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General Aspects of Signal Transduction and Cancer Therapy

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Summary

This chapter should serve as an introduction into the field of intracellular signal transduction. The biological role of signal transduction pathways will be presented

Cancer Signaling: From Molecular Biology to Targeted Therapy, First Edition.
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Companion Website:www.wiley.com/go/wagener/cancersignaling

together with the mechanisms and the protein domains that are responsible for the direct transduction of signals between molecules. In the second part, we define and describe the major groups of anticancer drugs and their effects on different levels in a simplified model of tumorigenesis. We give examples of important classical drugs and explain their mode of action. Finally, the major mechanisms of drug resistance are described and compounds and approaches that can be used to prevent or circumvent this problem are mentioned.

1.1 General Principles of Signal Transduction

(Video: General aspects of signal transduction – enhanced ebook and closed website: signal_transduction_ebook.mp4)

1.1.1 Biological Signals have to be Processed

Levels of biological communication include communication between whole organisms, communication between organs within an organism, and communication between single cells. Mechanisms for intercellular communication are based on the transfer of signals between cells through direct contacts, by electrical signals, ions, small molecules, or macromolecules. Once a signal has reached a cell, the cell has to "decide" whether and how to react. For this reason, the cell has to process the incoming signal. Most signals are processed by intracellular signal transduction pathways. A signal transduction pathway is a biochemical cascade that connects the incoming signal with the cellular response. Such a pathway fulfills two major functions. First, it modulates the intensity of the originally extracellular signal. It can amplify, weaken, or extinguish the signal. Secondly, the pathway converts the signal into a form that allows and also prepares the cellular response. Examples for potential responses are proliferation, migration, differentiation, and apoptosis. It has to be noted that a signal can be transferred not only by the presence of a molecule but also by its absence. For example, normal cells react to the absence of growth factors by activation of signal transduction pathways that activate apoptosis.

1.1.2 What is a Signal Transduction Pathway?

A signal transduction pathway consists of factors, receptors, adapter proteins, enzymes, second messengers, and transcription factors, which together form a hierarchical sequence of signaling events. Most frequently, the signal transfer from one molecule to another is performed by direct contact and subsequent covalent or noncovalent modification resulting in conformational change of at least one of the interacting partners.

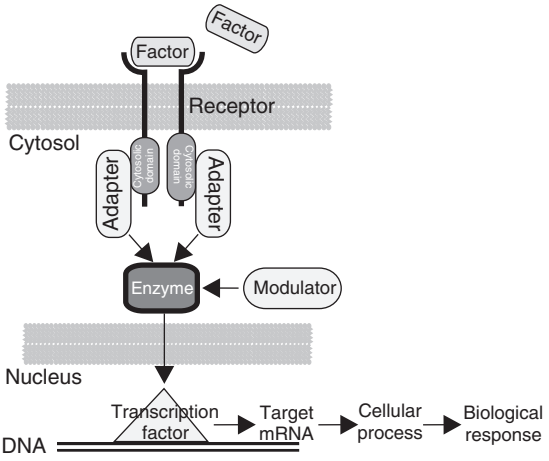


Figure 1.1 Simplified version of a general signal transduction pathway. A signal in the form of an extracellular factor binds to a membrane-bound receptor. The receptor transfers the signal through the membrane onto its cytosolic domain, which transfers the signal to an adapter protein. The adapter transfers the signal to a cytosolic protein, which is frequently an enzyme, for example, a kinase. The activity of this enzyme might be altered by a modulator. The enzyme regulates a nuclear transcription factor, which regulates the expression of target genes. The activities of the translated gene products mediate a cellular process such as proliferation or migration, which results in the biological response to the original signal. (Wagener and Müller, 2009), with permission.

A typical pathway begins with the binding of an extracellular ligand to a membrane-bound receptor. The receptor transports the signal through the plasma membrane into the cell by altering the activity of molecules on the cytosolic side (Figure 1.1). The signal is transferred through the cytoplasm via macromolecules or small molecules. In most pathways, only one or a few enzymes are necessary at this level due to their ability to amplify a signal by several orders of magnitude. Finally, the signal reaches the cell nucleus, where the activity of a transcription factor is altered. As a consequence, this factor promotes or inhibits the expression of distinct genes. The transcribed mRNA is translated and the resulting proteins mediate the biological answer to the original signal.

