBM) cells that were responsive to temozolomide, as opposed to GBM cells that exhibited resistance to temozolomide (Table 6.1) (St-Coeur et al. 2015). With certain exceptions, the observed augmentation of phospholipid levels seems to be associated with enhanced cellular resistance.

Adaptation to Bioenergetic Conditions

The phenomenon of altered energetics is widely recognized as a distinguishing characteristic of cancer. Bioenergetic adaptation serves as a mechanism by which cancer cells can effectively respond to the challenges posed by drug treatments. The ovarian cancer cells that are sensitive to cisplatin exhibit a state of active glycolysis. Nonetheless, the cisplatin-resistant counterparts can increase the flow of glycolysis when exposed to an ATP synthase inhibitor or acquire more substantial oxidative phosphorylation (OXPHOS) compensation when glycolysis is inhibited. The findings of the study suggest that ovarian cancer cells that are resistant to treatment can transition between OXPHOS and glycolysis metabolic pathways (Dar et al. 2017). The study found that cells with resistant chronic myelogenous leukemia (CML) exhibited an increase in the production of creatine and its conversion to phosphocreatine (Dewar et al. 2010). The elevation in phosphocreatine levels potentially offers a supplementary energy reservoir, enabling cells to evade cell death induced by imatinib (Fig. 6.4) (Puchades-Carrasco and Pineda-Lucena 2017).

Glucose and Glycolysis Dependence

Tumor cells exhibit a preference for utilizing glucose as the primary fuel source for the aerobic glycolysis pathway. Sorafenib functions as a potent inhibitor of tyrosine kinases. The metabolism of leukemia cells that have developed resistance to sorafenib was reprogrammed to enhance the demand for glucose and reduce the flow of glucose into the PPP (You et al. 2019). Imatinib is a pharmacological agent that inhibits the tyrosine kinase activity of the breakpoint cluster region gene-Abelson murine leukemia viral oncogene homolog (BCR-ABL). However, the metabolic alterations observed in chronic myeloid leukemia (CML) cells that develop resistance to imatinib display conflicting results. A preliminary investigation revealed that BCR-ABL-positive cells that were resistant to imatinib exhibited

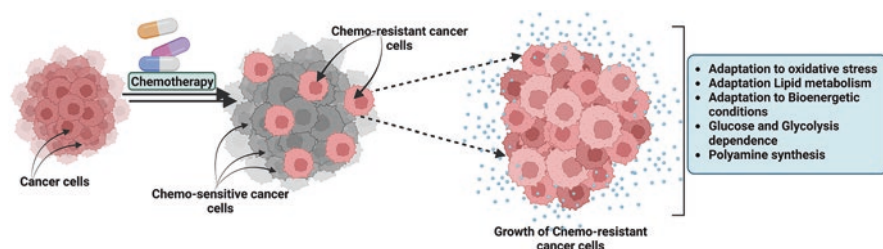


Fig. 6.4 The figure depicts the survival of resistant cancer cells in the presence of chemotherapy by adapting to various processes that help in their survival under drug pressure

augmented glucose uptake, glycolysis, and lactate production when subjected to imatinib treatment, in contrast to imatinib-sensitive cells (Kominsky et al. 2009).

In a study conducted by Dewar et al., it was discovered that cells exhibiting resistance to CML demonstrated a decrease in consumption of glucose as well as lactate production (Dewar et al. 2010). The factors contributing to the lack of consistency in the results remain unclear. Flavopiridol is a pharmacological compound that functions as a broad-spectrum inhibitor of cyclin-dependent kinases, thereby promoting programmed cell death specifically in cancerous cells. Human prostate cancer cells that were resistant to flavopiridol exhibited an increase in glycolysis and a reduced susceptibility to apoptosis triggered by cisplatin and docetaxel (Li et al. 2017). Adriamycin, also known as doxorubicin, functions as an inhibitor of topoisomerase II. In contrast to the responsive cells, the central metabolism of leukemia cells that have developed resistance to Adriamycin exhibits notable alterations. These changes are characterized by an elevated reliance on glucose and a diminished reliance on exogenous glutamine, as well as reduced uptake of pantothenic acid and a decline in the rate of fatty acid β -oxidation (Staubert et al. 2015).

Polyamine Synthesis

Polyamines are widely present in all living cells and play a crucial role in promoting the growth of eukaryotic cells (Nowotarski et al. 2015). The synthesis of polyamines has been shown as a notable metabolic alteration in platinum-resistant ovarian carcinoma cells (Poisson et al. 2015). The metabolomics assessment conducted on the supernatant of tumor-associated macrophages (TAMs) in colorectal cancer revealed that the administration of 5-fluorouracil (5-FU) resulted in the increased secretion of putrescine. This elevated secretion of putrescine was found to be associated with the development of resistance in tumor cells against apoptosis induced by 5-FU. The reduction or suppression of ornithine decarboxylase, an essential enzyme involved in the synthesis of putrescine, resulted in decreased levels of putrescine. This, in turn, prevented the resistance to 5-FU mediated by TAMs and enhanced the growth inhibition of cancerous cells induced by 5-FU (Zhang et al. 2016a).

The metabolic alterations brought about by chemotherapy may exhibit a dynamic response (Klawitter et al. 2009; Sasada et al. 2013). The application of 5-FU in the treatment of gastric cancer cells for a brief period leads to a decrease in cellular proline levels and an increase in glutamate levels. This phenomenon could potentially be linked to the upregulation of proline dehydrogenase (PRODH), an enzyme that facilitates the conversion of proline into glutamate, thereby promoting glutamate production (Sasada et al. 2013). However, the impact on proline and glutamate levels is comparatively lower in cells that have developed resistance to 5-FU. This resistance is characterized by the absence of upregulation of PRODH expression upon 5-FU treatment. The enzyme PRODH initiates the initial stage of proline degradation, leading to the generation of superoxide and the conversion of proline into glutamate. The activity of PRODH is induced as a result of genetic stress caused by the blockage of nucleotide synthesis by 5-FU, leading to the generation of mitochondrial superoxide (Sasada et al. 2013). This phenomenon plays a key role in the drug resistance.

Typically, the metabolic adaptation of cancer cells that exhibit resistance encompasses various aspects, such as redox regulation, bioenergetics, lipid metabolism, glycolysis, and polyamine synthesis (Fig. 6.4). The various mechanisms by which different chemotherapeutic agents combat tumors can result in distinct metabolic changes associated with resistance. Nevertheless, certain medications have the potential to cause comparable alterations in metabolism. The cells that exhibited resistance to Adriamycin and cisplatin demonstrated several common differential metabolites when compared to their drug-sensitive counterparts (Zhang et al. 2016b). Furthermore, the application of various drug combinations to cellular systems can result in distinct metabolic modifications. An illustrative instance involves the administration of a combination of cisplatin along tamoxifen to human breast cancer cells, resulting in a substantial decrease in cellular phosphocholine levels. Conversely, the combination of cisplatin and Adriamycin has been observed to elevate the levels of lactate (Maria et al. 2017). Despite the ongoing challenges in predicting metabolic changes, several studies have endeavored to determine plasma markers in cancer individuals as a means of predicting resistance to drugs (Wu et al. 2016).

6.5 Role of Oncogenes and Tumor Suppressor Genes in Metabolism Reprogramming

The phenomenon of tumor metabolic reprogramming can be attributed to the modification of intracellular signaling cascades, which are influenced by alterations occurring in oncogenes and tumor suppressor genes. Indeed, a majority of cancers possess activating mutations in oncogenes and/or inactivating mutations in tumor suppressor genes. These genetic alterations play a crucial role in determining the metabolic phenotype of tumors and contribute to the development of cancer by conferring transformed cells with proliferative advantages compared to noncancerous cells. Multiple oncogenes, such as hypoxia-inducible factor 1 (HIF1), MYC,

protein kinase B (PBK or Akt), mechanistic target of rapamycin (mTOR), and phosphoinositide-3-kinase (PI3K), have been identified as crucial players in the control of tumor metabolic reprogramming (Qiu and Simon 2015; Cairns et al. 2011; Chen and Russo 2012).

HIF1

The hypoxia-inducible factors, namely, HIF1, HIF2, and HIF3, serve as the primary regulators of transcriptional homeostatic responses in the context of oxygen deprivation. The expression of HIF1 is observed in a wide range of tissues, whereas the expression of HIF2 and HIF3 is limited to specific tissues (Gordan and Simon 2007). The HIF factors consist of a HIF α subunit that is dependent on oxygen levels and a HIF β subunit that is expressed continuously. The activity of HIF is rigorously controlled through a series of production and oxygen-dependent proteasomal breakdown cycles. HIF α subunits are subject to continuous synthesis, and their level of stability is modulated in response to the presence or absence of oxygen (Gordan and Simon 2007). In normal oxygen conditions, the HIF α subunits undergo hydroxylation on proline residues within the oxygen-dependent degradation (ODD) domain. This hydroxylation process is facilitated by prolyl hydroxylase enzymes (PHDs). Following hydroxylation, the HIF α subunits are ubiquitinated by the tumor suppressor protein von Hippel-Lindau (VHL) before being degraded in the proteasome (Fig. 6.5) (Gordan and Simon 2007; Dang et al. 2008). In conditions characterized by low oxygen levels (hypoxia), the enzyme action of PHDs is reduced. This reduction in activity is attributed to the oxidation of ferrous ions inside the active regions of PHDs by ROS released from inadequate respiration in the mitochondria. Consequently, the interaction between PHDs and VHL is prevented (Dang et al. 2008; Guzy and Schumacker 2006). As a result, HIF α subunits that are stable in nature combine with HIF β subunits to form heterodimers, which then migrate to the nucleus. In the nucleus, these heterodimers bind to particular consensus sequences known as hypoxia response elements (HRE) located in the promoter region of genes

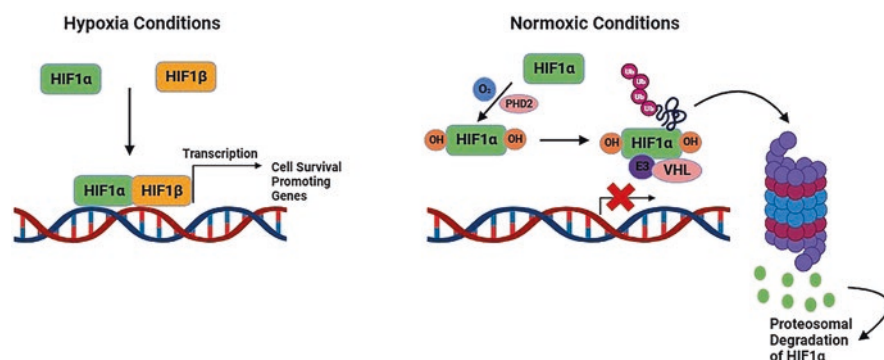


Fig. 6.5 The figure depicts HIF1 α regulation under hypoxia and normoxia conditions

that respond to hypoxia. This binding leads to the stimulation of transcription, facilitating the cellular adaptation to low oxygen levels (Semenza 2009).

Solid tumors often experience hypoxia as a consequence of the rapid proliferation of tumor cells, which leads to the outgrowth of the tumor beyond its existing vascular network. This, in turn, causes a restricted diffusion of oxygen within the tumor. Cells primarily activate response mechanisms via the stabilization and buildup of HIF α to adjust to the hypoxic microenvironment and promote cell survival. This process predominantly facilitates invasion, angiogenesis, and metabolic reprogramming (Majmundar et al. 2010; Semenza 2010). In numerous instances, it has been observed that HIF α levels are elevated in various types of human cancers, and this elevation is associated with unfavorable clinical outcomes (Semenza 2010). It is noteworthy to mention that tumor cells have been observed to display elevated levels of HIF1 α even in the presence of normal oxygen levels, a phenomenon commonly referred to as pseudo-hypoxia (Majmundar et al. 2010). An instance of this can be observed in the stimulation of RAS or SRC oncogenic signaling, which facilitates the accumulation of normoxic HIF1 α by inhibiting prolyl hydroxylation (Chan et al. 2002).

The activation of HIF1 leads to an increase in the availability of oxygen and nutrients to tumors by stimulating angiogenesis and erythropoiesis through the overexpression of VEGF as well as erythropoietin (EPO), respectively (Courtney et al. 2015). Furthermore, as a component of the molecular pathways linked to the Warburg effect, the HIF1 amplifies the process of glycolysis and the formation of lactate by stimulating transporters of glucose as well as the glycolytic enzymes (Iyer et al. 1998). In line with the intensified aerobic glycolysis, HIF1 hinders the mitochondrial-mediated oxidation of pyruvate, which is the ultimate outcome of glycolysis, by suppressing the function of pyruvate dehydrogenase (PDH) via the activation of pyruvate dehydrogenase kinase 1 (PDHK1) (Kim et al. 2006). In addition, it has been observed that HIF1 promotes the improvement of electron transport chain efficiency by inducing the expression of COX4I2, thereby replacing the less efficient isoform 1 (COX4I1). This substitution leads to an increase in ATP synthesis and a decrease in the production of ROS (Yung et al. 2013). Furthermore, HIF1 promotes anabolic activities, including glycogen production, by enhancing the enzymes implicated in its biosynthesis process (Pescador et al. 2010; Pelletier et al. 2012; Khan et al. 2024).

MYC

Along with MYCL and MYCN, the MYC oncogene is a member of the MYC gene family. Nevertheless, MYC is the sole isoform that exhibits ubiquitous expression across a wide array of tissues, whereas MYCN and MYCL are typically expressed solely during the developmental stages (Meyer and Penn 2008). The MYC protein functions as a versatile transcription factor that regulates various cellular processes, including the proliferation of cells, progression of the cell cycle, growth of cells, apoptosis, metabolism, differentiation, and response to stress. This regulation is

achieved through the transcriptional control of specific target genes (Meyer and Penn 2008; Gabay et al. 2014). MYC has been observed to bind to the promoter regions of around 10–15% of the genes that are currently known. This binding activity has been found to regulate the expression of genes that encode both proteins and noncoding RNA molecules belonging to various functional categories (Meyer and Penn 2008; Patel et al. 2004). The dysregulation of MYC expression occurs in numerous human malignancies through mechanisms such as chromosomal translocation or amplification of genes. Furthermore, the dysregulation of MYC protein and MYC mRNA can contribute to tumorigenesis by facilitating uncontrolled cell proliferation, suppressing cell differentiation, promoting metabolic adaptation, inducing angiogenesis, impairing cell adhesion, and causing genomic instability (Gabay et al. 2014; Wahlström and Henriksson 2015; Adhikary and Eilers 2005).

The MYC gene has been observed to stimulate glycolysis by activating certain glycolytic genes, including HK2, GAPDH, ENO1, and PK, as well as glucose transporters such as SLC2A1, SLC2A2, and SLC2A4 (Dang et al. 2006; Osthus et al. 2000). Furthermore, MYC serves to enhance the synthesis and outflow of lactate, hence upregulating the gene expression of LDHA as well as the lactate transporter MCT1 (Adhikary and Eilers 2005; Gordan et al. 2007; Rimpi and Nilsson 2007).

The cells that have undergone transformation demonstrate an elevated reliance on glutaminolysis and an increased need for glutamine, which is regulated by the MYC gene (Wise et al. 2008; Gao et al. 2009). The principal oncoprotein, MYC, has been characterized as the primary factor that initiates a transcriptional program facilitating glutaminolysis and initiating cellular dependence on glutamine as a bioenergetic substrate (Wise et al. 2008). Tumor cells exhibit a dependency on glutamine, which prompts them to reconfigure their intermediate metabolism in order to sustain the integrity of the mitochondrial tricarboxylic acid (TCA) cycle (Wise et al. 2008). Additionally, it has been observed that the elevated levels of MYC play a significant role in the enhancement of mitochondrial biogenesis as well as function. This leads to an increase in the rate of utilization of oxygen and the generation of energy necessary for the rapid proliferation of cells (Wahlström and Henriksson 2015; Li et al. 2005; Zhang et al. 2007; Morrish et al. 2003). A high rate of glutaminolysis leads to the efficient generation of NADPH, which is essential for meeting the demands of cellular proliferation (Wise et al. 2008; DeBerardinis et al. 2007). The survival of cells is dependent on the breakdown of glutamine caused by MYC in situations where there is limited availability of glucose and oxygen (Le et al. 2012). In addition, it has been observed that cells exhibiting elevated levels of MYC are particularly sensitive to the suppression of mitochondrial oxidative metabolism (Morrish et al. 2008). Furthermore, it has been observed that MYC also plays a role in enhancing the absorption of glutamine by upregulating the level of expression of glutamine transporters, including SLC1A5 and SLC7A5 (Gao et al. 2009; Dang 2013). Significantly, the enhancement of glutaminolysis by MYC occurs through the transcriptional repression of miR-23a and miR-23b, which are microRNAs. This repression leads to increased production of the target protein, GLS1 (glutaminase) (Gao et al. 2009). GLS1 serves as the initial enzyme in the glutaminolysis pathway, facilitating the transformation of glutamine into glutamate. This

conversion is essential for the subsequent oxidation of glutamate in the TCA cycle, as well as for the production of proteins or glutathione. MYC has the ability to induce the use of the TCA cycle for the production of intermediates required for macromolecular synthesis, utilizing both glucose and glutamine as carbon sources (Le et al. 2012). Nevertheless, there have been reports indicating that cells with elevated levels of MYC exhibit a strong dependence on mitochondrial oxidative phosphorylation and experience a 2- to 4-fold rise in glutaminolysis, with only a modest 1.2-fold increase in glycolysis (Murphy et al. 2013).

PI3K/Akt Signaling Pathway

The overstimulation of PI3K/Akt signaling is frequently detected in human malignancies due to its crucial role in cellular processes such as growth, proliferation, survival, and metabolic reprogramming (Thorpe et al. 2015). PI3Ks are a group of lipid kinases that play a crucial role in integrating many pro-survival signals, including cytokines, hormones, growth factors, and other environmental stimuli. These signals are subsequently translated into intracellular cues, which in turn trigger downstream signaling pathways that are both dependent and independent of Akt (Thorpe et al. 2015). Akt is a protein kinase that mostly undergoes regulation after PI3K activation and by successive phosphorylation at Ser473 and Thr308 (Liu et al. 2014a; Altomare and Testa 2005). It may be fascinating to molecularly target elements of this route for the treatment of cancer because constitutive stimulation of Akt, a critical node in the PI3K/Akt signaling pathway, is frequently observed in human tumors (Altomare and Testa 2005).

The FOXO transcription factors are known to be the primary targets of Akt, a protein kinase that plays a crucial role in various physiological processes. These transcription factors have been found to regulate important biological functions such as cell cycle progression, cell growth, cell survival, DNA repair, programmed cell death (apoptosis), cellular differentiation, metabolism, resistance to oxidative stress, and tumor suppression pathways (Greer and Brunet 2005; Wątroba et al. 2012; Eijkelenboom and Burgering 2013; Yeo et al. 2013; Shats et al. 2013). Mammalian cells encode four distinct FOXO proteins, namely, FOXO1, FOXO3a, FOXO4, and FOXO6. These proteins are expressed ubiquitously, with FOXO6 being mainly expressed in brain cells (Nho and Hergert 2014; Fu and Tindall 2008). Posttranslational modifications, including phosphorylation, acetylation, and ubiquitination, play a vital role in governing the movement of FOXO proteins to the cell's nucleus (Lam et al. 2013). Once in the nucleus, these modifications enable the activation of transcription through the binding of FOXO proteins to gene regulatory areas (Eijkelenboom et al. 2013). The primary mechanism underlying the regulation of FOXO transcription involves the phosphorylation of FOXO by Akt, resulting in the inhibition of its ability to bind to DNA and the facilitation of its association with the chaperone proteins 14-3-3. The association elicits the nuclear exclusion, cytoplasmic buildup, and destruction of FOXO factors through the ubiquitin-proteasome pathway, hence facilitating cell survival (Brunet et al. 1999; Biggs et al. 1999).

When oxidative stress occurs, FOXO proteins become activated and dissociated from 14-3-3 proteins via the signaling pathway mediated by JNK (Jun N-terminal kinase) (Greer and Brunet 2005; Eijkelenboom and Burgering 2013; Calnan and Brunet 2008; Wang et al. 2014a).

The FOXM1 transcription factor plays a pivotal role in governing cellular proliferation and the progression of the cell cycle, exhibiting elevated expression levels across various cancer types. FOXM1, a member of the forkhead subfamily, regulates various biological processes such as angiogenesis, cell differentiation, DNA damage repair, senescence, and tissue homeostasis. This regulation grants FOXM1 oncogene-like characteristics (Gomes et al. 2013). Recent studies have indicated that FOXO3a has the ability to suppress the expression of FOXM1. Furthermore, these two proteins are known to compete for binding to comparable DNA sequences, and they also share a considerable number of target genes. However, it is important to note that they act as antagonists in their regulatory functions (Lam et al. 2013; Karadedou et al. 2012; Yung et al. 2013; Jiang et al. 2013). FOXO3a and FOXM1 proteins are indirectly affected by various standard cytotoxic chemotherapy agents, such as cisplatin or gefitinib. These drugs exert their effects by activating FOXO3a and indirectly repressing FOXM1 via the inhibition of the PI3K/Akt signaling pathway (Liu et al. 2014b; Kwok et al. 2010; McGovern et al. 2009; Fernandez de Mattos et al. 2008; Cortés et al. 2014; Fang et al. 2011). The potential dysregulation of the PI3K/Akt/FOXO3a axis has been implicated in the development of drug resistance, since it may enhance DNA repair mechanisms. Additionally, this dysregulation may contribute to the proliferation, maintenance, and survival of cancer cells through the overexpression of FOXM1 (Lam et al. 2013; Gomes et al. 2013).

6.6 Targeting Metabolism in Cancer Cells

Antimetabolites are a pharmacological category of drugs that possess a structural resemblance to nucleotide metabolites, yet exhibit distinct variations that effectively impede metabolic processes. The clinical research on the efficacy of aminopterin in the treatment of pediatric leukemia served as a catalyst for the subsequent advancement of antimetabolites (Farber et al. 1948; Luengo et al. 2017). The discovery of aminopterin resulted in the development of methotrexate and pemetrexed, which are two folate analogs that inhibit the process of nucleotide de novo synthesis (Stine et al. 2022). Methotrexate is administered as part of the chemotherapy regimen for the treatment of juvenile acute lymphoblastic leukemia. A phase III clinical investigation has demonstrated its efficacy, revealing a cure rate of over 80% (Sakura et al. 2018). The utilization of 5-fluorouracil (5-FU), a pyrimidine analog, has been established as a therapeutic approach for gastrointestinal cancer (Stine et al. 2022). During the process of DNA synthesis, the compound 5-fluorouracil (5-FU) exerts an inhibitory effect on the biosynthesis of thymidine nucleotides. Purine analogs, such as 6-mercaptopurine and 6-thioguanine, inhibit the biosynthesis of purines (Stine et al. 2022). Gemcitabine and cytarabine, which are nucleoside analogs, are incorporated into replicating DNA internucleoside competitively, hence restricting the

elongation of nucleotide chains. These compounds have been extensively utilized in the therapeutic management of leukemia (Wang et al. 2014b; Lv et al. 2019). In recent times, endeavors have been made to specifically target metabolic enzymes or proteins that exhibit differential expression in cancer. A particular instance, STF-31 was specifically engineered with the intention of decreasing the activity of GLUT1. However, it has been observed that STF-31 also acts as an inhibitor for nicotinamide phosphoribosyltransferase, an enzyme that plays a crucial role in the manufacture of NADH (Adams et al. 2014). Glutor is a GLUT inhibitor that selectively targets GLUT1, GLUT2, and GLUT3 transporters (Reckzeh et al. 2019). Nevertheless, Glutor in isolation is inadequate in eradicating cancer cells. The combined administration of Glutor and a GLS inhibitor at low concentrations exhibited a synergistic effect in suppressing the growth of HCT116 cells, indicating a promising approach for achieving dual inhibition (Reckzeh et al. 2019). Moreover, a potent inhibitor of MCT1 known as AZD3965 has successfully undergone a phase 1 clinical trial involving individuals diagnosed with solid tumors in advanced stages (Plummer et al. 2018). The results indicate that this pharmaceutical compound exhibits potential therapeutic efficacy in people characterized by elevated MCT1 expression, and ongoing investigations are underway to further explore its clinical applications (Plummer et al. 2018). An antagonist of glutamine transport, ASCT260, was developed to restrict the absorption of glutamine by a specific glutamine transporter. V-9302, a compound that mimics glutamine, has demonstrated potential as an inhibitor of cell growth and inducer of death *in vitro* (Schulte et al. 2018). Specifically, this study found that V-9302 exhibited these effects in over 50% of the cell lines examined. Nevertheless, a study conducted *in vivo* showed that V-9302 exhibited unintended effects on additional transporters, hence constraining further exploration of this compound as a potential therapeutic agent (Bröer et al. 2018). However, a significant obstacle in tackling dysregulated metabolism in cancer lies in achieving selectivity. In addition, the utilization of single-drug therapy is frequently associated with considerable toxicity. This is due to the administration of high drug concentrations during chemotherapy induction, which heightens the probability of long-term side effects in pediatric patients and inferior clinical outcomes in adult individuals (Pei et al. 2020). The utilization of a solitary pharmacological intervention may also lead to the persistence of disease, indicating the presence of resistance and the potential for recurrence (Joshi et al. 2021; Lee et al. 2017c). Numerous studies have demonstrated that the integration of conventional chemotherapeutic treatments with novel approaches such as molecular or drug repurposing holds promise in facilitating effective therapeutic interventions to tackle these challenges (Jones et al. 2018; Pei et al. 2020; James et al. 2020).

6.7 Conclusion

The pivotal determinant of chemotherapy failure lies in drug resistance, a phenomenon intricately linked to the metabolic adaptability of cancer cells. Resistant cells exhibit a capacity to withstand oxidative stress and maintain intracellular redox

homeostasis by upregulating glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH) production. Additionally, these cells enhance fatty acid oxidation and phospholipid biosynthesis to secure an ample energy supply and preserve cell membrane integrity. Bioenergetic adaptation offers an alternative energy source for resistant cells to endure drug intervention. Certain resistant cells demonstrate a heightened dependence on glucose and glutamine, utilizing glycolysis and glutaminolysis to fuel energy requirements and intermediates crucial for proliferation. Polyamines play essential roles in this context, with tumor-associated macrophages (TAMs) secreting putrescine to induce drug resistance in tumor cells. The adaptive mechanisms vary based on different chemotherapeutic agents, attributed to genetic mutations or deregulation that amplifies glucose and glutamine uptake, augments metabolic pathways, reinforces antioxidant capacity, and induces mitochondrial reprogramming. This metabolic plasticity provides an avenue to sensitize resistant cells to chemotherapy by regulating cell metabolism. However, two critical issues need addressing. Firstly, much of the data is derived from two-dimensional cell cultures, necessitating the exploration of alternative research models such as organoids and animal models, combined with clinical data. Secondly, the diverse metabolic responses to chemotherapeutics, influenced by factors like glutathione levels and breast cancer subtype, emphasize the need for a nuanced understanding of drug resistance-related metabolic reprogramming to inform the development of novel therapeutic strategies. Integrating omics technologies like transcriptomics and proteomics will contribute to unraveling the intricate mechanisms of metabolic reprogramming in response to chemotherapy, distinguishing drivers from bystanders, and refining our comprehension of this complex interplay.

MCQs

1. **What is metabolic reprogramming in the context of tumor cells?**
 - A. A change in the genetic code of tumor cells
 - B. Alterations in the structure of cell membranes
 - C. Modifications in metabolic processes to support cellular proliferation
 - D. Changes in the cell's reproductive cycle

Answer C: Modifications in metabolic processes to support cellular proliferation

2. **Why is metabolic adaptation considered as a fundamental characteristic of malignancies?**
 - A. It enhances cellular respiration
 - B. It disrupts the synthesis of macromolecules
 - C. It provides ATP and intermediates for accelerated cell division
 - D. It decreases the demand for nutrients

Answer C: It provides ATP and intermediates for accelerated cell division

3. **How are alterations in cellular metabolism linked to tumor progression?**
 - A. They hinder cellular proliferation
 - B. They promote resistance to chemotherapeutic agents

- C. They reduce the reliance on glucose
- D. They decrease the overall energy production

Answer B: They promote resistance to chemotherapeutic agents

4. How can fast or fast-mimicking diets impact cancer therapy?

- A. They decrease the sensitivity of tumor cells
- B. They promote tumor growth
- C. They enhance the efficacy of cancer therapy
- D. They have no impact on cellular metabolism

Answer C: They enhance the efficacy of cancer therapy

5. What is one of the hallmarks of cancer, according to Otto Warburg's study in 1956?

- A. Apoptosis resistance
- B. Dysregulated metabolism
- C. DNA damage repair
- D. Enhanced cellular adhesion

Answer B: Dysregulated metabolism

6. In cancer cells, what metabolic process is employed for energy generation even in the presence of oxygen?

- A. Oxidative phosphorylation
- B. Glycolysis
- C. Citric acid cycle
- D. Fatty acid oxidation

Answer B: Glycolysis

7. What do cancer cells prioritize during metabolic reprogramming after chemotherapy exposure?

- A. Synthesis of nucleotide blocks
- B. Inhibition of ATP production
- C. Reduction of membrane skeletons
- D. Induction of apoptosis

Answer A: Synthesis of nucleotide blocks

8. What is a characteristic manifestation of dysregulated metabolism in cancer?

- A. Reduced glucose absorption
- B. Decreased glycolysis
- C. Elevated glucose uptake
- D. Inhibited synthesis of metabolic enzymes

Answer C: Elevated glucose uptake

9. Which protein is recognized as a crucial tumor suppressor with influence over glycolysis and oxidative phosphorylation?

- A. NF- κ B
- B. PFKP
- C. p53
- D. PKM2

Answer C: p53

10. How does p53 inhibit glycolysis in cancer cells?

- A. By promoting glucose absorption
- B. By suppressing glucose transporters
- C. By activating PKM2
- D. By enhancing glycolytic enzymes

Answer B: By suppressing glucose transporters

11. Which glycolytic enzyme exhibits elevated expression levels in various cancer types and prevents excessive accumulation of cytoplasmic calcium?

- A. PKM2
- B. PFK1
- C. GLUT1
- D. PGM55

Answer A: PKM2

12. How does an acidic tumor microenvironment contribute to cancer progression?

- A. Inhibits glycolysis
- B. Promotes apoptosis
- C. Impairs drug resistance
- D. Facilitates metastasis, vascularization, and resistance

Answer D: Facilitates metastasis, vascularization, and resistance

13. What compensatory mechanism do cancer cells exhibit when the glycolysis pathway is obstructed?

- A. Activation of RAS pathway
- B. Upregulation of TCA cycle
- C. Redirection of glycolytic flux into the PPP
- D. Inhibition of OXPHOS activity

Answer C: Redirection of glycolytic flux into the PPP

14. Why is NADPH essential for cancer cells with resistance to treatment?

- A. To inhibit the synthesis of macromolecules
- B. To decrease GSH reductase activity
- C. To induce early cell cycle arrest
- D. To protect cells against oxidative damage

Answer D: To protect cells against oxidative damage

15. What is a potential consequence of the transition from glycolysis to lipid metabolism in anticancer therapy?

- A. Inhibition of AURKB signaling
- B. Increase in early cell cycle arrest
- C. Modification of global methylation status
- D. Decreased reliance on amino acids

Answer C: Modification of global methylation status

16. What is the primary anti-tumor mechanism of cisplatin in cancer therapy?

- A. Inhibition of proteasome activity
- B. Induction of cell apoptosis through DNA damage
- C. Stimulation of unfolded protein response (UPR)
- D. Augmentation of antioxidant activity

Answer B: Induction of cell apoptosis through DNA damage

17. **How does cysteine contribute to cancer cell survival and resistance to platinum-based chemotherapy drugs?**

- A. By inhibiting glucose metabolism
- B. By sequestering platinum molecules
- C. By promoting early cell cycle arrest
- D. By inducing endoplasmic reticulum stress (ERS)

Answer B: By sequestering platinum molecules

18. **Why is the regulation of cysteine bioavailability considered a strategic approach in countering resistance to hypoxia and carboplatin?**

- A. Cysteine inhibits tubulin assembly
- B. Cysteine is a significant supplier of pyruvate
- C. Cysteine reduces ROS levels
- D. Cysteine impedes the activity of NFE2-related factor 2 (NRF2)

Answer B: Cysteine is a significant supplier of pyruvate

19. **What is the role of T790M mutation in cells resistant to erlotinib?**

- A. Decreases levels of ROS
- B. Upregulates the expression of enzymes involved in GSH synthesis
- C. Enhances tubulin assembly
- D. Inhibits the activity of proteasomes

Answer B: Upregulates the expression of enzymes involved in GSH synthesis

20. **What is a distinguishing characteristic of cancer cells concerning altered energetics?**

- A. Increased sensitivity to drug treatments
- B. Bioenergetic adaptation
- C. Enhanced apoptosis response
- D. Reduced glycolysis activity

Answer B: Bioenergetic adaptation

21. **What is the primary function of Adriamycin in inhibiting cancer cell growth?**

- A. Induction of tyrosine kinase activity
- B. Inhibition of topoisomerase II
- C. Promotion of glycolysis
- D. Reduction in glutamine levels

Answer B: Inhibition of topoisomerase II

22. **How does the enzyme PRODH contribute to drug resistance in cancer cells?**

- A. Inducing apoptosis
- B. Generating superoxide
- C. Reducing proline levels
- D. Blocking nucleotide synthesis

Answer B: Generating superoxide

23. **What is the primary mechanism for controlling the activity of HIF under normal oxygen conditions?**

- A. Ubiquitination by VHL
- B. Hydroxylation by PHDs

C. Oxidation of ferrous ions

D. Mitochondrial respiration

Answer B: Hydroxylation by PHDs

24. How does MYC contribute to mitochondrial biogenesis and function?

A. By inhibiting oxygen utilization

B. By downregulating glutamine transporters

C. By promoting mitochondrial oxidative phosphorylation

D. By suppressing NADPH generation

Answer C: By promoting mitochondrial oxidative phosphorylation

25. How do standard cytotoxic chemotherapy agents, such as cisplatin, affect FOXO3a and FOXM1?

A. Activate both FOXO3a and FOXM1

B. Repress both FOXO3a and FOXM1

C. Activate FOXO3a and repress FOXM1

D. Repress FOXO3a and activate FOXM1

Answer C: Activate FOXO3a and repress FOXM1

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Epigenetic Alterations as an Adaptive Response to Chemotherapy

7

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Abstract

The growing field of cancer research has revealed the complex interaction between epigenetic modifications and the development of drug resistance in response to chemotherapy. A heritable alteration in gene expression that is unrelated to variations in the sequence of nucleotides is known as epigenetic regulation. Histone modification, noncoding RNA regulation, and DNA methylation have all been thoroughly investigated as common epigenetic regulatory mechanisms. The chapter explores the molecular intricacies of DNA methylation and histone modifications, elucidating their influence on the establishment and maintenance of drug resistance in various cancer types. Epigenetic alterations not only serve as pathways for the evolution of drug-resistant phenotypes but also emerge as potential therapeutic targets for overcoming treatment resistance. By understanding the complex regulatory functions of epigenetic alterations, researchers can uncover novel strategies for therapeutic intervention. Emphasizing

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the pivotal roles of DNA methylation and histone modifications in cancer drug resistance opens new avenues for targeted interventions, ultimately paving the way for more effective and personalized cancer therapies.

Keywords

Epigenetics · Drug resistance · DNA methylation · Histone modifications · HDACs · HMTs

7.1 Introduction

In eukaryotic cells, DNA is organized into chromatin, which consists of nucleosomes. These nucleosomes are composed of four core histones (H2A, H2B, H3, and H4) that form an octamer, around which approximately 147 base pairs of DNA are coiled (McGinty and Tan 2013). The N-terminal tails and globular histone protein are prone to various posttranslational modifications (PTMs) that comprise the nucleosome. The concept of “epigenetics” states heritable gene expression changes without any modification to the order of nucleotides in DNA (McGinty and Tan 2013; Sadakierska-Chudy and Filip 2015). The intricate molecular mechanisms underlying developmental biology and cellular differentiation have conventionally been attributed primarily to genetic factors. The relationship between epigenetic alterations and the emergence and progression of cancer has drawn increased attention as the branch of epigenetics has grown (Khan et al. 2023a). Epigenetic modifications, such as methylation of DNA and histone modification, affect chromatin structure and hence gene expression (Chen and Yan 2021). Epigenetic regulatory enzymes are altered in numerous types of cancer, and mutations in these enzymes are closely associated with the malignant phenotype (Ilango et al. 2020; Khan et al. 2023b; Singh et al. 2017; Wani et al. 2021). Targeting the epigenetic regulators offers a viable method for reversing drug resistance [6]. Inhibitors targeting such mutated enzymes are now entering preclinical and clinical trials. The processes of DNA methylation, histone modification, and regulation by noncoding RNA are essential mechanisms in gene expression regulation that have been studied vastly (Hussain et al. 2022; Hogg et al. 2020). Acetylation and methylation constitute the extensively researched epigenetic modifications that have been recognized to considerably impact gene expression, causing activation of oncogene and/or repression of tumor suppressor genes, resulting in the emergence of cancer therapy resistance (Chen et al. 2015; Rajan et al. 2020). Apart from acetylation and methylation, the current focus in cancer research includes regulations due to microRNA (miRNAs), sumoylation, ubiquitylation, and phosphorylation (Biswas and Rao 2017; Mortazavi et al. 2022).

Since cancer is a dreadful disease, resistance to therapies is a key factor in its lethality (Mir et al. 2023; Khan et al. 2022a). Epigenetic mechanism plays a crucial role in tumor resistance to various medications. Numerous new medications

targeting epigenetic processes are being developed, demonstrating the huge promise of using epigenetic modulators to combat cancer therapy resistance. In the future, an emphasis on epigenetics will be crucial for gaining an improved comprehension of the phenomena of cancer treatment resistance and designing innovative therapeutics.

7.2 Drug Resistance as a Result of Epigenetic Alteration

Epigenetic alterations are attributed to a major contribution to drug resistance in cancer therapy. This phenomenon is influenced by two different forms of epigenetic changes, including DNA methylation and histone modifications (Ilango et al. 2020) (Fig. 7.1).

Role of DNA Methylation in Drug Resistance

DNA methylation is adding a methyl group to cytosine residues, mostly found in CpG islands upstream of gene promoters. Nonetheless, methylation may take place at different locations across the genome (Jones 2012).

In cancer cells, when the multidrug resistance gene (MDR1) undergoes demethylation, it causes the cells to acquire a trait that makes them resistant to several drugs (Emran et al. 2022). Additionally, the cells experience a decrease in

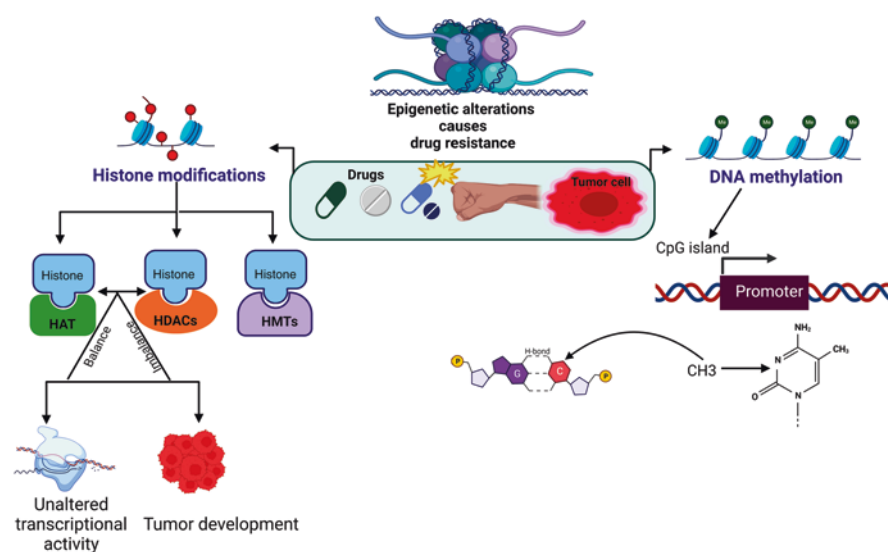


Fig. 7.1 Graphical representation of drug resistance involving epigenetic alterations

the accumulation of anti-tumor drugs. Epigenetic processes can also impact the DNA repair system, specifically the mismatch repair system. For instance, alterations like mutations or excessive methylation in genes such as hMLH1 may have a role in the growth of colorectal cancer (Jubb et al. 2001). DNA methylation inhibition using drugs like 5-aza-2'-deoxycytidine (decitabine or DAC) can sensitize tumors to other drugs such as carboplatin and cisplatin (Linnekamp et al. 2017). Likewise, the demethylation of the hMLH1 promoter gene induced by DAC (decitabine) can repair the mismatch repair system and improve the responsiveness of colorectal cancer cells to 5-FU (5-fluorouracil) (Morita et al. 2007). Therefore, combining epigenetic drugs with conventional chemotherapeutic agents has shown effectiveness in treating drug-resistant tumors and cancer cells. Here are a few key mechanisms by which DNA methylation contributes to drug resistance:

1. **Suppression of drug targets:** In some cases of chemotherapy resistance, tumor suppressor genes, like p16INK4a or TP53 methylation, lead to their reduced expression which in turn reduces the response to treatment (Esteller 2002).
2. **Altered drug metabolism:** DNA methylation can influence gene expression associated with drug metabolism, including drug-metabolizing catalysts and drug transporters. Methylation-induced downregulation of these genes can lead to decreased activation of drugs or modifications in the dispersion of drugs within cells. As a consequence, this can result in reduced drug efficacy and ultimately the emergence of drug resistance (Shen et al. 2012).
3. **Epigenetic regulation of DNA repair:** DNA repair mechanisms are of utmost importance in safeguarding the integrity of the genome and repairing DNA damage caused by drugs. DNA methylation can have an impact on the gene's expression associated with DNA repair processes, such as mismatch repair genes like hMLH1 and MSH2 (Pasanen et al. 2020). When these genes undergo hypermethylation, their expression can be impaired, resulting in a diminished DNA repair capacity (Jin and Robertson 2012).
4. **Epigenetic Regulation of signaling pathways:** DNA methylation can modulate the activity of signaling pathways that are engaged in drug response and survival of cells. Methylation-mediated alterations in key regulatory genes can disrupt these signaling pathways, leading to resistance to targeted therapies. For instance, in certain cases of the development of drug resistance against inhibitors targeting the epidermal growth factor receptor (EGFR) in cancer treatment, epigenetic modification within the EGFR promoter region may result in reduced receptor expression and weaken the drug's effectiveness (Boeckx et al. 2013). Understanding the significance of DNA methylation in the development of drug resistance is essential for developing effective therapeutic strategies. Epigenetic modifications, including DNA methylation, can be reversible, making them potential targets for epigenetic therapies aimed at restoring drug sensitivity in resistant cells.

