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Christoph Wagener, Carol Stocking,
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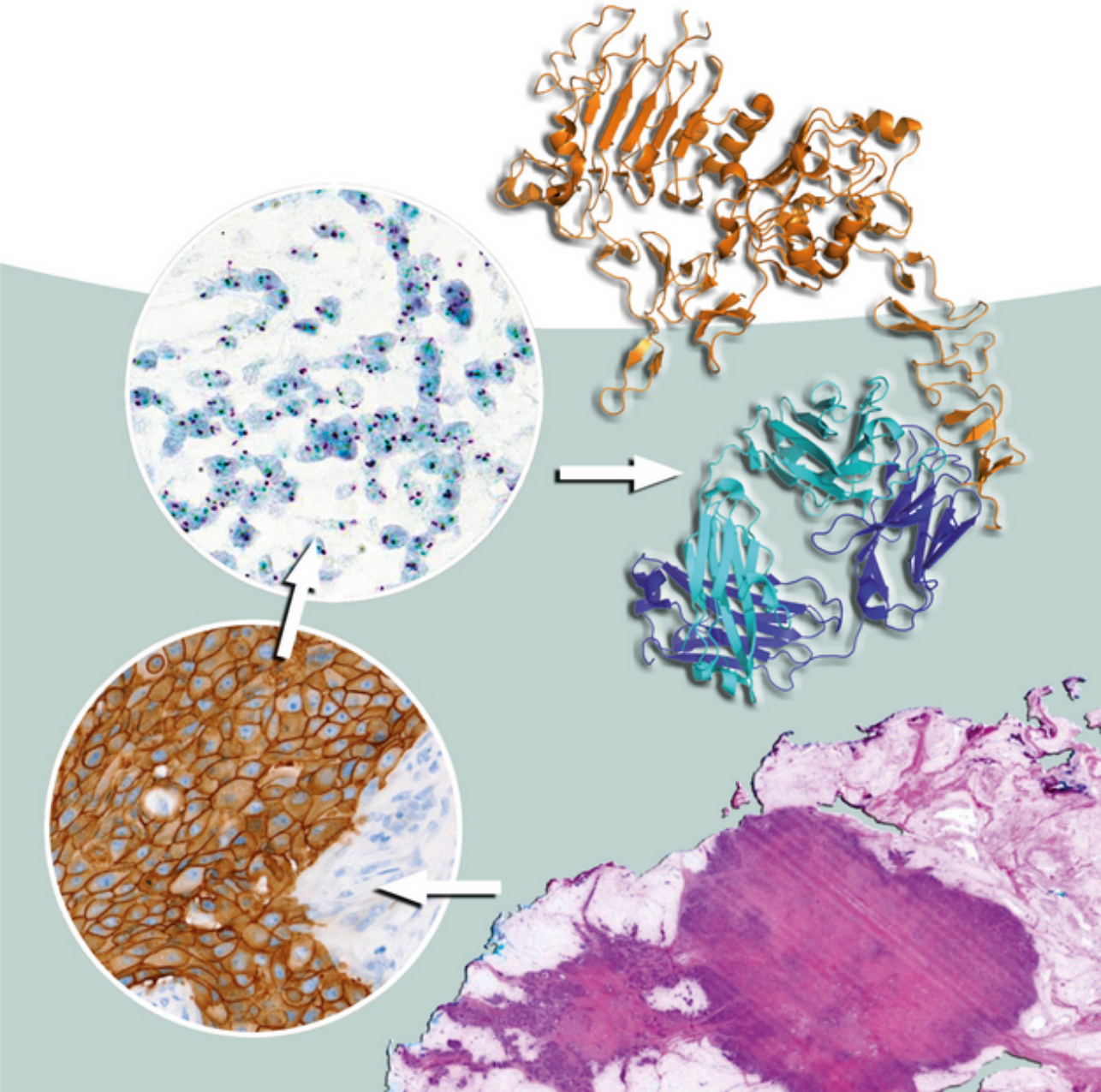
Cancer Signaling

From Molecular Biology to Targeted Therapy



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Cover
In a breast cancer specimen (white/violet), the amplification of the *ERBB2* gene is demonstrated on the protein level by immunohistochemistry (brown membrane staining of tumor cells) and on the DNA level by in situ hybridization (dark blue dots). Based on these results, the patient is eligible for therapy by a HER2/Neu antibody. The three-dimensional structure of the antibody (blue/green) in complex to the HER2/Neu receptor (orange) is shown.