In this book, we describe the different pathways in the same way as this "master pathway," namely as direct and straight cascades. In this manner, we aim to clearly illustrate their important properties, their biological effects, as well as the potential sites and mechanisms of drugs interfering with them. We are aware that this approach reflects a highly simplified view, which is far from the *in vivo* processes in a living cell. Actually, every real signaling pathway consists of multifaceted parallel or antiparallel cascades, manifold branches, feedback loops, bypasses, and connections to other pathways, which are permanently or temporarily active. Thus, one has to keep in mind that despite the simplified representation, a signaling pathway has to be regarded as a complex signaling network or at least as a part of such a network, rather than as a simple and isolated linear cascade.

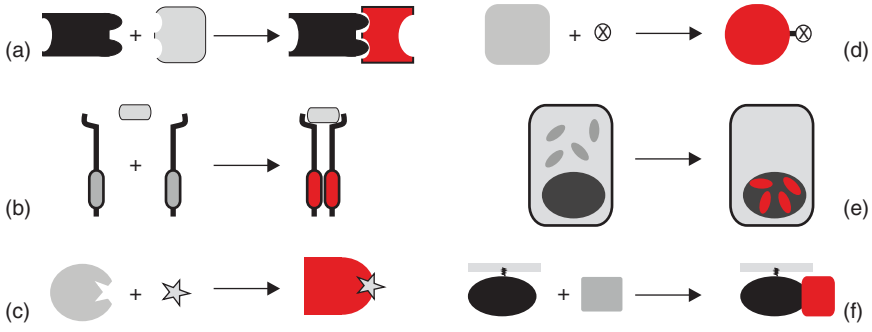


Figure 1.2 Mechanisms of signal transduction. Direct interaction of two different proteins (a), dimerization of monomers (b), interaction with a small molecule (star, c), covalent modification (d), translocation into another compartment (e), or translocation to the inner side of the cell membrane (f) might result in alteration of conformation and activity (red). (Wagener and Müller, 2009), with permission.

1.1.3 Mechanisms of Direct Signal Transduction

Signals are primarily transduced within a pathway by the direct contact of two molecules, usually proteins. There are different mechanisms for this type of signal transfer (Figure 1.2):

1) Many signals are transduced by the noncovalent interaction of two different proteins. Such a protein–protein interaction (PPI) can lead to a conformational change or to an altered activity of one or both interacting partners. An example for such a signal transfer is the interaction between the adapter protein growth factor receptor-bound protein 2 (GRB2) and the GTPase exchange factor son of sevenless-1 (SOS-1), which is part of the mitogen-activated protein kinase (MAPK) signaling pathway (Chapter 7). This binding leads to a conformational change of SOS-1 and to its ability to catalyze the nucleotide exchange of a small G-protein.
2) Secondly, the signal can be transduced by a PPI that is a homo- or a hetero-oligomerization of protein monomers. As an example, the homodimerization of the monomers of a receptor tyrosine kinase, such as the platelet-derived growth factor receptor (PDGFR), leads to the autophosphorylation of both monomers and subsequently to their activation. In the example of PDGFR, the tyrosine kinase activity of the two monomers increases by several orders of magnitude.
3) Third, the noncovalent binding of a small molecule to a protein can cause a conformational change and an altered activity of a protein. An example for such a signal transfer is the activation of the protein kinase A (PKA) by binding of the second messenger cAMP.
4) Fourth, a signal can be transduced by PPI following the covalent modification and activation of one of the interacting proteins. The most frequent covalent

protein modifications are phosphorylations of serine, threonine, and tyrosine residues by kinases. Example is the activating phosphorylation of serines/threonines of MAPK/ERK kinase (MEK) 1/2 by the kinase BRaf. Other covalent modifications leading to conformational changes and to signal transfers are acetylation and the attachment of single amino acids or peptides.