Drug Resistance and Histone Modifications

Histone Acetyltransferases (HATs) in Drug Resistance

Histone modifications, specifically histone acetylation, have been implicated in drug resistance in cancer (Fantin and Richon 2007). HATs and HDACs regulate histone acetylation levels. Keeping HATs and HDACs in balance is crucial for the proper transcriptional activity of genes and the structure of chromatin (Peserico and Simone 2011). Dysregulation of this balance can contribute to tumor development. The acetyl group is transferred from acetyl coenzyme A to lysine residues on histone tails by HATs (Fan et al. 2015) (Fig. 7.2). This process results in chromatin's unfolding by removing the lysine's positive charge. Several families of HATs, like the MYST family, the p300/CREB-binding protein family, and the GNAT family, are involved in this process (Panigrahi 2012). For instance, increased levels of histone acetyltransferase 1 (HAT1) have been associated with increased resistance in castration-resistant pancreatic cancer and prostate cancer towards drugs such as enzalutamide and gemcitabine (Capone et al. 2023). HAT1 facilitates the transcription of PVT1 and enhances EZH2 protein stability, ultimately leading to an escalation in resistance to the drug (Wang et al. 2023).

Suppression of lysine acetyltransferase 6A (KAT6A), which belongs to the MYST family, may trigger apoptosis and increase ovarian cancer cell's responsiveness to cisplatin treatment (Wiesel-Motiuk and Assaraf 2020). Another histone acetyltransferase, KAT2A, has been associated with tamoxifen resistance in breast cancer by decreasing p53 levels (Wiesel-Motiuk and Assaraf 2020). In prostate cancer cells, reducing KAT2A through knockdown techniques enhances their sensitivity to abiraterone (Lu et al. 2021). P300, a HAT, has a double role in drug resistance.

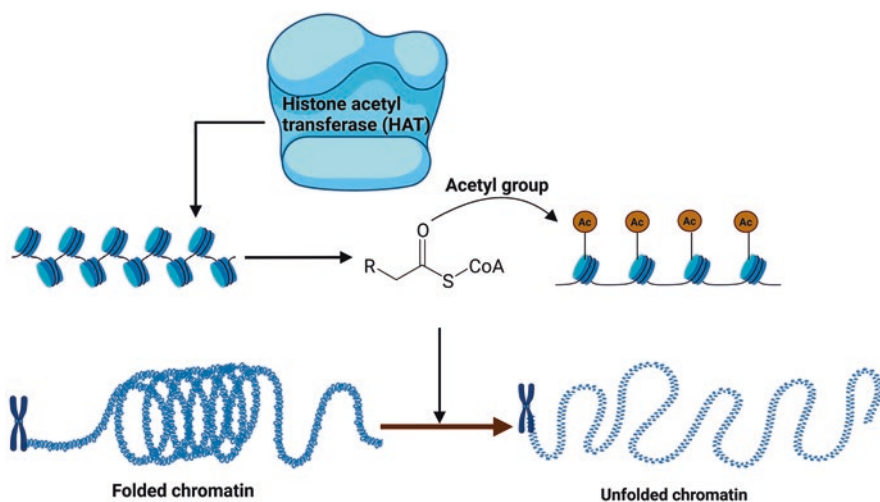


Fig. 7.2 Mechanism of histone acetyltransferase (HATs) involved in the formation of unfolded chromatin

When P300 is lost in pancreatic cancer, PORCN inhibition may not be effective, while suppression of P300 results in doxorubicin resistance of bladder cancer cells (Zhong et al. 2022). However, upregulation of P300 promotes resistance to apatinib in gastric cancer cells and resistance of melanoma cells to BRAF inhibitors (Wang et al. 2023).

The use of small-molecule inhibitors to target histone acetyltransferases (HATs) is a potential treatment strategy for overcoming resistance to drugs in cancer. Numerous HAT inhibitors have been created, primarily focusing on CBP/P300 (Chen et al. 2015). The efficacy of these inhibitors in treating hematologic malignancies, prostate cancer, non-small cell lung cancer, and breast cancer is now being investigated in clinical studies. The safety and maximum tolerated dosage of EP31670, a dual inhibitor of BET and CBP/P300, in advanced solid tumors are also being assessed in phase I clinical investigation (Wang et al. 2023). To combat medication resistance and enhance cancer treatment, targeting HATs with inhibitors shows promising results.

Histone Deacetylases (HDACs) and Drug Resistance

HDACs which are crucial for many cellular processes and the emergence of diseases have a profound impact on the control of gene expression. HDACs remove acetyl groups from histone and non-histone lysine sites, unlike histone acetyltransferases (HATs), which cause chromatin condensation and decrease gene transcription activity (Bassett and Barnett 2014). HDACs are desirable targets for preventing drug resistance due to their abnormal expression being connected to the development of drug resistance in tumors. Presently, the FDA has approved several HDAC inhibitors for clinical use in cancer treatment including romidepsin, belinostat, panobinostat, and vorinostat (Peserico and Simone 2011; Shah 2019) (Fig. 7.3).

These inhibitors are being tested in conjunction with other targeted medicines such as mTOR inhibitors, PI3K inhibitors, EGFR inhibitors, and immune checkpoint inhibitors in both preclinical and clinical investigations since they have demonstrated potential in clinical trials (Yu et al. 2022).

Histone Methyltransferases

Histone methyltransferases (HMTs) are enzymes responsible for adding methyl groups to histone proteins. Histone lysine methyltransferases and histone arginine methyltransferases are the two classes of HMTs. HMTs catalyze the transfer of methyl groups to lysine or arginine residues on histones using S-adenosyl-L-methionine as the methyl group donor (Abdelraheem et al. 2022) (Fig. 7.4).

In mammals, the histone arginine methyltransferases family has nine members (PRMT1-9), while the histone lysine methyltransferases family consists of enzymes like EZH2, G9a, DOT1L, and SETD2 (Boehm and Ott 2017). Depending on how many methyl groups are added, as well as their location, different forms of methylation can occur. For lysine residues, mono-, di-, and trimethylation (Kme1, Kme2, Kme3) can take place, while arginine residues can undergo mono- and dimethylation (Rme1/MMA, Rme2). The PRMT family exhibits two types of dimethylation:

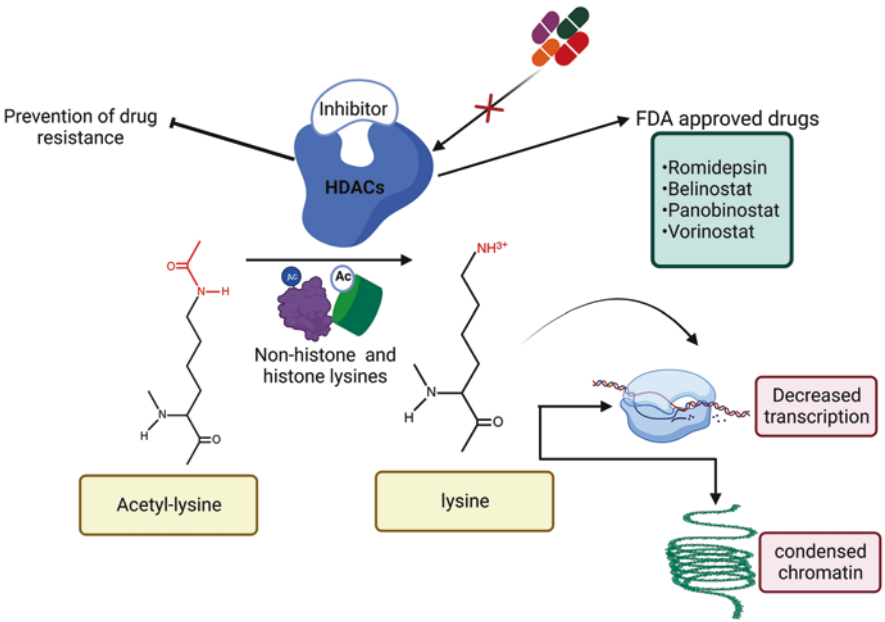


Fig. 7.3 Mechanistic revelation of HDACs’ involvement in cancer drug resistance and as an important target of drug discovery with important FDA-approved drugs

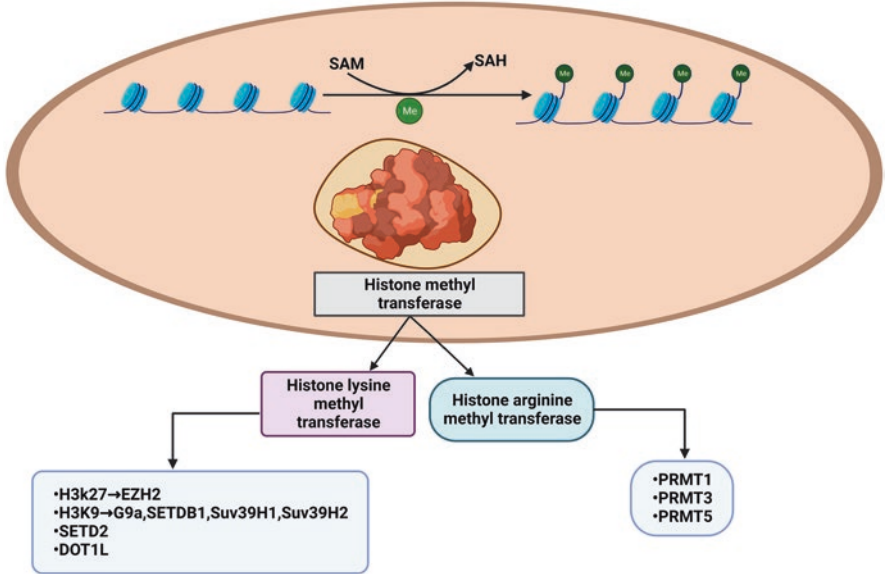


Fig. 7.4 Mechanism of histone methyl transferase and different HMTs involved in drug resistance

asymmetric dimethylarginine (Rme2a or ADMA) catalyzed by PRMT1-4,6,8 and symmetric dimethylarginine (Rme2s or SDMA) catalyzed by PRMT5,9 (Maron 2021). Notably, HMTs methylate non-histone proteins in addition to histone proteins, thereby influencing various biological functions. For instance, SMYD2 catalyzes the methylation of p53 at lysine 370, leading to the inhibition of its activities (Eggert 2017). PRMT1 methylates Smad6 at specific arginine residues. Methylation can induce conformational changes in proteins, altering downstream gene expression and regulating diverse biological processes. HMTs target the lysine and arginine residues on histones and have regulatory effects by using SAM as the methyl group donor (Katsuno et al. 2018). By catalyzing lysine residues at certain locations, KMTs have a role in downstream gene expression regulation and tumor growth. For instance, via regulating the expression of homeobox-related genes, the MLL gene, which encodes a histone methyltransferase, is essential for stem cell self-renewal and tissue development (Yang et al. 2020). PRMTs are crucial for alternative splicing, RNA processing, post-transcriptional regulation, differentiation, cell proliferation, apoptosis, and the formation of tumors. For instance, PRMT1-catalyzed ADMA stimulates genes linked to cell proliferation in breast cancer (Li et al. 2020), whereas PRMT5-catalyzed SDMA suppresses genes linked to cell cycle progression (Li et al. 2020). The transcription elongation factor SPT5 can be methylated by PRMT1 and PRMT5, which alters how it interacts with RNA polymerase II (Kwak et al. 2003). Moreover, HMTs have the capacity to methylate arginine or lysine residues on non-histone proteins, thereby impacting cellular functions and the development of cancer. A prime example is the methylation of p53 at lysine 370 by SMYD2, which influences transcription. In conclusion, HDACs and HMTs are important regulators of gene expression and have been linked to several cellular functions and disease processes, including cancer (Coimbra 2015; Khan et al. 2023c). Targeting these enzymes has become a promising therapeutic approach, such as with HDAC inhibitors. Understanding the functions and mechanisms of HDACs and HMTs provides insights into their potential as targets for cancer treatment and overcoming drug resistance.

Cancer Drug Resistance Due to KMTs

Lysine methyltransferases (KMTs) play a critical role in cancer drug resistance, specifically H3K27 and H3K9 methyltransferases (Tian et al. 2013) (Fig. 7.4).

Drug resistance due to H3K27 KMTs: One of the prominent KMTs involved in the methylation of H3K27 is EZH2, which catalyzes the formation of H3K27me3 (Pan et al. 2018). In general, methylation facilitated by EZH2 is associated with gene repression, which can block pro-apoptotic pathways or enhance anti-apoptotic pathways in tumor cells, ultimately promoting resistance to drugs (Ozyerli-Goknar and Bagci-Onder 2021). However, it is still unclear what precise molecular processes underlie EZH2 overexpression and how they contribute to medication resistance. In multiple myeloma, ovarian cancer, and other cancers, several studies have discovered noticeably greater levels of EZH2 expression in drug-resistant cells as compared to susceptible cells. It has been found that EZH2 expression is

upregulated in drug-resistant ovarian cancer and has been linked to the presence of c-Myc, a transcription factor that controls both cell division and apoptosis. In drug-resistant cells, c-Myc suppresses the activity of miR-137 by recruiting EZH2 (Ozyerli-Goknar and Bagci-Onder 2021), resulting in increased EZH2 expression and subsequent drug resistance. Another study in breast cancer demonstrated that EZH2 confers tamoxifen resistance by silencing the expression of GREB1, an estrogen receptor alpha (ER α) co-factor, through direct DNA methylation (Sukocheva et al. 2022).

Drug resistance due to H3K9 KMTs: KMTs that modify H3K9, including G9a, SETDB1, Suv39H1, and Suv39H2, have been linked to drug resistance as well. G9a, the most prevalent lysine methyltransferase (KMT), is responsible for catalyzing histone H3 methylation at lysine 9, leading to the formation of H3K9me1 and H3K9me2 (Li et al. 2019). Moreover, G9a has been connected to cisplatin resistance in head and neck squamous cell carcinoma (HNSCC) (Liu et al. 2017). The high expression of G9a was found to be correlated with reduced sensitivity to cisplatin treatment in HNSCC patients (Liu et al. 2017). Mechanistically, G9a facilitates the mono-methylation of the promoter region of the glutamate-cysteine ligase catalytic (GCLC) subunit, leading to increased glutathione synthesis and subsequent resistance to platinum drugs (Yang et al. 2020); EZH2 and GLP (G9a-like protein) were found to work together with G9a in the process of methylating the histone 3 lysine residues in HNSCC. However, there was conflicting evidence on the relationship between GLP, EZH2, and cisplatin sensitivity in HNSCC (Poulard et al. 2021). G9a promotes cisplatin resistance by catalyzing mono-methylation through the GCLC promoter, rather than through cisplatin-related transporters, as suggested by subsequent mechanistic studies (Yang et al. 2020). The methylation prompted GCLC transcriptional activation, resulting in glutathione upregulation in cells. Glutathione and cisplatin were shown to increase cancer cell resistance to platinum-based drugs and lessen the damage of DNA that cisplatin causes. Chang et al. utilized RNAseq, gene manipulation, and other techniques to demonstrate that G9a suppresses the expression of miR-145-5p by activating H3K9me2, resulting in increased expression of HER3 in tumor cells (Chang et al. 2019). It has also been revealed that G9a boosts H3K9me2 and decreases H3K9ac in the PTEN promoter region, which suppresses PTEN transcription (Bhat et al. 2019).

Fas, a death receptor superfamily member, is responsible for inducing apoptosis in cells and is commonly found on tumor cell surfaces (Khan et al. 2022b). FasL, expressed on activated cytotoxic T cells, plays a vital role in cancer immunosurveillance (Villalobos et al. 2018). In metastatic colon tumor cells, it was discovered a decrease in Fas expression and a high presence of H3K9me3 in the promoter region (Paschall et al. 2015). Consequently, these cells exhibit resistance to drozitumab, a death receptor 5 agonist, and can evade apoptosis (Kang et al. 2011). However, the anti-apoptotic response can be reversed by utilizing the Suv39H1 inhibitor verticillin A (Li et al. 2019). These examples demonstrate how KMTs, including G9a and Suv39H1, regulate drug resistance by methylating key genes on the H3K9 chromatin regulatory subunit (Yang et al. 2020).

Other KMTs and Drug Resistance

Apart from KMTs that modify H3K9 and H3K27, several enzymes that modify other sites have been implicated in drug resistance. The significance of certain KMTs in drug resistance and their mechanisms in diverse malignancies are highlighted in this section.

SETD2: SETD2 is the sole enzyme among KMTs responsible for synthesizing H3K36me3 (Sankaran 2016). SETD2 is an interesting gene that suppresses tumor growth. Drug resistance is frequently caused by loss-of-function SETD2 mutations in nonsolid tumors. For instance, in the acute leukemia relapse, it was found that leukemia cells with inactive mutant SETD2 exhibit downregulation of S- and G2/M-checkpoint regulation signals, affecting replication of DNA and mitosis. The loss of these checkpoints accelerates leukemia development and contributes to resistance against cytarabine-based chemotherapy (Yang et al. 2020). In renal cell carcinoma, SETD2 deletion has been linked to sunitinib resistance by reducing the signaling of the pro-survival protein MCL-1 downstream of pathways of GSK3 β and ERK (Frismantas 2016). Furthermore, SETD2 deletion has been linked to cisplatin resistance in non-small cell lung cancer by inhibiting ERK activation, enhancing Bcl-xL expression, and fostering cisplatin resistance (Kim et al. 2019).

DOT1L: DOT1L, a mammalian KMT, plays a role in H3K79 methylation (mono-, di-, and trimethylation). Its involvement in drug resistance has been noticed in cisplatin-resistant ovarian cancer, where DOT1L regulates downstream genes (Yang et al. 2020). According to the proposed mechanism, CCAAT/enhancer-binding protein (C/EBP) improves the ability of DOT1L to bind to target genes, enhancing resistance to cisplatin (Wang et al. 2021). These downstream genes are involved in cell survival, DNA damage repair, and drug delivery. Additionally, human HNSCC has been associated with DOT1L and treatment resistance. By altering the miR-10b promoter via H3K79me1, the increase of DOT1L brought on by matrix hyaluronan causes overexpression of RhoGTPases and survival proteins which in turn develops cisplatin resistance in HNSCC (Boroughs and DeBerardinis 2015).

7.3 PRMT-Related Drug Resistance in Cancer

While the role of protein arginine methyltransferases (PRMTs) in cancer drug resistance is not extensively studied, PRMT1 and PRMT5 have been investigated to some extent. PRMT1 and PRMT5 have been implicated in various cellular processes and cancer-related signaling pathways (Kim and Ze'ev 2020). However, further research is needed to fully elucidate their involvement in cancer drug resistance.

Drug Resistance Involving PRMT1

PRMT1, which accounts for more than 75% of PRMT catalytic activity, has been implicated in cetuximab resistance in colorectal cancer and HNSCC (Ning et al. 2023). PRMT1 catalyzes methylation of arginine residues (R198 and R200) in the extracellular domains of EGFR in colorectal cancer improving the receptor's ability to bind to EGFR and encouraging receptor dimerization (Liao 2015). This methylation-induced EGFR signaling activation leads to cell proliferation and resistance to cetuximab, an EGFR-targeted drug (Liao 2015). In HNSCC, Snail-induced epithelial-mesenchymal transition triggers cetuximab resistance by upregulating lymphotoxin- β expression, which activates PRMT1. Subsequently, PRMT1 methylates EGFR, enhancing its ligand binding capacity and dimer formation, thereby promoting drug resistance (Hsu et al. 2017). Additionally, it has been discovered that cisplatin-resistant ovarian cancer cells have enhanced PRMT1 modification. Through the NF- κ B pathway, PRMT1 activates genes linked to the senescence-associated secretory phenotype (SASP), giving resistance to cisplatin (Musiani et al. 2020).

Drug Resistance Involving PRMT5

PRMT5, a member of the PRMT family, is involved in catalyzing symmetric dimethylation and suppresses gene transcription. When it comes to breast cancer, PRMT5 induces the stemness of cancer cells by promoting FOXP1 expression (Musiani et al. 2020). PRMT5 recruits H3R2me₂s, SET1, and H3K4me₃ to the FOXP1 promoter, facilitating stem cell-related gene activation (Chiang et al. 2017). Additionally, in glioblastoma (GBM), PRMT5 causes symmetric dimethylation of heterogeneous nuclear ribonucleoprotein 1, which enhances the binding of hnRNP A1 to cyclin D1 and c-Myc IRESs and results in resistance to mTOR inhibitors (Holmes et al. 2019). Hepatocyte nuclear factor 4 (HNF4) gene transcription is suppressed in liver cancer cells by PRMT5 and H4R3me₂s binding to the gene's promoter region. EMT and stemness in liver cells are inhibited by the tumor suppressor HNF4 (Zheng et al. 2019). The development of cancer stem cells and treatment resistance in liver cancer are both aided by PRMT5's suppression of HNF4. The involvement of PRMT5 in drug resistance might change based on the kind of tumor, emphasizing its intricate roles in various situations (Zheng et al. 2019).

7.4 Regulating HMTs in Tumors to Combat Drug Resistance

HMTs and cancer drug resistance relationship has gained significant attention. The concept of drug resistance reversal by modulating HMT activity has emerged as a potential strategy. This section focuses on recent advancements in using HMT inhibitors to reverse drug resistance, particularly targeting KMTs (lysine

methyltransferases) and their effects on H3K27 and H3K9 methylation (Yang et al. 2020). HMT inhibitors, such as EZH2 inhibitors and G9a inhibitors, are effective in overcoming medication resistance in several cancer types (Yang et al. 2020).

Drug Resistance Reversal by KMT Inhibitors

- **Small molecular drugs:** A typical treatment for metastatic renal cell carcinoma is the receptor tyrosine kinase inhibitor, sunitinib, although, with time, it frequently results in medication resistance. Studies have indicated that increasing the drug dose partially reverses resistance, suggesting epigenetic regulation of sunitinib resistance. EZH2, a well-studied KMT, has been found to trigger kinases' phosphorylation, causing drug resistance (Cardoso et al. 2021). EZH2 inhibition using the EPZ011989 inhibitor activates tumor suppressors, reduces phosphorylation, and reverses drug resistance (Duan et al. 2020).
- **Classic chemotherapeutic drugs:** Cisplatin and 5-FU, the classic chemotherapeutic drugs, exhibit severe drug resistance. Ovarian cancer, for instance, commonly develops resistance to cisplatin due to increased cellular drug efflux that results in reduced intracellular platinum levels (Mihanfar et al. 2019). EZH2 overexpression contributes to the copper transporter 1 degradation, which is associated with platinum resistance (Sun et al. 2018). Inhibition of EZH2 using the GSK126 inhibitor significantly increases copper transporter 1 level, potentially reversing drug resistance (Samaržija et al. 2022). Another study in small cell lung cancer (SCLC) recommended that EZH2 regulation of CDKN1C (P57Kip2) through H3K27me3 catalysis leads to drug resistance (Deb et al. 2014). Inhibiting EZH2 with GSK126 increases CDKN1C expression, enhancing drug sensitivity.
- **Immunotherapeutic drugs:** Immunotherapeutic drugs have emerged as a promising treatment modality, but drug resistance often develops over time (Vanneman and Dranoff 2012). In studies involving mice treated with anti-CTLA-4 or IL-2 immunotherapy, it was observed that increased production of TNF- α (tumor necrosis factor- α) and the accumulation of T cells resulted in heightened expression of EZH2 in melanoma cells (Zingg et al. 2017). This process hindered immunogenicity and antigen presentation, leading to drug resistance. Even so, by lowering H3K27me3 levels and PD-L1 expression and successfully reversing drug resistance in melanoma cells, the administration of the EZH2 inhibitor GSK503 showed promising results (Hou et al. 2022).

Reversal of Drug Resistance by Targeting H3K9 KMTs

Targeting KMTs involved in H3K9 methylation has been explored as a significant approach to overcoming drug resistance. G9a was discovered to be an activator of the AKT signaling pathway in research on EGFR-TKI resistance in non-small cell lung cancer (NSCLC) via modifying H3K9me2 levels in the PTEN promoter region (Wang et al. 2018; Wadhwa et al. 2020). This process led to reduced acetylation and

subsequent drug resistance. Inhibiting G9a using UNC0638 demonstrated significant growth inhibition of drug-resistant cancer cells and induced apoptosis (Agarwal and Jackson 2016). Similarly, in cisplatin-resistant HNSCC, G9a inhibition with UNC0638 led to reduced GCLC expression, resulting in alterations in intracellular glutathione levels and drug resistance being overcome (Liao et al. 2023). The application of specific inhibitors of G9a, such as BIX-01294, has shown promising results in re-sensitizing glioblastoma multiforme (GBM) cells to temozolomide (Ciechomska et al. 2016). Moreover, hydralazine-mediated inhibition of G9a and H3K9me2 has been demonstrated to reverse gemcitabine resistance in cervical cancer (Candelaria et al. 2012).

Drug Resistance Reversal by Targeting Other KMTs

- **SETD2 and H3K36 methylation:** SETD2 is a KMT that catalyzes H3K36 methylation and has been associated with anticancer effects (Rogawski et al. 2016). Interestingly, unlike other KMTs that promote cancer development and drug resistance, inducing methylation through SETD2 has shown potential in inhibiting cancer growth and reversing drug resistance. Leukemias frequently exhibit deletions and loss-of-function mutations of SETD2 (Yang et al. 2022). Tumor cells with SETD2 alterations exhibit reduced DNA damage response and decreased sensitivity to cytotoxic chemotherapeutic drugs. The loss of H3K36me3 in proteins involved in homologous recombination and mismatch repair, such as LEDGF and MSH6, is thought to be the cause of this condition (Li 2021). It can be difficult to restore SETD2 function after mutation or loss; however, JIB-04's inhibition of KDM4A (an H3K9 and H3K36 demethylase) has shown promise in reversing drug resistance by elevating H3K36me3 levels (Sheng et al. 2019).
- **DOT1L and H3K79 methylation:** DOT1L is a unique enzyme that catalyzes H3K79 methylation and has been implicated in reversing drug resistance by regulating estrogen receptor alpha (ER α) expression (Alexandrova et al. 2022). ER α expression and regulation are crucial factors in breast cancer cells' resistance to hormonal drugs. Nassa et al. discovered that using the DOT1L inhibitor EPZ004777 reduced DOT1L binding to the C-terminal of ER α (Alexandrova et al. 2022). In drug-resistant cells, mono-, di-, and trimethylation of H3K79 were decreased in the linked genes' transcriptional domains (Farooq et al. 2016). Consequently, this drug reversed resistance to hormonal drugs in breast cancer.

7.5 Contribution of Epigenetic Regulation in the Detoxification of Drugs

Numerous studies have been conducted on the pathology of cancer, and it has become clear that variations in the drug-metabolizing expression of genes are linked to differential DNA methylation in different types of tumor cells. Phase I enzymes like CYP7B1 (Olsson et al. 2007) and CYP1A1 (Sharma et al. 2010), phase II

enzymes like NAT1 (Kim et al. 2008) and SULT1A1 (Kwon et al. 2006), and phase III transporters like SLC22A3 (Chen et al. 2013) and ABCC6 (Yu et al. 2007) are a few instances of drug-metabolizing genes modulated by cancer-related DNA methylations. Certain genes, such as ALDH1A2 (Kim et al. 2005) and CYP24 (Chung et al. 2007), show decreased expression and hypermethylation at promoter regions in certain tumor cells, whereas several transporters, including SLC19A1 (Ferreri et al. 2004) and ABCG2 (Bram et al. 2009), display elevated expression that is correlated with promoter hypomethylation in specific tumor cells, resulting in a drug-resistant phenotype of malignancies. It's been demonstrated that modifications in enhancer methylation associated with cancer can affect drug-metabolizing gene activation in addition to promoter sites. For instance, in prostate carcinoma, enhancers' hypermethylation inhibits CYP1A1's susceptibility to dioxin (Okino et al. 2006). It's interesting to note that histone repressive mark H3K9me2 and methylation of the promoter suppress CYP24A1, a cytochrome P450-related family enzyme, in prostate carcinoma (Luo et al. 2010). For hormone-responsive breast tumors, the methylation pattern of the drug-metabolizing enzyme (DME) promoters determines the prognosis and effectiveness of treatments. For example, in both tamoxifen-treated and nontreated individuals, the promoter methylation level of the P450 family enzyme CYP1B1, which metabolizes tamoxifen, has been demonstrated to indicate survival and outcome (Widschwendter et al. 2004). N-acetyl transferase 1 (NAT1), a phase II DME that controls resistance to tamoxifen in breast carcinoma, is another instance of hypermethylation in this scenario (Kim et al. 2010).

7.6 Drug Efflux Pump Overexpression and the Significance of Epigenetics

Therapeutic resistance is known to be largely caused by a lower intracellular drug buildup as a result of elevated drug efflux pumps. The tumor's inherent resistance to medications or the resistance that develops after the first round of therapy can both be attributed to the tumor's elevated level of drug efflux pumps. The main transmembrane transporters in charge of drug outflow are members of the ABC transporter superfamily, which comprises ABCG2, MDR1, and MRP1 (Vasiliou et al. 2009). A wide range of pharmaceutical drugs, including anthracyclines, alkylating compounds, vinca alkaloids, etc., can be expelled by these drug efflux pumps. Because of their resistant character, CSCs have been shown to express such drug efflux pumps at the highest levels inside the tumor mass (Cho and Kim 2020). According to the latest research, the two main stemness factors, Sox2 and Oct4, indirectly augment the expression of ABCG2 in CSCs by suppressing SMAR1 through the HDAC1 recruitment, which results in the deacetylation of the SMAR1 promoter. However, SMAR1 suppresses ABCG2 effectively via HDAC2 recruitment on its promoter when Oct4 and Sox2 are not present (Bhattacharya et al. 2020). It has also been observed that hypomethylation of promoter is linked to the increased production of ABC transporters in CSCs (Sameer et al. 2024). Therefore, encouraging DNMT function in CSCs may be a useful strategy for

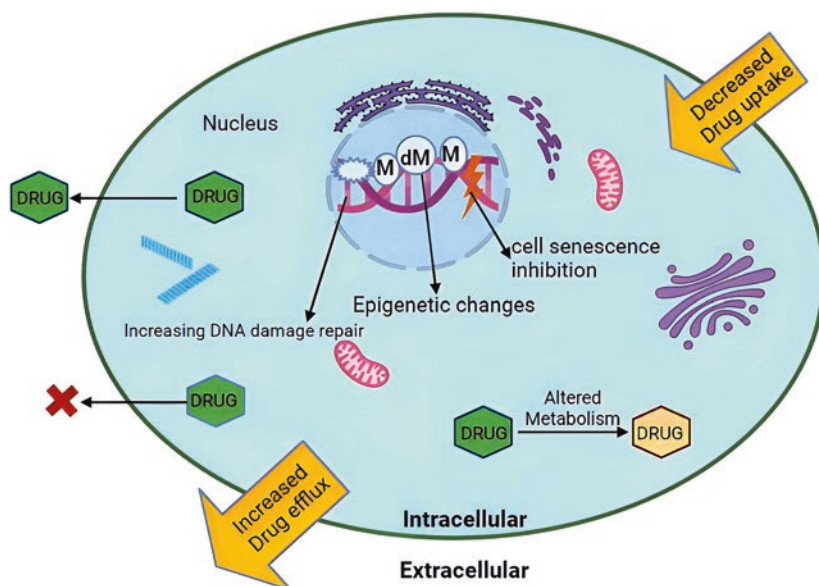


Fig. 7.5 The figure depicts the role of epigenetics in the efflux of drugs

chemosensitizing them. For instance, afatinib-induced DNMT activity increases the ABCG2 promoter's hypermethylation and decreases the expression of ABCG2 in drug-resistant breast carcinoma cells, as shown by a study conducted by Wang et al. (Wang et al. 2014). The ABCG2 promoter's methylation is increased, and its expression is repressed when DNMT activity is induced in brain CSCs, according to a different research study conducted by Martin et al. (Martin et al. 2013). Furthermore, hypomethylation of the promoter is implicated in both the decreased folate carrier SLC19A1 and the augmented ABCG2 expression levels, and this is favorably correlated with the induction of drug resistance (Ferreri et al. 2004; Bram et al. 2009). It is noteworthy that in TNBC, the tumor suppressor as well as the chromatin, ZMYND8, forms transcription suppressor complex with EZH2 and KDM5C that raises the H3K27me3 mark on the promoter regions of the drug efflux pumps ABCB1, ABCC1, and ABCC2, thus suppressing the expression of these genes (Mukherjee et al. 2020). These data suggest that CSCs can enhance drug resistance by altering the chromatin structure and DNA epigenetically to increase the level of expression of the drug efflux pump (Fig. 7.5).

7.7 The Players in Epigenetics Preventing Apoptosis

One of the primary characteristics of cancer is its ability to evade apoptosis, which allows cancer cells to survive when exposed to cytotoxic as well as genotoxic stressors (Hanahan and Weinberg 2011; Khan et al. 2023d; Minto et al. 2021). Resistance to apoptosis greatly influences resistance to therapy because the majority of

present-day therapy treatments, such as chemotherapy, work by activating apoptotic signaling pathways (Pfeffer and Singh 2018; Nalli et al. 2019). The balance of pro- and anti-apoptotic signals and the expression of genes linked to apoptosis are the factors that influence the development of apoptotic resistance (Ozyerli-Goknar and Bagci-Onder 2021). In cancerous cells, the various pro- and anti-apoptotic signaling factors are epigenetically controlled at the transcription and posttranslational levels. For example, apoptosis resistance develops when anti-apoptotic factors like Bcl-2, Bcl-xl, and IAPs, etc. are expressed or activated more (Fernald and Kurokawa 2013). Similar to this, resistance to apoptosis is encouraged by the suppression of pro-apoptotic genes including Bax, Bid, Puma, Noxa, Bim, etc. Furthermore, it has been observed that Akt and its transcription regulators, STAT and NF- κ B, continue to be significantly highly expressed in a number of advanced malignancies, suggesting a potential role for them in treatment resistance (Fernald and Kurokawa 2013; Hassan et al. 2020).

Treatment resistance and tumorigenesis are linked to distinct histone variations and their changes. For instance, it has been found that the histone variation H2AZ is highly expressed in a number of solid tumors, which aids in the growth of the tumor (Vardabasso et al. 2014). Apoptotic cell death is stimulated by H2AZ knockdown by repressing Bcl-2 and elevating pro-apoptotic markers Bak, Caspase 3, and Caspase 9 (Yang et al. 2018). Treatment resistance and the advancement of cancer are frequently linked to atypical histone modifications. Prior research has demonstrated that the process of apoptosis involves the deubiquitylation of H2A; phosphorylation of H2A, H2B, H3, and H4; and dephosphorylation of histone H1 (Liao et al. 2023). By enhancing the expression of several DNA repair genes (such as BRCA 1/2, MGMT, etc.) and activating H3K4Me3, tumor cells evade drug toxicity-mediated apoptosis (Ozyerli-Goknar and Bagci-Onder 2021). The investigation conducted on the NCI-H460 human lung carcinoma cell line demonstrated that the combined activity of ZBP-89 and HDAC3 leads to the suppression of the p16^{INK4a} promoter, which in turn controls the processes of senescence as well as apoptosis (Feng et al. 2009). In breast cancer, the gene CDKN1C, which encodes the tumor suppressor p57^{KIP2}, is suppressed by EZH2-mediated modification of histone H3K27, specifically through trimethylation (H3K27me3). This suppression results in the development of a highly resistant phenotype (Yang et al. 2009). The inhibition of the p21WAF1 promoter in bladder cancer cells is facilitated by histone deacetylation, which is carried out by HDACs (Richon et al. 2000). The consequence of this is the emergence of resistance to apoptosis, a process that can be efficiently counteracted by specifically targeting the cells using HDAC inhibitors (Richon et al. 2000). Furthermore, particular alterations to histones can result in the recruitment of protein complexes that regulate the equilibrium between cellular demise and viability. The phosphorylation of H2A.X-Y142 inhibits the MDC1-mediated recruitment of proteins involved in DNA repair to the site of DNA damage (yH2AX) (Stucki 2009). As a result, this process encourages the formation of pro-apoptotic complexes at the location, ultimately leading the cell towards apoptosis (Stucki 2009; Stucki et al. 2005). During the development of therapeutic resistance, this route undergoes

changes in the cancerous cells, enabling them to survive even after exposure to DNA damaging chemicals such as chemotherapy.

The methylation state of DNA also has a crucial impact on regulating the destiny of apoptotic cells in different types of malignancies. The CpG islands located on promoters of tumor suppressor and pro-apoptotic genes retain a state of hypermethylation due to the action of DNMT in cancerous cells (Ehrlich 2009; Khan et al. 2023e). The hypermethylation of such genes hinders the programmed cellular death process, causing the tumor cells to develop a resistant phenotype. For example, it has been observed that the promoter regions of the pro-apoptotic indicators Bcl-2 and Bik exhibit persistent hypermethylation in prostate cancer patients (Wang et al. 2005; Murphy et al. 2011). Furthermore, in the case of multiple myeloma, the promoters of Bax, Bad, Bak, and Puma exhibit a phenomenon known as hypermethylation (Pompeia et al. 2004). The promoter of Bim exhibits hypermethylation and subsequent suppression in chronic myeloid leukemia (San José-Eneriz et al. 2009). The resistance phenotype in bladder cancer, glioblastoma, hepatocellular carcinoma, retinoblastoma, small-cell lung cancer, and neuroblastoma is caused by the methylation and suppression of Caspase 8 and Caspase 10, which are important drivers of the apoptotic cascade (Khan et al. 2022a; Malekzadeh et al. 2009; Cho et al. 2010; Hervouet et al. 2010; Shivapurkar et al. 2002; Harada et al. 2002). The promoter hypermethylation in gastric carcinoma leads to the inhibition of BNIP3, Bcl-2/10, and HRK expression (Xu et al. 2011; Sugita et al. 2011; Obata et al. 2003). The p53 protein, which is a key regulator of tumor growth, has a crucial function in triggering the process of programmed cell death. In individuals diagnosed with acute lymphatic leukemia, the p53 gene exhibits hypermethylation and inhibition, resulting in the inefficacy of treatment protocols (Agirre et al. 2003). Furthermore, the suppression of hypermethylated cancer 1 (HIC1) in cancerous cells results in the deactivation of p53, hence impeding the route of apoptosis induced by DNA damage (Chen et al. 2005). The expressions of MGMT, MLH1, BRCA1, APC, and APAF1 are known to be reduced in cancer cells due to hypermethylation associated with their promoters (Esteller 2007; Furukawa et al. 2005). The inhibition of FAS expression by hypermethylation on its promoter has been found to play a role in the development of cutaneous T cell lymphoma as well as resistance to apoptosis (Wu and Wood 2011). These results suggest that the complex participation of the epigenetic process plays a crucial role in regulating apoptosis in cancerous cells. This indicates that epigenetic remodeling is essential in making cancer cells resistant to apoptosis.

7.8 Conclusion

In conclusion, the chapter on “Epigenetic Alterations as an Adaptive Response to Chemotherapy” has traversed a comprehensive exploration of the dynamic landscape of epigenetic modifications in the context of drug resistance. Commencing with an introduction that set the stage for understanding the adaptive nature of cancer cells, the subsequent sections investigated specific aspects of epigenetic

regulation. The elucidation of the role of DNA methylation revealed its influence as a modulator of gene expression, shaping the resistance profile of cancer cells to therapeutic agents. The involvement of histone modifications added another layer of complexity, emphasizing the impact of chromatin dynamics on drug responsiveness. The exploration of epigenetic regulation's contribution to detoxification mechanisms and its pivotal role in preventing apoptosis highlighted the specific strategies cancer cells employ to withstand therapeutic challenges. Furthermore, the involvement of epigenetics in the efflux of drugs showcased the active participation of epigenetic modifications in the cellular defense mechanism against chemotherapy agents. Finally, the discussion on targeting epigenetic regulators offered a glimpse into potential strategies to overcome drug resistance, providing a hopeful avenue for future research and therapeutic innovations in the persistent pursuit of effective cancer treatments. Together, these insights underscore the need for a holistic understanding of epigenetic adaptations in cancer cells, paving the way for more targeted and efficacious approaches to counter drug resistance.