Professor Dr. Axel Niendorf, Pathologie Hamburg-West, Germany, kindly provided the breast cancer images. The structure image was designed and kindly provided by Dr. Ingrid Vetter, Max-Planck-Institut für molekulare Physiologie, Dortmund, Germany. The image is based on the entry 1N8Z in http://www.rcbs.org by Cho et al. (2003, Nature 421, 756ff).

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We dedicate our work to all young researchers and students who have chosen to face the challenges and enjoy the satisfactions of scientific and medical careers, with the intention of contributing to the fight against cancer in order to improve, prolong and save lives.

Contents

Preface

1
History of Cancer Signaling Research

Of all the various fields of biomedical science, research on intracellular signaling provides some of the best examples for the successful transfer of basic science discoveries into the clinic. Findings from signaling research, together with new techniques of prevention, diagnosis, radiation, and surgery, have helped to increase the cure rates of all cancer patients from below 10% in the 1930s to over 60% today. It has been a long road from the discovery at the beginning of the twentieth century of chromosomal aberrations in tumor genomes, providing the first molecular insight into tumorigenesis, to the modern-day targeted and personalized anticancer therapy based on over 100 years of molecular and biochemical advances.

The first successful steps in the fight against cancer were observed using broad and unspecific cytostatic drugs. In 1947, the first partial remission of pediatric leukemia in a 4-year-old girl was achieved by using the drug aminopterin. Until this time, children with acute leukemia usually died within weeks of being diagnosed. Aminopterin is a competitive analog of the vitamin folic acid that is a necessary cofactor in the synthesis of nucleobases, the building stones of DNA and RNA.

In 1949, the US Food and Drug Administration (FDA) approved nitrogen mustard, the first chemotherapeutical drug, for the treatment of Hodgkin's lymphoma. Originally developed as a weapon gas, nitrogen mustard kills (cancer) cells by modifying their DNA by alkylation. Nitrogen mustard and its derivatives paved the way for many other alkylating and nonalkylating cytostatic and cytocidal anticancer drugs.

In 1958, the first therapeutic use of a combination of different cytostatic drugs was found to prolong the survival times of leukemia patients. In 1965, it was reported that a specific combination of chemotherapeutics could cure 50% of all patients with Hodgkin's lymphoma. Combination therapy is still used today to lower the risk of side effects and resistance.

During the 1960s, scientists in a hospital in Philadelphia identified a chromosomal aberration linked to certain forms of myeloid and lymphoid leukemia. Thirty years later, a fusion protein generated by the so-called Philadelphia chromosome became the target of one of the first specific and targeted cancer treatments with the kinase inhibitor imatinib (Gleevec).

The 1970s ushered in the era of oncogenes. Researchers found that tumor-causing viruses carry mutated forms of mammalian genes. This finding led to the identification of the first proto-oncogene *KRAS* in 1979. The Ras protein was characterized as an intracellular protein that sends signals from the inner cell membrane into the nucleus. As the first protein with specific signaling activity involved in tumorigenesis, Ras opened a new chapter of cancer research, namely cancer signaling research. Over the following years, the identification of other signaling proteins led to the finding that intracellular signaling pathways control all biological processes and all cellular functions, such as proliferation, differentiation, and migration.

In the 1980s, the first tumor suppressor gene *TP53* was discovered. The corresponding protein is a paradigm for a tumor suppressing protein, acting as a regulator and an inhibitor of several important pathways of intracellular signaling that control proliferation, cell death, and DNA repair. Also during the 1980s, scientists could show that tamoxifen lowers the risk of breast cancer relapse after surgery. Tamoxifen is a steroid hormone analog that functions as a signaling molecule at important knots of the intracellular signaling network.