5) The fifth principle of molecular signal transfer is the change in concentration of a protein, a small molecule, or an ion in a distinct cellular compartment. In this case, the signal is not transferred by interactions and conformational changes but rather by the concentration change of a molecule in a limited cellular region. At the new concentration, the molecule can induce an effect that it could not do at the original concentration. An example is the increase in the concentration of β-catenin, a transcription-factor-activating protein, in the nucleus of cells with an activated Wnt pathway (Chapter 11). The high nuclear β-catenin concentration leads to the activation of the transcription factor TCF-4. An example for an altered ion concentration is the increase in the cytosolic calcium concentration after activation of the PI3K/AKT pathway (Chapter 8). This increase leads to the activation of calcium-dependent enzymes.
6) Finally, the altered localization of a protein within the same cellular compartment can lead to its conformational change and thus to signal transduction. Example is the recruitment of the kinase BRaf to the inner side of the plasma membrane by the active Ras protein in cells with the activated MAPK pathway (Chapter 7). At the membrane, BRaf interacts with ceramides and other membrane factors, which leads to its activation.

1.1.4 The Interactome Gives Insight into the Signaling Network

Most signals are transferred from protein to protein by their direct interaction that might be followed by covalent or noncovalent modification. By the use of a high-throughput version of the double-hybrid screen, a PPI map of nearly 6000 human proteins was generated. The screen yielded more than 3000 specific interactions between more than 1700 different proteins (Stelzl *et al.*, 2005). Because most PPIs serve to transfer signals, this map is equivalent to a view into the signaling network of a human cell.

Mathematical approximation indicated that the so-called interactome comprises more than 650 000 different PPIs between 25 000 proteins (Stumpf *et al.*, 2008). Because most PPIs lead to the transfer of a signal, the interactome is equivalent to a view into the signaling network of a living cell. The virtual display of the manifold interactions between the tumor suppressor protein cyclin-dependent kinase inhibitor 1 (CDKN1A or p21) and its interacting protein partners shows a tiny detail and the complexity of this signaling network (Figure 1.3). A simplified version of many signal transduction pathways that are important in tumor development and their interplays is shown in Figure 1.4.