MCQs

1. **What is the primary function of multidrug resistance (MDR) transporters in cancer cells?**
 - A. Promote drug absorption into the cells
 - B. Enhance drug efficacy
 - C. Reduce intracellular drug concentration
 - D. Prevent drug detoxification

Answer C: Reduce intracellular drug concentration

2. **How do epigenetic alterations contribute to drug resistance in cancer cells?**
 - A. Enhancing drug efficacy by increasing gene expression
 - B. Inhibiting drug absorption by downregulating transporters
 - C. Causing mutations in drug targets
 - D. Influencing gene expression and silencing tumor suppressor genes

Answer D: Influencing gene expression and silencing tumor suppressor genes

3. **Which enzyme family is responsible for histone acetylation in drug resistance?**
 - A. Histone methyltransferases (HMTs)
 - B. Histone deacetylases (HDACs)
 - C. Histone acetyltransferases (HATs)
 - D. Protein arginine methyltransferases (PRMTs)

Answer C: Histone acetyltransferases (HATs)

4. **Which histone acetyltransferase has been associated with tamoxifen resistance in breast cancer?**
 - A. HAT1
 - B. KAT2A
 - C. KAT6A
 - D. p300

Answer B: KAT2A

5. **In which cancer type has the increased expression of EZH2 been associated with drug resistance to cetuximab?**

- A. Ovarian cancer
- B. Colorectal cancer
- C. Lung cancer
- D. Melanoma

Answer B: Colorectal cancer

6. **Which KMT enzyme catalyzes H3K79 methylation and has been implicated in reversing drug resistance by regulating estrogen receptor alpha (ER α) expression?**

- A. DOT1L
- B. SETD2
- C. G9a
- D. PRMT1

Answer A: DOT1L

7. **How can DNA demethylation agents be potentially utilized to overcome drug resistance in cancer treatment?**

- A. By reducing drug dosages to counteract resistance mechanisms
- B. By promoting the expression of drug target genes through methylation
- C. By increasing the expression of drug efflux pumps through methylation
- D. By reactivating silenced tumor suppressor genes through demethylation

Answer D: By reactivating silenced tumor suppressor genes through demethylation

8. **Which enzyme is responsible for adding methyl groups to DNA during the process of DNA methylation?**

- A. DNA polymerase
- B. DNA ligase
- C. DNA methyltransferase
- D. RNA polymerase

Answer D: DNA methyltransferase

9. **How can DNA methylation patterns in cancer cells lead to acquired drug resistance?**

- A. DNA methylation prevents drug molecules from entering cancer cells.
- B. DNA methylation alters the primary sequence of drug target proteins.
- C. DNA methylation triggers drug-induced apoptosis in cancer cells.
- D. DNA methylation alters gene expression, promoting drug resistance mechanisms.

Answer D: DNA methylation alters gene expression, promoting drug resistance mechanisms.

10. **Which enzyme is primarily responsible for adding acetyl groups to histone proteins?**

- A. DNA methyltransferase
- B. Histone acetyltransferase (HAT)
- C. Histone deacetylase (HDAC)
- D. RNA polymerase

Answer B: Histone acetyltransferase (HAT)

11. How do microRNAs (miRNAs) regulate gene expression in the context of epigenetics?

- A. By directly modifying DNA sequences
- B. By recruiting DNA methyltransferases to target genes
- C. By binding to mRNA and promoting its degradation or inhibiting translation
- D. By facilitating chromatin condensation through histone phosphorylation

Answer C: By binding to mRNA and promoting its degradation or inhibiting translation

12. Which of the following statements accurately describes the role of epigenetics in drug efflux?

- A. Epigenetics has no impact on drug efflux.
- B. Epigenetic modifications can alter the expression of drug efflux transporters.
- C. Epigenetics only affects drug metabolism, not drug efflux.
- D. Epigenetics can directly bind to drugs and prevent their efflux.

Answer B: Epigenetic modifications can alter the expression of drug efflux transporters.

13. Which of the following is an example of an epigenetic modification that can influence drug efflux?

- A. DNA methylation
- B. Drug binding to efflux transporters
- C. Drug degradation in the liver
- D. Drug interactions with cellular receptors

Answer A: DNA methylation

14. How can DNA methylation affect drug efflux?

- A. DNA methylation increases drug efflux by enhancing transporter activity.
- B. DNA methylation decreases drug efflux by suppressing transporter expression.
- C. DNA methylation has no impact on drug efflux.
- D. DNA methylation directly interacts with drugs to prevent efflux.

Answer B: DNA methylation decreases drug efflux by suppressing transporter expression.

15. Besides DNA methylation, what other epigenetic modification can impact drug efflux?

- A. Histone acetylation
- B. RNA splicing
- C. Protein phosphorylation
- D. Lipid metabolism

Answer A: Histone acetylation

16. Which of the following research areas is focused on understanding the role of epigenetics in drug efflux?

- A. Pharmacokinetics
- B. Pharmacodynamics
- C. Pharmacogenomics
- D. Epigenomics

Answer D: Epigenomics

17. **Which cellular component is primarily responsible for epigenetic regulation in drug detoxification?**

- A. Ribosomes
- B. Mitochondria
- C. Nucleus
- D. Golgi apparatus

Answer C: Nucleus

18. **Which class of epigenetic modifications is involved in regulating drug detoxification?**

- A. Acetylation and methylation of histones
- B. Replication of DNA strands
- C. Translation of mRNA into proteins
- D. Phosphorylation of lipids

Answer A: Acetylation and methylation of histones

19. **How do epigenetic modifications influence drug metabolism and detoxification processes?**

- A. By directly altering the drug's chemical structure
- B. By changing the expression of drug transporters
- C. By inducing mutations in drug-metabolizing enzymes
- D. By affecting the solubility of drugs in the bloodstream

Answer B: By changing the expression of drug transporters

20. **Which of the following is NOT an example of a drug efflux pump that can be regulated by epigenetic mechanisms?**

- A. P-glycoprotein (P-gp/ABCB1)
- B. Multidrug resistance-associated protein 1 (MRP1/ABCC1)
- C. Breast cancer resistance protein (BCRP/ABCG2)
- D. Cytochrome P450 (CYP450) enzyme

Answer D: Cytochrome P450 (CYP450) enzyme

21. **Epigenetic regulation of drug efflux pumps can be reversed by using:**

- A. Antiviral medications
- B. Epigenetic inhibitors
- C. Antibiotics
- D. Chemotherapy

Answer B: Epigenetic inhibitors

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Autophagy Plays a Dual Role in Drug Resistance

8

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Abstract

Autophagy is a crucial mechanism that maintains the balance of cellular homeostasis by removing faulty or unneeded proteins as well as damaged or aged organelles in cells. Autophagy is triggered by the formation of autophagosomes, which sequester and enclose anomalous constituents. Subsequently, autophagosomes combine with lysosomes to efficiently recycle or eliminate degradative cargo. Autophagy regulation plays a dual role in both suppressing and promoting

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cancer in many types of malignancies in a context-dependent manner. In addition, autophagy regulates the characteristics of tumor formation, the spread of cancer, the occurrence of cancer stem cells, and the resistance to drugs used in cancer treatment. Autophagy regulators are employed to modify autophagy for the purpose of anticancer therapy. However, the dual functions of autophagy hinder their effectiveness in this therapy, thereby serving as the primary cause of treatment failure. The chapter provides a concise overview of the mechanisms behind autophagy and its correlation with carcinogenesis, metastasis, and resistance to anticancer drugs. Ultimately, we examine the potential and efficacy of addressing autophagy as a prospective therapeutic approach in anticancer treatment resistance.

Keywords

Autophagy · Chemoresistance · Apoptosis · Cancer · Multidrug resistance

8.1 Introduction

Autophagy is a biological activity that is widely preserved throughout different organisms. Its major function is to maintain the balance within cells by breaking down and reusing proteins that are damaged or have a lengthy lifespan, as well as getting rid of misfolded proteins and aberrant organelles (Onorati et al. 2018; Parzych and Klionsky 2014; Khan et al. 2024; Khan et al. 2022a). Furthermore, autophagy is carefully controlled to safeguard cells against many forms of cellular stress, such as DNA damage, starvation, hypoxia, and exposure to chemotherapy (Khan et al. 2023a). It also fulfills the metabolic needs of cells, ensuring the proper functioning of organelles and cellular signaling pathways (Fig. 8.1). Autophagy is a controlled process of breaking down cellular components using autophagosomes,

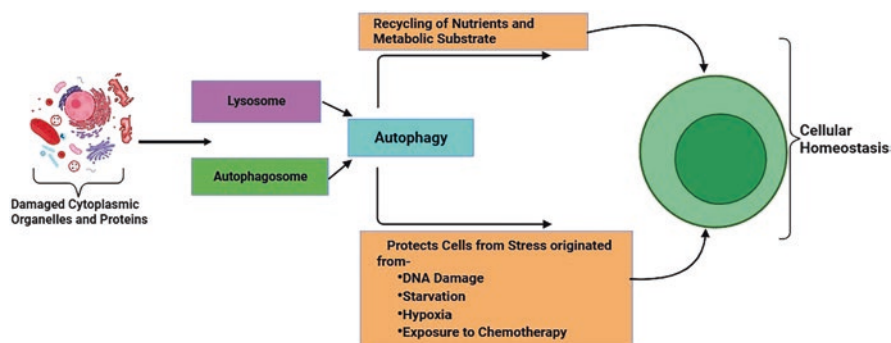


Fig. 8.1 The figure depicting the role of autophagy in maintaining cellular homeostasis by recycling damaged proteins and organelles

which are double-membrane vesicles. These autophagosomes then merge with lysosomes to produce autolysosomes, where the recycling of cellular components takes place (Parzych and Klionsky 2014; Glick et al. 2010). Autophagy plays a crucial role in maintaining good health and triggering the onset of illnesses. Aberrant autophagy is linked to a range of conditions, such as neurological disorders (Guo et al. 2018), cardiovascular illnesses (Mialet-Perez and Vindis 2017), type II diabetes (Sarparanta et al. 2017), and carcinoma (Yun and Lee 2018; Nalli et al. 2019).

Autophagy has a function in suppressing tumor growth during the development and progression of cancer. The inhibitory effects occur through the removal of aberrant cells and organelles, as well as the reduction of the proliferation of cells and genetic instability in cancer (Dong et al. 2019; Endo et al. 2017; Roshani-Asl et al. 2020). Furthermore, autophagy serves to support the metabolic needs of rapidly dividing cancer cells and contributes to the development of resistance to chemotherapy (Lozy and Karantza 2012; Xiong et al. 2019). It is a mechanism that is largely conserved throughout evolution and serves to meet metabolic requirements and maintain homeostasis by recycling intracellular components or undergoing self-degradation (Khan et al. 2023a; White et al. 2015). It is triggered in several cellular stress situations, including hunger, cellular injury, and the accumulation of malfunctioning proteins (Yun and Lee 2018; Khan et al. 2023b). Autophagy can be categorized into three types: microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA). Macroautophagy has a role in segregating cytoplasmic cargo into phagophores, leading to the creation of autophagosomes that consist of double-membrane vesicles (Yorimitsu and Klionsky 2005). Microautophagy is a form of autophagy where cytosolic components are directly engulfed by the lysosomal membrane through the capture of cargo (Oku and Sakai 2018). CMA is a kind of autophagy that specifically targets and transports cargos, which are bound to chaperone proteins like HSC70, across the lysosomal lumen. This process is facilitated by a lysosomal membrane receptor called lysosomal-associated membrane 2A (LAMP-2A) (Kaushik and Cuervo 2018; Cordani et al. 2017).

8.2 Autophagy as a Double-Edged Sword in Treatment Resistance

Autophagy serves a maintenance function by recycling unessential proteins and impaired organelles in healthy cells. Recycling by autophagy reliably gives tumor cells a survival advantage during their development (Long and McWilliams 2020). Nevertheless, prolonged and excessive autophagy may unpredictably result in caspase-independent autophagic death of cells. Autophagy, triggered by metabolic as well as therapeutic stressors, can lead to the development of drug resistance (Chang and Zou 2020). It serves a dual purpose as both a protector and an executor, with the ability to activate survival or death pathways.

Tumor Drug Resistance: Autophagy as a Guardian

Comprehensive research suggests that increased autophagy not only promotes the life of tumors but also contributes to their resistance to drugs across various types of tumors. While the precise pathways via which autophagy enhances drug resistance in tumors remain incompletely comprehended, significant indications are beginning to emerge. Evidently, the activation of autophagy by therapeutic agents facilitates the metabolic processes of tumor cells by recycling impaired proteins as well as organelles. This, in turn, hinders DNA damage and leads to the development of resistance to cancer drugs (White 2016). Recently, convincing evidence has demonstrated that capsaicin triggers the stimulation of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and PARP-1 (poly (ADP-ribose) polymerase-1) through the development of autophagy-regulated DNA damage response, mediated by ATM (Yoon et al. 2012). Autophagy also triggers an intensified DNA damage response through the homologous recombination (HR) repair pathway, which is a primary mechanism for mending DSBs (Anand et al. 2020). In addition, the activation of autophagy by epirubicin (EPI) can enhance the expulsion of drugs from cells by P-glycoprotein (P-gp), which is encoded by MDR genes. This leads to a reduction in the concentration of drugs inside the cells. Furthermore, it downregulated the NF- κ B signaling pathway resulting in a reduced rate of apoptosis. Consequently, this leads to the development of resistance to EPI (Fig. 8.2) (Zhang et al. 2016; Hassan et al. 2020). MDR genes encode a significant protein called MRP1, which is also increased by ER stress-induced autophagy to enhance the removal of drugs from inside the cells (Lim et al. 2010). During temozolomide treatment, the detoxifying enzyme ALDH1A3, which plays a role in acquired drug resistance, is likewise controlled by autophagy (Wu et al. 2018). High-mobility group B1 (HMGB1), a prototypical damage-associated molecular pattern (DAMP), is released during

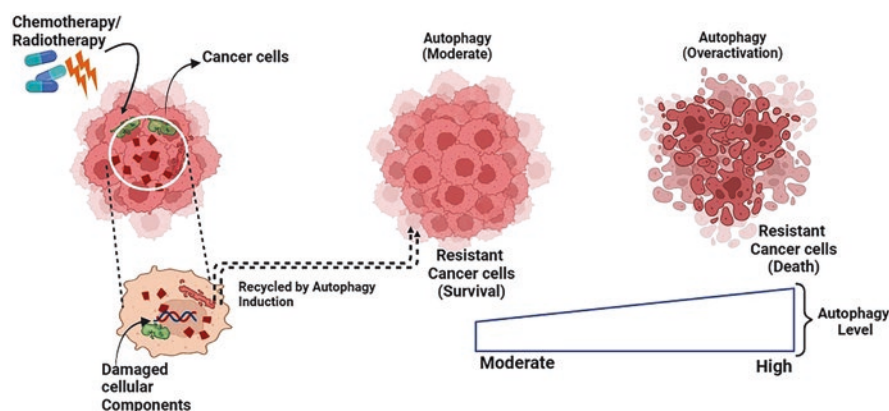


Fig. 8.2 The figure depicts the effect of the level of autophagy on drug resistance. A moderate level of autophagy promotes the survival of resistant cancer cells, while excessive autophagy results in cell death

induced autophagy and has a role in promoting therapeutic resistance in colorectal cancer, ovarian cancer, and lung cancer (Chang and Zou 2020; Tang et al. 2010; Anderson et al. 2021). Thus, autophagy-mediated drug resistance is a complex phenomenon that encompasses various factors, including gene repair, renewal of cytoplasmic material, changes in drug concentration and metabolism, and modifications in the activity and expression of crucial proteins.

The emergence of drug resistance is significantly influenced by alterations in apoptotic and survival signals. In cells that are resistant to TNF-related apoptosis-inducing ligand (TRAIL), the process of cytoprotective autophagy breaks down a component of the active caspase-8 enzyme, which is a crucial protein involved in the mitochondrial apoptosis pathway (Hou et al. 2010). The tumor suppressor p53 initiates the process of autophagy, while the resulting active autophagy subsequently inhibits p53 (White 2016; Mintoo et al. 2021). According to most reports, induced autophagy is followed by the Beclin-1/Bcl-2 complex disruption. As a result, Bcl-2 is liberated from the complex, which promotes the survival of cells (Yang et al. 2020; Khan et al. 2022b). Accordingly, autophagy-promoting drug resistance can be attributed to three types of molecular alterations: deactivation of pro-apoptotic proteins, stimulation of anti-apoptotic effectors, and amplification of survival signals (Silva et al. 2020). Autophagy specifically enhances the ability of cells to withstand stress-induced damage, protecting against therapeutic stress and preventing the activation of apoptotic signals (Fig. 8.2). Both of these processes are necessary for inducing cell death, and autophagy helps to prevent this, resulting in tumor resistance to apoptosis (Wirawan et al. 2012).

Tumor Drug Resistance: Autophagy as an Executioner

In addition to promoting cell survival, autophagy can also trigger a form of cell death known as autophagic cell death. This type of cell death occurs when autophagy continues to occur or is sustained, resulting in extensive formation of autophagic vacuoles in the cytoplasm and the characteristic appearance of vacuolated cells. Autophagic cell death is distinct from apoptosis (type I cell death) and necrosis (type III cell death). Excessive expression of autophagy genes can significantly contribute to the process of cell death through autophagy. For example, when Beclin-1 is deliberately activated, it triggers an excessive process of autophagy and facilitates human synovial sarcoma cells' (SW982) death (Zhu et al. 2018). The buildup of p62/SQSTM1 caused by the plant compound resveratrol activates autophagic cell death in imatinib-resistant CML-chronic myelogenous leukemia cells (Puissant et al. 2010). In ovarian cancer cells treated with metformin, the ATG5-ATG12 complex builds and triggers autophagic cell death (Fig. 8.2) (Moon et al. 2016).

Autophagic cell death, which is regulated by autophagy genes, is influenced by specific circumstances and can occur in cells that are resistant to apoptosis, especially in cells lacking pro-apoptotic proteins (Platini et al. 2010). Furthermore, autophagic cell death can be utilized as an alternate method of cell death when cells are unable to undergo apoptosis, even in cases of treatment resistance in tumors

(Fig. 8.2) (Towers et al. 2019). The determination of whether autophagy serves a pro-survival or pro-death function can be heavily influenced by signaling thresholds. The strength of oncogenic Ras signaling is associated with the result of either senescence or autophagic cell death. The enforced activation of Ras increases the production of the BH3-only protein Noxa and Beclin-1, which in turn promotes autophagic cell death (Elgendy et al. 2011). Increased expression of prolidase triggers extensive autophagy, resulting in cell death by enhancing ATG7, Beclin-1, and LC3A/B (Zareba et al. 2020). In addition, numerous signaling pathways are simultaneously triggered to fulfill the purpose of excessive autophagy. The flavonoid baicalein, known for its proven anticancer activities, stimulates the activation of AMPK/ULK1 and reduces the expression of mTORC1 complex components, leading to the initiation of autophagic cell death (Aryal et al. 2014).

It is worth mentioning that the elevated levels of caspase-10, as well as cFLIPL in myeloma cells, prevent autophagic death of cells by breaking and deactivating BCLAF1, which encodes Bcl-2-associated transcription factor 1 (Lamy et al. 2013). The reason for these inconsistencies (supporting survival and promoting death utilizing the same autophagy machinery) lies in a quantitative correlation where upstream signaling molecules influence cellular reactions by regulating the relative limit of autophagy flow in response to stimuli from the outside. Enhancing autophagy flow beyond a certain level (resulting in autophagic death of cells) or reducing autophagy flux (by inhibiting autophagy for survival) could be a promising approach for combating drug resistance in tumors mediated by autophagy.

8.3 Autophagy-Activated Survival Mechanisms in Chemoresistance

Autophagy is a cellular mechanism that involves the degradation of damaged or unwanted cellular components through the lysosomal pathway. This process plays a key role in maintaining cellular homeostasis and enhancing cell survival under various stress conditions (Parzych and Klionsky 2014; Wani et al. 2021). In cancer, autophagy has been revealed to have dual roles in tumor development and progression. On one hand, autophagy can promote tumor cell survival by providing bioenergetic support and removing toxic cellular components. On the other hand, autophagy can also act as a tumor-suppressive mechanism by inducing cell death under certain conditions (Aman et al. 2021).

Chemoresistance is a major therapeutic challenge in cancer treatment. It is often associated with the activation of survival mechanisms that promote tumor cell survival and proliferation. Autophagy has been implicated in chemoresistance as a mechanism by which cancer cells can survive and adapt to chemotherapy-induced stress. In this context, autophagy serves as a pro-survival mechanism by promoting the degradation of damaged proteins and organelles, thus reducing the accumulation of toxic metabolites that can cause cell death (Rezayatmand et al. 2022; Khan et al. 2023c).

Several studies have shown that autophagy can be activated in response to various chemotherapy agents, including cisplatin, doxorubicin, and etoposide (Xia et al. 2021; Wadhwa et al. 2020). Chemotherapy-induced autophagy has been associated with the upregulation of autophagy-related genes (ATGs) and the regulation of signaling pathways such as AMPK/mTOR and PI3K/Akt. Inhibition of autophagy has been shown to sensitize cancer cells to chemotherapy-induced cell death, suggesting that autophagy activation may contribute to chemoresistance (Fig. 8.3) (Khan et al. 2022b; Xia et al. 2021; Debnath et al. 2023).

In addition to promoting survival under chemotherapy-induced stress, autophagy can also contribute to chemoresistance by promoting cancer stem cell (CSC) survival. CSCs are a subpopulation of cancer cells that are highly resistant to chemotherapy and have been implicated in tumor relapse and metastasis (Nazio et al. 2019; Khan et al. 2023d). Autophagy has been shown to regulate CSC survival by promoting the maintenance of stem cell properties and preventing differentiation. Inhibition of autophagy has been shown to sensitize CSCs to chemotherapy-induced cell death, suggesting that autophagy may be a potential therapeutic target to overcome chemoresistance in CSCs (Fig. 8.3) (Nazio et al. 2019; Wang et al. 2022).

In the early stages of autophagy, Beclin-1/ATG6 is typically implicated in the trafficking of different vesicles within the cell. In addition to interacting with the Uvrag-Vps38 complex to control vacuolar sorting of proteins, the ATG6-Vps34

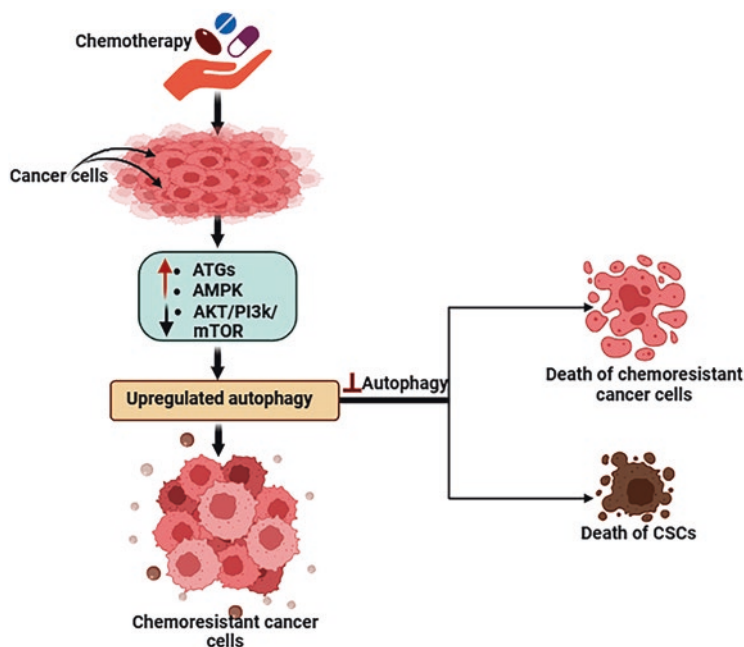


Fig. 8.3 The figure shows that chemotherapy-induced autophagy promotes the survival of resistant cancer cells and CSCs and that inhibition of autophagy sensitizes the resistant cancer cells and CSCs

complex additionally engages with ATG14L to encourage the development of autophagosomes. According to a recent investigation, cytokines as well as ligand-receptor degradation are related to the complex including UVRAG (Thoresen et al. 2010). In a recent study, it was shown that MicroRNA-30a downregulation contributes to chemoresistance of osteosarcoma cells by stimulating Beclin-1-mediated autophagy (Xu et al. 2016). The emergence of resistance to drugs is significantly influenced by the direct control of Beclin-1. In vitro, tamoxifen responsiveness was enhanced by Beclin-1 inhibition in ER-positive breast carcinoma cells, and patients having ER-positive breast carcinoma who have reduced Beclin-1 expression have a more favorable outcome (Gu et al. 2017; Ahmad et al. 2022).

In conclusion, autophagy plays a crucial role in promoting the survival of cancer cells under chemotherapy-induced stress and contributes to chemoresistance by promoting CSC survival. Targeting autophagy may offer a potential therapeutic strategy to overcome chemoresistance in cancer. Nevertheless, further studies are required to completely elucidate the complex role of autophagy in cancer and to identify optimal strategies for autophagy inhibition in cancer therapy.

8.4 Autophagy-Regulating Factors in Promoting Drug Resistance

Autophagy is a dynamic process that is regulated by a complex network of signaling pathways and factors. Several factors have been identified as being involved in the regulation of autophagy and promoting drug resistance in cancer cells (He and Klionsky 2009). Here are some of the key factors involved in this process:

1. **mTOR:** mTOR is a crucial regulator of autophagy that inhibits autophagy under normal conditions. However, in cancer cells, mTOR signaling is often deregulated and leads to the activation of pro-survival pathways, including autophagy (Paquette et al. 2018). Activation of mTOR signaling has been linked to resistance to chemotherapy and targeted therapies in several types of cancer (Burris 2013). Therefore, mTOR inhibitors are regarded as a beneficial addition to chemotherapeutics or targeted cancer therapy, either as a treatment choice for patients who have relapsed or as a combination treatment to stop or delay the emergence of resistance caused by persistent mTOR signaling. The utilization of second-generation, ATP-competitive, catalytic mTORC1/mTORC2 inhibitors, due to their broad effects, may be constrained by more severe toxicities than first-generation mTOR inhibitors, known as rapalogs, which impede phosphorylation of only some mTORC1 targets (Gremke et al. 2020). Given that mTOR plays a significant role in cancer drug resistance and that mTOR inhibitors have only had limited clinical effectiveness, Gremke et al. have found innovative therapeutic approaches for cancerous cells that exhibit mTOR-dependent drug resistance (Gremke et al. 2020). Given the crucial function of mTOR as a rheostat of the metabolism of cells, they looked at medicines that target energy metabolism, which is reprogrammed in cancer cells to create the massive quantities of bio-

mass required for continued growth. The “Warburg effect” or aerobic glycolysis is a typical change in cancer cells that upregulates glycolysis even in the presence of oxygen (Chen et al. 2015). This specific dependency of fast-proliferating cells on biosynthetic pathways that utilize intermediates produced from glycolysis is thought to be the cause of this distinctive metabolic state, which is considered a hallmark of cancer (Gremke et al. 2020; Ilagan and Manning 2016).

2. **Beclin-1:** Beclin-1 is a novel protein with only a Bcl-2-homology (BH)-3 domains. Beclin-1 null mice have an embryonic phenotype that is much more severe compared to that of other autophagy gene-deficient mice, which pass away at an early stage of development (E7.5 or earlier) with problems with pro-amniotic canal closure (Oberstein et al. 2007). Many human and murine tissues express Beclin-1, which is primarily found in cytoplasmic structures like the ER, mitochondria, and the perinuclear membrane. Beclin-1 is found in the cytoplasm, cytoplasmic membrane, and nucleus of human colon cancer cells. BH3 domain (amino acids 114–123) at the N-terminus, central coiled-coil domain (CCD, amino acids 144–269), and evolutionarily conserved domain (ECD, amino acids 244–337) are the three structural domains that have been found in Beclin-1 (Kang et al. 2011). Beclin-1 is a critical regulator of autophagy and is involved in the initiation of autophagy. It has been shown that high expression of Beclin-1 is associated with increased autophagy and drug resistance in various types of cancer. In 1998, Beth Levine’s team used a yeast two-hybrid screen to clone the human autophagy gene Beclin-1, which is an ortholog of the yeast protein Atg6/vacuolar protein sorting (VPS)-30. When Beclin-1 interacts with the class III phosphoinositide 3-kinase (PI3KC3)/Vps34, autophagic proteins are important for localization to a pre-autophagosomal structure (PAS). The core complex of Beclin-1-Vps34-Vps15 is made up of them all (Kang et al. 2011; Vega-Rubín-de-Celis 2019).
3. **p53:** The tumor suppressor protein p53 has been shown to regulate autophagy and contribute to drug resistance in cancer. p53 can induce autophagy in response to various cellular stresses, including chemotherapy. Inhibition of p53-dependent autophagy has been shown to sensitize cancer cells to chemotherapy-induced cell death (Borrero and El-Deiry 2021).
4. **Bcl-2 family proteins:** The Bcl-2 family of proteins is involved in the regulation of apoptosis and autophagy. Anti-apoptotic Bcl-2 family members, such as Bcl-2 and Bcl-XL, have been shown to inhibit autophagy and promote drug resistance in cancer cells (Kale et al. 2018).
5. **MAPK and JNK:** The mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) signaling pathways are involved in the regulation of autophagy and drug resistance in cancer cells. The activation of these pathways has been linked to the induction of autophagy and drug resistance in various types of cancer (Zhou et al. 2015).
6. **HIF-1 α :** Hypoxia-inducible factor 1-alpha (HIF-1 α) is involved in the regulation of cellular adaptation to hypoxia and stress conditions. HIF-1 α has been shown to induce autophagy and promote drug resistance in cancer cells under hypoxic conditions (Ikeda and Takeya 2021).

7. **Reactive oxygen species (ROS):** Reactive oxygen species (ROS) are involved in the regulation of autophagy and drug resistance in cancer cells. ROS can activate autophagy and promote the survival of cancer cells under stress conditions (Prasad et al. 2017; Khan et al. 2023e).

In summary, autophagy is regulated by a complex network of factors that contribute to drug resistance in cancer cells. Strategies that target these factors could potentially overcome drug resistance and improve cancer treatment outcomes.

8.5 Autophagy's Role in Multidrug Resistance (MDR) in Tumors

According to Bhardwaj et al.'s research (Bhardwaj et al. 2018), there was a considerable rise in the expression of ATG5, LC3, and Beclin-1 in the tissues of tumors, and there was a positive correlation between these expression levels and the MDR1 gene. This implies that autophagy plays a role in the emergence and progression of MDR. Nonetheless, recent studies have provided evidence that the function of autophagy in the development and carcinogenesis of tumors is complex. Tumor occurrence, progression, and tumor cells' resistance to chemotherapy are all influenced by autophagy (Mathew et al. 2007). As a defense mechanism for MDR, autophagy can be triggered during anticancer therapy. Thus, blocking autophagy can increase tumor cells' susceptibility to chemotherapy, which in turn increases the damaging effects of chemotherapeutic agents. On the other hand, autophagy can also result in autophagic cell death (Sui et al. 2013). As a result, autophagy may enhance the therapeutic efficacy of tumor MDR if used correctly in the treatment of tumors.

MDR Is Mediated by Autophagy

The ABC transporter and the incidence of MDR are tightly associated. Consequently, studies support the creation of medications that control ABC transporters as chemotherapeutic agents to combat MDR (Amawi et al. 2019). Nevertheless, the clinical therapeutic benefits of ABC transporter modulators are still not optimal. Moreover, MDR exhibits a wide range of intricate phenotypes. Based on the available clinical data, several researchers have attempted to connect autophagy with MDR (Kumar et al. 2012). The gene expression level of ABCB1 was found to be negatively associated with Raptor's expression levels and positively associated with LC3, Beclin-1, and Rictor, in tumor samples from patients with colorectal cancer that survived for 5 years (Shuhua et al. 2015). There is evidence to show that autophagy plays a key role in the emergence and progression of MDR.

Autophagy Facilitates the Generation and Advancement of MDR

Esophageal cancer, lung cancer, liver cancer, kidney cancer, ovarian cancer, prostate cancer, breast cancer, pancreatic cancer, stomach cancer, bladder cancer, colorectal cancer, and glioma have all been shown to benefit from autophagy in terms of tumor survival (Chiu et al. 2018; Soltany-Rezaee-Rad et al. 2014; Zhan et al. 2016; Zhang et al. 2013; Blessing et al. 2017; Yang et al. 2011, 2018; Han et al. 2014; Niu et al. 2020; Fan et al. 2014; Yao et al. 2021; Fu et al. 2019).

Studies have indicated that MDR is generated and developed subsequent to autophagy. Patients with worse prognoses have higher levels of autophagy, which implies that autophagy may facilitate the emergence of multidrug resistance.

MDR Generation Is Enhanced by Beclin-1

Lu et al.'s research investigation (Lu et al. 2018) revealed that individuals having multiple myeloma (MM) who expressed profilin1 (PFN1) had a worse prognosis. PFN1 has the ability to bind itself to the Beclin-1 complex and stimulate autophagy. The research discovered that augmented expression of PFN1 aided in the process of autophagy and generated BTZ (bortezomib) resistance in MM in addition to promoting proliferation. The findings imply that PFN1 may play a role in BTZ resistance by taking part in the formation of the Beclin-1 complex, which promotes autophagy, even though autophagy inhibition achieved by preventing the formation of the Beclin-1 complex can revert resistance towards BTZ (Lu et al. 2018). Furthermore, MDR could be mediated by HMGB1. HMGB1 stimulates autophagy in reaction to anticancer drugs. By triggering the MAPK/ERK signaling cascade during its transfer from the cell's nucleus to the cytoplasm, it can promote the assembly of the Beclin-1-PI3KC3 complex, which in turn induces autophagy (Pan et al. 2014). Furthermore, autophagy triggered by HMGB1 contributes to the chemoresistance of several types of malignancies (Xu et al. 2018).

MDR Development Is Aided by ROS and Autophagy

ROS are produced in excess by cancer cells during carcinogenesis, and this triggers autophagy and the response to oxidative stress in stromal cells. Antioxidant defense systems and autophagy induction in the stroma can shield cancerous cells from cellular harm and ultimately death (Zhou et al. 2013; Singh et al. 2017). Furthermore, ketone bodies and L-lactate, two high-energy metabolites that can promote tumor anabolism as well as mitochondrial biosynthesis, are abundant in the autophagy-mediated matrix (Zhou et al. 2013). According to some reports, ketone bodies and lactate can operate as chemical stimulants for tumor cells, promoting their development and metastasis. In addition, MDR cancerous cells have higher ROS levels and antioxidant/scavenging enzyme activity than non-MDR tumor cells and healthy cells (Cort et al. 2016). These findings suggest a tight relationship between ROS and tumor cell MDR.

8.6 Autophagy as a Hopeful Therapeutic Target for Drug Resistance

Autophagy functions as a safeguard mechanism by eliminating impaired organelles and proteins. Conversely, excessive autophagy acts as an inhibitory mechanism by triggering the autophagic death of cells. Additionally, autophagy has a role in the development of tumors, the advancement of cancer, and the ability to withstand anticancer treatments (Kumar et al. 2015). Autophagy is stimulated by cancer treatments, including chemotherapy, radiotherapy, and targeted treatments, mostly through its cytoprotective role in triggering multidrug resistance (MDR) against stress responses caused by the therapy (Wang et al. 2018; Taylor et al. 2018; Hao et al. 2019). Hence, inhibiting autophagy restores sensitivity in cancerous cells and enhances the therapeutic efficacy of anticancer treatments. Autophagy, in contrast, serves as the mechanism for the autophagic death of cells, which is distinct from apoptosis.

Blocking Autophagy Enhances the Effectiveness of Therapy

Presently, research on suppressing autophagy frequently utilizes the methods of genetically silencing ATGs and employing pharmaceutical inhibitors. Small interfering RNA (siRNA) is commonly used to silence genes involved in autophagy, such as Beclin-1, ATG5, ATG7, ATG8, and ATG12, in order to suppress the autophagy process and increase the susceptibility of drug-resistant tumors (Zeng et al. 2015; Fan et al. 2010; Chang et al. 2013; Janku et al. 2011). On the other hand, pharmaceutical agents like bafilomycin A1 (BafA), 3-methyladenine (3-MA), and chloroquine (CQ) are typically employed to hinder the formation of autophagosomes (Fig. 8.4) (Fan et al. 2010; Chang et al. 2013; Bellodi et al. 2009) and enhance the sensitivity of resistant tumor cells to chemotherapy treatment. 3-MA functions as a potent inhibitor of class III PI3K, i.e., VPS34. Consequently, it effectively suppresses the process of autophagy at its initial stage. On the other hand, BafA, an inhibitor of vacuolar H⁺-ATPase, and CQ, an inhibitor of the fusion between autophagosomes and lysosomes, are used in the later stages of autophagy to prevent the breakdown of cellular cargo (Fig. 8.4). Chemical inhibitors targeting the receptor tyrosine kinase (RTK)/class I PI3K/AKT/mTOR cascades have demonstrated efficacy as anti-tumor therapy. Dasatinib and imatinib, both classified as TKIs, are frequently recommended for the treatment of CML. Nevertheless, a substantial proportion of patients do not get satisfactory responses to these medications. Pharmacologically inhibiting autophagy by employing CQ, or suppressing the activity of ATG5 and ATG7, can greatly increase the death of imatinib-resistant chronic myeloid leukemia (CML) cells (Bellodi et al. 2009). The combination of dasatinib plus autophagy inhibitors led to the near-total elimination of phenotypical as well as functioning CML stem cells (Bellodi et al. 2009). The combination of NVP-BEZ235, a dual class I PI3K/mTOR inhibitor, plus autophagy inhibitors enhances the process of apoptosis in drug-resistant malignancies (Fan et al. 2010;

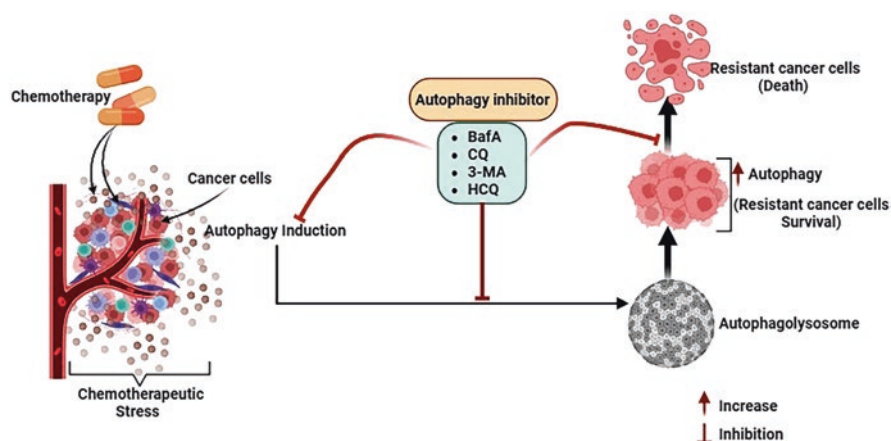


Fig. 8.4 The figure demonstrates that inhibition of the process of autophagy at various steps sensitizes resistant cancer cells and induces death

Chang et al. 2013). Perifosine acts as an alkyl phospholipid that functions by inhibiting AKT activation and demonstrating anticancer effects. Perifosine, when combined with either CQ or NH₄Cl, resulted in increased apoptosis and suppression of tumor development (Sun 2010). The combination of temsirolimus (mTOR inhibitor) with hydroxychloroquine (HCQ) (autophagy inhibitor) increased the death of cells in individuals with metastatic solid tumors and melanoma in a phase I clinical trial (Rangwala et al. 2014).

Preclinical models demonstrated that certain proteasome inhibitors induce autophagy through the buildup of misfolded proteins, specifically causing endoplasmic reticulum (ER) stress. Consistent with the perspective, the suppression of autophagy caused by proteasome inhibitors (bortezomib and NPI-0052) utilizing 3-MA or CQ resulted in an augmented rate of death of cells in prostate carcinoma cell models (Zhu et al. 2010). When compared with animals that have the normal genetic makeup, the proteasome inhibitor epoxomicin showed a stronger ability to fight against tumors in mice that have a malfunctioning autophagy process due to the Beclin-1+/- mutation (Janku et al. 2011). In head and neck squamous cell carcinoma, the use of autophagy inhibitor CQ along with the next-generation proteasome inhibitors oprozomib and carfilzomib resulted in the induction of autophagy and increased cell death (Zang et al. 2012). CQ promotes a gradual building of proteins specifically in lysosomes, whereas bortezomib triggers a quick accumulation of proteins in the cytosol, leading to the formation of aggresomes (Carew et al. 2010). Accumulation occurrences frequently result in mitochondrial dysfunction, which is characterized by the production of cytochrome c along with the stimulation of Apaf-1-containing apoptotic complexes (Srivastav et al. 2020). Therefore, the simultaneous suppression of proteasome and autophagy can result in a more potent anticancer effect.

Tumor cells exhibit a form of drug resistance that can be attributed to the cell cycle, which is the process by which cells divide. This insensitivity to chemotherapeutic medications is a result of the cell cycle process. Isoliquiritigenin has demonstrated anticancer effects in various types of cancers by causing G2 as well as M phase arrest and suppressing cell division (Chen et al. 2017). The application of 3-MA to inhibit autophagy triggered by isoliquiritigenin amplifies its anticancer efficacy in ES-2 cells (ovarian carcinoma cells) (Chen et al. 2017). Silibinin induces cell cycle arrest and subsequent cell death in the majority of cancer types (Jahanafrooz et al. 2018). Similarly, the suppression of autophagy enhances the susceptibility of SW480 and SW620 cells to silibinin-induced cell death (Kauntz et al. 2011). Vincristine, a significant chemotherapeutic drug that targets the cell cycle, triggers apoptosis in gastric carcinoma cells. Additionally, the use of siRNA to inhibit Beclin-1 or ATG5 enhances the sensitivity of tumor cells resistant to vincristine (Zhan et al. 2012). The most intriguing feature of cell cycle-inhibiting drugs is their ability to induce protective autophagy through one or more distinct signaling pathways in different types of cells. This will make autophagy more effective in eliminating drug resistance in cancers. Cetuximab, an antibody that inhibits the activity of EGFR, caused programmed cell death and autophagy. Knockdown of Beclin-1 or ATG7 or the administration of CQ made cancer cells more responsive to cetuximab-induced programmed cell death (Li and Fan 2010). Cetuximab functioned by reducing the expression of Bcl-2, which facilitated the interaction between Beclin-1 and VPS34.