The 1990s can be regarded as the key decade of cancer signaling research. Many more tumor suppressor genes were discovered. Mutations in such genes, e.g., *APC* and *BRAC1*, were shown to play important roles in the development of inherited and sporadic forms of the two most common cancer types, carcinomas of the colon and breast, respectively. The function of these tumor suppressors could be linked to important intracellular signaling pathways. Also during the 1990s, many new anticancer drugs of the second generation were approved. These drugs do not inhibit tumor growth by specific killing of the cell or by attacking DNA or RNA, but rather by interfering with biological processes that are the outputs of intracellular signaling. A good example is the family of taxanes, which inhibit dynamics of microtubule and thereby block the mitotic process, and are still in use today against ovarian and breast cancer. In 1997, the FDA approved the first anticancer drug of the third generation, rituximab (Rituxan). Rituximab was not only the first drug of molecularly targeted therapy, it was also the first monoclonal antibody used in medical therapy. Rituximab targets a B-cell surface receptor that initiates important signaling cascades regulating proliferation.

During the early 2000s, Trastuzumab was introduced into the therapy of metastatic breast cancer. Trastuzumab binds to HER2/Neu, a homolog of the receptor for the epidermal growth factor (EGF), and inhibits the transfer of growth-activating signals from the cell membrane into the cell. In 2001, the FDA approved imatinib (Gleevec) after just three months of review – the fastest approval in FDA history. Imatinib is the first drug proven to counteract a

molecular defect on the so-called Philadelphia chromosome. It has since become the standard care for patients with chronic myeloid leukemia (CML). Its high efficacy and easily administered pill form enables most patients to live with CML as a chronic but manageable disease. Imatinib inhibits BCR-ABL, which is a constitutively activated kinase positioned in a central node of intracellular signaling. Other targeted drugs were also approved after the turn of the millennium, including gefitinib (Iressa), which blocks the intracellular enzymatic activity of the EGF receptor involved in driving lung cancer growth and spread.

During the late 2000s, tumor-genome sequencing projects started answering questions of how many and which genes are involved in the development of common cancers. Hundreds of the 25 000 protein-coding genes in the human genome show somatic mutations in human tumors. Today, there is strong evidence that mutations in close to 400 genes can be classified as driver mutations, that is, they causally contribute to cancer development. Nearly all cancer genes encode proteins with important functions in signaling pathways. The most prominent group is that of protein kinases, composed of both cytosolic and receptor protein kinases. Furthermore, most of the cancer genes functionally cluster within specific signaling pathways, such as the MAPK pathway, the Wnt pathway, and the PI3K/AKT pathway.

2 Outlook

Molecularly targeted tumor therapy is a rational approach based on the aberrant intracellular signaling implicated in tumorigenesis and tumor progression. However, although great successes have been reported, resistance to therapy remains a sobering phenomenon. After initial spectacular effects, tumors often recur within a year. Interconnecting and parallel signaling pathways coupled to tumor cell heterogeneity are the major reasons for therapy resistance. In order to overcome resistance to targeted therapies, the underlying molecular mechanisms need to be completely and rigorously understood. Since the spectrum of gene mutations and activated pathways can vary between tumor cells at the same location or with the same histological type, predictive diagnostic tests are essential to choose the adequate drug(s) for each individual patient. Comparable to the successful shift to combinatorial application of cytostatic and cytocidal drugs observed in the late 1950s, choosing drug combinations that target different signaling pathways is likely to be more successful than single drugs.

Resistance and ineffective response to targeted therapies have led to some disappointment in the public eye. However, one should consider that it took nearly a century from the first reports of the minor effects of chemotherapeutic drugs to the effective combination therapies applied today. Novel analytical tools, such as deep and whole-genome sequencing, allow for the first time an in-depth analysis of molecular aberrations in tumors. Using these tools, potential therapeutic targets for the rational design of clinical trials can be identified. Although we are still

waiting for the results of more recent clinical trials, it is safe to assume that it will probably not take another century until significant progress has been achieved. Combinatorial therapy has successfully been used to combat AIDS; nevertheless, cancer is much more complex than HIV.

In addition to therapy resistance, the costs of targeted therapies are a great concern to all. In order to deliver effective therapies to all patients, costs must correlate to the budgets available.

3
Intention of this Book

We wrote this book in the hope that it will serve as a useful introduction to a fascinating research field for students, physicians, and researchers. We do hope that the information provided in the book will help facilitate the transfer of results from basic research into the clinic.