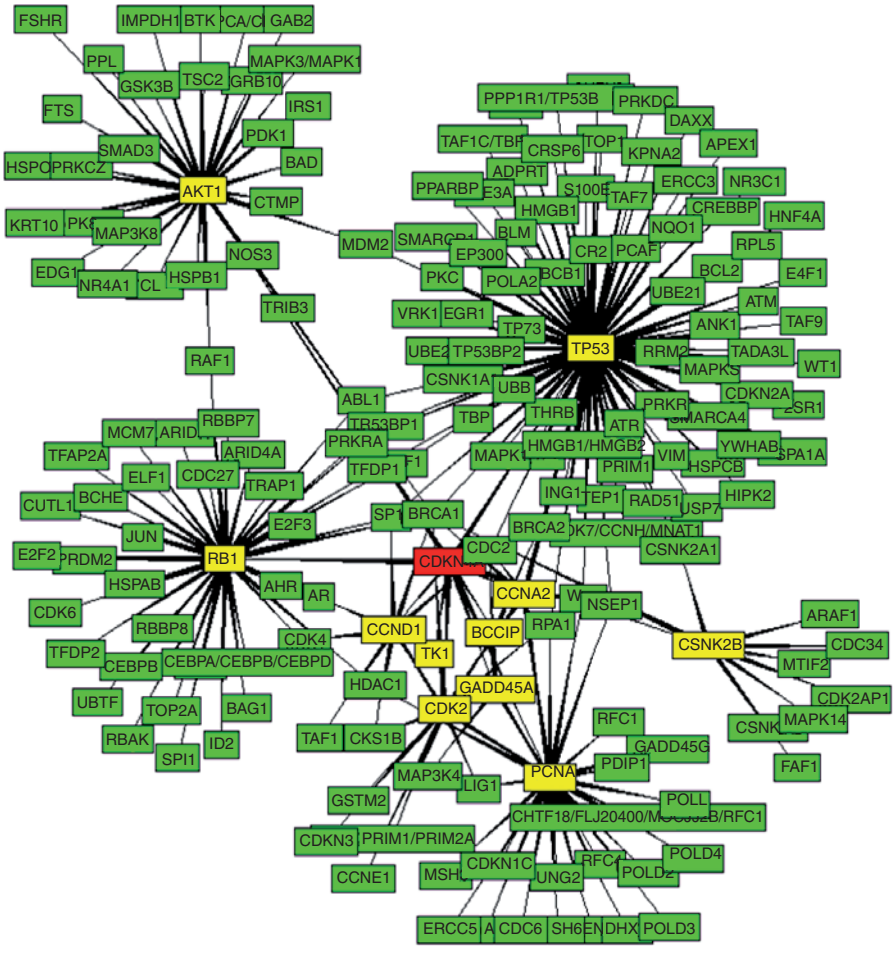


Figure 1.3 Protein interaction partners (yellow) of cyclin-dependent kinase inhibitor 1 (CDKN1A) (red) that are directly or indirectly involved in DNA repair and their protein interaction partners (green). (Wagener and Müller, 2009), with permission.

1.1.5
Protein Domains for Protein–Protein Interaction and Signal Transduction

PPIs are mediated by typical protein domains, which are sequential and structural sections of a protein. The database Gene Ontology classifies protein domains according to intracellular localization, molecular function, and the controlled cellular process (www.geneontology.org). Many signaling proteins carry conserved domains that are responsible for the interaction with a specific ligand (Pawson and Nash, 2000, 2003; Pawson, Raina, and Nash, 2002). When the sequence of a newly identified protein is compared with the database and such domains are recognized, it is possible to predict potential interacting partners and thereby its

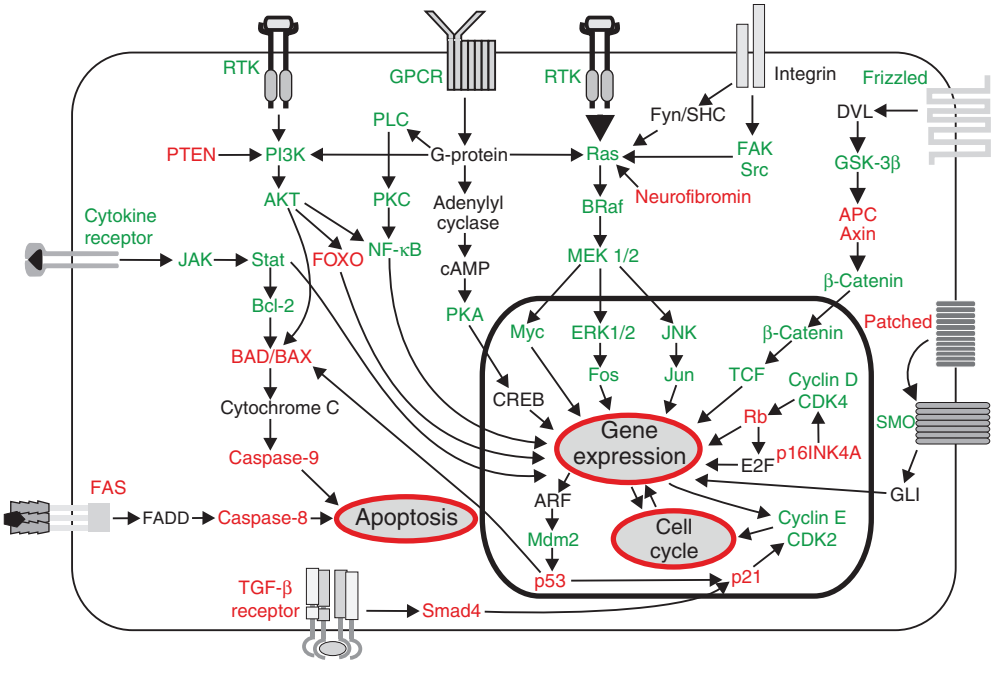


Figure 1.4 Simplified representation of important signaling pathways in tumor cells. Green, proto-oncoproteins; red, tumor suppressor proteins. Arrows indicate interaction and effects, which might be activating or inhibiting. (Wagener and Müller, 2009), with permission.

Table 1.1 Protein domains for protein–protein interaction in signal transduction.