Additionally, miRNAs have demonstrated their significance as a crucial therapeutic approach in the regulation of drug resistance across a diverse range of cancer types. The ATG12 expression was decreased by miR-23b-3p in order to sensitize vincristine-resistant gastric cancer cell SGC7901 by inhibiting autophagy (YiRen et al. 2017). The inhibition of autophagy, mediated by USP22, by miRNA-29c, resulted in an increase in the death of pancreatic carcinoma cells caused by gemcitabine (Huang et al. 2018). A recent study (Cheng et al. 2018) demonstrated that miR-409-3p improved the responsiveness of ovarian carcinoma cells to cisplatin by disrupting the FIP200-mediated autophagy. Therefore, the utilization of miRNA to target autophagy could present an appealing and promising strategy for conquering treatment resistance.

Overcoming Drug Resistance Can Be Achieved by Promoting Autophagic Cell Death

Multiple studies have demonstrated that autophagy is essential for efficiently eliminating tumor cells in specific situations. In this situation, autophagy is referred to as autophagic cell death, which is considered to be a form of cell death that involves autophagy, rather than cell death caused by autophagy (Macintosh and Ryan 2013). In fact, certain drugs like bortezomib, rapamycin, and butyrate have been shown to

promote the formation of autophagosomes (Levy and Thorburn 2011). Additionally, the enforced expression of autophagy-related genes like ATG3, ATG4, ATG5, ATG9, and Beclin-1 has been shown to promote autophagic cell death (Fulda and Kögel 2015). These drugs and gene expressions can also work together with additional chemotherapy drugs to effectively combat cancer in specific types of cells.

mTOR serves as the primary controller of autophagy and is a crucial target for regulating autophagy. In adenoid cystic carcinoma cells receiving cancer chemotherapy, the pharmacologic inhibitor rapamycin improved isoliquiritigenin-induced autophagic as well as apoptotic cell death (Fig. 8.5) (Chen et al. 2012). Propachlor and everolimus, both mTOR inhibitors, acted synergistically to promote cell death in prostate carcinoma cells by triggering the process of autophagic cell death (Tai et al. 2012). Moreover, the administration of RAPA, an mTOR inhibitor that triggers autophagy, resulted in enhanced cell mortality in U251 glioma cells treated with temozolomide (Fig. 8.5) (Li et al. 2018). However, inhibiting mTOR doesn't always result in autophagic death; instead, it results in protective autophagy. For example, because an mTOR inhibitor is a prominent stimulant of autophagy, HCQ alleviated temsirolimus resistance and greatly increased anticancer action with tolerability and acceptability in cancer individuals with advanced solid tumors as well as melanoma (Rangwala et al. 2014).

A proteasome inhibitor, bortezomib has become a widely accepted and effective treatment for multiple myeloma (Driscoll et al. 2013). Nevertheless, bortezomib exhibits limited efficacy in a significant number of patients, who frequently acquire medication tolerance. A recent investigation has demonstrated that the induced expression of ATG5 can effectively overcome the resistance of hematological cancers to bortezomib (Driscoll et al. 2013). The compound marchantin M, which is a new inhibitor of proteasomes, has the ability to stimulate autophagic cell death in prostate cancerous cells by directly affecting the PI3K/AKT/mTOR pathway (Jiang et al. 2013). Therefore, the induction of autophagic death of cells may potentially overcome therapeutic resistance towards proteasome inhibitors.

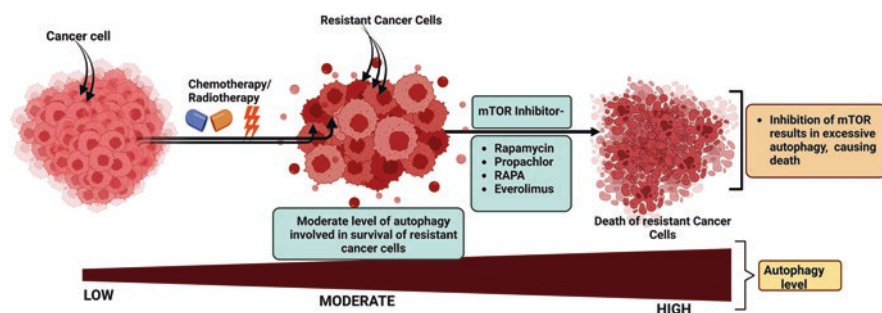


Fig. 8.5 The figure depicts the importance of the level of autophagy in the survival of cancer cells. Increased autophagy activation by targeting mTOR results in the death of resistant cancer cells

8.7 Conclusion

From a clinical viewpoint, the occurrence of treatment resistance leading to frequent tumor growth is disappointing for both researchers and medical professionals. It is imperative to elucidate the mechanisms that develop treatment resistance in malignancies. The suppression of autophagy has been demonstrated to enhance the effectiveness of anti-tumor treatments and conquer resistance to therapy in several types of cancer cells, both in laboratory settings and in vivo settings. As a result, it has gained significant attention from numerous researchers. Nevertheless, as autophagy research progresses, it has become evident that autophagy has a dual function in tumor treatment resistance, with both pro-death and pro-survival effects. This ambiguity has led to differing interpretations among experts. Autophagy in established malignancies enhances metabolic processes to deactivate drugs and promote drug resistance. Indeed, specific anticancer medications can likewise trigger excessive or prolonged autophagy, resulting in the death of cancerous cells. The diverse characteristics of autophagy are being used to develop various effective treatments for distinct types of malignancies. Further preclinical investigations across several biological disciplines are vital to enhance comprehension of the dual function of autophagy in cancer and advance towards clinical studies for cancer treatment including autophagy regulation. Targeting autophagy is essential for the development of effective treatment approaches in anticancer therapy. Furthermore, it is necessary to conduct clinical investigations to assess the effectiveness of anticancer treatments that involve various combinations of autophagy regulators and anticancer medicines.

MCQs

1. **What is the primary function of autophagy in cells?**

- A. DNA repair
- B. Synthesis of new proteins
- C. Breaking down and recycling damaged proteins and organelles
- D. Cellular respiration

Answer C: Breaking down and recycling damaged proteins and organelles

2. **How is autophagy triggered in cells?**

- A. Exposure to sunlight
- B. Cellular injury
- C. Cell division
- D. Oxygen abundance

Answer B: Cellular injury

3. **Which of the following is NOT a type of autophagy?**

- A. Macroautophagy
- B. Microautophagy
- C. Chaperone-assisted autophagy
- D. Nanoautophagy

Answer D: Nanoautophagy

4. **What are autophagosomes?**

- A. Single-membrane vesicles
- B. Triple-membrane vesicles
- C. Double-membrane vesicles
- D. Spherical protein aggregates

Answer C: Double-membrane vesicles

5. **What is the primary role of autophagy in cancer development?**

- A. Promoting genetic instability
- B. Enhancing cell proliferation
- C. Suppressing tumor growth through the removal of aberrant cells and organelles
- D. Inducing chemotherapy resistance

Answer C: Suppressing tumor growth through the removal of aberrant cells and organelles

6. **Which autophagy type directly engulfs cytosolic components by lysosomal membrane capture?**

- A. Macroautophagy
- B. Microautophagy
- C. Chaperone-mediated autophagy
- D. Autophagosome-mediated autophagy

Answer B: Microautophagy

7. **What is the function of chaperone-mediated autophagy (CMA)?**

- A. Segregating cytoplasmic cargo into autophagosomes
- B. Direct engulfment of cytosolic components by lysosomal membrane capture
- C. Targeting and transporting cargo bound to chaperone proteins across the lysosomal lumen
- D. Fusing autophagosomes with lysosomes

Answer C: Targeting and transporting cargo bound to chaperone proteins across the lysosomal lumen

8. **Prolonged and excessive autophagy may lead to:**

- A. Increased cell survival
- B. Caspase-independent autophagic cell death
- C. Enhanced DNA damage repair
- D. Accelerated cell division

Answer B: Caspase-independent autophagic cell death

9. **What is the role of capsaicin in autophagy-regulated DNA damage response?**

- A. Inhibiting autophagy
- B. Stimulating DNA repair enzymes
- C. Triggering caspase-independent cell death
- D. Activating DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and PARP-1

Answer D: Activating DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and PARP-1

10. **How does autophagy enhance drug expulsion from cells during epirubicin (EPI) treatment?**

- A. Downregulating DNA repair pathways
- B. Increasing drug concentration inside cells
- C. Activating P-glycoprotein (P-gp) encoded by MDR genes
- D. Inducing apoptosis

Answer C: Activating P-glycoprotein (P-gp) encoded by MDR genes

11. **What does ALDH1A3 control during temozolomide treatment, contributing to acquired drug resistance?**

- A. Autophagy pathways
- B. DNA repair mechanisms
- C. Metabolic processes
- D. Drug expulsion from cells

Answer B: DNA repair mechanisms

12. **How does autophagy influence resistance to tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)?**

- A. Inhibiting caspase-8
- B. Enhancing TRAIL sensitivity
- C. Activating p53
- D. Disrupting the Beclin-1/Bcl-2 complex

Answer A: Inhibiting caspase-8

13. **In addition to promoting cell survival, autophagy can also trigger:**

- A. Apoptosis
- B. Necrosis
- C. Autophagic cell death
- D. Senescence

Answer C: Autophagic cell death

14. **Autophagic cell death is distinct from:**

- A. Apoptosis
- B. Senescence
- C. Necrosis
- D. All of the above

Answer D: All of the above

15. **What plant compound causes the build-up of p62/SQSTM1, leading to autophagic cell death in imatinib-resistant CML cells?**

- A. Resveratrol
- B. Metformin
- C. Baicalein
- D. Beclin-1

Answer A: Resveratrol

16. **In ovarian cancer cells treated with metformin, what complex builds up and triggers autophagic cell death?**

- A. Beclin-1/Bcl-2 complex
- B. ATG5-ATG12 complex

- C. mTORC1 complex
- D. AMPK/ULK1 complex

Answer B: ATG5-ATG12 complex

17. **The strength of oncogenic Ras signaling is associated with the result of either senescence or:**

- A. Apoptosis
- B. Necrosis
- C. Autophagic cell death
- D. Proliferation

Answer C: Autophagic cell death

18. **Which signaling pathways are activated during chemotherapy-induced autophagy?**

- A. NF- κ B and JAK/STAT
- B. AMPK/mTOR and PI3K/Akt
- C. Wnt and Notch
- D. TGF- β and MAPK

Answer B: AMPK/mTOR and PI3K/Akt

19. **Chemotherapy-induced autophagy has been associated with the upregulation of:**

- A. Apoptosis-related genes
- B. Autophagy-related genes (ATGs)
- C. Metabolism-related genes
- D. DNA repair genes

Answer B: Autophagy-related genes (ATGs)

20. **How does autophagy contribute to chemoresistance in cancer stem cells (CSCs)?**

- A. Promoting differentiation
- B. Inhibiting survival mechanisms
- C. Sensitizing CSCs to chemotherapy-induced cell death
- D. Promoting the maintenance of stem cell properties

Answer D: Promoting the maintenance of stem cell properties

21. **What is the role of mTOR in autophagy regulation under normal conditions?**

- A. Activates autophagy
- B. Inhibits autophagy
- C. Induces apoptosis
- D. Promotes glycolysis

Answer B: Inhibits autophagy

22. **In cancer cells, deregulated mTOR signaling is associated with:**

- A. Suppression of pro-survival pathways
- B. Inhibition of autophagy
- C. Activation of pro-survival pathways, including autophagy
- D. Downregulation of glycolysis

Answer C: Activation of pro-survival pathways, including autophagy

23. Beclin-1 is primarily found in cytoplasmic structures such as:

- A. Golgi apparatus
- B. Nucleus
- C. Mitochondria
- D. Endoplasmic reticulum (ER)

Answer C: Mitochondria

24. p53 has been shown to regulate autophagy and contribute to:

- A. Glycolysis
- B. Apoptosis
- C. Drug resistance in cancer
- D. Angiogenesis

Answer C: Drug resistance in cancer

25. Anti-apoptotic Bcl-2 family members, such as Bcl-2 and Bcl-XL, inhibit autophagy and promote:

- A. Glycolysis
- B. Senescence
- C. Drug resistance in cancer cells
- D. Apoptosis

Answer C: Drug resistance in cancer cells.

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Intracellular Compartments and Drug Resistance

9

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Abstract

Intracellular compartments play a pivotal role in cellular function, housing diverse organelles that contribute to essential processes such as protein synthesis, energy production, and waste disposal. The intricate organization of these compartments creates distinct microenvironments that impact cellular responses to external stimuli, including drug exposure. Understanding the interplay between intracellular compartments and drug resistance is crucial for advancing therapeutic strategies. One prominent example is the endoplasmic reticulum (ER), a mul-

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tifunctional organelle involved in protein folding and lipid synthesis. The ER's role in drug resistance is underscored by its participation in the unfolded protein response (UPR), a cellular stress response mechanism. Drug-induced ER stress can activate the UPR, promoting cell survival and contributing to resistance against certain chemotherapeutic agents. Likewise, mitochondria's involvement in drug resistance is multifaceted, encompassing alterations in energy production, reactive oxygen species (ROS) generation, and apoptotic signaling. Mitochondrial dysfunction can confer resistance to apoptosis-inducing drugs, allowing cancer cells to evade programmed cell death. Modulation of lysosomal function has also been linked to resistance against various anticancer drugs. Nuclear compartments, including the nucleolus and nuclear envelope, contribute to drug resistance by influencing gene expression and DNA repair mechanisms. A comprehensive understanding of intracellular compartments and drug resistance has substantial implications for therapeutic development. Targeting specific organelles or cellular processes involved in resistance mechanisms provides a promising avenue for overcoming treatment challenges.

Keywords

Intracellular compartments · Drug resistance · Cellular homeostasis · Therapeutic strategies · Unfolded protein response (UPR) · Cellular recycling

9.1 Introduction

Intracellular compartments play a pivotal role in the orchestration of cellular functions, serving as dynamic arenas where molecular processes are finely regulated (Chen et al. 2020). Understanding the intricacies of these compartments is essential for unraveling the complexities of cellular behavior and, notably, for addressing challenges in the field of pharmacology (Galy et al. 2023). One of the critical issues faced in modern medicine is the emergence and persistence of drug resistance, a phenomenon that compromises the efficacy of numerous therapeutic interventions (Nussinov et al. 2021). As we delve into the realm of intracellular compartments, it becomes evident that these structures are not mere architectural entities within the cell but rather dynamic entities that actively participate in cellular processes. The endoplasmic reticulum, Golgi apparatus, mitochondria, lysosomes, and various vesicular structures collectively form a sophisticated network that governs the synthesis, modification, and transport of biomolecules (Fig. 9.1) (Shao et al. 2023). Each compartment specializes in specific functions, contributing to the overall homeostasis and functionality of the cell (Zheng et al. 2023). Against this backdrop, the interplay between intracellular compartments and drug resistance comes to the front (Wang et al. 2022). Drug resistance, be it inherent or acquired, poses a formidable challenge in the treatment of various diseases, including cancer, bacterial

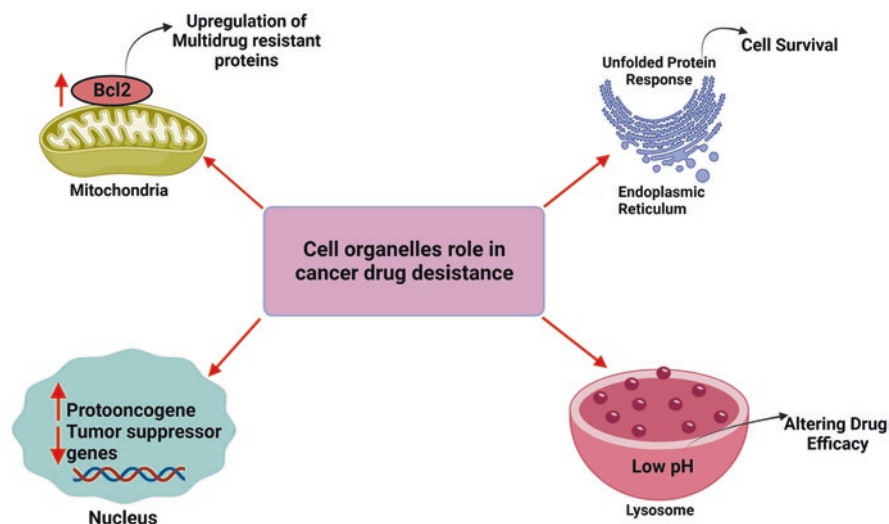


Fig. 9.1 The above diagram depicts the various factors that are dysregulated in chemoresistant cancer cells

infections, and viral illnesses (Tsimberidou et al. 2020). The ability of cells to evade the effects of therapeutic agents stems from a myriad of factors, and the role of intracellular compartments in this phenomenon is increasingly recognized (Fig. 9.1). The endocytic pathways, for instance, are integral to the internalization of drugs, influencing their bioavailability and subcellular distribution (Cole et al. 2020). Lysosomes, as the cellular recycling centers, play a dual role by sequestering drugs and contributing to drug metabolism (Geisslinger et al. 2020). Moreover, the endoplasmic reticulum and its associated signaling pathways are implicated in mediating cellular responses to drug-induced stress, influencing the fate of drug-treated cells (Sisinni et al. 2019). As we explore the relationship between intracellular compartments and drug resistance, it becomes evident that the cellular microenvironment is a dynamic landscape where pharmacological interventions must navigate intricate pathways (Münz 2021). This comprehensive understanding is imperative for devising strategies that can overcome or circumvent drug resistance, thereby enhancing the effectiveness of therapeutic interventions. In this extensive exploration, we will dissect the molecular mechanisms underpinning drug resistance within the context of intracellular compartments. From the role of drug efflux pumps to the modulation of cellular signaling cascades, we will scrutinize the diverse strategies employed by cells to withstand the attack of therapeutic agents (Lee et al. 2020). Furthermore, we will investigate the emerging technologies and methodologies that enable researchers to probe the intricacies of intracellular compartments and their involvement in drug resistance (Song et al. 2020).

9.2 Cell Organelles as a Major Contributor to Chemoresistance

Cell organelles are pivotal in the development of chemoresistance, a formidable challenge in treating diseases, especially cancer. Chemoresistance, a phenomenon where cells become less responsive to chemotherapy drugs, results in treatment failure. The mitochondria, recognized as the cell's powerhouse, are key contributors to chemoresistance due to their central role in energy production. Mitochondrial dysfunction can trigger a metabolic shift towards glycolysis, enabling cancer cells to evade chemotherapy-induced apoptosis (Genovese et al. 2021). Endoplasmic reticulum (ER) stress and unfolded protein response (UPR) pathways within the ER also contribute to chemoresistance (Khan et al. 2022a). Activation of these pathways during chemotherapy-induced stress promotes cell survival and drug resistance (Genovese et al. 2021). Lysosomes, responsible for cellular waste degradation, play a role in chemoresistance by sequestering drugs and hindering their effective action (Khan et al. 2022b). Drug efflux pumps in the plasma membrane, associated with organelles like endosomes, expel chemotherapeutic agents from the cell, reducing drug concentration (Fultang et al. 2020). Autophagy, a cellular recycling process mediated by lysosomes, protects cancer cells from chemotherapy-induced damage (Khan et al. 2023a). Understanding these intricate organelle-related mechanisms is crucial for developing strategies to overcome chemoresistance and enhance chemotherapy success.

1. *Cytosol*: Cytosol, the semi-fluid component of the cell's cytoplasm, plays a pivotal role in the development of chemoresistance in cancer and various other diseases. This resistance to chemotherapy, a common challenge in the field of medicine, can be attributed to several key factors within the cytosol (Fig. 9.2):
 - *Metabolic adaptation*: Cancer cells often display altered metabolism, favoring glycolysis over oxidative phosphorylation even in the presence of oxygen, a phenomenon known as the Warburg effect (Dai et al. 2020). This metabolic shift is largely regulated in the cytosol and contributes to chemoresistance by providing energy and biosynthetic precursors necessary for cell survival and proliferation.
 - *Detoxification pathways*: The cytosol is home to various enzymes and pathways responsible for detoxifying and eliminating chemotherapeutic drugs. One of the primary mechanisms is the glutathione-dependent detoxification pathway (Khan et al. 2023b), where glutathione molecules in the cytosol bind to and neutralize reactive drug metabolites, rendering them ineffective.
 - *Efflux transporters*: The cytosol hosts drug efflux transporters, such as P-glycoprotein (P-gp), which actively pump chemotherapeutic agents out of the cell (Abd El-Aziz et al. 2021). Overexpression of these transporters in the cytosol can significantly reduce intracellular drug concentrations, contributing to chemoresistance.
 - *DNA repair and genomic stability*: Key components of DNA repair mechanisms, such as various repair enzymes and checkpoints, operate within the cytosol (Li et al. 2021). When damaged DNA is successfully repaired, cancer

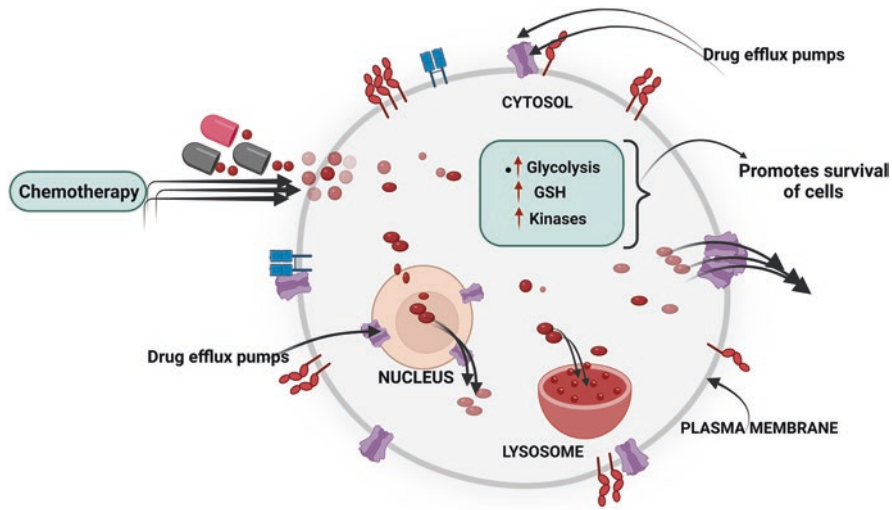


Fig. 9.2 The figure shows the general overview of various organelles and cytosol in chemoresistance

cells can escape the lethal effects of chemotherapy, making them more resistant to treatment.

- *Autophagy*: Autophagy, a cellular process for recycling and removing damaged cellular components, is controlled in part by cytosolic factors (Khan et al. 2023c). It can serve as a survival mechanism by recycling nutrients, thereby promoting the survival of cancer cells during chemotherapy-induced stress.
- *Signaling pathways*: Cytosolic signaling pathways play a role in mediating the cellular response to chemotherapy (Huang and Zhou 2021). For example, the activation of various kinases in the cytosol can trigger pro-survival signals, leading to the evasion of drug-induced cell death.
- *Microenvironment influence*: The cytosol, being a central hub of cellular activity, is influenced by the tumor microenvironment (Schulz et al. 2019). Factors such as hypoxia, nutrient availability, and interactions with neighboring cells can modulate the cytosol's response to chemotherapy and promote chemoresistance.

In summary, the cytosol is a major contributor to chemoresistance due to its involvement in diverse cellular processes that collectively enable cancer cells to withstand the cytotoxic effects of chemotherapy. Understanding these mechanisms within the cytosol is crucial for developing strategies to overcome chemoresistance and improve the efficacy of cancer treatment.

2. *Nucleus*: The nucleus of a cell plays a crucial role in chemoresistance. Several factors related to the nucleus contribute to this phenomenon (Fig. 9.3):

- *DNA repair mechanisms*: Chemotherapy drugs often work by damaging the DNA of rapidly dividing cells, including cancer cells. The nucleus contains

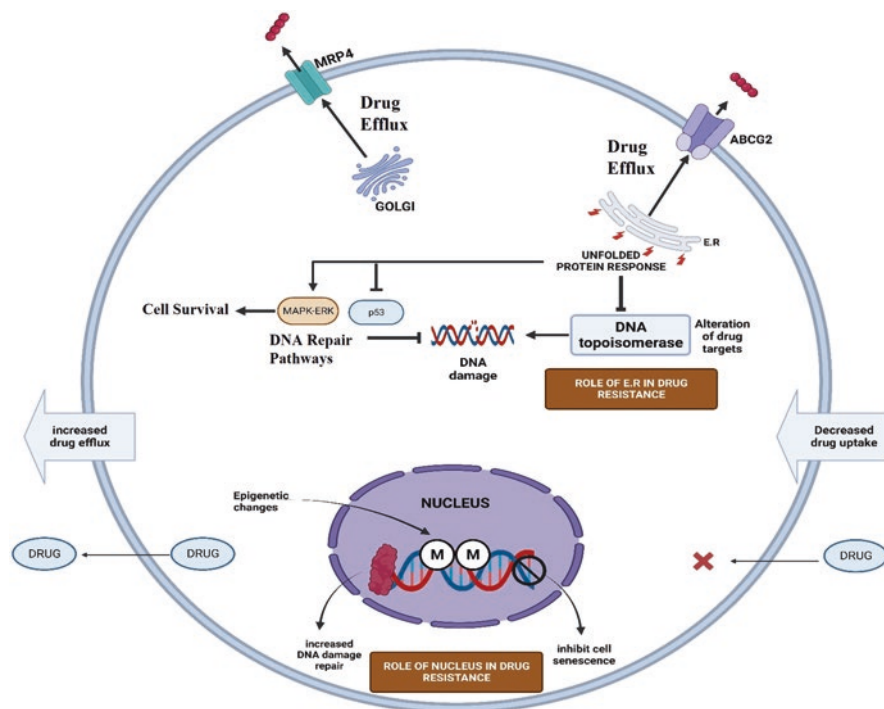


Fig. 9.3 The figure depicts the role of nucleus in chemoresistance

DNA repair mechanisms that can fix this damage, allowing cancer cells to recover and continue to replicate (Gillespie et al. 2023).

- *Altered gene expression*: Changes in the expression of genes within the nucleus can lead to the overproduction of proteins that protect cancer cells from the effects of chemotherapy drugs. These proteins can promote cell survival and drug resistance (Ciocan-Cartita et al. 2020).
 - *Enhanced drug efflux*: The nucleus can control the expression of transport proteins that pump chemotherapy drugs out of the cell, reducing their effectiveness. This is often seen in multidrug resistance, where cancer cells actively remove multiple types of chemotherapy agents (Buechel and Pinkett 2020).
 - *Epigenetic modifications*: Nucleus-related epigenetic changes can lead to alterations in gene regulation, making cancer cells less responsive to chemotherapy. This can involve changes in DNA methylation, histone modifications, and noncoding RNAs (Koo et al. 2023).
 - *Cellular dormancy*: Some cancer cells within a tumor can enter a state of dormancy, where they are less susceptible to chemotherapy. The control of this process is influenced by signals from the nucleus (Rossari et al. 2020).
3. *Mitochondria*: Mitochondria can play a significant role in chemoresistance, especially in cancer cells. They are involved in several mechanisms that contribute to resistance to chemotherapy. Some key factors include:

- *ATP production*: Mitochondria are the primary site of ATP production in cells. Chemotherapy often targets rapidly dividing cells, but cancer cells can adapt by increasing their mitochondrial ATP production, which helps them survive and resist treatment (Fu et al. 2021).
 - *ROS regulation*: Mitochondria are a major source of reactive oxygen species (ROS). In some cases, cancer cells with dysfunctional mitochondria can be less susceptible to chemotherapy-induced oxidative stress, leading to resistance (Fu et al. 2021; Khan et al. 2023d).
 - *Apoptosis regulation*: Mitochondria are central to the regulation of apoptosis or programmed cell death. Chemoresistance can result from alterations in the mitochondrial pathway of apoptosis, allowing cancer cells to evade death signals triggered by chemotherapy (Ngoi et al. 2020; Minto et al. 2021).
 - *Energy metabolism*: Mitochondria can undergo metabolic changes in cancer cells, such as the Warburg effect, which shifts energy production from oxidative phosphorylation to glycolysis. This altered metabolism can make cancer cells more resilient to chemotherapy (Dai et al. 2020).
 - *Drug efflux*: Mitochondria can be involved in the efflux of drugs from cancer cells, reducing their effectiveness. Proteins in the mitochondrial membrane can pump drugs out of the mitochondria and the cell (Giddings et al. 2021). Understanding these mitochondrial mechanisms is crucial in developing strategies to overcome chemoresistance in cancer treatment. Recent research suggests promising approaches like mitochondrial-targeted therapies and combination treatments to address this issue (Zhou et al. 2019).
4. *Lysosomes*: These are recognized for their role in cellular waste disposal and are increasingly acknowledged as major contributors to chemoresistance in cancer therapy (Halaby 2019). Several key points highlight the crucial involvement of lysosomes in this phenomenon (Fig. 9.4):
- *Drug sequestration*: Lysosomes act as cellular storage compartments, sequestering chemotherapeutic agents and reducing their concentration in the cytoplasm, hindering their anticancerous activity (Halaby 2019).
 - *pH-dependent drug activation*: Lysosomes, with their acidic environment, can inadvertently activate or degrade drugs that require specific pH conditions, altering their efficacy (Cheng et al. 2021).
 - *Autophagy and drug resistance*: Lysosomes are closely tied to autophagy, promoting chemoresistance in cancer cells by removing damaged structures or toxic substances, and aiding cell survival after chemotherapy (Khan et al. 2023b; Zamanian et al. 2023).
 - *Lysosomal membrane permeability*: Changes in lysosomal membrane integrity impact the release of toxic substances, contributing to cell death resistance and reducing drug effectiveness (Hraběta et al. 2020).
 - *Lysosomal exocytosis*: Cancer cells utilize lysosomal exocytosis, releasing lysosomal contents into the extracellular space, promoting cell survival, and creating a chemoresistant microenvironment within tumors (Halaby 2019; Wadhwa et al. 2020).

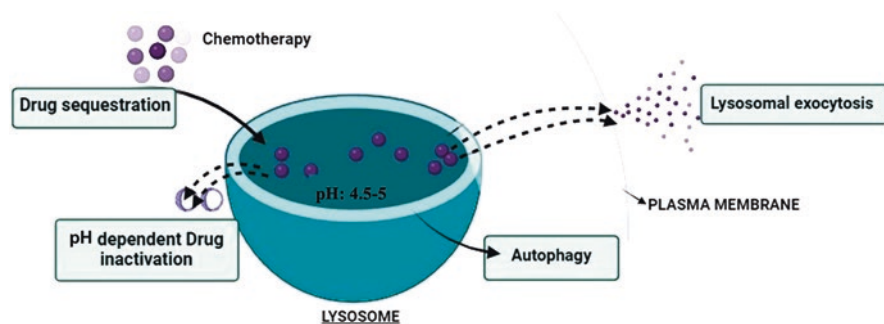


Fig. 9.4 The above diagram depicts the different roles of lysosomes in drug resistance

- *Lysosome-mediated multidrug resistance (MDR)*: Lysosomes play a pivotal role in multidrug resistance by facilitating drug efflux from the cell through lysosomal transporters (Geisslinger et al. 2020).

In conclusion, lysosomes are significant contributors to chemoresistance through their roles in drug sequestration, pH-dependent drug activation, autophagy regulation, membrane integrity, exocytosis, and the development of multidrug resistance (Luzio et al. 2007). Understanding and targeting these lysosomal processes are essential for improving cancer treatment effectiveness (Luzio et al. 2007).

5. *Endoplasmic reticulum*: The endoplasmic reticulum (ER) has emerged as a major contributor to chemoresistance, a formidable challenge in the treatment of various cancers (Avril et al. 2017). This crucial organelle plays a multifaceted role in rendering cancer cells less responsive to chemotherapy. Several factors related to the endoplasmic reticulum contribute to this phenomenon (Fig. 9.3):
 - *Drug detoxification*: The ER is involved in drug detoxification processes, such as the metabolism and elimination of chemotherapeutic agents. It can upregulate enzymes like cytochrome P450s, which can break down and inactivate drugs, reducing their effectiveness (Iyanagi 2007).
 - *Drug efflux*: The ER can influence drug efflux pumps, such as P-glycoprotein, that pump drugs out of the cell (Mutoh et al. 2006). This can lead to reduced drug accumulation within cancer cells, making them less susceptible to chemotherapy.
 - *Unfolded protein response (UPR)*: Chemotherapy-induced ER stress can trigger the unfolded protein response, leading to cell survival and resistance (Sisinni et al. 2019; Khan et al. 2023c). The UPR helps cancer cells adapt to the stress caused by chemotherapy.
 - *Apoptosis inhibition*: The ER is also involved in apoptosis regulation. In chemoresistant cells, the ER can inhibit apoptotic pathways, allowing cancer cells to evade cell death triggered by chemotherapy (Akman et al. 2021). Understanding and targeting the ER's role in chemoresistance is an active area of research to improve the effectiveness of cancer treatments (Wu et al. 2018).

6. *Golgi apparatus*: The Golgi apparatus is a crucial organelle in eukaryotic cells, responsible for processing, modifying, and packaging various molecules, including proteins and lipids (Núñez-Olvera et al. 2020). It plays a significant role in contributing to chemoresistance in several ways (Fig. 9.3):

- *Drug detoxification*: The Golgi apparatus is involved in modifying and detoxifying drugs or cytotoxic compounds. Enzymes within the Golgi can alter the chemical structure of drugs, making them less effective or promoting their elimination from the cell. This detoxification process can reduce the efficacy of chemotherapy drugs (Yang et al. 2022; Zhang et al. 2022).
- *Efflux pump regulation*: The Golgi apparatus is responsible for the synthesis and trafficking of membrane proteins, including ATP-binding cassette (ABC) transporters. ABC transporters function as efflux pumps, expelling drugs from the cell. When these transporters are overexpressed or properly localized by the Golgi, they can enhance the efflux of chemotherapy drugs, leading to reduced intracellular drug concentrations and chemoresistance (Alexa-Stratulat et al. 2019).
- *Posttranslational modifications*: Many proteins involved in drug response are glycosylated and modified in the Golgi. Altered glycosylation patterns can impact protein function and localization. Chemoresistant cells often exhibit changes in glycosylation that affect the function of drug targets, rendering them less susceptible to treatment (Xi et al. 2022).
- *Protein trafficking*: The Golgi controls the trafficking of various proteins, including those involved in apoptosis, DNA repair, and cell cycle regulation. Chemoresistance can result from the Golgi apparatus promoting the proper localization of anti-apoptotic and pro-survival proteins, ultimately preventing cell death and allowing the cell to evade the effects of chemotherapy (Liu et al. 2021; Liu et al. 2023).
- *pH regulation*: The Golgi apparatus maintains a slightly acidic pH, which is crucial for its functions. This acidic environment can contribute to chemoresistance by altering the protonation state of weakly basic chemotherapeutic agents, reducing their cellular uptake and inhibiting their cytotoxic effects (Halcrow et al. 2021).

In summary, the Golgi apparatus is a major contributor to chemoresistance through its role in drug detoxification, regulation of efflux pumps, posttranslational modifications, protein trafficking, and pH regulation. Understanding the mechanisms by which the Golgi contributes to chemoresistance is essential for developing more effective cancer treatments.

9.3 Crosstalk Between Cell Organelles and Cell Metabolism in Therapeutic Resistance

Cellular metabolism and its intricate crosstalk with various cell organelles play a pivotal role in therapeutic resistance, a phenomenon that complicates the treatment of various diseases, including cancer (Gonçalves et al. 2021). Cellular metabolism

is the sum of all biochemical reactions that occur within a cell to sustain life. This metabolic network is highly dynamic and regulated, with a multitude of pathways and reactions interlinked to support the fundamental processes necessary for cell survival, growth, and division (Vaidya et al. 2022). Cell organelles, the specialized subunits within a cell, also play a significant role in these metabolic processes (Vaidya et al. 2022). Therapeutic resistance occurs when cells adapt to treatment, rendering drugs less effective or entirely ineffective. Understanding the crosstalk between cell organelles and metabolism is essential for developing strategies to overcome therapeutic resistance.

The central player—mitochondria: Mitochondria, often referred to as the “powerhouses” of the cell, are the primary site for energy production through oxidative phosphorylation (Braun 2020). These double-membraned organelles have their DNA and machinery for generating ATP. In the context of therapeutic resistance, mitochondria are central players. Dysfunctional mitochondria can alter cellular metabolism and contribute to resistance against therapies that target bioenergetic pathways (Genovese et al. 2021). Additionally, damaged mitochondria can affect apoptotic pathways, enabling cells to evade programmed cell death induced by treatment (Fig. 9.5).

Multifunctional endoplasmic reticulum: The endoplasmic reticulum (ER) is another organelle with a multitude of functions (Khan et al. 2023c). It plays a critical role in protein synthesis, folding, and lipid metabolism. Crosstalk between the ER and metabolism is particularly evident in conditions of stress, such as the unfolded protein response (UPR) (Sisinni et al. 2019). The UPR is activated when misfolded proteins accumulate in the ER, and this can promote therapeutic resistance by altering metabolic pathways and promoting cell survival (Sicari et al. 2019).

The intricate dance of autophagy: Autophagy is a cellular process by which damaged organelles and proteins are engulfed in autophagosomes and subsequently

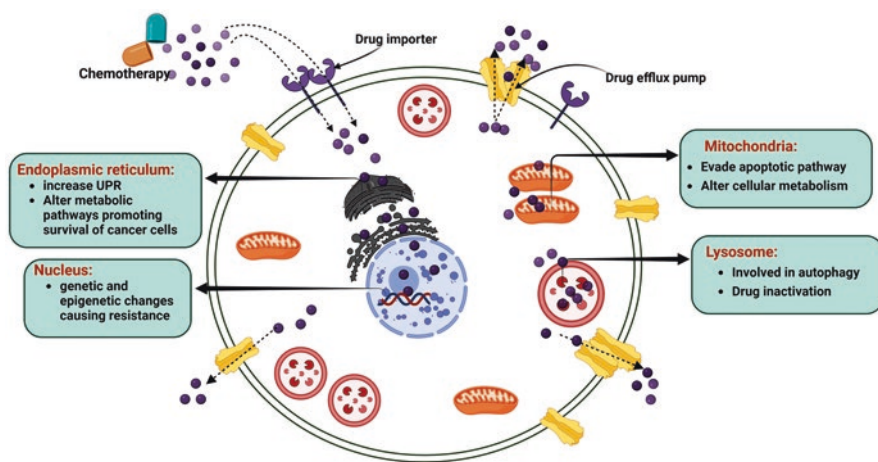


Fig. 9.5 The diagram shows the crosstalk between different organelles and their contribution to chemoresistance

degraded in lysosomes (Khan et al. 2023e). This process is tightly connected with metabolism. Autophagy is often upregulated in response to cellular stress, including exposure to therapeutic agents (Qi and Chen 2019). While it can promote cell survival under these conditions, it may also lead to therapeutic resistance by allowing cells to recover from drug-induced damage.

Lysosomes and cellular recycling: Lysosomes are acidic organelles containing various hydrolytic enzymes (Khan et al. 2022b). They play a crucial role in cellular recycling and the breakdown of cellular components. Lysosomes are also involved in programmed cell death and can influence the sensitivity of cells to therapy. The altered lysosomal function can contribute to therapeutic resistance by impairing both apoptotic and autophagic pathways (Fig. 9.5) (Geisslinger et al. 2020; Hraběta et al. 2020).

Nucleus—the genomic control center: The nucleus is the command center of the cell, housing the genetic material Alex Gamma (2013). It controls gene expression, and changes in the nucleus can significantly impact cell metabolism. Mutations and epigenetic modifications that occur in the nucleus can lead to altered metabolic pathways, which can, in turn, influence therapeutic responses (Kim et al. 2019; Sameer et al. 2024). Resistance can develop when changes in nuclear genetic material allow cells to bypass drug-induced damage.

Glycolysis and Warburg effect: Glycolysis is a metabolic pathway that occurs in the cytoplasm and is responsible for the breakdown of glucose to produce ATP (Dai et al. 2020). Cancer cells often exhibit increased glycolysis, a phenomenon known as the Warburg effect (Vaupel and Multhoff 2021). This shift in energy production can confer therapeutic resistance, as glycolysis can provide an alternative energy source when the mitochondria are compromised by therapy (Fig. 9.5).