4
Selection of Topics and Readers' Comments

In this book, we summarize the current knowledge of cancer signaling research. We are aware that we have not comprehensively enumerated all important findings, and we apologize for failing to cite each and every colleague who has made significant contributions to this field. Our intention was to give a short and concise overview of the important but fast-evolving field of cancer research, rather than writing an exhausting review of the scientific literature. The selection of presented topics is based on our own assessment of the relevant findings and topics discovered over the past decades.

Because nobody is perfect and some aspects in the cancer signaling field change faster than our perception, we appreciate any comments, suggestions, and corrections for this and all upcoming editions.

5
Structures of Chapters

After the introductory chapters on general aspects of intracellular signaling, we describe the molecular background of tumor relevant biological processes, such as cell birth, cell aging, and cell death. In the third part of the book, examples of important signaling pathways and their molecular players are presented. All chapters are structured similarly. After a short summary, we present general aspects of the pathway to provide a basis for the following presentation of molecular details, including key proteins and their functions. In the last part of each chapter, we introduce current therapeutic strategies and some examples

for specific drugs or drug candidates that interfere with the functions of the discussed proteins. For an up-to-date overview of clinical trials, the reader is referred to http://www.clinicaltrials.gov, a service of the U.S. National Institutes of Health. Each chapter ends with a short outlook, in which we summarize open questions and upcoming therapies.

6
Figures and Video Animations

We have set particular attention on the visual presentation to help convey complicated features and interactions. Protein functions, signaling pathways, and biological processes are presented in simplified figures within the text. In addition, several subjects are presented in short animations as part of this book. Because we want these video clips to be clear and understandable, the clips were held simple and are restricted to selected aspects. The basic message is understandable without reading the corresponding chapter in the book. Nevertheless, a viewer of a clip will not regret reading the corresponding book chapter, because he will find valuable additional information and links. Vice versa, readers of the book might use the clips as short animated summaries of the written information.

A few more video clips are found on our Onkoview channel on YouTube (https://www.youtube.com/user/Onkoview/videos) and also on the home page of our book entitled "*Molekulare Onkologie*" published in German language in 2009 (http://www.onkoview.com).

7
Nomenclature and Abbreviations

Names and abbreviations of genes and proteins are those recommended by UniProt (http://www.uniprot.org). The abbreviations deviate from the recommendations in so far as no hyphens were used in the abbreviations of proteins.

June 2016

Hamburg and Zweibrücken
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List of Abbreviations

5-FU	5-fluorouracil
ABC	activated B-cell
ACL	ATP-citrate lyase
ACTH	adrenocorticotropic hormone
ADAM	a disintegrin and metalloprotease
AFP	alpha-fetoprotein
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
AMPK	adenosine-monophosphate-activated kinase
APAF	apoptosis protease-activating factor
APC	adenomatous polyposis coli protein
APRIL	a proliferation-inducing ligand
Arm	armadillo repeat
ASA	acetylsalicylic acid
AT	Ataxia telangiectasia
BAF	B-cell-activating factor
BAFF	B-cell-activating factor of tumor necrosis factor family
BANGs	BMPs, activins, Nodal, GDFs
BCC	basal cell carcinoma
BCL	B-cell lymphoma
Bcl-2	B-cell lymphoma 2
BCMA	B-cell maturation antigen
BCR	breakpoint cluster region
BER	base excision repair
BH3	Bcl-2 homology domain 3
BLNK	B-cell linker protein
BrdU	bromodeoxyuridine
BTK	Bruton's kinase
BTRC	β-transducin repeat-containing protein
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CARD	caspase-associated recruitment domain
caspase	cysteinyl aspartate cleaving protease