Domain	Examples for proteins with domain	Ligand of domain
SH2	Src, GRB2, RasGAP	Phosphotyrosine
SH3	Src, GRB2, crk	Proline-rich sequences
PTB	SHC-transforming protein, IRS-1, X-11a	Phosphotyrosine
14-3-3	Cdc25, BAD, BRaf, PKC	Phosphoserine
PH	PLCδ, PKB, ATK	Phosphatidylinositol

potential functions. The domains SH2, SH3, PTB, 14-3-3, and PH mediate most of the interactions between signaling proteins (Table 1.1).

SH domains were named after their homology to the three major domains of the protein kinase Src: the catalytic SH (Src homology) 1 domain, the cytoplasmic SH2 domain, and the SH3 domain. The SH2 domain, which stretches over approximately 100 amino acids, is found in many intracellular signaling proteins. SH2 domains bind directly to other proteins with phosphorylated tyrosine residues, including phosphorylated cytoplasmic domains of receptor protein tyrosine kinases (Chapter 5). Proteins with SH2 domains can be classified in

two groups: first, proteins with enzymatic or enzyme-activating activity such as the phosphatidylinositol phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), the phospholipase C (PLC) gamma, members of the Src family of nonreceptor tyrosine kinases, and the RasGTPase-activating protein (RasGAP); secondly, the so-called adapter molecules such as GRB2, crk, and SHC, which themselves have no enzymatic activity but serve rather as bridging proteins between proteins with phosphotyrosine domains and other signaling proteins. Structural analysis has shown that the binding pocket of an SH2 domain is necessary for its high-affinity interaction with phosphorylated tyrosines. Amino acids that are located carboxy-terminal to the tyrosines of the interacting partner determine binding specificity.

The PTB (phosphotyrosine-binding) domain is functionally related to the SH2 domain, though it has a different three-dimensional structure. Additionally, the PTB domain recognizes specific amino acids in the amino-terminal neighborhood of a phosphorylated tyrosine. PTB domains have been identified, for example, in IRS-1 (insulin receptor substrate-1) and the adapter protein Shc. The finding that Shc carries both a PTB and a SH2 domain confirms that there are functional differences between these two domains. Interactions of SH2 and PTB domains with their partners often play a role in the first steps of cytosolic signal transduction, which includes the transfer of the signal from the cytosolic receptor domain to a cytosolic protein.

Further downstream in the signaling pathway, other domains are responsible for PPIs. The best-known domain that mediates PPIs in the cytosol is the 60–70 amino-acid-long SH3 domain. SH3 domains bind preferentially to left-handed protein helices, which are rich in prolines. Besides the signaling proteins Src and GRB2, many proteins of the cytoskeleton carry SH3 domains. This finding indicates that the SH3 domain is involved not only in dynamic and fast-regulated signaling pathways but also in events that play a role in migration and interaction of cells.

Finally, many signaling proteins include the 100 amino-acid-long Pleckstrin homology (PH) domain. Although PH domains of different proteins show only low sequence homologies, their three-dimensional structures are very similar. PH domains are found, for example, in protein kinase B (PKB) and PLC and bind to the phospholipids PIP2 (phosphatidylinositol (4,5)-bisphosphate) and PIP3 (phosphatidylinositol (3,4,5)-trisphosphate).

1.1.6
Functions of Mutated Proteins in Tumor Cells

All important cellular processes and activities are regulated by signaling pathways. Tumorigenesis, metastasis, and tumor progression are caused by deregulated and dysfunctional pathways that regulate important properties of tumor cells, such as proliferation, cell adhesion, cell migration, or apoptosis. Signaling dysfunction might result from activation of proto-oncogenes or from inactivation of tumor suppressor genes. Products of these genes play key roles in signal transduction

Table 1.2 Proto-oncoproteins in signaling pathways.

Function of protein	Examples
Growth factor	Wnt, PDGF, FGF-3, FGF-4
Receptor tyrosine kinase	Egfr, CSF-1-R, Kit, Met, PDGFR, Ret, HER2 (erbB2)
Receptor without kinase activity	Mas, c-Mpl
Plasma-membrane-associated nonreceptor tyrosine kinase	Src, Fgr, Fyn, Yes
Nonreceptor tyrosine kinase	Abl, Sck, c-Fes, JAK-1, JAK-2
Kinase activator	Cyclin D1
Serine/threonine kinase	BCR, BRaf
Membrane-associated G-protein	HRas, KRas, NRas
Regulator of transcription factors	β-Catenin, Mdm2
Transcription factor	erbA, C-ets-1, c-Fos, FRA-1, FRA-2, AP-1 (c-Jun), Myc, Pax-1, c-Rel, TAL-1
Mitochondrial membrane protein	Bcl-2

Table 1.3 Tumor suppressor proteins in signaling pathways.