9.4 Organelle-Specific Expression of Drug Transporters and Chemoresistance

Organelle-specific expression of drug transporters is crucial in the development of chemoresistance, where cancer cells become resistant to chemotherapy drugs (Shao et al. 2023). Understanding the mechanisms behind this expression is pivotal for advancing cancer treatment strategies. The ATP-binding cassette (ABC) transporters, key players in organelle-specific drug transport, are membrane proteins responsible for actively effluxing various drugs from cells (Alexa-Stratulat et al. 2019; Munoz et al. 2007). These transporters are found in cellular compartments like the plasma membrane, endoplasmic reticulum (ER), and mitochondria (Hee Choi and Yu 2014; Liu 2019). In the context of the ER, drug transporters like P-glycoprotein (P-gp) contribute to the efflux of chemotherapeutic agents, reducing their concentration within the ER lumen (Battaglia et al. 2023). This interference with drug-induced ER stress affects the cytotoxic effects of some chemotherapeutic drugs (Khan et al. 2023c; Wu et al. 2018). Modulating these transporters presents a potential avenue for enhancing drug sensitivity in cancer cells (Nagy et al. 2023). Mitochondria, the powerhouse of the cell, also play a critical role in

chemoresistance. ABC transporters in the mitochondrial membrane, such as ABCB1 and ABCG2, actively pump out drugs, limiting their accumulation within the mitochondria (Huang et al. 2023). Disrupting these transporters could sensitize cancer cells to mitochondrial-targeted therapies. The nuclear envelope contains transporters influencing drug accessibility to the nucleus. The nuclear pore complex (NPC) acts as a gateway for molecule transport into and out of the nucleus (Raices and D'Angelo 2012). Specific transporters in the nuclear envelope can affect the nuclear concentration of chemotherapeutic agents, impacting their ability to target DNA and induce cell death (Long et al. 2022).

In conclusion, organelle-specific expression of drug transporters significantly contributes to chemoresistance development in cancer cells. Targeting these transporters in specific cellular compartments holds promise for improving chemotherapy efficacy and overcoming resistance. Continued research in this field is essential to unravel the complexities of organelle-specific drug transport and develop innovative strategies for enhanced cancer treatment outcomes.

Cell membrane: The cell membrane, a crucial component of cellular architecture, plays a pivotal role in drug transport and chemoresistance (Huang and Sadée 2006). Within this dynamic lipid bilayer, specific drug transporters such as P-glycoprotein (P-gp) and multidrug resistance-associated protein 1 (MRP1) act as gatekeepers, influencing the intracellular concentrations of various drugs (Jacqueroux et al. 2020; Begicevic and Falasca 2017). P-gp, a well-known efflux pump, actively pumps a broad spectrum of drugs out of the cells, reducing their accumulation within the cellular interior (Sharom 2014). This efflux action is a defense mechanism that cells employ against xenobiotics, but it also poses a significant challenge in chemotherapy. The overexpression of P-gp in cancer cells, for instance, can lead to decreased intracellular drug levels, thereby diminishing the efficacy of chemotherapeutic agents (Wojtkowiak et al. 2011; Wilkens 2015). This reduction in drug concentration is a major contributor to chemoresistance, as the therapeutic impact of the drugs is compromised. Similarly, MRP1, another transporter residing in the cell membrane, contributes to chemoresistance by facilitating the efflux of diverse drugs (Munoz et al. 2007; Cole and Deeley 2006). Understanding the intricate interplay between these drug transporters and the cell membrane is crucial for devising strategies to overcome chemoresistance and enhance the effectiveness of anticancer therapies.

Endoplasmic reticulum: The endoplasmic reticulum (ER), a complex membranous network within cells, emerges as a critical player in drug transport and chemoresistance (Khan et al. 2023c). One prominent drug transporter associated with the ER is ATP-binding cassette subfamily G member 2 (ABCG2) (Wakabayashi-Nakao et al. 2009). This transporter, also known as breast cancer resistance protein (BCRP), is responsible for actively pumping various drugs out of the ER and other cellular compartments (Choudhuri and Klaassen 2006). ABCG2's role in drug transport extends beyond the ER, affecting the overall intracellular distribution of drugs. However, its overexpression in cancer cells contributes significantly to chemoresistance (Mao and Unadkat 2015). The heightened levels of ABCG2 can limit the accumulation of drugs within the ER, disrupting the pharmacokinetics of

chemotherapeutic agents (Wakabayashi-Nakao et al. 2009; Stacy et al. 2013). As a consequence, the effectiveness of these drugs is compromised, and cancer cells become less susceptible to treatment. The ER, with its intricate involvement in protein synthesis and drug metabolism, becomes a focal point in understanding the mechanisms of chemoresistance (Avril et al. 2017). Deciphering the nuances of drug transporters like ABCG2 within the ER is crucial for developing targeted interventions to overcome resistance and enhance the therapeutic outcomes of anticancer treatments.

Mitochondria: Mitochondria play a multifaceted role in cellular function, including drug transport and chemoresistance (Mani et al. 2023). Within the intricate landscape of mitochondria, a significant drug transporter is ATP-binding cassette subfamily B member 1 (ABCB1), also known as P-glycoprotein (Shtil et al. 2000). This transporter, primarily recognized for its role at the cell membrane, has been identified within the mitochondria, suggesting a previously overlooked dimension of drug transport (Dartier et al. 2017). The expression of ABCB1 in the mitochondria raises intriguing questions about its impact on drug dynamics within this organelle (Abd El-Aziz et al. 2021; Vaidyanathan et al. 2016; Engle and Kumar 2022). The overexpression of ABCB1 in the mitochondria has been associated with chemoresistance, where the increased presence of this transporter may influence drug transport processes. Mitochondria, critical for cellular energy production and apoptosis regulation, become an unexpected player in the realm of drug resistance (Khan et al. 2023a). The intricate interplay between ABCB1 and mitochondrial drug transport sheds light on potential mechanisms contributing to resistance (Abd El-Aziz et al. 2021; Choudhuri and Klaassen 2006; Sauna et al. 2007; Katayama et al. 2014).

Golgi apparatus: The Golgi apparatus, a central hub in the cellular secretory pathway, assumes a crucial role not only in protein modification and packaging but also in drug transport, thereby influencing chemoresistance (Zhang et al. 2022). Among the notable drug transporters associated with the Golgi apparatus is multi-drug resistance-associated protein 4 (MRP4) (Marin et al. 2018). MRP4, a member of the ATP-binding cassette (ABC) transporter superfamily, participates in the active efflux of a diverse range of drugs and their metabolites. The intriguing aspect lies in its presence within the Golgi apparatus, suggesting a potential influence on drug distribution within the cell. The Golgi's involvement in posttranslational modification and sorting of proteins makes it a strategic location for drug transporters to exert their effects (Zhang et al. 2022; Liu et al. 2021). The expression of MRP4 in the Golgi raises questions about its impact on the intracellular trafficking of therapeutic agents. It implies that the Golgi apparatus serves not only as a processing center for cellular products but also as a dynamic player in the modulation of drug response. Unraveling the specific role of MRP4 in the Golgi and its implications for drug distribution is integral to comprehending chemoresistance mechanisms, paving the way for targeted strategies to enhance the effectiveness of chemotherapeutic interventions.

Lysosome: Lysosomes, often regarded as the cell's recycling centers, emerge as key players in drug transport and chemoresistance, with lysosomal membrane proteins (LMPs) taking center stage as critical drug transporters within these acidic

organelles (Khan et al. 2022b; Wani et al. 2021). LMPs are integral components of the lysosomal membrane, facilitating the transport of various substances, including drugs, into and out of lysosomes. The unique acidic environment of lysosomes is essential for their role in the degradation and recycling of cellular components. However, this characteristic milieu can also impact drug sequestration within lysosomes. Alterations in lysosomal drug sequestration, driven by changes in LMP expression or function, can significantly affect drug metabolism and contribute to chemoresistance (Soukupová and Rudolf 2019). Lysosomes play a pivotal role in drug processing and degradation, and any disruptions in this process can influence the overall efficacy of therapeutic agents. The implications of lysosomal drug transport extend beyond the individual organelle, affecting the broader landscape of cellular drug response. A comprehensive understanding of the interplay between LMPs and lysosomes in drug transport is essential for unraveling the complexities of chemoresistance and devising strategies to optimize drug delivery and enhance the outcomes of anticancer treatments.

Nucleus: The nucleus, the command center of the cell housing genetic material, assumes a pivotal role in drug transport and chemoresistance, particularly through the involvement of nucleoside transporters such as equilibrative nucleoside transporter 1 (ENT1) and equilibrative nucleoside transporter 2 (ENT2). These transporters, situated on the cell membrane, are responsible for facilitating the entry of nucleosides into the cell (Endo et al. 2007). Nucleosides are crucial components of DNA and RNA synthesis, and their efficient transport is essential for various cellular processes, including the response to chemotherapeutic agents (Damaraju et al. 2003). Changes in the expression of nucleoside transporters can significantly impact drug entry into the nucleus, thereby influencing the efficacy of chemotherapy (Damaraju et al. 2003). Altered expression levels of ENT1 and ENT2 have been associated with chemoresistance, as modifications in nucleoside transporter function can affect the intracellular concentrations of drugs targeting DNA replication or repair (Mackey et al. 1998). The nucleus, being the primary site of action for many chemotherapeutic agents, becomes a focal point in understanding and overcoming resistance mechanisms (Toledo et al. 2023). Deciphering the intricate interplay between nucleoside transporters and nuclear drug entry is critical for optimizing drug delivery strategies, enhancing the effectiveness of chemotherapy, and addressing challenges posed by chemoresistance in the context of nuclear targeting.

In conclusion, delving into the intricacies of organelles and their associated drug transporters provides a profound understanding of the mechanisms underpinning chemoresistance in cells (Ross 2000). The cell membrane, with key players like P-glycoprotein (P-gp) and multidrug resistance-associated protein 1 (MRP1), showcases how active efflux mechanisms can reduce intracellular drug concentrations, laying the foundation for resistance (Cole and Deeley 2006; Jiye and Jianting 2011). Exploring the endoplasmic reticulum's role, particularly with ATP-binding cassette subfamily G member 2 (ABCG2), sheds light on how drug transport dynamics within this organelle contribute to chemoresistance (Mao and Unadkat 2015; Stacy et al. 2013). Mitochondria, traditionally recognized for energy production, reveal a new dimension with ATP-binding cassette subfamily B member 1 (ABCB1),

suggesting that mitochondrial drug transport significantly impacts resistance mechanisms (Abd El-Aziz et al. 2021; Vaidyanathan et al. 2016). The Golgi apparatus, featuring multidrug resistance-associated Protein 4 (MRP4), emerges as a strategic player in drug distribution within cells, influencing chemotherapeutic outcomes (Owatari et al. 2007). Lysosomes, governed by lysosomal membrane proteins (LMPs), highlight the importance of the acidic environment in drug sequestration and its role in chemoresistance (Circu et al. 2017). Finally, the nucleus, governed by nucleoside transporters like ENT1 and ENT2, illustrates how changes in transporter expression influence nuclear drug entry, affecting the cellular response to chemotherapy (Senyavina et al. 2016). Altogether, comprehending the orchestration of these organelles and transporters provides a holistic view of the intricate web of cellular processes contributing to chemoresistance. This knowledge becomes the cornerstone for developing targeted interventions, optimizing drug delivery, and ultimately overcoming the formidable challenges posed by chemoresistance in the realm of cancer treatment.

9.5 Organelle-Targeted Treatments in Cancer Drug Resistance

In the intricate landscape of cellular biology, organelles stand as integral components orchestrating various functions essential for the survival and proper functioning of a cell (Alberts 2010). These specialized subunits, from the energy-producing mitochondria to the protein-synthesizing endoplasmic reticulum, contribute harmoniously to cellular processes. Recognizing the pivotal role organelles play in the cellular milieu, researchers have increasingly turned their attention to developing strategies that specifically target these cellular structures in the context of cancer treatment (Rahman et al. 2022). This approach stems from the understanding that dysregulation or malfunctioning of organelles is often a hallmark of cancer cells (Warburg 1956). By honing in on these specific intracellular compartments, scientists aim to disrupt the aberrant processes that sustain cancer cell survival and proliferation. The concept of organelle-targeted treatments heralds a new era in precision medicine, offering a nuanced and sophisticated approach to combatting cancer at the cellular level (Chen et al. 2003). This strategy holds the promise of not only enhancing the efficacy of anticancer drugs but also potentially mitigating the development of drug resistance, thus opening avenues for more tailored and effective therapeutic interventions in the ongoing battle against cancer (Table 9.1) (Wallace 2012).

To meet the needs for energy in cells, the mitochondrial ETC combines the production of macromolecules like nucleotides as well as amino acids with intermediates from different pathways of metabolism, including the FAO and TCA cycle (Anderson et al. 2018; Ma et al. 2020; Pesini et al. 2014). Because disruption of the ETC invariably results in apoptosis and upsets the cellular redox equilibrium, it offers a potential method of eradicating cancerous cells (Pang et al. 2018). Mitochondrial complex I (CI), the largest complex of the ETC, transfers TCA

Table 9.1 The table provides an overview of various organelles, the components targeted within each organelle, and strategies employed to overcome drug resistance in cancer cells

Organelle	Targeted component	Strategies to overcome drug resistance
Nucleus	DNA and chromatin	<ul style="list-style-type: none"> • Disrupting DNA repair pathways to enhance the efficacy of DNA-damaging agents • Targeting DNA methylation and histone modifications to regulate gene expression and restore sensitivity to drugs
Endoplasmic reticulum (ER)	Unfolded protein response (UPR)	<ul style="list-style-type: none"> • Regulating UPR signaling to overcome adaptive responses and enhance cell death induced by ER stress • Developing drugs that selectively target the ER and induce apoptosis
Mitochondria	Electron transport chain	<ul style="list-style-type: none"> • Disrupting mitochondrial function to induce apoptosis in cancer cells, overcoming resistance • Altering mitochondrial metabolism to sensitize cells to chemotherapy and reduce drug resistance
Cell membrane	Efflux pumps	<ul style="list-style-type: none"> • Blocking drug efflux pumps to prevent the expulsion of chemotherapeutic agents from cancer cells • Utilizing nanoparticles to enhance drug delivery and bypass efflux mechanisms
Lysosomes	Acidification	<ul style="list-style-type: none"> • Disrupting lysosomal function to impair drug sequestration and enhance drug efficacy • Targeting lysosomal-mediated autophagy to sensitize cells to chemotherapy and reduce drug resistance

cycle-derived electrons from NADH to the UbQ and maintains the proton gradient on the MIM (mitochondrial inner membrane). Several inhibitors including tamoxifen, piericidin, ME-344, and metformin that directly target the respiratory complex I have gained attention as potential antitumor therapeutics (Marinello et al. 2019; Lee et al. 2019). Metformin, an antidiabetic drug has now been repurposed as an anticancer drug and was observed to inhibit CI (Marinello et al. 2019; Lee et al. 2019). By disrupting CI and bioenergetics, metformin consistently made lymphoma more sensitive to isocitrate dehydrogenase (IDH) mutant antagonists and improved lymphoma responsiveness to AZD3965, an MCT1 (monocarboxylate transporter) inhibitor (Beloueche-Babari et al. 2017). Plant-derived compound resveratrol effectively inhibited ETC complexes, particularly CIII, to cause apoptosis and disrupt several cellular functions in primary as well as resistant carcinoma cells (Chu et al. n.d.; Yan et al. 2015; Rauf et al. 2018).

The conversion of glutamine (Gln) into several metabolites provides a significant source of carbon for replenishing the TCA cycle (Cenigaonandia-Campillo et al. 2021; Xiang et al. 2015). Many kinds of drugs that target Gln metabolism in the mitochondria are currently being explored as potential cancer treatments. GLS (glutaminase) inhibitors have demonstrated a positive antitumor impact in preclinical carcinoma models. For instance, it has been shown that the GLS inhibitor

bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES) inhibits the growth of a variety of tumor types (Rajeshkumar et al. 2017; Seltzer et al. 2010).

UPR activation in cancer cells undergoing chemotherapy-induced stress contributes to pro-survival signaling, promoting cell survival and resistance to drug-induced apoptosis. By activating the UPR sensors IRE1 α , ATF6, PERK, and their main regulator GRP78, cancer cells utilize the UPR system to promote drug resistance (Avril et al. 2017). In recent years, small-molecule UPR antagonists have been discovered that specifically inhibit the UPR sensors IRE1 α , ATF6 α , and PERK as well as their regulators or effectors, including PDIA1 and eIF2 α (Doultsinos et al. 2017). Their possible usage in conjunction with chemotherapy may significantly increase the effectiveness of anticancer medications. For example, pancreatic carcinoma cells treated with ISRIB, a medication that counteracts phosphorylation of eIF2 α , showed an upsurge in gemcitabine-mediated cell death (Palam et al. 2015). The PDI antagonist 16F16, which is connected to the ATF6 α pathway, restores leukemia cells' resistance to imatinib, probably via inhibiting PDIA5 (Higa et al. 2014). On the other hand, pharmacological agents that modulate UPR have demonstrated encouraging *in vitro* outcomes. For example, glioma cells are resensitized to TMZ by epigallocatechin gallate, a medication that targets GRP78 specifically (Bhattacharjee et al. 2015).

It has been demonstrated that multidrug-resistant cells become more susceptible to chemotherapeutics when the acidity of their lysosomes is disrupted (Halaby 2019). One potential method of counteracting the buildup of weak chemotherapy bases in lysosomes is to administer lysosome alkalinizing drugs, including the vesicular H⁺-ATPase antagonist bafilomycin A1 (Ouar et al. 2003). Using lipophilic medications, which are scavenged in lysosomes but can still cause LMP (lysosomal membrane permeabilization), is an additional strategy to eliminate lysosomal drug sequestration (Shiraishi et al. 1986). Chloroquine was employed in one investigation to get resistant non-small-cell lung carcinoma cells to respond to cisplatin (Circu et al. 2017). Chloroquine has been demonstrated in another study to increase topotecan's cytotoxic impacts by blocking autophagy (Wang et al. 2011). In conclusion, the targeted modulation of cell organelles represents a promising frontier in the battle against cancer drug resistance.

9.6 Conclusion

The complicated relationship between intracellular compartments and drug resistance underscores the complexity of combating diseases at the cellular level. The dynamic interplay within these compartments, such as endosomes, lysosomes, and the endoplasmic reticulum, significantly influences drug efficacy and resistance mechanisms. Understanding the molecular intricacies that govern drug transport, metabolism, and cellular response is paramount in developing targeted therapeutic strategies. Moreover, the heterogeneity of intracellular environments poses a formidable challenge in designing drugs that can effectively navigate and exert their desired effects. Strategies aimed at modulating drug delivery systems, enhancing

cellular uptake, and circumventing resistance mechanisms within these compartments hold promise for overcoming treatment hurdles. The emergence of multidrug resistance further emphasizes the need for a comprehensive approach. Combinatorial therapies, exploiting multiple targets within distinct intracellular compartments, may represent a viable strategy to mitigate resistance and enhance treatment outcomes. Continued research into the specific molecular mechanisms orchestrating drug resistance within intracellular compartments is essential for advancing our understanding and developing innovative therapeutic interventions. In conclusion, unraveling the complexities of intracellular compartments and their role in drug resistance provides a foundation for designing more effective and targeted treatments. The integration of advanced technologies and interdisciplinary collaboration will be instrumental in navigating the intricate landscape of cellular biology to ultimately improve patient outcomes in the face of evolving drug resistance challenges.

MCQs

1. **In which intracellular compartment does protein synthesis primarily occur?**

- A. Nucleus
- B. Endoplasmic reticulum
- C. Golgi apparatus
- D. Mitochondria

Answer B: Endoplasmic reticulum

2. **Which organelle is responsible for the breakdown of cellular waste and debris?**

- A. Nucleus
- B. Lysosome
- C. Ribosome
- D. Peroxisome

Answer B: Lysosome

3. **Where are lipids synthesized within the cell?**

- A. Nucleus
- B. Endoplasmic reticulum
- C. Golgi apparatus
- D. Mitochondria

Answer B: Endoplasmic reticulum

4. **Which intracellular compartment contains the cell's genetic material?**

- A. Nucleus
- B. Endoplasmic reticulum
- C. Golgi apparatus
- D. Lysosome

Answer A: Nucleus

5. **What is the primary function of the mitochondria in a cell?**

- A. Protein synthesis
- B. Energy production

- C. Lipid synthesis
- D. Waste breakdown

Answer B: Energy production

6. **Which organelle is involved in the modification and packaging of proteins for secretion?**

- A. Nucleus
- B. Endoplasmic reticulum
- C. Golgi apparatus
- D. Lysosome

Answer C: Golgi apparatus

7. **Multidrug resistance in cancer often involves overexpression of what type of proteins?**

- A. Transporters
- B. Enzymes
- C. Receptors
- D. Hormones

Answer A: Transporters

8. **How do cancer cells develop resistance to chemotherapy drugs over time?**

- A. Increased drug sensitivity
- B. Enhanced DNA repair mechanisms
- C. Decreased cell division
- D. Reduced drug metabolism

Answer B: Enhanced DNA repair mechanisms

9. **Which intracellular compartment is targeted by antiviral drugs to inhibit viral replication?**

- A. Nucleus
- B. Endoplasmic reticulum
- C. Golgi apparatus
- D. Viral envelope

Answer A: Nucleus

10. **Which of the following is a common strategy to overcome drug resistance in cancer treatment?**

- A. Higher drug dosage
- B. Combination therapy
- C. Decreased treatment duration
- D. Single drug approach

Answer B: Combination therapy

11. **What is the primary function of the endoplasmic reticulum in drug metabolism?**

- A. Protein synthesis
- B. Lipid synthesis
- C. Drug detoxification
- D. Energy production

Answer C: Drug detoxification

12. **The Golgi apparatus is involved in the modification and sorting of which cellular components?**

- A. Proteins
- B. Lipids
- C. Nucleic acids
- D. Carbohydrates

Answer A: Proteins

13. **Which mitochondrial complex is the largest and transfers TCA cycle-derived electrons from NADH?**

- A. Complex II (CII)
- B. Complex III (CIII)
- C. Complex I (CI)
- D. Complex IV (CIV)

Answer C: Complex I (CI)

14. **What is the potential anticancer effect of metformin, an antidiabetic drug?**

- A. Promotion of cell growth
- B. Inhibition of isocitrate dehydrogenase (IDH)
- C. Disruption of mitochondrial complex IV
- D. Inhibition of mitochondrial complex I (CI)

Answer D: Inhibition of mitochondrial complex I (CI)

15. **Which plant-derived compound inhibits electron transport chain (ETC) complexes, particularly CIII, leading to apoptosis in carcinoma cells?**

- A. Tamoxifen
- B. Piericidin
- C. Resveratrol
- D. ME-344

Answer C: Resveratrol

16. **What is the significance of glutamine (Gln) conversion in cancer cells?**

- A. Reducing apoptosis
- B. Enhancing cell growth
- C. Providing a carbon source for TCA cycle replenishment
- D. Promoting cellular redox equilibrium

Answer C: Providing a carbon source for TCA cycle replenishment

17. **What role does UPR activation play in cancer cells undergoing chemotherapy-induced stress?**

- A. Inducing cell proliferation
- B. Promoting drug-induced apoptosis
- C. Enhancing drug resistance
- D. Inhibiting pro-survival signalling

Answer C: Enhancing drug resistance

18. **Which UPR sensors are activated in cancer cells, contributing to pro-survival signaling during chemotherapy-induced stress?**

- A. ATF4 and ATF6 α
- B. IRE1 α , ATF6 α , and PERK

C. GRP78 and PDIA1

D. eIF2 α and PDIA5

Answer B: IRE1 α , ATF6 α , and PERK

19. **What is the potential impact of small-molecule UPR antagonists on cancer cells?**

A. Inducing drug resistance

B. Promoting UPR activation

C. Inhibiting pro-survival signaling

D. Increasing the effectiveness of anticancer medications

Answer D: Increasing the effectiveness of anticancer medications

20. **Which pharmacological agent resensitizes glioma cells to TMZ by targeting GRP78 specifically?**

A. ISRIB

B. Epigallocatechin gallate

C. 16F16

D. PDIA1 inhibitor

Answer B: Epigallocatechin gallate

21. **How do lysosome alkalizing drugs, such as bafilomycin A1, contribute to overcoming drug resistance in multidrug-resistant cells?**

A. Inducing lysosomal membrane permeabilization

B. Increasing lysosomal acidity

C. Enhancing drug sequestration in lysosomes

D. Disrupting lysosomal acidity

Answer D: Disrupting lysosomal acidity

22. **What is one potential consequence of administering lipophilic medications in the context of lysosomal drug sequestration?**

A. Decreased lysosomal membrane permeabilization

B. Enhanced drug resistance

C. Induction of autophagy

D. Lysosomal membrane permeabilization

Answer D: Lysosomal membrane permeabilization

23. **In the context of the cell membrane, which drug transporter contributes to chemoresistance by actively pumping a broad spectrum of drugs out of cells?**

A. ABCB1

B. Multidrug resistance-associated protein 1 (MRP1)

C. P-glycoprotein (P-gp)

D. ABCG2

Answer C: P-glycoprotein (P-gp)

24. **What is the primary role of lysosomes in chemoresistance, and how do they hinder drug action?**

A. ATP production

B. DNA repair

- C. Drug sequestration
- D. Apoptosis regulation

Answer C: Drug sequestration

25. How can the nucleus contribute to multidrug resistance by controlling the expression of certain proteins?

- A. Enhanced drug concentration
- B. Increased drug sequestration
- C. Enhanced drug efflux
- D. Activation of DNA repair mechanisms

Answer C: Enhanced drug efflux

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Gut Microbes: Role in Cancer and Cancer Drug Resistance

10

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Abstract

The gut microbiota is becoming more and more of a research area in many diseases, including cancer, obesity, diabetes, brain disease, rheumatoid arthritis, and cardiovascular disease. The human digestive tract contains about 100 trillion microorganisms. Cancer is the most prevalent malignancy in the world. The likelihood of survival can be increased by an accurate, early diagnosis and the necessary medical care. Recent studies have demonstrated that the microbiome has an impact on cancer. Different microbial signatures with various patterns have been found in the cancer, depending on the stage and biological subgroups. In cancer, the gut microbiota has been shown to modulate the efficacy of anticancer drugs. The changed gut microbiota is linked to resistance to immunological checkpoint

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inhibitors (ICIs) and chemotherapy treatments, whereas the addition of certain species of bacteria can restore the responsiveness to anticancer medications. Various evidence suggested the potential of gut microbiota manipulation to increase the effectiveness of anticancer medications. In this book chapter, we focused on the gut microbiota population and its relationship to cancer therapy resistance, with a particular emphasis on its potential to serve as a biomarker for the disease. Despite the important results from preclinical models and patient clinical data, a deeper comprehension of the interactions between microbiota and cancer therapy aids in the identification of novel strategies for cancer prevention, the stratification of patients for more effective treatment, and the reduction of treatment complications.

Keywords

Cancer · Microbiome · Drug resistance · Cancer therapeutics · Immunotherapy · Biomarker

10.1 Introduction

After heart disease, the second most common cause of death is cancer worldwide. In women, breast cancer cases of approximately 287,850 were diagnosed in 2022 alone (Sung et al. 2021; Turashvili and Brogi 2017). In 15–20% of cancer cases, the microorganisms are responsible for contributing to cancer development as shown by various studies (Morales-Sánchez and Fuentes-Pananá 2014; Khan et al. 2022a). The body of a human is a complicated system of symbiosis with a wide range of host cells (human) and diverse microbial cells (Chow et al. 2010) (Fig. 10.1). The microbes are widely distributed in our body, including the gastrointestinal tract, consisting of a wide range of microbial colonies. In our body, the count of these microbes is around 100 trillion, and these microbes play an important role in the disease and health (Sender et al. 2016; Gilbert et al. 2018; Lynch and Pedersen 2016; Allen and Sears 2019). Identification of microbial signatures is possible with the advent in the techniques involved in sequencing. In cancer also, these dysbiotic microbial signatures took part in the modulation of the commencement and disease progression (Kadosh et al. 2020). Various studies provide valuable evidence related to the composition and regulation of the microbiome. Initiation of the formation of tumors and in vivo promotion of tumor growth is also under the influence of diverse microbes as reported in these studies (Parida et al. 2021; Hwang et al. 2020; Routy et al. 2018; Daillère et al. 2016; Guo et al. 2020). Using the following three main mechanisms, most of the microbes persuade cancer: (1) by controlling the immune system of host, (2) by controlling the metabolism of the host cell, and (3) by alteration of the balance between the proliferation of the cell and death. The pathway in the host cell, Wnt/ β -catenin, which is responsible for the cell growth and the polarity is shifted by these microbiota by altering the balance and is reflected in the progression of the tumor (de Sousa E Melo and Vermeulen 2016). Various proteins—Avra

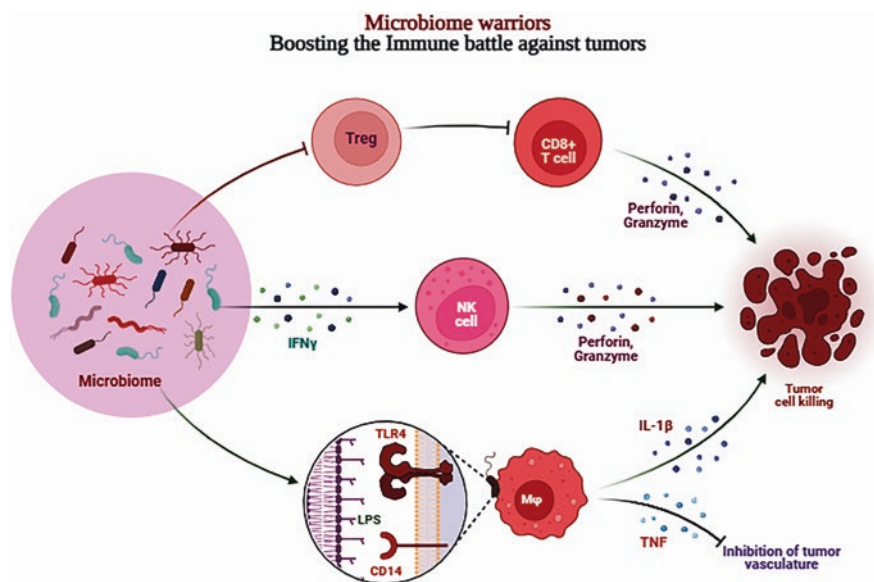


Fig. 10.1 The figure depicts the complex interaction between cancer, the immune system, and the gut microbiota

(*Salmonella typhi*), CagA (*Helicobacter pylori*), and FadA (*Fusobacterium nucleatum*)—are responsible for causing hepatobiliary, stomach, and colorectal cancer by activation of the β -catenin (Guo et al. 2020).

The microbiota in order to kill aggressive bacteria as the mechanism for survival in its surroundings secretes bacterial toxins which are also responsible for mediating the breaks in double-stranded DNA. They also lead to carcinogenesis by mediating DNA damage at the cellular level (Mellemegaard and Gaarslev 1988). Some of the bacterial toxins that mediate the breaks in the double-stranded DNA comprise *B. fragilis* secreting *Bacteroides fragilis* toxin (BFT), cytolethal expansive toxin (CDT) from ϵ - γ -*Proteobacteria*, and colibactin from *Enterococcaceae* (Oliero et al. 2022). The immune system of the host cell and the inflammation process are also mediated and controlled by the microbiota. The microbiota stimulates both immune responses including innate and adaptive (Grivennikov 2013) (Fig. 10.2). In cancer immunotherapy, it is apparent that the microbiota and the gut act as a double-edged sword, as the cancer treatment side effects and the efficacy can be altered by the gut microbiota (Li et al. 2021). The microbiome in the gut contributes towards the modulation of the tumor microenvironment locally via affecting the mucosal immunity and remodeling the tissue or by promoting the inflammation thereby inducing the tumor and hence contributing to the commencement and the succession of the tumor (Fulbright et al. 2017).

Nevertheless, it has also been reported that the regulation of the anticancer immune response, the protection of the host cells from inflammation which is tactless, and the protection of the tumor microenvironment are under the influence of

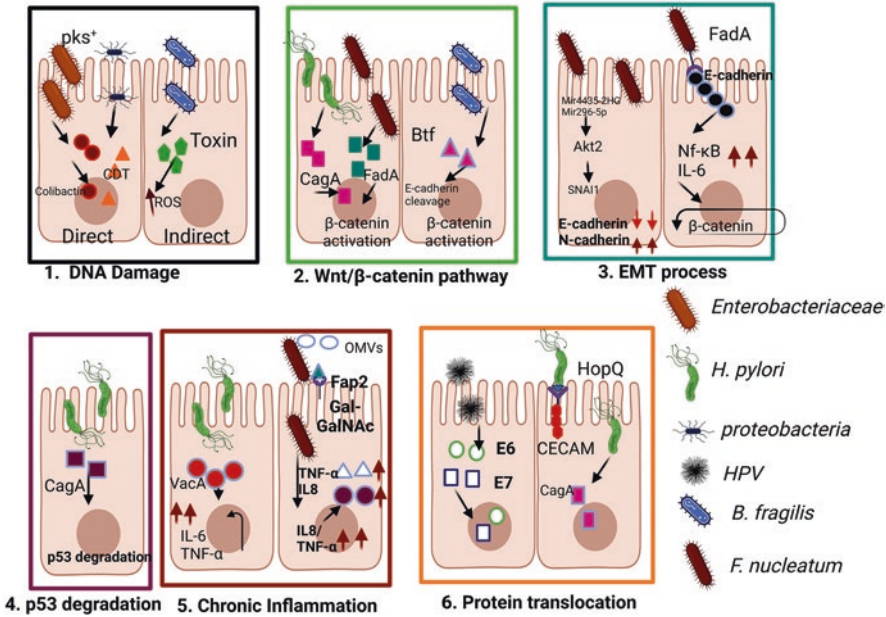


Fig. 10.2 The figure illustrates the various molecular pathways that the intratumor microbiota may use to encourage cancer progression

few gut microbes. In this book chapter, we discussed the extensive outlook of the gut microbiome and the cancer. By influencing the growth of tumors and the therapeutic response, the gut microbiome has influenced cancer immunotherapy directly and indirectly.

10.2 The Population of Microbiota in the Human Gut

Inside the digestive tract, the microbial population varies in the gut microbiota. Few bacterial species are present in the stomach and in the small intestine (Hillman et al. 2017). In contrast, the highest microbial density of any microbial community that is studied so far and associated with humans is present in the colon which constitutes between 300 and 1000 varied species (Shapira 2016). These are abundantly present in the intestine, and as a result, they also constitute 60% of feces as dry mass. An estimated 99% of gut bacteria are anaerobic, while the bacteria that are aerobic achieve elevated densities in the cecum. In comparison to the human genome, the gut flora has a hundred times the count of genes (Qin et al. 2010).

The bacterial phyla that are more dominant in the gut of humans comprise *Actinomycetota*, *Bacteroidota*, *Pseudomonadota*, and *Bacillota* (*Firmicutes*) (Khanna and Tosh 2014). The genera to which the bacteria mostly belong include *Clostridium*, *Faecalibacterium*, *Bacteroides*, *Peptococcus*, *Ruminococcus*, *Eubacterium*, *Bifidobacterium*, and *Peptostreptococcus* (Guarner and Malagelada

2003; Beaugerie et al. 2003). To a lesser extent, *Lactobacillus* and *Escherichia* genera are present (Guarner and Malagelada 2003; Quigley 2013). The bacteria which constitutes around 30% of the gut bacteria is the species of the genus *Bacteroides* indicating that the specific genus is crucial for the host functioning (Sears 2005). The gut also comprises the genera of fungi of *Saccharomyces*, *Penicillium*, *Candida*, *Trametes*, *Aspergillus*, *Bullera*, *Galactomyces*, *Sclerotinia*, and *Pleospora* among others (Cui et al. 2013). The stomach has particular bacteria that inhabit therein comprised mainly of *Staphylococcus*, *Peptostreptococcus*, *Lactobacillus*, and *Streptococcus*. The *Helicobacter pylori* bacterium is spiral in shape, gram-negative, and is responsible for establishing the gastric mucosa, thereby responsible for causing peptic ulcer disease, acts as a potential gastric cancer carcinogen, and gastritis (Wroblewski et al. 2010). Due to the influence of the stomach and proximity, fewer microorganisms are present in the small intestine. Mainly the microorganisms which are present in the intestine are bacteria which are rod-shaped and gram-positive cocci. *Enterobacteriaceae* (gram-negative bacteria) are present in the distal part of the small intestine and are supported by the alkaline conditions (Tannock 1995). In diverse functions of the intestine, the bacterial flora plays wide role by gut utility and the development via providing various signals that are regulatory in nature, while as the bacterial overgrowth in small intestine can lead to intestine failure (Andrew et al. 2007). There is an abundance of bacteria in the large intestine. Obligate anaerobes such as *Bifidobacterium* and *Bacteroides* constitute around 99% of the large intestine (Maier et al. 2014). The relationship between humans and the gut flora is a mutualistic, symbiotic relationship rather than merely a commensal (co-existence which is not harmful) (Haque and Haque 2017). Microorganisms play a wide range of functions which include hormonal production that directs the host for fat storage; fermentation of the energy substrates that are unused; immune system training by acetates and propionates which are the end products of metabolism; and regulation of gut development by producing vitamins (e.g., vitamin K and biotin) for the host (Rowland et al. 2018). Gut microbiota imbalance and the various modifications in its gene collection or microbiome are allied with obesity. Nevertheless, in a few conditions, few bacterial species cause disease by causing infection or by enhancing the host's risk for cancer (Fig. 10.3) (Davis 2016; Kho and Lal 2018).

Microbiota as a Potential Biomarker in Cancer

The gut microbiota has gained increasing attention in recent years as a potential biomarker and modulator of cancer development, progression, and treatment response (Veizant et al. 2021). Research suggests that alterations in the gut microbiota composition, known as dysbiosis, may contribute to cancer development and affect the efficacy of cancer therapies (Vivarelli et al. 2019).

Here are a few key points regarding the gut microbiota as a potential biomarker in cancer:

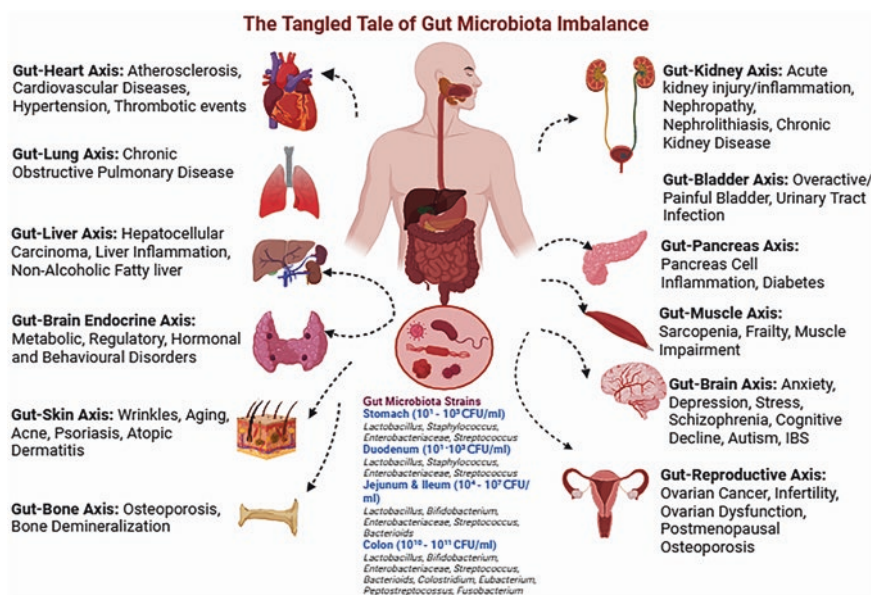


Fig. 10.3 Gut microbial strains and negative health outcomes of gut microbial dysbiosis

Cancer risk assessment: Certain microbial signatures or dysbiotic patterns have been associated with an increased risk of developing certain types of cancer (Bhatt et al. 2017). For example, studies have shown that individuals with colorectal cancer often exhibit distinct microbial compositions compared to healthy individuals. These microbial changes may serve as potential biomarkers for cancer risk assessment (Zwezerijnen-Jiwa et al. 2023).

Cancer diagnosis: The gut microbiota has the potential to be used as a diagnostic tool for cancer. Studies have demonstrated that specific microbial profiles can distinguish between cancer patients and healthy individuals (Zhou et al. 2021). Additionally, the detection of certain microbial metabolites or products in body fluids, such as blood or urine, may also aid in cancer diagnosis (Sarhadi and Armengol 2022).

Treatment response: The gut microbiota can influence the response to cancer treatment, including chemotherapy, immunotherapy, and radiotherapy (Ma et al. 2019; Khan et al. 2023a). Studies have shown that specific microbial compositions can enhance or hinder the effectiveness of cancer therapies (Bhatt et al. 2017). By analyzing the gut microbiota before treatment, it may be possible to predict treatment response and personalize therapeutic strategies (Ting et al. 2022).

Prognosis and survival: Emerging evidence suggests that the gut microbiota composition may have prognostic value in cancer. Studies have found associations between certain microbial signatures and patient outcomes, such as overall survival and recurrence rates. However, more research is needed to establish the significance

of these associations and their clinical applicability (Ting et al. 2022; Oliva et al. 2021).

It's important to note that while there is growing evidence supporting the role of gut microbiota as a biomarker in cancer, further research is needed to establish robust and reliable associations (Bhatt et al. 2017). The field of microbiota-based biomarkers is still in its early stages, and more studies are required to validate findings, standardize methodologies, and understand the underlying mechanisms (Hajjo et al. 2022). Nonetheless, the potential of the gut microbiota as a biomarker in cancer holds promise for improving cancer prevention, diagnosis, and treatment in the future (Bultman 2016).