CCC	cholangiocellular carcinoma
CCRCC	clear cell renal cell carcinoma
CDK	cyclin-dependent kinase
CDKI	cyclin-dependent kinase inhibiting protein
CENP-C	centromere protein-C
cIAP	cellular inhibitor of apoptosis
CIN	chromosomal instability
Cip/Kip	CDK-interacting protein/cyclin-dependent kinase inhibitor protein
CK	casein kinase
CLL	chronic lymphocytic leukemia
CML	chronic myeloid leukemia
CMML	chronic myelomonocytic leukemia
COX	cyclooxygenase
DAG	diacylglycerol
DAMPs	damage-associated molecular pattern molecules
DD	death domain
DHF	dihydrofolate
DKK	Dickkopf-related protein
DLBCL	diffuse large B-cell lymphoma
DSB	double-strand break
DUB	de-ubiquitinylating
DVL	disheveled
EBV	Epstein – Barr virus
ECM	extracellular matrix
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
eIF4	eukaryotic translation initiation factor 4
ELISA	enzyme-linked immunosorbent assay
EMT	epithelial – mesenchymal transition
EPO	erythropoietin
ERBB	avian erythroblastosis oncogene B
ERK	extracellular signal-regulated kinase
FAK	focal adhesion kinase
FAP	familial adenomatous polyposis
FAT domain	FRAP-ATM-TRAP domain
FDA	food and drug administration
FGF	fibroblast growth factor
FIH1	Factor inhibiting HIF1α
FISH	fluorescence *in situ* hybridization
FKBP12	FK-506-binding protein of 12 kD
FOXO	forkhead box O
FRB	FKBP12-rapamycin-binding
FTase	farnesyl transferase
FTI	farnesyl transferase inhibitor

FZD	Frizzled
G phase	gap phase
GAP	GTPase-activating protein
GATOR1	GAP activity toward Rag 1
GBD	GTPase-binding domain
GCB	germinal center B-cell like
GDNF	glial cell line-derived growth factor
GEF	guanine nucleotide exchange factor
GF	growth factor
GIST	gastrointestinal stroma tumor
Glut	glucose transporter
GLUT1	glucose transporter 1
GOF	gain-of-function mutation
GPCR	G-protein-coupled receptor
GRB	growth factor receptor-bound
GRB10	growth factor receptor-bound protein 10
GSI	γ-secretase inhibitor
GSK	glycogen synthase kinase
GSK-3β	glycogen synthase kinase 3β
HAT	histone acetylase
HD domain	heterodimerization domain
HDAC	histone deacetylase
HEAT	Huntington-EF3-A subunit of PP2A-TOR1
HER	human EGF receptor
HGF	hepatocyte growth factor
HIF	hypoxia-inducible factor
HIS	haploinsufficiency
HNPCC	hereditary nonpolyposis colorectal cancer
HR	homologous recombination
HRS	Hodgkin–Reed–Sternberg
HSP90	heat shock protein 90
HTLV	human T-cell leukemia virus
IDH	isocitrate dehydrogenase
IFT	intraflagellar transport
IGF	insulin-like growth factor
IGF1	insulin-like growth factor 1
IGFBP	IGF-binding protein
IKK	IkB kinases
IkB	inhibitor of kB
INK4	inhibitors of kinase CDK4
IP3	inositol-1,4,5-trisphosphate
IRS1	insulin receptor substrate 1
ITAM	immunoreceptor tyrosine-based activation motif
IWP	inhibitor of Wnt production

JAK-STAT	Janus kinase and signal transducer and activator of transcription
LAP	latency-associated protein
LDH	lactate dehydrogenase
LKB1	liver kinase B1
LNR	Lin12/Notch repeats
LOF mutation	loss-of-function mutation
LOH	loss of heterozygosity
LOX	lysyl oxidase
LRP	low density lipoprotein receptor-related protein
LTBP	latent TGFβ-binding protein
M phase	mitosis phase
M1	phase 1 of mortality
MALT	mucosa-associated lymphoid tissue
MAPK	mitogen-activated protein kinase
mar	marker chromosome
MB	medulloblastoma
MDR	multidrug resistance
MEK	MAPK/ERK kinase
MEKK	MEK Kinase
MMP2	matrix metalloproteinase 2
MMR	mismatch repair
MMTV	mouse mammary tumor virus
MNNG	*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine
MOMP	mitochondrial outer membrane permeabilization
MSI	microsatellite instability
mTOR	mechanistic target of rapamycin
MTX	methotrexate
NEC fragment	Notch extracellular fragment
NEMO	NF-κB essential modulator
NES	nuclear export sequence
Neu	neural tumor
NF1	neurofibromatosis type 1
NF-κB	nuclear factor kappa B
NGF	nerve growth factor
NHEJ	nonhomologous end joining
NIC fragment	Notch intracellular fragment
NLR	nucleotide oligomerization domain-like receptor
NLS	nuclear localization sequence
NMDA	*N*-methyl-D-aspartate
NOD	nucleotide oligomerization domain
NRR	negative regulatory region
NSAID	nonsteroidal anti-inflammatory drug
NSCLC	non-small-cell lung cancer
NTMIC fragment	Notch transmembrane and intracellular fragment