Function of protein	Example
Growth factor antagonist	WIF-1
Adapter protein	Axin
Kinase inhibitor	p21
GTPase activator	Neurofibromin
Transcription factor	p53
Transcriptional repressor	EVI-9 (Bcl-11A)

(Tables 1.2 and 1.3, Figure 1.4). Proto-oncogenes can be activated by mutation, amplification, or overexpression, whereas tumor suppressor genes can be inactivated by mutation, deletion, or inhibited expression.

Futreal and coworkers conducted a census of all genes that have been found to be mutated in human tumors (Futreal *et al.*, 2004). The list is updated regularly on http://cancer.sanger.ac.uk/cancergenome/projects/census/. More than 400 human genes, making 1% of all, are implicated via mutation in cancer. Of these, approximately 90% have somatic mutations in cancer, 20% bear germline mutations that predispose to cancer, and 10% show both somatic and germline mutations. Expression products include all major functions of signaling proteins, for example, growth factors and their receptors, adapter proteins, kinases, and transcription factors. Strikingly, more than half of all mutated proteins belong to only three functional groups. Nearly 10% of all tumor relevant proteins are kinases, while only 3% of all proteins in a normal cell are kinases. Secondly, mutations in transcription factors occur at an incidence 10 times higher than that predicted by their numbers. Finally, proteins that are necessary for detection and

repair of DNA mutations are significantly overrepresented among the mutated proteins.

On the other hand, some protein functions are very rarely mutated in comparison to their proportion in the proteome of a normal cell. An example is the group of G-protein-coupled membrane receptors, for example, the Rhodopsin-like seven-transmembrane domain receptors. Mutations in only 1 of 10 different members of this family have been identified. In conclusion, though mutations in many different proteins are responsible for the differences between normal and tumor cells, these mutations affect only few protein functions.

1.2 Drugs against Cancer

1.2.1 Terms and Definitions

Nearly all drugs used in anticancer therapy can be classified into five groups according to their major effects: cell-killing drugs, process-blocking drugs, molecule-interfering drugs, DNA-repairing drugs, and resistance-inhibiting drugs. Certainly, this classification is not completely strict. For example, some modifiers of methylation and acetylation bind to specific molecules and affect signal transduction and also inhibit the process of transcription.

Anticancer therapy with the cell-killing and process-blocking drugs is widely known as *chemotherapy*, abbreviated CTX or chemo. Because the term *chemotherapy* was originally used for all therapies with synthetic chemicals (chemotherapeutics) independently of the treated disease, this term is ambiguous. Instead, we use the phrase cytostatic therapy or anticancer therapy. It has to be mentioned that cytostatic describes only one aspect of a drug, namely its inhibitory effects on cell proliferation. Strictly speaking, cytostatic drugs do not include drugs with cell-killing (cytocidal) activities. Such activities may base on cytotoxic, apoptosis inducing, or starving effects. A correct, though uncommon, term covering the therapy by nearly all anticancer drugs would be "cytostatic and cytocidal therapy."

1.2.2 The Steps from a Normal Cell to a Tumor

An ideal anticancer drug should affect all tumor cells, whereas it should preserve all normal cells. Thus, the drug has to attack tumor cells at one or more distinct features that normal cells do not possess. The most important features can be summarized in a simplified cascade of tumorigenesis, which show the differences between a tumor cell and a normal cell at different levels (Figure 1.5). The ultimate cause for the transition of a normal cell into a tumor cell is mutation of its DNA. As described earlier in this chapter, mutations might lead to altered levels or altered activities of proteins that play important roles in signal transduction pathways.