Gut Microbiota that Facilitates the Development and Progression of Cancer

While the gut microbiota is generally beneficial for human health, certain microbial imbalances or specific bacteria within the gut microbiota have been associated with the facilitation of cancer development and progression (Durack and Lynch 2019). Here are some examples:

***Fusobacterium nucleatum*:** *Fusobacterium nucleatum* is a bacterium commonly found in the oral cavity and gastrointestinal tract (Han 2015). It has been associated with colorectal cancer and has been found to promote tumor growth and metastasis. *F. nucleatum* can interact with immune cells, promote inflammation, and facilitate tumor cell adhesion and invasion (Wu et al. 2019).

***Helicobacter pylori*:** *Helicobacter pylori* is a bacterium known to cause chronic gastritis, and peptic ulcers, and is a risk factor for gastric cancer (Wroblewski et al. 2010). Chronic infection with *H. pylori* can induce inflammation, damage the gastric mucosa, and lead to genetic alterations in host cells, increasing the risk of developing gastric cancer (Kumar et al. 2021; Piscione et al. 2021).

***Escherichia coli*:** Certain strains of *Escherichia coli* (*E. coli*), particularly those harboring a pathogenicity island called polyketide synthases (pks), have been associated with colorectal cancer (Yang et al. 2019; Lucas et al. 2020). These strains can produce a genotoxin called colibactin, which causes DNA damage in host cells, promoting the development of cancer (Wang and Fu 2023).

***Bacteroides fragilis*:** While *Bacteroides fragilis* is a normal component of the gut microbiota, specific strains producing a toxin called *Bacteroides fragilis* toxin (BFT) have been linked to colorectal cancer (Valguarnera and Wardenburg 2020). BFT can disrupt the integrity of the intestinal epithelium, promote inflammation, and contribute to tumor formation (Cheng et al. 2020).

Streptococcus gallolyticus (formerly known as *Streptococcus bovis*): *Streptococcus gallolyticus* is a bacterium associated with colorectal cancer, particularly in individuals with inflammatory bowel disease (Kumar et al. 2017; Taddese et al. 2021). It has been hypothesized that *S. gallolyticus* may promote tumor formation through its ability to adhere to and colonize damaged intestinal tissues and induce inflammation (Taylor et al. 2023).

It's important to note that the presence of these bacteria does not guarantee the development of cancer, and other factors, such as an individual's genetic predisposition and overall health, play a role as well (Anand et al. 2008). Additionally, the mechanisms by which these bacteria contribute to cancer development are still being studied, and more research is needed to fully understand their specific roles (Zheng et al. 2020; Sedighi et al. 2019). Overall, while the gut microbiota is essential for human health, imbalances or specific bacteria within the microbiota can potentially promote cancer development and progression through various mechanisms, including inflammation, DNA damage, and disruption of the gut barrier (Zhang et al. 2015).

Interplay Between Microbiome and Treatment Resistance in Cancer

The interplay between the microbiome and treatment resistance in cancer is an area of active research. Emerging evidence suggests that the gut microbiome can influence the response to cancer treatment, including chemotherapy, immunotherapy, and targeted therapy (Ting et al. 2022; Sevcikova et al. 2022). Here are some ways in which the microbiome may impact treatment resistance:

Chemotherapy: The gut microbiome can affect the efficacy and toxicity of chemotherapy drugs. Certain bacteria in the gut can metabolize or modify chemotherapy drugs, altering their effectiveness (Ervin et al. 2020; Wani et al. 2021). For example, studies have shown that certain bacteria can deactivate or reduce the bioavailability of chemotherapeutic agents, leading to treatment resistance (Mansoori et al. 2017). On the other hand, some bacteria can enhance the efficacy of chemotherapy by activating prodrugs or producing metabolites that have anticancer effects (Sedighi et al. 2019).

Immunotherapy: The gut microbiome plays a crucial role in modulating the response to immunotherapy, particularly immune checkpoint inhibitors (ICIs) (Li et al. 2022). The gut microbiota can influence the activation of the immune system and the effectiveness of ICIs (Aghamajidi and Maleki Vareki 2022). Specific bacteria in the gut have been associated with improved response to immunotherapy, while others have been linked to resistance. Microbes in the gut can impact the diversity and function of immune cells, alter the tumor microenvironment, and affect the production of immune-modulating metabolites (Zheng et al. 2020).

Targeted therapy: The gut microbiome can potentially influence the response to targeted therapy, which aims to inhibit specific molecular targets in cancer cells (Ting et al. 2022). The microbiome may affect the absorption, metabolism, and elimination of targeted drugs, influencing their efficacy and toxicity (Li et al. 2016). Additionally, microbial metabolites and signaling molecules produced in the gut can interact with the pathways targeted by the therapy, potentially modulating treatment response (Descamps et al. 2019).

Treatment-related toxicity: The gut microbiome can influence the development and severity of treatment-related side effects (Song and Bai 2021). For example,

certain bacteria can produce enzymes that activate prodrugs into toxic metabolites, leading to increased chemotherapy-induced toxicity (Sedighi et al. 2019). On the other hand, a healthy and diverse gut microbiota may protect against treatment-related toxicities by enhancing the repair mechanisms in the gut and supporting overall immune function (Mukherjee et al. 2018).

Understanding the complex interactions between the gut microbiome and cancer treatment is an area of ongoing research. Strategies such as fecal microbiota transplantation (FMT) and the use of probiotics or prebiotics to modulate the gut microbiota are being explored as potential approaches to enhance treatment response and reduce treatment resistance (Fong et al. 2020). However, more research is needed to determine the specific mechanisms involved and to develop reliable strategies for manipulating the microbiome to improve cancer treatment outcomes.

Cyclophosphamide: Cyclophosphamide is a chemotherapy drug commonly used in the treatment of various cancers (Patel et al. 2023). The interplay between the gut microbiota and cyclophosphamide treatment has been a subject of research, and emerging evidence suggests that the gut microbiota can influence the efficacy and toxicity of cyclophosphamide (Ting et al. 2022). Here are some key points regarding this interplay:

Metabolism of cyclophosphamide: The gut microbiota can metabolize cyclophosphamide, converting it into various metabolites that can have different effects on the body (Chen et al. 2021). Certain bacteria, such as *Enterococcus hirae* and *Eggerthella lenta*, possess enzymes that can activate cyclophosphamide into its active form, which is responsible for its anticancer activity. The presence of these bacteria may enhance the effectiveness of cyclophosphamide (Fig. 10.4) (Hannachi and Camoin-Jau 2021).

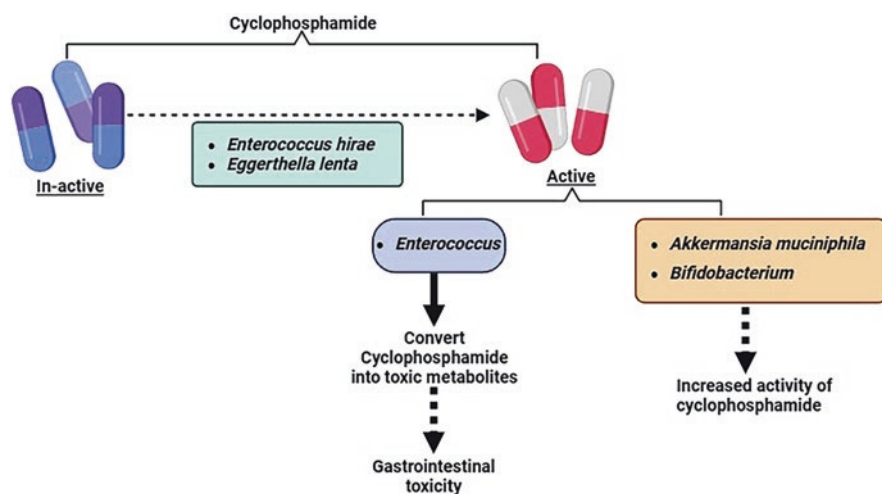


Fig. 10.4 The above figure shows the role of various microbes in the activation of cyclophosphamide as well as its conversion to toxic metabolites

Impact on efficacy: Studies in animal models and some clinical observations suggest that specific gut microbial communities can enhance the efficacy of cyclophosphamide treatment (Xu and Zhang 2015). For example, certain bacteria, like *Akkermansia muciniphila* and certain strains of *Bifidobacterium*, have been associated with improved responses to cyclophosphamide in preclinical cancer models (Fig. 10.4). These bacteria may modulate the immune system and contribute to the antitumor effects of cyclophosphamide (Fong et al. 2020; Qi et al. 2022).

Influence on toxicity: Cyclophosphamide can cause side effects, such as gastrointestinal toxicity and immunosuppression. The gut microbiota has been implicated in mediating these toxicities (Boussios et al. 2012). Certain bacteria, such as the genus *Enterococcus*, can activate cyclophosphamide into toxic metabolites that contribute to gastrointestinal toxicity (Daillère et al. 2016). Additionally, dysbiosis or alterations in the gut microbiota composition may lead to increased susceptibility to cyclophosphamide-induced toxicity (Fig. 10.4) (DeGruttola et al. 2016).

Modulation of microbiota: Strategies to modulate the gut microbiota have been explored to potentially improve the efficacy and reduce the toxicity of cyclophosphamide. For example, preclinical studies have shown that the use of probiotics or specific bacteria can modify the gut microbiota composition and enhance the anti-tumor effects of cyclophosphamide (Fong et al. 2020). Additionally, fecal microbiota transplantation (FMT) from responders to cyclophosphamide treatment into non-responders has demonstrated improved treatment outcomes (Xu et al. 2022).

It's important to note that while the interplay between the gut microbiota and cyclophosphamide treatment shows promise, more research is needed to establish consistent associations and to better understand the underlying mechanisms (Bhatt et al. 2017). Factors such as individual variations in the gut microbiota and the complex interactions between different microbial species can influence the outcomes. Nevertheless, these findings highlight the potential of the gut microbiota as a modifiable factor that may impact the response to cyclophosphamide treatment in cancer patients (Vivarelli et al. 2019).

Platinum-based derivatives: Platinum-based derivatives, such as cisplatin and carboplatin, are widely used in cancer chemotherapy, particularly for the treatment of solid tumors (Zhang et al. 2022a; Khan et al. 2023b, c; Wadhwa et al. 2020). The interplay between the gut microbiome and platinum-based derivative treatment is an emerging area of research (Wiertsema et al. 2021). While the specific mechanisms are not yet fully understood, here are some points regarding the potential interplay between the gut microbiome and treatment resistance in cancer:

Metabolism of platinum-based derivatives: The gut microbiome can potentially influence the metabolism and activation of platinum-based derivatives (Rottenberg et al. 2021). Some studies suggest that specific bacterial enzymes can activate or modify platinum-based drugs, affecting their pharmacokinetics and therapeutic efficacy (Ting et al. 2022). Conversely, the gut microbiome might also play a role in inactivating or detoxifying these drugs, potentially leading to treatment resistance (Dasari and Tchounwou 2014).

Impact on drug distribution: The gut microbiome can potentially influence the distribution and availability of platinum-based derivatives within the body.

Alterations in the gut microbiome composition may affect drug absorption, transport, and distribution, potentially impacting the drug's bioavailability and efficacy (Zhang et al. 2021). Understanding these interactions may provide insights into treatment response variations.

Modulation of the tumor microenvironment: The gut microbiome can indirectly influence the tumor microenvironment, which can impact the response to platinum-based chemotherapy (Gopalakrishnan et al. 2018). Certain bacteria in the gut can modulate the immune system and systemic inflammation, which can influence the effectiveness of chemotherapy. Additionally, the gut microbiome can produce metabolites that may influence the tumor microenvironment and affect treatment response (Aghamajidi and Maleki Vareki 2022).

Gut microbiome and treatment-related toxicity: Platinum-based derivatives can cause significant treatment-related toxicities, including gastrointestinal toxicity and nephrotoxicity. The gut microbiome has been implicated in mediating these toxicities (Oh et al. 2021). Dysbiosis or alterations in the gut microbiota composition might affect the metabolism and detoxification of platinum-based drugs, potentially impacting treatment-related toxicities (Enright et al. 2016).

Modulation of the microbiome: Strategies aimed at modulating the gut microbiome have been explored to potentially enhance the efficacy and reduce toxicity of platinum-based chemotherapy (Chrysostomou et al. 2023). These include the use of probiotics, prebiotics, and fecal microbiota transplantation (FMT) to influence the gut microbiota composition. Preclinical studies have shown that certain microbial communities or interventions can impact treatment response and toxicity profiles in platinum-based chemotherapy (Fong et al. 2020).

It's important to note that while there is growing evidence of the interplay between the gut microbiome and platinum-based derivative treatment, more research is needed to establish consistent associations and to elucidate the underlying mechanisms. The complexity of the gut microbiome and individual variations in microbial composition necessitate further investigation. Nonetheless, understanding and manipulating the gut microbiome may hold promise for optimizing platinum-based chemotherapy outcomes in cancer patients.

Gemcitabine: The interplay between the gut microbiome and gemcitabine, a chemotherapy drug commonly used to treat various cancers, is an emerging area of research (Fu et al. 2021). While our understanding is still evolving, here are some key points regarding the potential interplay between the gut microbiome and gemcitabine treatment:

Metabolism of gemcitabine: The gut microbiome can potentially influence the metabolism and effectiveness of gemcitabine (Toschi et al. 2005). Studies suggest that certain bacteria in the gut can metabolize gemcitabine, leading to its inactivation or modification. These microbial-mediated metabolic processes may affect the drug's pharmacokinetics, bioavailability, and therapeutic efficacy (Geller and Straussman 2018).

Impact on drug activation: Gemcitabine requires activation within cells to exert its anticancer effects. The gut microbiome could potentially influence the activation process by producing enzymes that can activate or deactivate the drug. However, the

specific microbial species involved in gemcitabine metabolism and their clinical relevance are not yet well established (Sevcikova et al. 2022).

Modulation of treatment response: The gut microbiome may indirectly impact the treatment response to gemcitabine by influencing the immune system and systemic inflammation (Belkaid and Hand 2014). Certain gut bacteria have been associated with immune modulation, and alterations in the gut microbiota composition might influence the tumor microenvironment and affect treatment efficacy (Aghamajidi and Maleki Vareki 2022).

Gut microbiome and treatment-related toxicity: Gemcitabine can cause toxicities, such as gastrointestinal side effects and myelosuppression. The gut microbiome has been implicated in mediating these toxicities (Gori et al. 2019). Dysbiosis or alterations in the gut microbiota may affect the metabolism, absorption, or detoxification of gemcitabine, potentially contributing to treatment-related toxicities (Toschi et al. 2005).

Modulation of the microbiome: Strategies aimed at modulating the gut microbiome have been explored to potentially improve the efficacy and reduce toxicity of gemcitabine treatment. Preclinical studies have shown that interventions such as probiotics, prebiotics, or fecal microbiota transplantation (FMT) can influence gut microbiota composition and impact treatment outcomes (Baldi et al. 2021). However, more research is needed to determine the clinical applicability and effectiveness of these approaches (Fong et al. 2020).

It's important to note that the interplay between the gut microbiome and gemcitabine treatment is still a relatively new area of research, and more studies are needed to establish robust associations and understand the underlying mechanisms. The complex nature of the gut microbiome and individual variations in microbial composition further complicate the analysis. Nonetheless, investigating and manipulating the gut microbiome may hold promise for optimizing gemcitabine chemotherapy outcomes in the future.

Anthracyclines: The interplay between the gut microbiome and anthracyclines, a class of chemotherapy drugs that includes doxorubicin and daunorubicin, is an area of emerging research (Cejas et al. 2024). While our understanding is still evolving, here are some key points regarding the potential interplay between the gut microbiome and anthracycline treatment:

Metabolism of anthracyclines: The gut microbiome can potentially influence the metabolism and efficacy of anthracycline drugs. Studies suggest that certain bacteria in the gut can metabolize anthracyclines, leading to their inactivation or modification (Li et al. 2016). These microbial-mediated metabolic processes may impact the drug's pharmacokinetics, bioavailability, and therapeutic effectiveness.

Impact on drug activation: Anthracyclines require intracellular activation to exert their cytotoxic effects. The gut microbiome could potentially influence the activation process by producing enzymes that activate or deactivate the drugs (Yin et al. 2022). However, the specific microbial species involved and their clinical relevance in anthracycline metabolism are not yet well established.

Modulation of treatment response: The gut microbiome may indirectly influence treatment response to anthracyclines by influencing the immune system and

systemic inflammation (Ciernikova et al. 2021). Certain gut bacteria have been associated with immune modulation, and alterations in the gut microbiota composition might impact the tumor microenvironment and treatment efficacy (Kunika and Rangrez 2023).

Gut microbiome and treatment-related toxicity: Anthracyclines can cause significant treatment-related toxicities, such as cardiotoxicity and gastrointestinal side effects. The gut microbiome has been implicated in mediating some of these toxicities (Huang et al. 2022a). Dysbiosis or alterations in the gut microbiota might affect the metabolism, absorption, or detoxification of anthracyclines, potentially contributing to treatment-related toxicities.

Modulation of the microbiome: Strategies aimed at modulating the gut microbiome have been explored to potentially improve the efficacy and reduce the toxicity of anthracycline treatment (Feng et al. 2020). Preclinical studies have shown that interventions such as probiotics, prebiotics, or fecal microbiota transplantation (FMT) can influence gut microbiota composition and impact treatment outcomes (Baldi et al. 2021). However, more research is needed to determine the clinical applicability and effectiveness of these approaches.

It's important to note that the interplay between the gut microbiome and anthracycline treatment is still a relatively new area of research, and more studies are needed to establish robust associations and understand the underlying mechanisms. The complex nature of the gut microbiome and individual variations in microbial composition further complicate the analysis. Nonetheless, investigating and manipulating the gut microbiome may hold promise for optimizing anthracycline chemotherapy outcomes in the future.

Taxanes: The interplay between the gut microbiome and taxanes, a class of chemotherapy drugs that includes paclitaxel and docetaxel, is an emerging area of research (Khan et al. 2023a, b; Hertz 2021; Smorenburg et al. 2001). While our understanding is still evolving, here are some key points regarding the potential interplay between the gut microbiome and taxane treatment:

Impact on treatment efficacy: The gut microbiome may influence the efficacy of taxane treatment. Studies in preclinical models have shown that specific gut bacteria can enhance the antitumor effects of taxanes. Certain bacteria, such as *Akkermansia muciniphila*, have been associated with improved taxane response and increased tumor sensitivity to these drugs (Sevcikova et al. 2022; Zhang et al. 2019). These bacteria may modulate the immune system, affect the tumor microenvironment, or interact with taxane metabolism.

Modulation of drug metabolism: The gut microbiome can potentially influence taxane metabolism and bioavailability. Some gut bacteria, such as *Eggerthella* spp., can metabolize taxanes, leading to changes in their pharmacokinetics and therapeutic efficacy (Alpuim Costa et al. 2021; Gardiner et al. 2015). Alterations in the gut microbiota composition might impact the levels of active drugs available systemically, potentially affecting treatment response.

Immune system modulation: The gut microbiome can influence the immune system, which plays a vital role in the response to taxane treatment. Certain gut bacteria can affect immune cell populations, modulate immune activation and

inflammation, and influence the tumor microenvironment (Belkaid and Hand 2014; Wu and Wu 2012). These immune-related effects may impact taxane efficacy and treatment response.

Modulation of treatment-related toxicity: Taxanes can cause treatment-related toxicities, such as peripheral neuropathy and gastrointestinal side effects (Velasco and Bruna 2015; Khan et al. 2022b, 2023d). The gut microbiome has been implicated in mediating some of these toxicities. Dysbiosis or alterations in the gut microbiota composition might impact the metabolism, absorption, or detoxification of taxanes, potentially contributing to treatment-related toxicities.

Modulation of the microbiome: Strategies aimed at modulating the gut microbiome have been explored to potentially enhance the efficacy and reduce the toxicity of taxane treatment. Preclinical studies have shown that interventions such as probiotics, prebiotics, or fecal microbiota transplantation (FMT) can influence gut microbiota composition and impact treatment outcomes (Ting et al. 2022). However, more research is needed to determine the clinical applicability and effectiveness of these approaches.

It's important to note that the interplay between the gut microbiome and taxane treatment is still an emerging field, and more studies are needed to establish robust associations and understand the underlying mechanisms. The complexity of the gut microbiome and individual variations in microbial composition further complicate the analysis. Nonetheless, investigating and manipulating the gut microbiome may hold promise for optimizing taxane chemotherapy outcomes in the future.

Fluoropyrimidine analogs: The interplay between the gut microbiome and fluoropyrimidine analogs, a class of chemotherapy drugs that includes 5-fluorouracil (5-FU) and capecitabine, is an emerging area of research (Gmeiner 2020; Thorn et al. 2011; Khan et al. 2023e; Singh et al. 2017a). While our understanding is still evolving, here are some key points regarding the potential interplay between the gut microbiome and fluoropyrimidine analog treatment:

Metabolism of fluoropyrimidine analogs: The gut microbiome can potentially influence the metabolism and efficacy of fluoropyrimidine analogs. Studies suggest that certain bacteria in the gut can metabolize 5-FU, leading to the production of metabolites that can modulate the drug's pharmacokinetics and therapeutic effectiveness (Gmeiner 2020; Spanogiannopoulos et al. 2022). These microbial-mediated metabolic processes may impact treatment response and resistance.

Impact on drug activation: Fluoropyrimidine analogs require activation within cells to exert their cytotoxic effects. The gut microbiome could potentially influence the activation process by producing enzymes that activate or inactivate the drugs (Huang et al. 2022b). The specific microbial species involved in fluoropyrimidine metabolism and their clinical relevance are still being investigated.

Modulation of treatment response: The gut microbiome may indirectly influence treatment response to fluoropyrimidine analogs by modulating the immune system and systemic inflammation (Belkaid and Hand 2014). Certain gut bacteria have been associated with immune modulation and alterations in the gut microbiota composition, which might impact the tumor microenvironment and treatment efficacy.

Gut microbiome and treatment-related toxicity: Fluoropyrimidine analogs can cause treatment-related toxicities, such as gastrointestinal side effects, myelosuppression, and hand-foot syndrome (Del Re et al. 2010; Kwakman et al. 2020; Hodroj et al. 2021). The gut microbiome has been implicated in mediating some of these toxicities. Dysbiosis or alterations in the gut microbiota composition might impact the metabolism, absorption, or detoxification of fluoropyrimidine analogs, potentially contributing to treatment-related toxicities.

Modulation of the microbiome: Strategies aimed at modulating the gut microbiome have been explored to potentially enhance the efficacy and reduce toxicity of fluoropyrimidine analog treatment. Preclinical studies have shown that interventions such as probiotics, prebiotics, or fecal microbiota transplantation (FMT) can influence gut microbiota composition and impact treatment outcomes (Hamamah et al. 2022). However, more research is needed to determine the clinical applicability and effectiveness of these approaches.

It's important to note that the interplay between the gut microbiome and fluoropyrimidine analog treatment is still a relatively new area of research, and more studies are needed to establish robust associations and understand the underlying mechanisms. The complex nature of the gut microbiome and individual variations in microbial composition further complicate the analysis. Nonetheless, investigating and manipulating the gut microbiome may hold promise for optimizing fluoropyrimidine analog chemotherapy outcomes in the future.

Probiotics Modulate Gut Microbiota Composition in Cancer

Probiotics have been studied for their potential to modulate gut microbiota composition in the context of cancer (Torres-Maravilla et al. 2021). Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host. They can help restore or maintain a healthy balance of gut bacteria, potentially impacting various aspects of health, including cancer (Das et al. 2022).

Here are some key points regarding the modulation of gut microbiota composition by probiotics in cancer:

Restoring dysbiosis: Cancer patients often experience dysbiosis, which refers to an imbalance or disruption in the gut microbiota composition. Probiotics can help restore a more balanced microbiota by promoting the growth of beneficial bacteria and inhibiting the growth of potentially harmful bacteria. This can help counteract dysbiosis associated with cancer and its treatments (Zhang et al. 2022b; Gagliardi et al. 2018).

Immune system modulation: Probiotics can influence the immune system's response, which is closely linked to cancer development and progression. By modulating the gut microbiota, probiotics can enhance immune function, stimulate anti-tumor immune responses, and potentially contribute to improved cancer outcomes (Wang et al. 2021).

Reduction of inflammation: Chronic inflammation is associated with an increased risk of cancer and poor treatment outcomes. Probiotics have shown the

potential to reduce inflammation in the gut by improving the gut barrier function and inhibiting pro-inflammatory signaling pathways. By mitigating inflammation, probiotics may help create a less favorable environment for cancer development (Górska et al. 2019; Francescone et al. 2014).

Metabolite production: The gut microbiota plays a role in the production of various metabolites that can affect cancer progression (Sameer et al. 2024; Rossi et al. 2020). Probiotics can influence the production of short-chain fatty acids (SCFAs) and other metabolites that have anti-inflammatory and anticancer properties (Markowiak and Śliżewska 2017). By promoting the production of beneficial metabolites, probiotics may contribute to a less favorable environment for tumor growth (Raman et al. 2013; Markowiak-Kopeć and Śliżewska 2020).

Modulation of chemotherapy response and side effects: Some studies suggest that probiotics may enhance the efficacy of chemotherapy and reduce its side effects. By modulating the gut microbiota composition, probiotics can potentially impact drug metabolism, bioavailability, and toxicities (Yin et al. 2022). However, the effects of probiotics on chemotherapy outcomes are still under investigation, and it is essential to consider potential interactions between probiotics and specific chemotherapy agents (Rodriguez-Arrastia et al. 2021).

It's worth noting that the effects of probiotics on the gut microbiota composition and cancer outcomes can vary depending on several factors, including the specific strains and formulations used, the individual's baseline microbiota, the type and stage of cancer, and other individual factors (Hemarajata and Versalovic 2013). Therefore, it is crucial to consult with healthcare professionals knowledgeable in the field of oncology before considering probiotic use as an adjunct to cancer treatment (Wei et al. 2018).

10.3 Modification of the Gut Microbiota to Improve Treatment Effectiveness

Modification of the gut microbiota is an area of active research with the aim of improving treatment effectiveness in cancer (Ma et al. 2019). Here are some strategies that have been explored:

Probiotics: Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits. Probiotics can be used to introduce beneficial bacteria into the gut, restore microbial balance, and potentially enhance treatment outcomes for cancer. Studies have investigated the use of specific probiotic strains to modulate the gut microbiota and improve chemotherapy efficacy, reduce treatment-related toxicities, and enhance the antitumor immune response (Kechagia et al. 2013; Hill et al. 2014).

Prebiotics: Prebiotics are nondigestible dietary fibers that selectively stimulate the growth and activity of beneficial bacteria in the gut (Davani-Davari et al. 2019; Carlson et al. 2018). By promoting the growth of specific beneficial bacteria, prebiotics can positively influence the gut microbiota composition and function. Prebiotic

supplementation has been explored as a strategy to enhance the efficacy of cancer treatment, reduce treatment-related toxicities, and improve the overall response to therapy (Deleemans et al. 2021).

Fecal microbiota transplantation (FMT): FMT involves transferring fecal material from a healthy donor into the gastrointestinal tract of a recipient (Gupta et al. 2016). FMT has been primarily used to treat recurrent *Clostridioides difficile* infection but is now being investigated for its potential to modulate the gut microbiota and improve treatment outcomes in cancer (Kim and Gluck 2019; Azimirad et al. 2020). FMT aims to restore a healthy microbiota composition and enhance the response to cancer treatment, including chemotherapy and immunotherapy (Yi et al. 2019). However, further research is needed to establish its safety and efficacy in the cancer setting.

Dietary interventions: Diet plays a crucial role in shaping the gut microbiota composition. Specific dietary interventions, such as high-fiber diets, plant-based diets, or diets rich in certain nutrients, can influence the gut microbiota and potentially improve treatment effectiveness in cancer (Leeming et al. 2019; Singh et al. 2017b). For example, dietary fiber can promote the growth of beneficial bacteria that produce short-chain fatty acids (SCFAs), which have been associated with anti-cancer effects (Silva et al. 2020; Mirzaei et al. 2021).

Antibiotics and microbiota modulators: The use of antibiotics and other microbiota modulators can impact the gut microbiota composition (Patangia et al. 2022). In some cases, selective antibiotic treatment has been employed to reduce specific harmful bacteria that may interfere with treatment effectiveness (Llor and Bjerrum 2014; Li et al. 2017). However, the use of antibiotics should be carefully considered, as they can also disrupt beneficial bacteria and impact immune function (Patangia et al. 2022; Langdon et al. 2016).

It's important to note that the field of microbiota modification for improving cancer treatment effectiveness is still evolving, and more research is needed to understand the optimal approaches, specific microbial signatures, and their clinical applications. The effects of these interventions may vary depending on individual factors, cancer type, treatment regimen, and other variables. Therefore, it is essential to consult with healthcare professionals knowledgeable in the field of oncology for personalized guidance and recommendations (Malone et al. 2020) (Fig. 10.5).

The use of antibiotics is successful in eliminating pathobionts, but because they also kill healthy bacteria, they can have an adverse effect on gut homeostasis and limit their use in the management of CRC. Probiotic development is encouraged by prebiotic activity. Probiotics have a variety of anticarcinogenic effects: (1) They can prevent pathogenic bacteria from colonizing the body. (2) They can improve barrier function by increasing the production of mucin and tight junction proteins. (3) They support homeostatic immune responses, helping to increase Treg cell anti-inflammatory responses and control pro-inflammatory cytokine release. (4) They support cancer cell death. By preventing NFATc3 activation, postbiotics limit tumor cell proliferation while also causing selective cytotoxicity against tumor cells. Finally, fecal microbiota transplantation (FMT) may be used to treat CRC by

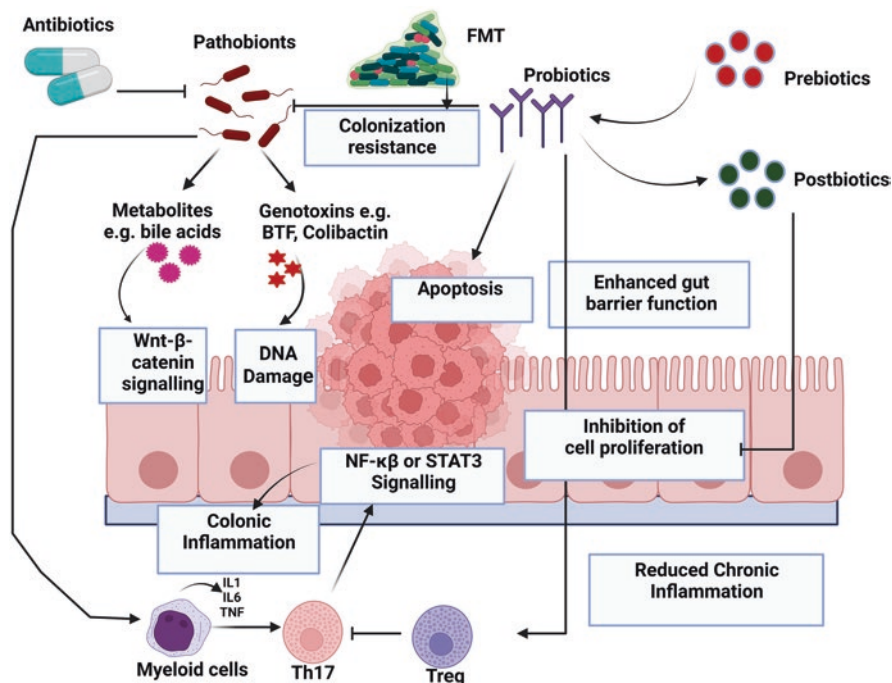


Fig. 10.5 Unbalanced host-microbe interactions help to activate pro-carcinogenic inflammatory pathways, which in turn help in cancer progression

restoring microbiome necrobiosis and triggering homeostatic immune responses. However, FMT has some risks, such as the introduction of new pathobionts and the spread of genes linked to disease.

10.4 Conclusion

The human gut is home to a vast and diverse population of microorganisms, collectively referred to as the gut microbiota or gut microbiome. These microorganisms include bacteria, viruses, fungi, and other microbes. While the exact composition of the gut microbiota can vary from person to person, there are certain bacterial species that are commonly found in the human gut. Bacteria make up the majority of the gut microbiota, and there are estimated to be trillions of bacteria residing in the human gut. The number of bacterial cells in the gut is believed to be around ten times greater than the total number of cells in the human body. The gut microbiota is incredibly diverse, with hundreds of different bacterial species. The most abundant phyla of bacteria in the gut are *Firmicutes* and *Bacteroidetes*, although there are other phyla present as well, such as *Actinobacteria* and *Proteobacteria*. The specific composition of the gut microbiota can be influenced by

various factors, including diet, lifestyle, genetics, and antibiotic use. Additionally, the gut microbiota is known to play a crucial role in human health, aiding in digestion, nutrient absorption, and immune system function and even influencing mental health. It's important to note that research on the gut microbiota is ongoing, and our understanding of its complexity and functions continues to evolve. Therefore, the information provided here represents a general understanding based on current knowledge up until September 2021.

MCQs

1. **What is the primary function of gut microbes in the human body?**

- A. Digesting proteins
- B. Synthesizing vitamins
- C. Regulating blood pressure
- D. Maintaining bone density

Answer B: Synthesizing vitamins

2. **How can gut microbes influence cancer development?**

- A. Enhancing immune system function
- B. Breaking down carcinogenic substances
- C. Producing inflammatory compounds
- D. All of the above

Answer D: All of the above

3. **Which type of cancer has been linked to imbalances in gut microbiota?**

- A. Lung cancer
- B. Breast cancer
- C. Colorectal cancer
- D. Leukemia

Answer C: Colorectal cancer

4. **What is the term for a community of microorganisms living in the digestive tracts of humans?**

- A. Microflora
- B. Mycelium
- C. Microbiome
- D. Mycorrhiza

Answer C: Microbiome

5. **The presence of which bacteria in the gut has been associated with a decreased risk of colorectal cancer?**

- A. *Escherichia coli* (*E. coli*)
- B. *Bifidobacterium*
- C. *Streptococcus*
- D. *Clostridium difficile*

Answer B: *Bifidobacterium*

6. **How can gut microbes contribute to cancer drug resistance?**

- A. By metabolizing drugs
- B. By promoting inflammation

- C. By suppressing the immune system
- D. All of the above

Answer A: By metabolizing drugs

7. What role do gut microbes play in the metabolism of certain cancer drugs?

- A. They deactivate the drugs.
- B. They enhance drug efficacy.
- C. They have no impact on drug metabolism.
- D. They increase drug toxicity.

Answer A: They deactivate the drugs.

8. Which of the following is NOT a potential mechanism by which gut microbes influence cancer drug response?

- A. Modulating drug absorption
- B. Producing enzymes that inactivate drugs
- C. Influencing drug distribution in the bloodstream
- D. Enhancing drug manufacturing

Answer D: Enhancing drug manufacturing

9. The interaction between gut microbes and cancer drugs is primarily mediated through which organ?

- A. Liver
- B. Kidneys
- C. Lungs
- D. Heart

Answer A: Liver

10. Which bacterial component has been implicated in promoting inflammation and cancer progression?

- A. DNA
- B. Lipopolysaccharide (LPS)
- C. Ribosomes
- D. Plasmids

Answer B: Lipopolysaccharide (LPS)

11. Question: The term “dysbiosis” refers to:

- A. A healthy balance of gut microbes
- B. Imbalance or maladaptive changes in gut microbial communities
- C. Microbial resistance to antibiotics
- D. Rapid microbial growth

Answer B: Imbalance or maladaptive changes in gut microbial communities

12. In addition to the gut, which other body part is a significant reservoir of microbes influencing cancer?

- A. Skin
- B. Brain
- C. Pancreas
- D. Spleen

Answer A: Skin

13. **Which of the following dietary components can influence the composition of gut microbiota?**

- A. Fiber
- B. Sugar
- C. Saturated fats
- D. All of the above

Answer D: All of the above

14. **The gut-brain axis refers to the bidirectional communication between the gut and:**

- A. Heart
- B. Brain
- C. Liver
- D. Lungs

Answer B: Brain

15. **Probiotics are live microorganisms that confer health benefits when consumed in adequate amounts. Which is a common example of a probiotic?**

- A. *Saccharomyces cerevisiae*
- B. *Escherichia coli* (*E. coli*)
- C. *Lactobacillus rhamnosus*
- D. *Clostridium difficile*

Answer C: *Lactobacillus rhamnosus*

16. **What is the potential role of gut microbiota in modulating the side effects of cancer treatment?**

- A. Aggravating side effects
- B. Mitigating side effects
- C. No impact on side effects
- D. Inducing new side effects

Answer B: Mitigating side effects

17. **Antibiotics can impact gut microbiota. How might this influence cancer patients undergoing treatment?**

- A. Enhancing drug effectiveness
- B. Exacerbating drug resistance
- C. Improving overall health
- D. Accelerating cancer cell growth

Answer B: Exacerbating drug resistance

18. **Which term describes the process by which gut microbes contribute to the activation of certain drugs?**

- A. Biotransformation
- B. Metabolism
- C. Acetylation
- D. Detoxification

Answer A: Biotransformation

19. **What is the significance of the “tumor microenvironment” in cancer development?**

- A. It refers to microbes within tumors.
- B. It influences the behavior of cancer cells.

- C. It is irrelevant to cancer progression.
- D. It determines the color of tumors.

Answer B: It influences the behavior of cancer cells.

20. The use of fecal microbiota transplantation (FMT) is being explored in cancer treatment. What is the primary purpose of FMT?

- A. To enhance drug metabolism
- B. To restore a healthy balance of gut microbes
- C. To induce inflammation
- D. To eradicate cancer cells

Answer B: To restore a healthy balance of gut microbes

21. What is the primary function of probiotics when administered in adequate amounts?

- A. Promoting chronic inflammation
- B. Inhibiting gut microbiota growth
- C. Conferring health benefits to the host
- D. Inducing dysbiosis

Answer C: Conferring health benefits to the host

22. How do probiotics contribute to countering dysbiosis associated with cancer and its treatments?

- A. Promoting the growth of harmful bacteria
- B. Inhibiting the growth of beneficial bacteria
- C. Restoring a more balanced microbiota
- D. Inducing inflammation in the gut

Answer C: Restoring a more balanced microbiota

23. What strategy involving probiotics has been explored to potentially improve chemotherapy outcomes?

- A. Enhancing chemotherapy side effects
- B. Inhibiting drug metabolism
- C. Modulating gut microbiota composition to improve chemotherapy efficacy
- D. Inducing toxicities related to treatment

Answer C: Modulating gut microbiota composition to improve chemotherapy efficacy

24. Which strategy involves transferring fecal material from a healthy donor into the gastrointestinal tract of a recipient?

- A. Probiotics supplementation
- B. Prebiotic intake
- C. Fecal microbiota transplantation (FMT)
- D. Dietary interventions

Answer C: Fecal microbiota transplantation (FMT)

25. How do prebiotics positively influence the gut microbiota in the context of cancer treatment?

- A. Suppressing beneficial bacteria
- B. Stimulating harmful bacteria
- C. Selectively stimulating the growth of beneficial bacteria
- D. No impact on gut microbiota composition

Answer C: Selectively stimulating the growth of beneficial bacteria

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Novel Strategies for Overcoming Drug Resistance

11

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and Fayaz Malik

Abstract

This book chapter explores novel strategies for overcoming drug resistance in cancer, focusing on advancements in drug delivery using nanoparticles (NPs) and CRISPR-based techniques. Nanoparticles offer targeted delivery, improving drug bioavailability and minimizing off-target effects. The chapter reviews recent developments in NP-based formulations designed to overcome resistance mechanisms, emphasizing their potential to enhance therapeutic efficacy. CRISPR technologies enable targeted modifications to the cancer cell genome, disrupting resistance-associated pathways and sensitizing cells to treatment. The chapter evaluates CRISPR-based interventions, discussing their potential to tackle intrinsic and acquired resistance mechanisms. By synthesizing these two cutting-edge approaches, this chapter presents an integrative view of how nanoparticle drug delivery and CRISPR-based strategies can synergistically address drug resistance challenges in cancer therapy.

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Keywords

Drug resistance · Cancer cells · Nanoparticles · Apoptosis · Efflux transporters · CRISPR/Cas9

11.1 Introduction

Developing novel and cutting-edge cancer treatments is a significant global issue (Siegel et al. 2021; Khan et al. 2023a; Wani et al. 2021). The therapeutic effectiveness of certain malignant cancers has significantly increased due to the development of personalized treatment plans and a rise in the variety of cancer therapy options. One common and established cancer treatment strategy is chemotherapy (Khan et al. 2023b). Chemotherapy has several distinct modes of action, but its primary purpose is to kill rapidly proliferating cells, both tumor and healthy ones (Khan et al. 2023c). This can have serious adverse effects, such as digestive reactions, loss of hair, and reduction of bone marrow (Zitvogel et al. 2008). Hence, the primary focus of an enormous amount of cancer-related study over recent decades has been the development of medications that exhibit enhanced specificity towards tumor cells, as opposed to normal cells. Despite significant advancements in precision treatment with the creation of targeted therapy, there are several inevitable side effects, and the persistent issue of drug resistance has posed ongoing challenges (Khan et al. 2022a). At present, cancer continues to be the second most prevalent cause of mortality, with existing treatments for numerous types of cancer proving to be insufficient. Therefore, an escalating number of research endeavors are focused on the development of targeted cancer therapies and strategies to overcome drug resistance.