PAK2	p21 activated kinase 2
PanIN	pancreatic intraepithelial neoplasia
PcG	Polycomb group
PDAC	pancreatic ductal adenocarcinoma
PDE	phosphodiesterase
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
PDK1	phosphoinositide-dependent kinase 1
PDPK1	3-phosphoinositide-dependent protein kinase 1
PEST domain	domain rich in proline (P), glutamic acid (E), serine (S), and threonine (T)
PH	Pleckstrin homology
PHD	prolyl hydroxylase domain
PI	phosphatidylinositol
PI3K	phosphatidylinositol-4,5-bisphosphate 3-kinase
PIP2	phosphatidylinositol-4,5-bisphosphate
PIP3	phosphatidylinositol-3,4,5-trisphosphate
PKB	protein kinase B
PKC	protein kinase C
PLC	phospholipase C
PMBL	primary mediastinal B-cell lymphoma
PP2A	protein phosphatase 2A
PPI	protein–protein interaction
PPIase	peptidyl-prolyl *cis–trans* isomerase
PPP	pentose phosphate pathway
PS	phosphatidylserine
PSA	prostate specific antigen
PTB	phosphotyrosine binding
PTEN	phosphatase and tensin homolog
pVHL	von Hippel–Lindau protein
R point	restriction point
RANK	receptor activator of NF-kB
RANKL	RANK ligand
RasGAP	RasGTPase-activating protein
Rb	retinoblastoma-associated protein
Rbx1	ring box 1
RHD	Rel homology domain
Rheb	RAS homolog enriched in brain
RICTOR	rapamycin-insensitive companion of mTOR
RIG	retinoic-acid-inducible gene
RIP	receptor-interacting protein
RKIP	Raf kinase inhibitor protein
ROCK	Rho-activated kinase
ROS	reactive oxygen species
RSV	Rous sarcoma virus

RTK	receptor protein tyrosine kinase
S phase	synthesis phase
S6K	ribosomal S6 protein kinase
SAHFs	senescence-associated heterochromatin foci
SAPK	Stress-activated protein kinase
SA-β-GAL	senescence-associated β-galactosidase
SCC	squamous cell carcinoma
SCF	stem cell factor
SCLC	small-cell lung cancer
SFRP	secreted frizzled-related protein
SH2	Src homology 2
SH3	Src homology 3
SOS	son of sevenless
SREB1/2	sterol-regulatory element-binding protein1/2
SSB	single-strand break
TACI	transmembrane activator and calcium modulator and cyclophilin ligand interactor
TAD	transactivation domain
TAK1	transforming growth factor-activated kinase-1
T-ALL	T-cell acute lymphoblastic leukemia
TCA cycle	tricarboxylic acid cycle
TCF/LEF	T-Cell Factor/Lymphoid-Enhancing Factor
TCGA	The Cancer Genome Atlas
TCR	T-cell receptor
TGF	transforming growth factor
TGFβ	transforming growth factor β
THF	tetrahydrofolate
TIAM1	T-cell lymphoma invasion and metastasis-1
TIS	therapy-induced senescence
TNF	tumor necrosis factor
TNFR	TNF receptor 1
TPA	12-*O*-tetradecanoylphorbol-13-acetate
TPR	translocated promoter region
TRADD	TNF receptor-associated protein with a death domain
TRAF	TNF receptor associated factor
TSC	tuberous sclerosis
TUNEL	TdT-mediated dUTP-biotin nick end labeling
VASP	Vasodilator-stimulated phosphoprotein
V-ATPase	vacuolar ATPase
VEGF	vascular endothelial growth factor
WIF	Wnt-inhibiting factor
WT	wild type