In recent decades, there has been a growing utilization of nanotechnology in the field of healthcare, encompassing many applications such as diagnostics, treatment, and targeted tumor therapy, with the aim of enhancing safety and efficacy. The utilization of nanoparticle (NP)-based delivery systems for drugs has demonstrated numerous benefits in the field of cancer therapy. These advantages include favorable pharmacokinetics, accurate localization of tumor cells, mitigation of adverse effects, and overcoming resistance to drugs (Dadwal et al. 2018; Palazzolo et al. 2018). Nanoparticles (NPs) employed in systems for drug delivery are often selected or engineered depending on their dimensions and properties, in accordance with the pathophysiology of the malignancies. In the context of cancer therapy, nano-carriers function by selectively targeting tumor cells via the carrier consequence of NPs and establishing the impact of the targeting material subsequent to absorption. Subsequently, drugs are administered to the tumor cells with the aim of eliciting cytotoxic effects. The nano-carriers contain drugs such as conventional chemotherapeutic agents and nucleic acids, suggesting their potential application in both cytotoxic and gene therapies (Chen et al. 2009). Furthermore, NPs can serve as a viable substrate for encapsulating poorly soluble medicines and facilitating their delivery into the circulatory system (Kipp 2004). In recent times, there have been reports on

the possibility of nanoparticle-based treatment in addressing multidrug resistance (MDR) in various kinds of cancer, such as breast cancer (Alimoradi et al. 2018), ovarian cancer (Wang et al. 2018a), and prostate cancer (Zhang et al. 2019). The integration of nanotechnology into the realm of medicine has resulted in a novel era of treatment for cancer, requiring further investigation into the synergistic potential of these interdisciplinary domains. Genome manipulation has emerged as a novel therapeutic strategy for the treatment of several types of malignancies. The CRISPR/Cas9 gene-editing method has received significant attention in recent times. By employing this methodology, it becomes feasible to modify the genome within malignant cells. The efficacy of the CRISPR/Cas9 method in cancer treatment has been demonstrated through experiments conducted on cellular as well as animal models. By enabling researchers to precisely modify the genetic makeup of cells, CRISPR/Cas9 facilitates the identification of key genes involved in resistance and opens avenues for the development of more effective therapeutic interventions.

11.2 Overcoming Drug Resistance with Drug Delivery Based on Nanoparticles (NPs)

Extensive research and utilization of nanotechnology in the field of cancer treatment have been observed, primarily due to the significant potential of nanoparticles as carriers for drug delivery. Nanoparticle-based drug delivery systems offer distinct advantages over conventional drugs, including enhanced stability and biological compatibility, improved permeability and retention impact, and specific targeting capabilities. The utilization of nanotechnology in the context of delivering drugs for cancer therapy has demonstrated a substantial impact in addressing the challenge of drug resistance (Fig. 11.1 and Table 11.1).

Efflux Transporters Targeting Via NPs

A plethora of studies have shown that some chemotherapeutic-loaded NPs can avoid anti-tumor drug contact with efflux transporters because NPs go into the cell primarily via endocytosis rather than diffusion and release the drugs at a perinuclear location inside the cell, far from the membranes of cells and efflux transports (Murakami et al. 2011). The drug delivery system utilizing nanoparticles has the capacity to modulate the regulation of drug release. For instance, previous studies have employed low pH levels and redox reactions as stimuli to induce drug release in nanoparticles (Yu et al. 2018; Kundu et al. 2019). In addition, NPs, such as polymers, have been observed to function as modulators of multidrug resistance (MDR) (Qin et al. 2018). An example of this is the utilization of micelles composed of an amphiphilic diblock polymer consisting of N-(2-hydroxypropyl)methacrylamide (HPMA) as well as poly(propylene oxide) block (PPO) to inhibit P-gp (Braunová et al. 2017).

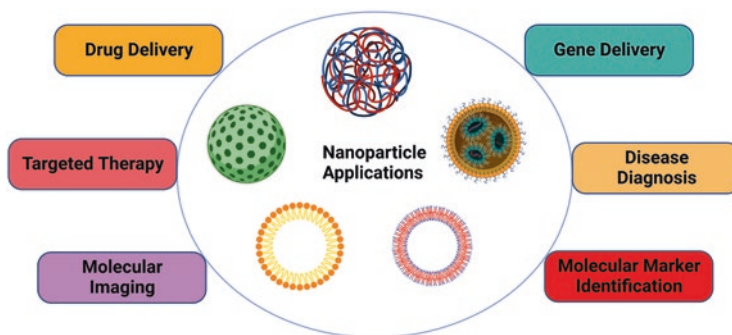


Fig. 11.1 The figure illustrates various types of nanoparticles and highlights their wide-ranging applications in cancer

Table 11.1 Combating drug resistance through the use of drug delivery systems based on nanoparticles

Targeting pathway	Mechanisms	Drugs	References
Apoptosis	Inhibiting anti-apoptotic pathways	<ul style="list-style-type: none"> • siRNA targeting Bcl-2 • Curcumin/pyrrolidine dithiocarbamate (NF-κB inhibitors) 	(Wang et al. 2006; Saad et al. 2008; Choi et al. 2019) (Fan et al. 2010; Misra and Sahoo 2011) (Prabha and Labhasetwar 2004) (Devalapally et al. 2007; van Vlerken et al. 2010)
	Activating pro-apoptotic pathways	<ul style="list-style-type: none"> • p53 gene therapy • Ceramide 	
Efflux transporters	Inhibiting efflux transporters	<ul style="list-style-type: none"> • siRNA targeting P-gp • COX-2 inhibitors 	(Patil et al. 2010; Navarro et al. 2012) (Zhang et al. 2019) (Murakami et al. 2011)
	Bypass efflux transporters	<ul style="list-style-type: none"> • Nanoparticle itself 	
Hypoxia	Inhibiting the activity of HIF-1 α	<ul style="list-style-type: none"> • HIF-1α inhibitors 	(Reddy et al. 2011) (Zhang et al. 2018) (Long et al. 2018)
	Indirectly downregulating HIF-1 α expression	<ul style="list-style-type: none"> • PI3K/mTOR/Akt pathway inhibitors • HSP90 inhibitors 	

Combination therapy represents an additional therapeutic approach for the management of drug-resistant malignancies. In order to address this issue, the utilization of nanoparticle (NP)-based combination therapies has proven effective in mitigating the challenges associated with variations in drug pharmacokinetics. This approach involves the incorporation of several therapeutic agents into a single drug carrier, thereby enhancing the efficacy of cancer therapy and combating drug resistance (Cuvier et al. 1992; Schneider and Hunke 1998; Allen et al. 2000; Susa et al. 2009). Furthermore, an alternative approach to address treatment resistance caused by efflux transporters would involve the inhibition of their production and

activity, in addition to bypassing them. This approach can be implemented by the development of nanoparticle formulations that incorporate both efflux pump inhibitors and chemotherapy drugs (Fig. 11.2) (Soma et al. 2000). Alternatively, another method involves the reduction of ATP supply to the efflux pump (Wang et al. 2018b). According to Sui and colleagues (2011) (Sui et al. 2011), there is evidence suggesting that COX-2 plays a role in P-gp-mediated MDR in cancers. Consequently, the utilization of a selective COX-2 inhibitor has the potential to decrease the expression of P-gp. Certainly, a recent investigation carried out by Zhang and colleagues in 2019 (Zhang et al. 2019) has substantiated that the simultaneous administration of COX-2 inhibitors and doxorubicin via NPs effectively counteracted the MDR exhibited by breast cancer cells. In addition, a number of studies have demonstrated that the simultaneous administration of P-gp-targeted siRNA and anticancer medications using NPs can effectively address drug-resistant tumors by suppressing the ABC transporters' expression (Patil et al. 2010; Navarro et al. 2012). In a recent investigation, it was found that the combination of miRNA-495 as well as doxorubicin in cancer cell membrane-coated silica NPs demonstrated efficacy in overcoming resistance to therapies in lung tumors (He et al. 2019).

Furthermore, Bai and colleagues in 2013 (Bai et al. 2013) conducted a study, whereby they observed that the utilization of NPs facilitated the transportation of drugs to the neovasculature of tumors. This approach proved effective in circumventing MDR caused by the expression of P-glycoprotein (P-gp) in cancer cells. The NPs were specifically designed to target kinase insert domain receptor (KDR) receptors, which are prominently present in the vasculature of tumors. The results of this study demonstrated that the system had superior efficacy in inhibiting tumor growth compared to the combination therapy of chemotherapy and P-gp inhibitors.

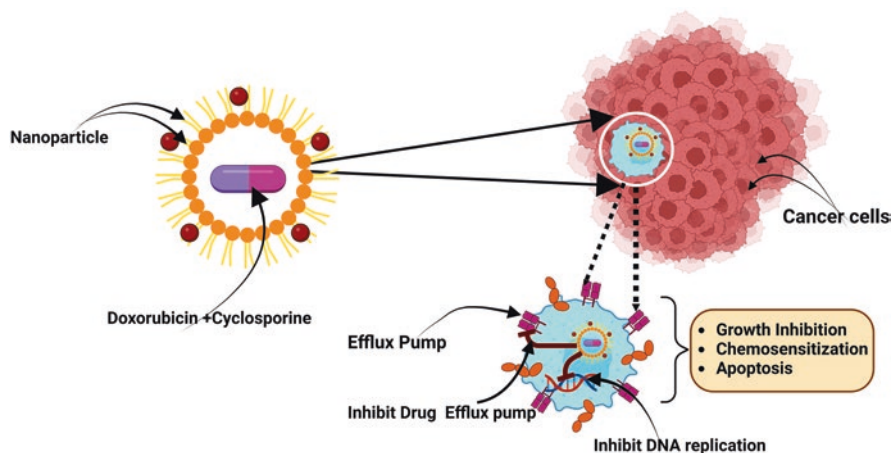


Fig. 11.2 The figure depicts an innovative strategy to overcome treatment resistance arising from efflux transporters by employing nanoparticle formulations

Nanoparticle formulations carry both efflux pump inhibitors and chemotherapy drugs simultaneously. This dual payload approach ensures that the inhibitors and therapeutic agents reach the target site together, allowing for coordinated and synergistic action.

Apoptotic Pathway Targeting Via NPs

The presence of malfunctioning apoptotic mechanisms allows cancer cells to avoid undergoing apoptosis, leading to enhanced cell survival and ultimately contributing to the development of treatment resistance in cancer (Khan et al. 2023b, c, d; Viktorsson et al. 2005). The malfunctioning apoptotic pathway is frequently activated by the dysregulation of Bcl-2 and NF- κ B (Hassan et al. 2020). The protein Bcl-2 has gained significant attention in scientific research due to its anti-apoptotic properties. It is prominently present in numerous types of malignancies and has been identified as a crucial factor in the development of drug resistance. Consequently, there is growing interest in exploring Bcl-2 as a possible target for counteracting drug resistance. There is a growing body of evidence suggesting that the simultaneous administration of Bcl-2-targeted siRNA and chemotherapeutics using NPs presents a viable strategy for addressing drug resistance in cancers (Chen et al. 2009; Wang et al. 2006; Saad et al. 2008; Choi et al. 2019). Furthermore, NP-based combination therapy has incorporated NF- κ B inhibitors such as pyrrolidine dithiocarbamate (PDTC) (Fan et al. 2010) and curcumin to target cancers (Misra and Sahoo 2011; Zhao et al. 2019).

Furthermore, the utilization of pro-apoptotic substances can be employed as a strategy to counteract medication resistance caused by the apoptotic pathway, in addition to the inhibition of anti-apoptotic factors. An instance of this is the augmentation of the effectiveness of therapy in several drug-resistant cancer models with the combination of ceramide and the chemotherapeutic agent paclitaxel (Devalapally et al. 2007; van Vlerken et al. 2010). In contrast, a new investigation has demonstrated that ceramide possesses the capability to reinstate the expression of wild-type p53 protein, a crucial tumor suppressor, through the modulation of alternative pre-mRNA splicing. The study conducted by Khiste and colleagues in 2020 (Khiste et al. 2020) demonstrates that NPs present a more efficient mechanism for delivering ceramide into cancerous cells harboring p53 missense mutations, a significant occurrence in the context of cancer. The restoration of p53 function or related tumor suppressors is regarded as a promising strategy for overcoming medication resistance in cancer, given the crucial role that p53 plays in apoptosis. Consequently, there has been additional investigation into the utilization of a NP-based delivery system for p53 gene therapy. The transfection of the p53 gene into breast and lung cancer cells has been documented in previous studies and also utilizing cationic solid lipid NPs and PLGA, respectively (Prabha and Labhasetwar 2004; Choi et al. 2008; Minto et al. 2021). The findings of this study demonstrate the successful production of apoptosis and the suppression of tumor growth.

In addition, certain drug delivery methods based on nanoparticle (NP) technology operate by impeding the activity of efflux pumps while also facilitating the

process of apoptosis. In their study, Cheng and colleagues in 2018 (Cheng et al. 2018) employed an amphiphilic cationic nanoparticle (NP) complex that encapsulated paclitaxel with the Bcl-2 convertor gene. The objective was to impede the proliferation of drug-resistant liver tumor cells. The study's results indicated that the NP complex had a detrimental effect on P-gp-mediated drug efflux and the initiation of apoptosis. This research is a groundbreaking investigation that effectively addressed medication resistance, encompassing both pump-mediated and non-pump-mediated mechanisms (Fig. 11.3). Furthermore, the combined administration of doxorubicin with resveratrol within NPs has demonstrated noteworthy cytotoxic effects on BC cells that are resistant to doxorubicin (Zhao et al. 2016). This cytotoxicity is achieved by triggering apoptosis, which is facilitated by the suppression of NF- κ B and Bcl-2 expression, as well as the reduction of efflux transporter expression (Zhao et al. 2016). Another separate investigation showed the efficacy of folic acid-conjugated planetary ball-milled nanoparticles that were utilized as carriers for resveratrol and docetaxel, targeting the therapy of MDR prostate cancer. The findings demonstrated a decrease in the expression of anti-apoptotic genes and inhibition of ABC-transporter indicators (Singh et al. 2018). In addition, NPs targeting specifically mitochondria have demonstrated an impact on both efflux transporters and apoptotic pathways. The specific localization of the targeting mitochondria resulted in a decrease in ATP synthesis, a crucial energy source for ABC transporters. Furthermore, another study demonstrated that the introduction of paclitaxel-loaded TPP-Pluronic F127-hyaluronic acid nanomicelles induced mitochondrial outer membrane permeabilization (MOMP). This phenomenon subsequently led to the liberation of cytochrome C as well as the stimulation of caspase-3 and caspase-9, ultimately triggering apoptosis in DR lung cancer cells (Wang et al. 2020).

Hypoxia Targeting Via NPs

Jing and coworkers in 2019 (Jing et al. 2019) have identified hypoxia as an additional contributing component to the development of multidrug resistance. The hypoxic condition frequently observed in certain cancer cells can be attributed to

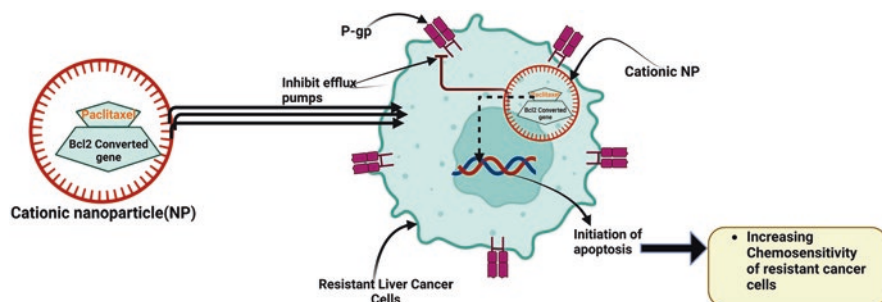


Fig. 11.3 The figure illustrates the transformative impact of nanoparticle (NP) complexes on addressing therapeutic resistance, particularly focusing on P-glycoprotein (P-gp)-mediated drug efflux and the induction of apoptosis

irregular blood arteries and the heightened oxygen requirements associated with the fast proliferation of these cells. There are multiple mechanisms via which hypoxia can cause medication resistance in cancers (Brahimi-Horn et al. 2011). In certain cases, cells that undergo gradual division in places with low oxygen levels have the ability to evade the damaging effects of chemotherapeutic drugs, including alkylating substances and antibiotics (Gordan and Simon 2007). Furthermore, the presence of hypoxia induces an oxygen gradient inside the tumor, consequently enhancing tumor heterogeneity and facilitating the development of a more malignant phenotype. Additionally, it has been demonstrated that hypoxia plays a role in the upregulation of drug efflux proteins (Khan et al. 2023b, e; Shu et al. 2005). HIF-1 α is a crucial participant in the process, and its excessive expression has been shown in numerous human malignancies (Vadde et al. 2017; Ahmad et al. 2022). Thus, the targeting of HIF-1 α is an additional therapeutic approach for circumventing medication resistance. Moreover, a thorough investigation has been conducted regarding the utilization of nanoparticles (NPs) in the management of hypoxia. One approach to impede a hypoxic environment involves the suppression of the HIF-1 α gene. Multiple studies have documented the efficacy of nanosystems including HIF-1 α siRNA in addressing medication resistance in cancer (Zhao et al. 2015; Hajizadeh et al. 2020; Luan et al. 2018). In addition, inhibitors of the HIF-1 α have demonstrated therapeutic effectiveness in mitigating medication resistance that arises from hypoxia (Reddy et al. 2011). Furthermore, the concept of indirectly reducing HIF-1 signaling has been previously contemplated, in addition to the direct suppression of HIF-1 activity. The expression of HIF-1 α can be regulated by the PI3K/Akt/mTOR system, and inhibiting this route leads to a downregulation of HIF-1 α expression. Consequently, this downregulation enhances the susceptibility of MDR cells to cancer treatment (Zhang et al. 2018). The utilization of various NPs such as PEGylated and non-PEGylated liposomes (Iwase and Maitani 2012) and PLGA-PEG (Tang et al. 2020) has been found to provide enhanced platforms for the implementation of combination therapies. Furthermore, the activity of HIF-1 transcription is dependent on the presence of HSP90 which is heat shock protein 90, and the expression of HIF-1 α can be reduced by inhibiting HSP90 (Semenza 2007). The study conducted by Long and coworkers in 2018 (Long et al. 2018) demonstrated a significant enhancement in the therapy of bladder cancer through the utilization of 17AAG-loaded NPs containing the HSP90 inhibitor.

11.3 CRISPR-Based Strategies in Overcoming Treatment Resistance

The CRISPR/Cas9 system in bacteria functions as an RNA-based immune system. This system has the capability to modify genes in eukaryotic cells, including those associated with multidrug resistance (MDR). To achieve this, a specific type of RNA known as single-guide RNA (sgRNA) is created to match the desired genetic sequence. Alongside sgRNA, an endonuclease called Cas9 is introduced into the target cell. The sgRNA serves as a guide for Cas9, directing it to the intended genetic

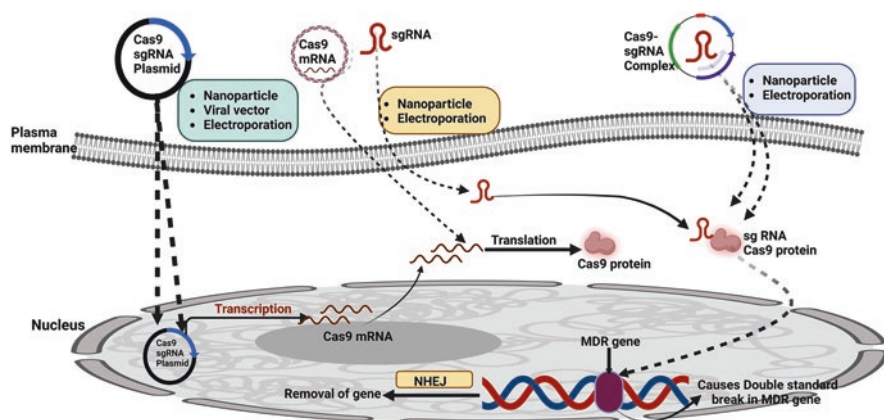


Fig. 11.4 This schematic representation illustrates the application of the CRISPR/Cas9 system to specifically target a multidrug resistance (MDR)-related gene within a cell

sequence. Once at the target site, Cas9 induces a double-strand break in the genetic sequence. Consequently, it becomes possible to either delete or insert the desired genetic sequence into the genes (Fig. 11.4) (Mohammadzadeh et al. 2020). In recent years, there has been a notable emphasis in scientific research on investigating the potential of CRISPR/Cas9 technologies in addressing drug resistance exhibited by cancer cells. Such studies have yielded intriguing findings that have instilled hope in the prospects of overcoming treatment resistance and achieving efficacious cancer therapies.

CRISPR/Cas9 Targeting of Efflux Transporters

One study conducted on KBV200 and HCT-8/V cancerous cells demonstrated that the application of the CRISPR/Cas9 system to target and eliminate the ABCB1 gene resulted in a notable augmentation in the intracellular buildup of doxorubicin (DOX) and a considerable improvement in chemosensitivity (Yang et al. 2016; Khan et al. 2022b). In a separate investigation involving BC cells that exhibited resistance to DOX, the researchers focused on the MDR1 gene and employed the CRISPR/Cas9 technology to suppress its expression. The findings of this study indicate a notable augmentation in the cytotoxic effects of DOX when administered to BC cells that had developed resistance, following treatment with Cas9-sgRNA plasmid (Ha et al. 2016). The study employed flow cytometric research to demonstrate that the utilization of the CRISPR/Cas9 system to target MDR1 in DOX-resistant BC cells resulted in a notable enhancement in intracellular drug accumulation. Specifically, the treated cells exhibited a fourfold increase in drug uptake when compared to the untreated cells (Ha et al. 2016). In a separate investigation pertaining to ovarian carcinoma cells, a total of three sgRNAs were devised and subsequently employed for the purpose of specifically targeting the fourth and fifth exons of the ABCB1

gene. The findings of this study indicate that the utilization of the CRISPR/Cas9 technology can effectively reduce the expression of the ABCB1 gene. Furthermore, the findings from the MTT experiment demonstrated a notable enhancement in the vulnerability of ovarian carcinoma cells to DOX (Norouzi-Barough et al. 2018). The findings of a meta-analysis also demonstrated that the utilization of CRISPR/Cas9 technology to target the ABCB1 gene might effectively reduce DOX resistance in drug-resistant osteosarcoma tumor cells (Liu et al. 2016a).

Targeting Metabolizing Enzymes

Glutathione S-transferase (GST) as well as glutathione plays significant roles in the processes of drug detoxification and chemotherapy resistance in cancerous cells. The involvement of GST in chemotherapy resistance may be attributed to its ability to augment the binding of chemotherapeutic agents with glutathione, thereby facilitating the detoxification of these drugs and mitigating apoptosis (Sameer et al. 2024). A study conducted on colorectal cancer cells demonstrated that the elimination of GST omega 1 (GSTO1) through the utilization of the CRISPR/Cas9 process resulted in an enhancement of the cytotoxic effects of chemotherapeutic drugs, such as cisplatin as well as oxaliplatin (Xu and Zhu 2020; Wadhwa et al. 2020).

Targeting DNA Repair Mechanisms

Enhancing the repair of DNA might represent another route of drug resistance in cancerous cells. Cyclin-dependent kinases (CDKs) play a significant role in this context, and their involvement in the development of drug resistance in cancerous cells has been substantiated. CDKs are a class of enzyme kinases that play a crucial role in the regulation of the cell cycle, cellular proliferation, and DNA repair processes (Khan et al. 2023d; Ahmad et al. 2022; Malumbres and Barbacid 2009; Johnson and Shapiro 2010; Singh et al. 2017). Research has demonstrated that the upregulation of such enzymes is observed in several types of malignancies, and the utilization of inhibitors targeting specific enzymes has exhibited highly encouraging outcomes in the therapeutic management of cancer. An instance of a CDK4/6 inhibitor that has obtained approval from the FDA as a pharmaceutical intervention for BC is palbociclib (Sherr et al. 2016). Nevertheless, an additional concern arises with the development of drug resistance against palbociclib, associated with the expression of CDK6. A study conducted on BC cells resistant to palbociclib revealed an upregulation of CDK6 expression. The findings of this investigation furthermore demonstrated that the utilization of the CRISPR/Cas9 method to eliminate CDK6 resulted in an augmented sensitivity to palbociclib and a decreased viability of cancer cells after palbociclib administration (Cornell et al. 2019). CDK11 has been observed to play a role in the development of drug resistance in cancerous cells. According to a research investigation conducted on ovarian cancerous cells, it was

observed that the protein CDK11 had a role in promoting cell proliferation, inhibiting programmed cell death, and conferring resistance to the chemotherapy drug paclitaxel (Liu et al. 2016b). A recent investigation on osteosarcoma cells demonstrated the potential for targeted disruption of CDK11 through the construction of sgRNA with the specific sequence TCCGAGACATTTGCTGGGGTGG, utilizing CRISPR/Cas9 system. The findings of this study revealed that the knockout of CDK11 via CRISPR/Cas9 resulted in a substantial rise in cell death and a reduction in the invasive properties of cancerous cells (Feng et al. 2015). BRCA1, a widely recognized and crucial tumor suppressor involved in DNA repair, holds significant importance in the field (Wu et al. 2010).

The incidence of BRCA1 mutations in breast as well as ovarian malignancies is approximately 1–7%, regardless of age or familial history (Balmana et al. 2011). The BRCA1 gene has been observed to mitigate the impact of certain chemotherapeutic medications by enhancing the activity of DNA repair mechanisms (Kennedy et al. 2004). These pharmaceutical compounds eliminate cancerous cells by initiating DNA damage. The presence of elevated levels of BRCA1 has been found to be correlated with the development of resistance to chemotherapy in cancerous cells (Taron et al. 2004). BRCA1 mutations, commonly referred to as BRCA1m, exhibit a significant degree of heterogeneity and present challenges in terms of targeted interventions. Nevertheless, it is feasible to target PARP1, which serves as the synthetic lethality partner of the BRCA1 gene, hence potentially augmenting chemosensitivity. The investigation of TNBC cells harboring mBRCA1 mutations revealed that the utilization of the CRISPR/Cas9 system to target PARP1 resulted in heightened responsiveness to chemotherapeutic agents, such as doxorubicin, docetaxel, and gemcitabine. Consequently, a reduced dosage of these medications is sufficient to attain therapeutic effectiveness (Mintz et al. 2020). Hence, the utilization of PARP1 inhibiting agents, CRISPR/Cas9 technology, as well as chemotherapy in combination has promise for the efficacious management of TNBC with mBRCA1. In a separate investigation pertaining to BC cells, a CRISPR/Cas9 system was devised to specifically target exon 12 HER2 and induce genetic alterations. The findings of this investigation demonstrated a notable inhibitory impact on the proliferation of cancerous cells when CRISPR/Cas9 technology and PARP inhibitors were used concurrently (Wang and Sun 2017).

The RECQL4 helicase protein is an additional component implicated in the processes of DNA repair as well as drug resistance. This protein has been observed to potentially augment the MDR1 (ABCB1) expression (Król et al. 2020). In a recent investigation conducted on glioma cells, researchers utilized the CRISPR/Cas9 method to specifically target and eliminate RECQL4 (Mo et al. 2016). The results demonstrated that this approach effectively augmented the cytotoxic effects of temozolomide (TMZ) on glioma cells. Consequently, the extent of DNA damage and various indicators of apoptosis, such as PARP-1 and phospho-H2AX, as well as cleaved caspases 3 and 7, exhibited a significant rise (Król et al. 2020). Hence, the utilization of CRISPR/Cas9 innovation to selectively target this protein is a viable therapeutic strategy for circumventing medication resistance in cancer.

Targeting Tumor Suppressor and Proto-oncogenes

Numerous studies have demonstrated that genetic alterations in oncogenes or tumor suppressor genes, such as KRAS and P53, significantly contribute to the development of treatment resistance in cancer cells (Abrams et al. 2010). The presence of these mutations has the potential to induce substantial alterations in the cellular signaling cascades that are related to reduced apoptosis, increased expression of ABC family transporter, and accelerated DNA repair (Healy et al. 2022; Sui et al. 2012; Daniyal et al. 2021; Sampath et al. 2001; Hientz et al. 2017; Nalli et al. 2019; Mir et al. 2023). Recent research has revealed that the utilization of the CRISPR/Cas9 system to target oncogenes and mutant tumor suppressors holds the potential for effectively addressing treatment resistance in cancerous cells (Aubrey et al. 2016). KRAS mutations have been identified as significant contributors to the origin, advancement, and development of drug resistance and therapeutic inefficacy in various malignancies, including colorectal cancer (CRC) (Liu et al. 2019; Siddiqui and Piperdi 2010). A compelling investigation was conducted on cells afflicted with CRC, wherein the successful manipulation of the KRAS gene was achieved through the strategic creation of a suitable sgRNA and the utilization of the CRISPR/Cas9 system. Furthermore, nanotechnology was employed to facilitate the effective transport of the CRISPR/Cas9 method. The findings of this study demonstrate that the utilization of CRISPR/Cas9 technologies to target KRAS can effectively augment the apoptotic effect of cetuximab. The results of the study also indicated that the application of CRISPR/Cas9 technology to specifically target KRAS in mice with KRAS-mutated colorectal cancer resulted in a notable reduction in tumor growth. Additionally, this approach synergistically increased the apoptotic effects of cetuximab treatment (Ryu et al. 2020). It is important to point out that cetuximab is a type of EGFR inhibitor that is employed in the treatment of colorectal cancer cases where the KRAS gene is unmutated (Wa 2008). Mutation in the p53 gene is a phenomenon that can appear in various types of cancers and has been implicated in the development of drug resistance (Cao et al. 2020). Recent studies have suggested that the utilization of the CRISPR/Cas9 approach to eliminate the mutated TP53 gene in osteosarcoma cells could potentially enhance the responsiveness to DOX, a commonly used chemotherapeutic agent, while simultaneously reducing the anti-apoptotic protein expression such as Bcl-2 as well as survivin (Tang et al. 2019).

CRISPR/Cas9 Targeting Multiple Receptors to Conquer Drug Resistance

The EGFR, also known as the epidermal growth factor receptor, is a type of receptor tyrosine kinase (RTK) that functions as a receptor for several members of the epidermal growth factor family. In typical circumstances, this receptor is implicated in the growth and maintenance of epithelial tissue. However, an upregulation of its expression, perhaps resulting from diverse mutations, plays a significant function in the initiation of tumor formation and the acquisition of resistance to therapeutic

drugs (Liu et al. 2020). EGFR mutations have been observed in a range of malignancies, including lung carcinoma as well as glioblastoma (Zheng 2017; Sigismund et al. 2018). The use of EGFR inhibitors as a treatment option for managing EGFR-mutant NSCL cancer has exhibited promising outcomes. Nevertheless, the issue of treatment resistance is a significant challenge (Tomasello et al. 2018). In a compelling investigation of NSCL cancer cells, researchers devised the CRISPR/Cas9 technology to specifically target EGFR harboring a single-nucleotide missense mutation (CTG > CCG). In this investigation, the combination treatment of sgRNA-EGFR and Cas9 was employed to target cancer cells, utilizing adenoviral vectors. The findings of this study demonstrate the effective utilization of CRISPR/Cas9 technologies in disrupting mutant EGFR, resulting in an observed augmentation of cellular apoptosis. In the context of *in vivo* analysis, mice exhibited a notable reduction in the size of tumors and an improvement in survival rate (Koo et al. 2017). In a separate investigation pertaining to renal cancer cells, a pair of sgRNA molecules and homology-directed repair (HDR) templates were employed to selectively target exon 2 and induce the disruption of EGFR gene expression through the utilization of CRISPR/Cas9 technique. The findings of this investigation demonstrated that the co-administration of sunitinib and the disruption of EGFR by CRISPR/Cas9 technology yielded a notable impact in reducing the cancer cell proliferation. Conversely, the influence of sunitinib on cancerous cells with unaltered EGFR expression was comparatively diminished (Liu et al. 2020). The urokinase plasminogen activator receptor (uPAR) is a protein that is frequently upregulated in different forms of cancer. The primary activity of this glycosyl-phosphatidyl inositol (GPI)-linked membrane receptor protein is to target the proteolytic function of urokinase. Urokinase has a role in the degradation of ECM components. The uPAR protein has been implicated in the promotion of epithelial-mesenchymal transition (EMT) and stemness, as well as the facilitation of cancer cell invasion, metastasis, and resistance to therapeutic drugs (Rizzo and Porta 2017; Gonias and Hu 2015).

In one study of multidrug-resistant cancer cells (HCT8/T and KBV200), the uPAR gene was knocked out effectively using CRISPR/Cas9 technology. The results of the MTT test in this study showed that in cancerous cells with knocked out uPAR, IC50 of chemotherapy drugs, including 5-FU, cisplatin, DOX, and docetaxel, was significantly reduced, indicating drug resistance attenuation and enhancement of cancer cell chemosensitivity (Li Santi et al. 2021).

Targeting CD44 Via CRISPR/Cas9

CD44 is widely recognized as a significant indicator of resistance to therapy and serves as a surface marker for cancer stem cells (Cain et al. 2011). Hyaluronic acid, a component of the ECM, is receptive to this glycoprotein. Research conducted on ovarian cancer cells has indicated that there is an upregulation of CD44 expression in cancer cells that are resistant to drugs. Furthermore, it has been observed that CD44 plays a significant role in the development of resistance to paclitaxel (Gao et al. 2015). CD44 has been implicated in the development of drug

resistance in cancer cells through multiple mechanisms. These mechanisms include the reduction of ubiquitination as well as degradation of ABCB1, the upregulation of anti-apoptotic proteins such as Bcl-xL, and the modulation of glucose metabolism by enhancing glycolysis and the pentose phosphate pathway (Cain et al. 2011; Ravindranath et al. 2015; Tamada et al. 2012). The findings of a research investigation on DR osteosarcoma cells showed that the utilization of the CRISPR/Cas9 approach to target CD44 resulted in a notable decrease in ABCB1 expression and a substantial enhancement in DOX sensitivity (Xiao et al. 2018). In a separate investigation, a study examining hepatocellular carcinoma cells similarly showed that the utilization of the CRISPR/Cas9 system to disrupt CD44 expression resulted in heightened susceptibility to sorafenib and 5-FU treatment (Asai et al. 2019). Furthermore, the findings of a meta-analysis revealed that the disruption of CD44 through the utilization of the CRISPR/Cas9 strategy exhibited the ability to impede metastasis, invasion, and spheroid formation in osteosarcoma cells (Liu et al. 2018). Hence, the investigation of the efficacy of CRISPR/Cas9 methods for targeting CD44 as a potential strategy for cancer therapy needs additional exploration.

Targeting MicroRNAs (miRNAs) and Long Noncoding RNAs (lncRNAs)

The investigation of the correlation between microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) in relation to the development of treatment resistance in cancer is a topic of significant research interest. Certain miRNAs and lncRNAs have shown potential as viable targets for therapeutic interventions (Ratti et al. 2020). The miR-371/372/373 cluster is a group of noncoding RNAs that have been observed to possess carcinogenic characteristics and play a role in the development of drug resistance (Yamaguchi et al. 2022). A recent investigation conducted on oral cancer cells has demonstrated the potential efficacy of CRISPR/Cas9 technology in eliminating the miR-371/372/373 cluster. The findings of this investigation demonstrated that this deletion greatly amplifies the apoptotic effects of cisplatin, resulting in a significant rise in the rate of late apoptosis in deleted subclones following a 48-hour exposure with 15 μ M cisplatin (Lin et al. 2020). A study demonstrated the inhibition of miR-21, a widely recognized onco-miRNA, in ovarian cancerous cells through the utilization of the CRISPR/Cas9 technique and lentiviral vector. The findings of this investigation demonstrated that the suppression of miR-21 expression through the utilization of the CRISPR/Cas9 methodology has the potential to enhance the apoptotic impacts of paclitaxel and mitigate the process of EMT in cells affected by ovarian cancer (Huo et al. 2017). Another study conducted by researchers demonstrated that the elimination of the promoter of the long noncoding RNA (lncRNA) gene in cells affected by triple-negative breast cancer, through the utilization of the CRISPR/Cas9 approach, exhibited the potential to augment the sensitivity of these cells to doxorubicin and paclitaxel (Shaath et al. 2021). The findings of this study indicate that the utilization of the CRISPR/Cas9 technique to target

miRNAs and lncRNAs implicated in cancer drug resistance holds promise as a potential strategy for surmounting drug resistance in many types of malignancies. However, additional investigation is needed to comprehensively explore and validate the efficacy of this method.

11.4 Conclusion

In conclusion, the book chapter illuminates a transformative trajectory in cancer therapeutics. The tandem exploration of “drug delivery based on nanoparticles (NPs)” and “CRISPR-based strategies” presents an integrative and cutting-edge approach to counteracting drug resistance. NPs showcase the potential of nanotechnology to revolutionize drug delivery, enhancing precision and efficacy while minimizing systemic side effects. Transitioning seamlessly, the exploration of CRISPR-based interventions unveils a genetic editing revolution that holds promise in disrupting resistance mechanisms at their core. Together, these innovative strategies offer a comprehensive toolkit to address the intricate challenges of drug resistance in cancer. The chapter serves not only as a demonstration of the steps made in personalized medicine but also as a roadmap for future research and clinical applications, accompanying a new era where tailored interventions hold the key to overcoming drug resistance and improving patient outcomes.

MCQs

1. **What is the primary purpose of chemotherapy in cancer treatment?**

- A. Targeting specific genetic mutations
- B. Enhancing immune system response
- C. Killing rapidly proliferating cells
- D. Promoting healthy cell growth

Answer C: Killing rapidly proliferating cells

2. **Why has there been a focus on developing medications with enhanced specificity towards tumor cells?**

- A. To increase drug resistance
- B. To mitigate adverse effects of chemotherapy
- C. To promote bone marrow reduction
- D. To enhance digestive reactions

Answer B: To mitigate adverse effects of chemotherapy

3. **What is a significant advantage of nanoparticle-based drug delivery systems in cancer therapy?**

- A. Inducing hair loss
- B. Facilitating bone marrow reduction
- C. Favorable pharmacokinetics and accurate tumor cell localization
- D. Increasing drug resistance

Answer C: Favorable pharmacokinetics and accurate tumor cell localization

4. **How do nano-carriers function in cancer therapy?**

- A. Targeting healthy cells
- B. Absorbing toxic substances
- C. Selectively targeting tumor cells and delivering drugs
- D. Inducing cytotoxic effects

Answer C: Selectively targeting tumor cells and delivering drugs

5. **How does combination therapy using nanoparticle-based therapies address challenges associated with drug resistance?**

- A. Incorporating multiple therapeutic agents into a single drug carrier
- B. Utilizing high pH levels as stimuli for drug release
- C. Reducing ATP supply to efflux pumps
- D. Releasing drugs primarily via diffusion

Answer A: Incorporating multiple therapeutic agents into a single drug carrier

6. **What role does COX-2 play in P-gp-mediated multidrug resistance (MDR) in cancers?**

- A. Enhancing the expression of P-gp
- B. Decreasing the expression of P-gp
- C. Suppressing the ABC transporters
- D. Inducing drug-resistant tumors

Answer A: Enhancing the expression of P-gp

7. **What is a significant contributor to the development of treatment resistance in cancer by allowing cancer cells to avoid undergoing apoptosis?**

- A. Dysregulation of Bcl-2 and NF- κ B
- B. Overexpression of p53
- C. Activation of efflux pumps
- D. Inhibition of apoptosis pathways

Answer A: Dysregulation of Bcl-2 and NF- κ B

8. **What is the crucial role of Bcl-2 in the context of drug resistance in cancer?**

- A. Inducing apoptosis in cancer cells
- B. Enhancing the effectiveness of chemotherapy
- C. Inhibiting the apoptosis of cancer cells
- D. Suppressing tumor growth

Answer C: Inhibiting the apoptosis of cancer cells

9. **What is a promising strategy for overcoming medication resistance in cancer, particularly involving the restoration of wild-type p53 functions?**

- A. Inhibition of NF- κ B
- B. NP-based delivery system for p53 gene therapy
- C. Activation of efflux pumps
- D. Dysregulation of Bcl-2

Answer B: NP-based delivery system for p53 gene therapy

10. **How did the combination of doxorubicin with resveratrol within NPs demonstrate cytotoxic effects on doxorubicin-resistant breast cancer cells?**

- A. Inducing ATP synthesis
- B. Activating efflux transporters

- C. Suppressing NF- κ B and Bcl-2 expression
- D. Inhibiting apoptosis pathways

Answer C: Suppressing NF- κ B and Bcl-2 expression

11. **What impact do nanoparticles targeting specifically mitochondria have on efflux transporters and apoptotic pathways?**
- A. Increase in ATP synthesis
 - B. Stimulation of caspase-3 and caspase-9
 - C. Activation of efflux pumps
 - D. Induction of alternative pre-mRNA splicing

Answer B: Stimulation of caspase-3 and caspase-9

12. **How does hypoxia contribute to medication resistance in cancer cells?**
- A. Enhancing drug effectiveness
 - B. Inducing slow division of cells
 - C. Evading damaging effects of chemotherapeutic drugs
 - D. Facilitating tumor heterogeneity

Answer C: Evading damaging effects of chemotherapeutic drugs

13. **What role does HIF-1 α play in hypoxia-induced medication resistance, according to the text?**
- A. Inhibiting drug efflux proteins
 - B. Facilitating tumor heterogeneity
 - C. Enhancing drug susceptibility
 - D. Upregulating drug efflux proteins

Answer D: Upregulating drug efflux proteins

14. **What therapeutic approach is suggested for circumventing medication resistance related to HIF-1 α expression?**
- A. Inhibitors of the PI3K/Akt/mTOR system
 - B. Activation of HSP90
 - C. Suppression of the hypoxic environment
 - D. Increased expression of HIF-1 α

Answer A: Inhibitors of the PI3K/Akt/mTOR system

15. **In the study involving KBV200 and HCT-8/V cancerous cells, what was the outcome of applying the CRISPR/Cas9 system to target and eliminate the ABCB1 gene?**
- A. Decreased intracellular buildup of doxorubicin (DOX)
 - B. Augmentation in chemosensitivity
 - C. Increased resistance to DOX
 - D. No significant change in drug accumulation

Answer B: Augmentation in chemosensitivity

16. **How did the researchers in the investigation on BC cells resistant to DOX employ the CRISPR/Cas9 technology to target the MDR1 gene?**
- A. Activation of MDR1 gene
 - B. Inhibition of DOX
 - C. Suppression of MDR1 gene expression
 - D. Induction of apoptosis

Answer C: Suppression of MDR1 gene expression

17. **What notable enhancement is observed in intracellular drug accumulation in DOX-resistant BC cells treated with the CRISPR/Cas9 system targeting MDR1?**

- A. No change in drug uptake
- B. Twofold increase in drug uptake
- C. Fourfold increase in drug uptake
- D. Decrease in drug uptake

Answer C: Fourfold increase in drug uptake

18. **How did the elimination of GST omega 1 (GSTO1) through the CRISPR/Cas9 process impact the cytotoxic effects of chemotherapeutic drugs?**

- A. Decreased cytotoxic effects
- B. No change in drug sensitivity
- C. Enhancement of cytotoxic effects
- D. Resistance to chemotherapy

Answer C: Enhancement of cytotoxic effects

19. **What role do cyclin-dependent kinases (CDKs) play in the context of drug resistance in cancerous cells?**

- A. Inhibiting programmed cell death
- B. Enhancing DNA repair processes
- C. Regulating cellular proliferation
- D. Both b and c

Answer D: Both B and C

20. **Which CDK4/6 inhibitor obtained FDA approval for pharmaceutical intervention in breast cancer (BC)?**

- A. Palbociclib
- B. Doxorubicin
- C. Paclitaxel
- D. Gemcitabine

Answer A: Palbociclib

21. **In the study on BC cells resistant to palbociclib, what was the impact of using the CRISPR/Cas9 method to eliminate CDK6?**

- A. Increased resistance to palbociclib
- B. Augmented sensitivity to palbociclib
- C. No change in viability of cancer cells
- D. Enhanced programmed cell death

Answer B: Augmented sensitivity to palbociclib

22. **What is the role of BRCA1 in chemotherapy resistance in cancerous cells?**

- A. Enhances activity of DNA repair mechanisms
- B. Inhibits CDK activity
- C. Promotes cell proliferation
- D. Suppresses apoptosis

Answer A: Enhances activity of DNA repair mechanisms

23. **In the investigation of TNBC cells with mBRCA1 mutations, what was the outcome of targeting PARP1 using the CRISPR/Cas9 system?**

- A. Decreased responsiveness to chemotherapeutic agents
- B. Increased dosage of medications required for therapeutic effectiveness

- C. Heightened responsiveness to chemotherapeutic agents
- D. No impact on chemosensitivity

Answer C: Heightened responsiveness to chemotherapeutic agents

24. **What is the effect of suppressing miR-21 expression in ovarian cancerous cells using the CRISPR/Cas9 methodology?**
- A. Decreased apoptotic impacts of paclitaxel
 - B. No change in EMT process
 - C. Increased sensitivity to paclitaxel
 - D. Enhanced angiogenesis

Answer C: Increased sensitivity to paclitaxel

25. **In triple-negative breast cancer cells, what potential outcome was observed when the promoter of the lncRNA gene was eliminated using CRISPR/Cas9?**
- A. Reduced sensitivity to doxorubicin and paclitaxel
 - B. Increased drug resistance
 - C. Enhanced cellular proliferation
 - D. Augmented sensitivity to doxorubicin and paclitaxel

Answer D: Augmented sensitivity to doxorubicin and paclitaxel

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Future Directions and Challenges in Overcoming Drug Resistance in Cancer

12

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Abstract

The chapter Future Directions and Challenges in Overcoming Drug Resistance in Cancer explores crucial aspects, primarily focusing on two key topics: biomarkers for the development of effective drugs and the integration of artificial intelligence (AI) and machine learning (ML) in drug development. The identification and validation of biomarkers play a pivotal role in tailoring targeted therapies and enhancing treatment efficacy by addressing drug resistance in cancer. Additionally, the chapter delves into the transformative potential of AI and ML in drug development, emphasizing their role in accelerating the discovery of novel compounds, optimizing drug design, and predicting patient responses. By examining these topics, the chapter provides valuable insights into the evolving landscape of cancer therapeutics, offering a forward-looking perspective on strategies to overcome drug resistance and improve treatment outcomes.

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12.1 Introduction

The formidable challenge of drug resistance in cancer has persistently thwarted efforts to achieve lasting therapeutic success (Cree and Charlton 2017). Despite significant advancements in our understanding of cancer biology and the development of innovative treatment modalities, the emergence of drug-resistant phenotypes poses a substantial hurdle in the effective management of various malignancies (Donnenberg and Donnenberg 2005). As we stand at the intersection of cutting-edge research and clinical application, it is imperative to explore future directions and confront the challenges that lie ahead in our relentless pursuit of conquering drug resistance. The intricate landscape of drug resistance in cancer encompasses a myriad of adaptive mechanisms employed by malignant cells to evade the cytotoxic effects of therapeutic agents (Dzobo et al. 2023). These mechanisms include, but are not limited to, alterations in drug efflux transporters, dysregulation of apoptotic pathways, genetic mutations, and the dynamic tumor microenvironment. Understanding the interplay of these factors is pivotal in devising strategies that not only target the existing resistance but also anticipate and counteract adaptive responses.

Future directions in overcoming drug resistance prominently feature the integration of advanced technologies and multidisciplinary approaches. The advent of precision medicine, fueled by genomics and personalized therapeutic strategies, holds promise in tailoring treatments to the unique genetic makeup of individual tumors (Dadwal et al. 2018; Khan et al. 2022a). Furthermore, the evolution of nanotechnology provides a platform for the development of targeted drug delivery systems, allowing for enhanced drug bioavailability and specific targeting of cancerous cells while minimizing collateral damage to healthy tissues. Artificial intelligence emerges as a pivotal player in this narrative, offering unprecedented capabilities in data analysis, pattern recognition, and predictive modeling. AI-driven algorithms examine through vast datasets, deciphering intricate genetic patterns, elucidating signaling pathways, and predicting the likelihood of resistance development (Liang et al. 2020). Machine learning algorithms, honed by exposure to diverse clinical scenarios, empower clinicians and researchers with insights that transcend the boundaries of traditional approaches. Precision medicine, propelled by AI, stands as a beacon of hope in the quest to tailor therapies with exquisite specificity to individual patients. Genomic profiling, coupled with AI analytics, enables the identification of subtle genetic variations that underlie drug resistance. This personalized approach promises to revolutionize treatment strategies, ensuring that interventions are not only effective but also dynamically adapted to the evolving genetic landscape of each patient's cancer (Liang et al. 2020).

Furthermore, the application of AI extends beyond the realm of genomics. Advanced imaging techniques, coupled with machine learning algorithms, refine

our ability to discern subtle changes in tumor morphology and microenvironment dynamics. This holistic understanding equips clinicians with real-time insights into the intricacies of resistance evolution, guiding timely therapeutic adjustments.

12.2 Biomarkers for the Development of Effective Drugs

In the field of drug research, clinical trials have traditionally utilized a toxicity-driven strategy to establish a predetermined threshold of toxicity. This technique aims to optimize drug exposure under the belief that it will concurrently enhance the antitumor effects. The advancement of cancer therapeutics has necessitated researchers to modify the technique of developing novel molecularly targeted medicines due to many factors. To begin with, targeted medicines are specifically engineered to obstruct a particular chemical or intracellular pathway, resulting in a comparatively restricted toxicity profile in comparison to cytotoxic chemotherapies. Consequently, a number of these therapeutic approaches fail to attain a “toxicity ceiling” or maximum tolerated dosage (MTD) in traditionally structured dose-escalation studies (Smith et al. 2014). Enhanced comprehension of the intracellular pathways that are essential to cancerous cells will additionally enable the assessment of pharmacodynamic (PD) effects through the measurement of downstream markers and alternative routes. This provides the opportunity to customize the dosage and administration schedule based on the effects of the PD, rather than focusing solely on probable adverse reactions. The desire to assess the effectiveness of treatments at an earlier stage in the treatment process has driven the exploration of new biomarker assays as potential intermediary endpoint biomarkers for measuring response (Smith et al. 2014; Wadhwa et al. 2020).

It is imperative to conduct a comprehensive evaluation of potential new biomarkers, encompassing both retrospective and prospective analyses (Hall et al. 2014). A diverse range of biomarkers, including pharmacodynamic, predictive, pharmacogenomic, pharmacokinetic, and intermediate endpoint biomarkers, need to be explored. These biomarkers would be integrated into intelligent, hypothesis-driven initial phase clinical studies with comprehensive outcome data. To assure the accuracy and repeatability of laboratory operations, the creation and assessment of any new biomarkers must follow the Clinical Laboratory Improvement Amendments (CLIA) and Good Clinical Laboratory Practice (GCLP) standards. This section focuses on the identification and examination of essential biomarkers that are crucial for the effective advancement of innovative molecularly targeted therapies.

Predictive Biomarkers

Predictive biomarkers are indicative of the probability of a positive response to particular anticancer drugs (Fig. 12.1) (Yap and Workman 2012). Predictive biomarkers encompass tumor-specific as well as surrogate biomarkers, playing a pivotal role in expediting the drug development trajectory. An instance of this can be observed

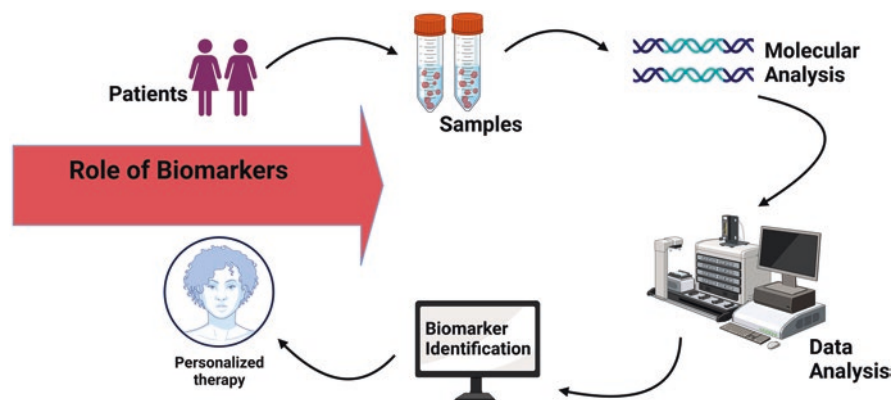


Fig. 12.1 The figure illustrates the pivotal role of biomarkers in guiding and optimizing cancer therapy

in the case of ERBB2 (HER2), which is a transmembrane tyrosine kinase found within cells and is expressed by the ERBB2 gene. The higher expression or amplification of HER2 in BCs serves as a valuable biomarker, playing a crucial role in identifying patients who are more likely to benefit from HER2-targeted medications. This has facilitated the development of various drugs such as trastuzumab (Dj 1987; Slamon et al. 2001), trastuzumab-DM1 (Burris et al. 2011; Krop et al. 2010), lapatinib (Spector et al. 2005), and pertuzumab (Baselga and Tabernero 2007). The oncogenic BCR-ABL gene fusion is a widely recognized predictive biomarker that is associated with antitumor response to the tyrosine kinase inhibitor imatinib in cases of chronic myelogenous leukemia (Talpaz et al. 2002). In recent times, biomarkers have been utilized to enhance or predict sensitivity to a specific targeted agent. Examples of such biomarkers include BRCA1 and BRCA2 mutations, which have been associated with the PARP inhibitor olaparib (Fong et al. 2009; Ledermann et al. 2012; Tutt et al. 2010; Audeh et al. 2010). Additionally, EML4-ALK fusions have been related to the ALK/MET inhibitor crizotinib (Kwak et al. 2010; Wani et al. 2021), while the V600E BRAF mutation has been associated with the BRAF inhibitor vemurafenib (Flaherty et al. 2010). Furthermore, EGFR wild-type advanced colorectal carcinoma has been found to be responsive to EGFR-targeted antibodies such as cetuximab (Van Cutsem et al. 2009) and panitumumab (Amado et al. 2008), and EGFR mutant metastatic NSCLC has shown sensitivity to small-molecule inhibitors like erlotinib (Zhou et al. 2011; Rosell et al. 2012) and gefitinib (Mok et al. 2009).

The word “enrichment biomarker” is employed to denote biomarkers that possess a robust scientific basis and preclinical data supporting their potential for anti-tumor response, although they have not undergone clinical confirmation (Yap et al. 2010). The enrichment biomarkers that are presently undergoing clinical trials have the potential to be clinically validated and subsequently evolve into predictive biomarkers in the years to come. Illustrative instances include PTEN loss or PIK3CA mutations as potential targets for PI3K-Akt-mTOR cascade inhibitors

Table 12.1 The list of biomarkers that have been identified in various types of malignancies, along with the corresponding drugs that have shown relevance in targeting these biomarkers

Cancer type	Biomarker	Drug	Mechanism of action of drug
Breast cancer	HER2	Lapatinib	HER2 and EGFR reversible tyrosine kinase inhibitor
		Trastuzumab	Humanized recombinant monoclonal antibody targeting HER2
Colorectal cancer	EGFR	Panitumumab	EGFR-specific humanized monoclonal antibody
Lung cancer	ALK	Ceritinib	ALK's tyrosine kinase inhibitor
Melanoma	MEK	Trametinib	MEK1/2 inhibitor
Melanoma	CTLA-4	Ipilimumab	Monoclonal antibody that targets CTLA-4 and increases T-cell activation

(NCT01449370; NCT01458067) and IGF mutations as potential targets for IGF-1R antibodies (NCT01562899; NCT01403974). Various biomarker panels, like the TruSeq Amplicon-Cancer Panel (TSACP), have been created to expedite the detection of significant biomarkers, specifically genetic alterations, for investigative purposes. These panels have the potential to serve as valuable point-of-care tests Table 12.1 (Chevrier et al. 2014).

Pharmacodynamics, Pharmacogenomics, and Pharmacokinetics

The utilization of molecularly targeted drugs has resulted in the need for biomarkers that can identify the effects of drugs on the target, route, and subsequent cellular processes (Yap et al. 2010; Workman et al. 2006). Consequently, it is imperative to conduct preclinical investigations in order to establish and assess biomarkers associated with PD, as this is crucial for the advancement of innovative treatment interventions targeting tumors. In a similar way, pharmacokinetic (PK) profiling plays a vital role in verifying the presence of active drug concentrations and in establishing the links between PK-PD drug patterns and toxicity (Yap and Workman 2012). This information can subsequently be utilized to guide Phase I research by establishing PK and PD thresholds to be targeted. The utilization of fresh tumor tissue continues to be widely regarded as the most reliable method for assessing biomarkers. However, there are less invasive alternatives for obtaining normal tissues, such as hair follicles, platelet-rich plasma, skin, and peripheral blood mononuclear cells. These alternative samples can be collected repeatedly over time, reducing the potential for variability between patients and within the same patient (Ern Ang et al. 2012). The interpatient variability in PK and PD after administering fixed dosages of targeted medicines may be influenced by individual pharmacogenomic variables. The presence of genetic variability among hosts might potentially influence the expression or functionality of proteins involved in drug metabolism, as well as alter the drug target itself. Consequently, this variability has the potential to affect the effectiveness and toxicity of treatments. The prevailing approach in clinical practice

involves adjusting the dosage for patients who encounter intolerable toxicities. Nevertheless, this approach entails the risk of undertreating a significant fraction of patients unless their dosage is increased (Smith et al. 2014).

Intermediate Endpoint Biomarkers

Intermediate endpoint biomarkers, also known as surrogate biomarkers, serve as indicators of treatment success at an earlier stage compared to the primary endpoint of the trial. Consequently, they can be used as substitutes for the clinical primary endpoints (Ferraldeschi et al. 2013). The utilization of well-established surrogate biomarkers can effectively expedite the process of medication approval, provide timely assessments of treatment efficacy, and reduce the financial burden and negative health outcomes associated with therapy. A multitude of biomarkers have been investigated in the context of surrogacy. However, the trials assessing their reliability frequently suffer from issues such as low reproducibility, inaccuracy, inconsistent application, or weak correlation with survival outcomes (Morris et al. 2013; Lee et al. 2011). The utilization of tumor markers, including PSA and CA125, as surrogate biomarkers, remains a subject of debate due to the inconclusive evidence on their prognostic value for survival (Morris et al. 2013; Lee et al. 2011). The use of progression-free survival (PFS) as a substitute for overall survival is a common practice, although its validity is a subject of debate in several types of malignancies, especially with the advent of targeted therapy (Oza et al. 2011; Saad et al. 2010; Giessen et al. 2013). In recent times, there has been an assessment of circulating tumor cells (CTCs) as a substitute biomarker in several types of cancer, such as castration-resistant prostate cancer (CRPC), breast cancer, lung cancer, and colon cancer (De Bono et al. 2008, 2011; Hou et al. 2009; Han et al. 2008; Krebs et al. 2011; Hayes et al. 2006; Nadal et al. 2012; Cohen et al. 2008; Scher et al. 2015; Khan et al. 2022b). The use of circulating plasma DNA as a biomarker shows promise in its ability to correlate with tumor behavior as well as shifts in cancer burden in several malignancies, including breast, gastrointestinal stromal, lung, and ovarian cancers (Dawson et al. 2013; Fackler et al. 2014; Newman et al. 2014; Maier et al. 2013; Martignetti et al. 2014). Previous research has reported the presence of measurable quantities of circulating plasma DNA in various types of cancer, including bladder, pancreatic, colorectal, gastroesophageal, melanoma, head and neck, and hepatocellular cancers (Bettegowda et al. 2014; Khan et al. 2023a). In essence, the utilization of improved preclinical models holds the potential to facilitate the elucidation of pertinent molecular pathways underlying both responsiveness and resistance. Genetically engineered mouse models (GEMM) and patient-derived tumor xenografts (PDX) are two experimental models commonly employed in laboratory settings to investigate surrogate indicators (Bidard et al. 2013). In recent times, notable advancements have been achieved in the utilization of PDX models to formulate hypotheses motivated by biomarkers. These hypotheses can subsequently be evaluated in clinical settings to ascertain the suitability of a treatment intervention for patients (Rosfjord et al. 2014).

Metabolomics and Proteomics

The cancer proteome, which refers to the comprehensive collection of proteins expressed by the cancerous cells, and the cancer metabolome, which encompasses the range of small-molecule metabolites generated by the disease, have the potential to provide valuable insights (Sreekumar et al. 2009; Khan et al. 2023b, c). Various techniques, including electrophoresis, mass spectrometry, and protein microarrays, can be employed to analyze metabolomics and proteomics signatures, as well as detect compounds that exhibit differential expression in specific types of cancer (Sreekumar et al. 2009; Álvarez-Chaver et al. 2014; Indovina et al. 2013; Chung and Baxter 2012; Khan et al. 2023d; Mintoo et al. 2021; Hassan et al. 2020). Mass spectrometry has the potential to be utilized for “metabolic phenotyping” through the mapping of interrelated networks of biochemical pathways, hence facilitating the discovery of potential biomarkers (Kaushik et al. 2014). Deep proteomics technologies, like liquid chromatography and high-resolution mass spectrometry (Nagaraj et al. 2011), can be employed to assess proteins with low abundance, even within a single cell type (Fig. 12.2).

Biomarkers for Functional Imaging

Magnetic resonance imaging (MRI) and computed tomography (CT) are widely recognized imaging technologies that play a crucial role in the structural assessment of tumor progression and the evaluation of therapeutic effectiveness in clinical settings. Nevertheless, their ability to evaluate lesions in a functional manner is limited, and they fail to tackle the specific molecular mechanisms occurring within the tumor. The evaluation and monitoring of several cellular processes, such as angiogenesis, cell

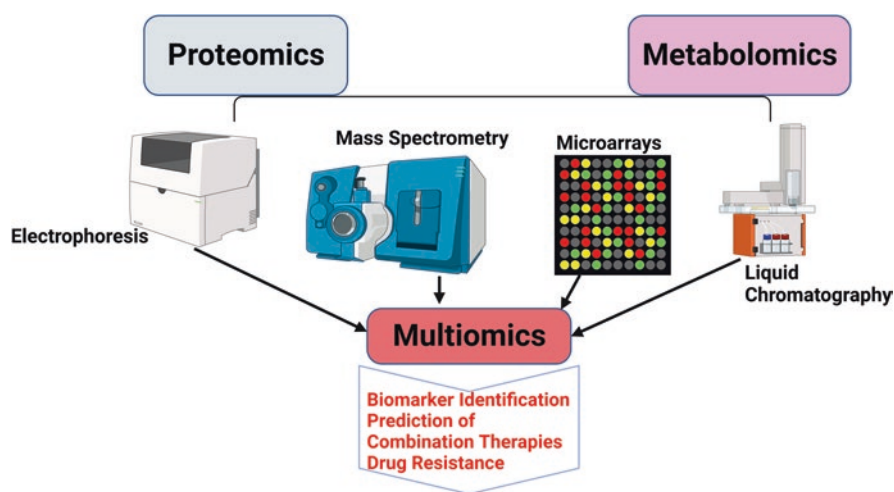


Fig. 12.2 The figure illustrates the integrated role of proteomics and metabolomics in biomarker identification and understanding drug resistance mechanisms in cancer

proliferation, infiltration, metabolism, metastasis, and apoptosis, can potentially be facilitated by the presence of newly developed functional imaging probes (Hassan et al. 2020; Rudin 2007; Ahmad et al. 2022; Khan et al. 2023e; Nalli et al. 2019). Functional imaging techniques, such as dynamic contrast-enhanced MRI (DCE MRI), 18 F-fluorodeoxyglucose positron emission tomography (FDG-PET), diffusion-weighted imaging MRI (DWI MRI), and magnetic resonance spectroscopy (MRS), are commonly utilized modalities for the measurement of cancer-specific molecules as well as signaling cascades (Seddon and Workman 2003). For example, PET tracers can be employed quantitatively to assess cell hypoxia, cellular proliferation, and apoptosis markers (Kurland et al. 2012). Additionally, compartmental modeling and dynamic image acquisition can be applied in conjunction with PET/CT to evaluate tumor perfusion and tracer extraction, as well as tissue metabolism (Muzi et al. 2012). Furthermore, recent advancements in MRI techniques have facilitated the evaluation of functional, dynamic, and metabolic alterations in the tumor vascular system by permeability or perfusion imaging approaches, such as DCE MRI, as well as the examination of the TME using DWI MRI (Paldino and Barboriak 2009; Gerstner and Sorensen 2011; Atri 2006). These technologies provide efficient evaluation of tumor response and the mechanisms by which drugs exert their effects, without the need for invasive procedures. However, the frequent utilization of functional MRI readouts as a reliable biomarker has been hindered by factors such as high expenses and inconsistencies in methodologies across different institutions and MRI platforms (Kurland et al. 2012).

Biomarkers play a pivotal role in advancing the development of effective drugs, particularly in overcoming drug resistance. Predictive biomarkers, such as those associated with pharmacokinetics, pharmacodynamics, and pharmacogenomics, offer valuable insights into how individuals may respond to specific treatments, enabling personalized medicine approaches. Intermediate endpoint biomarkers serve as measurable indicators during clinical trials, providing a bridge between the intervention and clinical outcomes. Metabolomics and proteomics contribute to a deeper understanding of biochemical changes, aiding in the identification of potential drug targets and assessing treatment efficacy. Moreover, the integration of biomarkers with functional imaging enhances our ability to visualize and comprehend drug responses in real time. This holistic approach, encompassing various biomarker categories, not only accelerates drug development but also addresses the challenge of drug resistance by tailoring therapies to individual characteristics and optimizing treatment strategies for improved patient outcomes.

12.3 Artificial Intelligence

AI and Anticancer Drug Development

AI has the potential to be utilized for the prediction of anticancer medication action and to assist in the creation of such drugs. The response modalities of various malignancies to identical medications might vary, and high-throughput screening

techniques frequently provide insights into the correlation between genetic heterogeneity in cancer cells and treatment efficacy. The random forest model developed by Lind and colleagues (Lind and Anderson 2019) was created through the integration of screening data and machine learning techniques. The predictive capacity of the model is capable of determining the efficacy of anticancer medications based on the specific mutational profile of the malignant cell genome. The drug sensitivity prediction model created by Wang et al. (Wang et al. 2018) was based on the utilization of elastic net regression, a machine learning technique. Several studies have demonstrated the efficacy of machine learning models in accurately predicting the medication responsiveness of patients diagnosed with gastric cancer (Li et al. 2019; Taninaga et al. 2019; Liu et al. 2019), ovarian cancer (Hossain et al. 2019; Paik et al. 2019; McDonald 2018), and endometrial cancer (Stanzione et al. 2021; Günakan et al. 2019). The model has identified a subset of patients who are anticipated to exhibit resistance to treatment. Specifically, this subset comprises individuals with ovarian cancer who have undergone tamoxifen therapy, patients with gastric cancer who have received 5-FU treatment, and individuals with endometrial cancer who have been administered paclitaxel. All of these patients were determined to have a negative prognosis. The findings of this study demonstrate the considerable potential of AI in accurately forecasting the sensitivity of anticancer medications. AI also assumes a significant function in the mitigation of therapeutic resistance in cancer, as evidenced by multiple investigations (Beck et al. 2020; Goldenberg et al. 2019; Leventakos et al. 2019). AI possesses the ability to rapidly comprehend the mechanisms underlying the development of drug resistance in cancer cells through the acquisition and analysis of extensive datasets pertaining to drug-resistant cancers. This capability holds promise for enhancing drug development processes and optimizing drug administration protocols.

Chemotherapy and AI

Within the domain of cancer treatment, AI places greater emphasis on investigating the interaction between medications and patients' responses. The primary accomplishments in the field of AI encompass the effective administration of chemotherapy drugs, the ability to forecast tolerance levels for such drugs, and the optimization of chemotherapy treatment plans (Fig. 12.3) (Chen et al. 2018; Levine et al. 2019; Zhu et al. 2012). AI has the potential to enhance and expedite the optimization process of combination chemotherapy. In a particular study, the investigators effectively ascertained the ideal dosage of ZEN-3694 as well as enzalutamide through the utilization of "CURATE.AI," an artificial intelligence platform developed by the National University of Singapore employing deep learning and other advanced methodologies. Consequently, this approach resulted in enhanced treatment effectiveness and tolerability when both medications were administered concurrently (Pantuck et al. 2018). Poly(ADP-ribose) polymerase (PARP) inhibitors have demonstrated potential in the treatment of breast cancer cells with homologous recombination (HR) deficit. Gulhan and colleagues (Gulhan et al. 2019) have created a

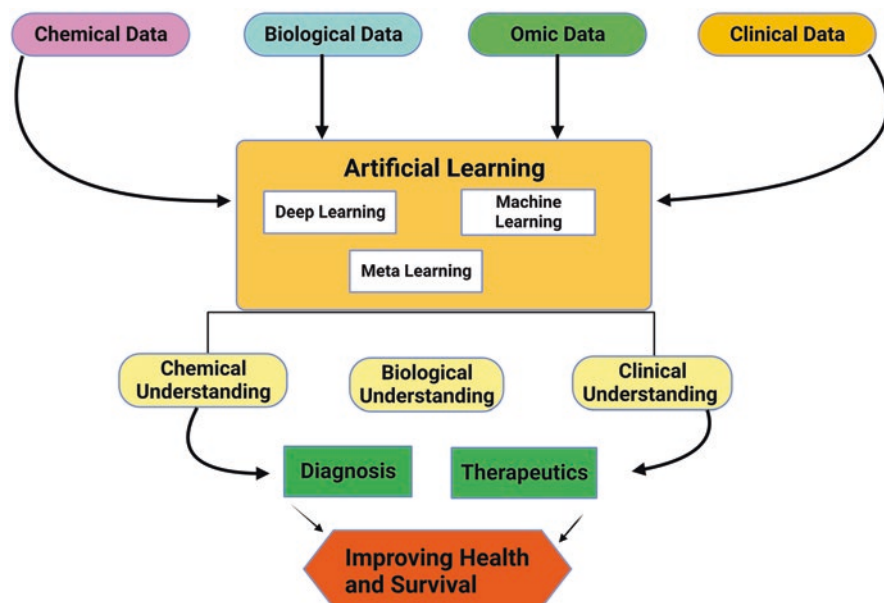


Fig. 12.3 This figure depicts the pivotal role of artificial intelligence (AI) in revolutionizing healthcare

deep learning-based screening system that demonstrates 74% accuracy in detecting cancer cells with HR deficiency. Furthermore, this system has the capability to identify the patients who will derive benefits from PARP inhibitors. Dorman and colleagues (Dorman et al. 2016) devised a machine learning system with the capability to forecast the responsiveness of breast cancer patients to chemotherapy treatment. The research investigation examined the correlation between chemotherapeutic drugs and the genetic composition of patients, successfully discerning the differential impacts of two specific chemotherapy agents, namely, Taxol and gemcitabine.

Radiotherapy and AI

In the case of cancer radiation therapy, the utilization of AI technology exhibits a higher degree of specificity. AI has the potential to assist radiologists in delineating target areas and automating the process of devising radiation regimens for therapy (Fiorino et al. 2020; Lou et al. 2019; Meyer et al. 2018). The study conducted by Lin and colleagues (Lin et al. 2019) employed a three-dimensional convolutional neural network (3D CNN) to accomplish the automated delineation of nasopharyngeal cancer. The achieved accuracy of 79% was found to be comparable to the performance of radiation specialists in this task. In their study, Cha and colleagues (Cha et al. 2017) integrated deep learning techniques with radiomics, a methodology for collecting picture characteristics from radiographic images, in order to develop a predictive model for assessing the treatment response in patients with bladder cancer. Babier and colleagues (Babier et al. 2018) devised automated software utilizing

deep learning technology, resulting in a significant reduction in the duration required for radiation therapy planning, which was condensed to a just few hours. The treatment plan produced by the AI software exhibits a level of comparability to the standard treatment plan of patients while also significantly reducing the time required for its generation.

Immunotherapy and AI

In the context of cancer immunotherapy, AI is mainly focused on assessing the efficacy of treatment and assisting healthcare professionals in modifying the treatment regimen (Jabbari and Rezaei 2019; Trebeschi et al. 2019; Abbasi 2019; Sameer et al. 2024). In their study, Sun and colleagues (Sun et al. 2018) established an AI platform utilizing machine learning techniques. The purpose of their platform was to effectively forecast the therapeutic efficacy of PD-1 inhibitors. The effectiveness of immunotherapy in patients with metastatic solid tumors who exhibit sensitivity to PD-1 inhibitors can be accurately assessed using this platform. A study conducted by Bulik-Sullivan and colleagues (Bulik-Sullivan et al. 2019) devised a machine learning approach that utilizes a database of human leukocyte antigen (HLA) mass spectrometry. This method aims to enhance the detection of cancer neoantigens and thus enhance the effectiveness of immunotherapy for cancer.

AI Minimizes Cancer Overtreatment

The algorithm created by Hu and colleagues (Hu et al. 2019) demonstrates the capability to evaluate digital images of the cervix in women and effectively detect pre-cancerous lesions, hence mitigating the issue of unnecessary therapy for patients. In their study, Bahl and colleagues (Bahl et al. 2018) devised a machine learning method aimed at mitigating the overutilization of treatment for suspected breast cancer lesions. The instrument possesses the capability to ascertain the likelihood of high-risk breast lesions progressing into cancer, aiding medical practitioners in making appropriate treatment choices and minimizing the need for unneeded surgical interventions.

AI and Clinical Decision-Making Systems

The utilization of deep learning technologies enhances the intelligence of cancer treatment decision-making processes. By leveraging large amounts of clinical information on cancer patients, artificial intelligence (AI) has the potential to identify the most optimal treatment plan for medical practitioners (Liu et al. 2018; Bogani et al. 2018; Golden 2017; Walsh et al. 2019; Blackledge et al. 2019). The Clinical Decision Support System (CDSS) created by Printz and colleagues (Printz 2017) uses deep learning technology to effectively analyze extensive clinical data extracted from medical records and provide cancer therapy recommendations. The study

provides evidence of the significance of artificial intelligence (AI) technology in enhancing the ability of oncologists to optimize cancer treatment programs for patients.

12.4 Machine Learning in the Creation of Anticancer Drugs

Models that predict how cancer cell lines and patients will react to novel medications or treatment combinations can be created by training machine learning algorithms on high-throughput screening data (Simon et al. 2020; Meng et al. 2020; Goecks et al. 2020). By generating and creating reverse synthesis paths for compounds using machine learning, scientists are speeding up the process of finding new drugs. A lot of data is produced during the entire process of developing a new medicine. In order to interpret chemical data and provide outcomes that can aid in the development of drugs, machine learning presents fantastic potential (Nascimento et al. 2019; Sharma and Rani 2020; Watson et al. 2019). We can process data gathered over decades or even more in a very short amount of time with the use of machine learning (Vamathevan et al. 2019). Furthermore, it will assist us in reaching better-informed conclusions than we would have to be experimenting and predicting (Koromina et al. 2019; Ballester 2019).

Deep learning, a distinctive technique within the field of machine learning, has demonstrated exceptional performance across various domains, notably in the realm of drug discovery (Xia et al. 2020; Baskin 2020; Stokes et al. 2020). These particular models possess distinct attributes that render them potentially more appropriate for intricate tasks involving the modeling of reactions to drugs using biological and chemical data. However, the utilization of deep learning in predicting drug responses has only been recently investigated. In recent times, deep learning has been employed to achieve remarkable progress in the domain of computer-based information extraction from images. The utilization of extensive datasets in the development of deep learning models has yielded intriguing prospects for drug repurposing. The research conducted by Kadurin and colleagues (Kadurin et al. 2017) serves as an illustrative instance. The researchers utilized the antagonistic autoencoder technique on the complete dose-response data collected from the NCI-60 cell line in order to construct a deep learning model.

12.5 Conclusion

In conclusion, the chapter underscores the critical importance of advancing research in two key domains: biomarkers for effective drug development and the integration of artificial intelligence (AI) and machine learning (ML) in drug discovery. The identification and utilization of biomarkers not only hold the promise of enhancing the precision of cancer treatments but also provide a foundation for personalized therapeutic approaches, crucial in overcoming drug resistance. Furthermore, the incorporation of AI and ML methodologies in drug development emerges as a

game-changer, expediting the discovery process and offering innovative solutions to the complex challenges posed by drug resistance in cancer. By addressing these pivotal aspects, the chapter lays the groundwork for a more targeted, efficient, and adaptive approach to cancer therapeutics, paving the way for breakthroughs in overcoming drug resistance and improving patient outcomes in the future.

MCQs

1. **What is the role of precision medicine in overcoming drug resistance?**

- A. Enhancing drug efflux transporters
- B. Targeting apoptotic pathways
- C. Tailoring treatments to individual tumor genetics
- D. Minimizing the role of nanotechnology

Answer C: Tailoring treatments to individual tumor genetics

2. **What is the role of artificial intelligence (AI) in addressing drug resistance?**

- A. Enhancing drug efflux mechanisms
- B. Analyzing genetic variations
- C. Inducing apoptotic pathways
- D. Minimizing the role of precision medicine

Answer B: Analyzing genetic variations

3. **How does AI contribute to overcoming drug resistance in the realm of genomics?**

- A. Identifying genetic variations underlying drug resistance
- B. Minimizing the role of personalized therapies
- C. Targeting apoptotic pathways
- D. Enhancing drug efflux mechanisms

Answer A: Identifying genetic variations underlying drug resistance

4. **In addition to genomics, where else does AI play a crucial role in understanding drug resistance?**

- A. Immunotherapy mechanisms
- B. Advanced imaging and microenvironment dynamics
- C. Nanoparticle drug delivery
- D. Traditional clinical approaches

Answer B: Advanced imaging and microenvironment dynamics

5. **Why has the traditional toxicity-driven strategy for clinical trials faced challenges in developing molecularly targeted medicines?**

- A. Lack of focus on drug exposure
- B. Limited understanding of intracellular pathways
- C. Inability to achieve a toxicity ceiling
- D. Restricted toxicity profile of targeted medicines

Answer C: Inability to achieve a toxicity ceiling

6. **What does the assessment of pharmacodynamic (PD) effects in drug development allow for?**

- A. Customizing dosage based on adverse reactions
- B. Tailoring treatments to tumor genetics

- C. Measuring downstream markers and alternative routes
- D. Focusing solely on probable adverse reactions

Answer A: Customizing dosage based on adverse reactions

7. What is the role of predictive biomarkers in drug development?

- A. Indicating the probability of a positive response to specific anticancer drugs
- B. Assessing pharmacodynamic effects
- C. Customizing dosage based on adverse reactions
- D. Expediting the detection of significant biomarkers

Answer A: Indicating the probability of a positive response to specific anticancer drugs

8. Which gene fusion is a widely recognized predictive biomarker associated with the response to the tyrosine kinase inhibitor imatinib?

- A. EGFR
- B. BCR-ABL
- C. HER2
- D. BRAF

Answer B: BCR-ABL

9. What term is used to describe biomarkers that have not undergone clinical confirmation but have a potential for antitumor response based on scientific evidence?

- A. Surrogate biomarkers
- B. Predictive biomarkers
- C. Enrichment biomarkers
- D. Intermediate endpoint biomarkers

Answer C: Enrichment biomarkers

10. Which biomarkers have been associated with the PARP inhibitor olaparib?

- A. BCR-ABL gene fusion
- B. BRCA1 and BRCA2 mutations
- C. EGFR wild-type
- D. V600E BRAF mutation

Answer B: BRCA1 and BRCA2 mutations

11. What is the primary purpose of conducting preclinical investigations related to pharmacodynamic (PD) biomarkers?

- A. Identifying genetic variability
- B. Establishing links between PK-PD drug patterns
- C. Assessing clinical primary endpoints
- D. Adjusting dosage for patients

Answer B: Establishing links between PK-PD drug patterns

12. How does pharmacogenomic variability influence the effectiveness and toxicity of treatments?

- A. It reduces the need for dosage adjustments.
- B. It has no impact on drug metabolism.
- C. It affects the expression or functionality of proteins involved in drug metabolism.

D. It increases the risk of undertreating patients.

Answer C: It affects the expression or functionality of proteins involved in drug metabolism.

13. **What is the purpose of adjusting the dosage in clinical practice for patients encountering intolerable toxicities?**

- A. To increase the risk of undertreatment
- B. To address individual pharmacogenomic variables
- C. To reduce the financial burden of therapy
- D. To improve treatment efficacy

Answer B: To address individual pharmacogenomic variables

14. **What do intermediate endpoint biomarkers serve as in clinical trials?**

- A. Indicators of treatment success at an earlier stage
- B. Primary endpoints for the trial
- C. Surrogates for genetic variability
- D. Genetic markers for drug resistance

Answer A: Indicators of treatment success at an earlier stage

15. **What does the cancer proteome refer to?**

- A. Collection of cancer drugs
- B. Comprehensive collection of proteins expressed by cancerous cells
- C. Range of small-molecule metabolites
- D. Structural assessment of tumors

Answer B: Comprehensive collection of proteins expressed by cancerous cells

16. **Which techniques can be employed to analyze metabolomics and proteomics signatures?**

- A. PCR and Western blotting
- B. Electrophoresis, mass spectrometry, and protein microarrays
- C. X-ray and ultrasound
- D. Immunohistochemistry

Answer B: Electrophoresis, mass spectrometry, and protein microarrays

17. **How can mass spectrometry be utilized for “metabolic phenotyping”?**

- A. Mapping genetic pathways
- B. Assessing tumor perfusion
- C. Analyzing interrelated networks of biochemical pathways
- D. Evaluating tumor response

Answer C: Analyzing interrelated networks of biochemical pathways

18. **What is a limitation of traditional imaging technologies like MRI and CT in evaluating tumors?**

- A. High cost
- B. Limited ability to assess lesions
- C. Inconsistencies in methodologies
- D. Lack of functional evaluation

Answer D: Lack of functional evaluation

19. Which functional imaging technique is commonly used for the measurement of cancer-specific molecules and signaling cascades?

- A. X-ray
- B. DCE MRI
- C. PCR
- D. Western blotting

Answer B: DCE MRI

20. What role do predictive biomarkers play in drug development?

- A. Providing measurable indicators during clinical trials
- B. Accelerating drug development
- C. Enabling personalized medicine approaches
- D. Bridging intervention and clinical outcomes

Answer C: Enabling personalized medicine approaches

21. How did the “CURATE.AI” platform enhance treatment effectiveness in a study?

- A. Increased drug dosage
- B. Reduced drug tolerance
- C. Improved response to combination chemotherapy
- D. Enhanced radiation therapy

Answer C: Improved response to combination chemotherapy

22. Which AI-based screening system demonstrated accuracy in detecting breast cancer cells with homologous recombination deficit?

- A. “CURATE.AI”
- B. Deep learning platform
- C. 3D CNN
- D. PD-1 inhibitors

Answer B: Deep learning platform

23. What is the primary focus of AI in the context of cancer immunotherapy?

- A. Diagnosing cancer types
- B. Automating surgical procedures
- C. Assessing treatment efficacy and modifying treatment regimens
- D. Enhancing radiotherapy effectiveness

Answer C: Assessing treatment efficacy and modifying treatment regimens

24. What role does deep learning technology play in the Clinical Decision Support System (CDSS)?

- A. Analyzing extensive clinical data and providing cancer therapy recommendations
- B. Assisting in surgical procedures
- C. Diagnosing cancer types
- D. Enhancing nutritional recommendations

Answer A: Analyzing extensive clinical data and providing cancer therapy recommendations

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