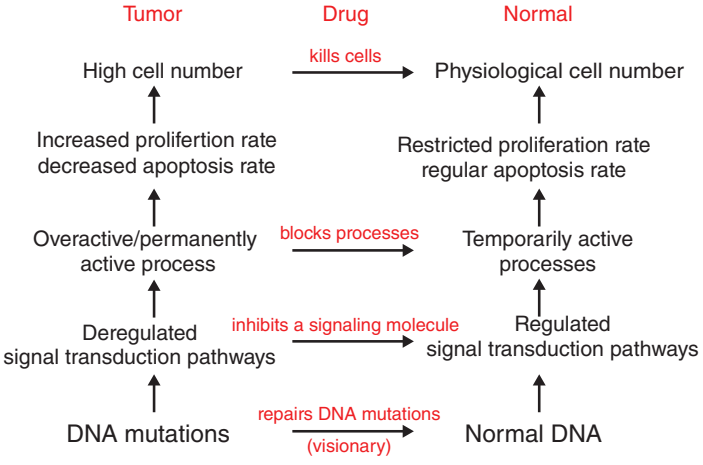


Figure 1.5 Mechanistic levels for the interference of anticancer drugs illustrated by a simplified cascade of tumorigenesis (left) and the corresponding cascade in normal cells (right). DNA mutations may cause deregulation of signaling pathways, which result in permanently active or overactive processes (e.g., transcription), in an increased proliferation rate and, at the end, in a higher cell number. Over the past decades, drug research moved from the top to the bottom of this cascade. Classical drugs kill cells or block processes underlying proliferation or apoptosis. Innovative drugs interfere with molecules involved in signaling pathways. DNA-repairing drugs are still visionary. Drugs circumventing or inhibiting drug resistance are not shown.

This results in deregulation of pathways. Signal transduction pathways control cellular processes, such as DNA replication, RNA transcription, chromosome segregation, and protein synthesis, which are the bases for proliferation, differentiation, apoptosis, and other biological functions. Deregulated signal transduction pathways might cause the permanent activation or overactivation of these processes. The resulting high proliferation rate or the low apoptosis rate might lead to an increased cell number and the formation of a tumor.

1.2.3 Interference Levels of Therapeutic Drugs

The five major groups of anticancer drugs affect different levels of this tumorigenic cascade and abolish the tumor specific features (Figure 1.5). A cell-killing drug attacks the cell in its entirety. A process-blocking drug interferes with a cellular process, such as gene transcription, proliferation, or apoptosis. Some general mechanisms and some major examples of these classical anticancer drugs are described in this chapter. More innovative drugs include molecule-interfering drugs, which bind to one specific molecule, which is involved in signal transduction, and change its activity or function. Such drugs are described in detail together with their modes of action in the chapters of the corresponding pathways.

DNA-repairing drugs, which are still visionary, might be able to convert the tumor cell back into a normal cell. The fifth group of drugs includes compounds that circumvent or inhibit drug resistance.

Over the past decades, the focus of anticancer drug research has moved from the top to the bottom of the tumorigenic cascade. Parallel to this movement, the specificity of the drugs increased from compounds attacking the cell in its entirety to those interfering with single molecules in signaling pathways.

1.2.4
Drugs Attacking the Whole Cell

During World War I, it was found that victims injured or killed by mustard gas had reduced numbers of white blood cells. This observation led to the beginning of systematic anticancer drug development during the 1940s. The mustard gas molecule sulfur mustard (bis(chloroethyl) sulfide) was chemically modified to mechlorethamine (bis(2-chloroethyl) methylamine) (Figure 1.6) and used to treat patients with Hodgkin's disease (Joensuu, 2008). Mechlorethamine (trade name Mustargen) is an alkylating agent causing irreversible DNA damage. Its two reactive ethyl groups bind covalently to the nitrogens in the rings of the nucleobases guanine and cytosine leading to permanent bonds between the two DNA strands. These bonds block the separation of the DNA double strand into single strands, which is necessary for transcription and replication, leading to cell death.

Because of its strong side effects, mechlorethamine is only used today in the treatment of few forms of leukemia and lymphoma. Nevertheless, the molecule served as the prototype structure for other therapeutics and can be regarded as the first anticancer drug of the cell-killing drug group. These first-generation drugs

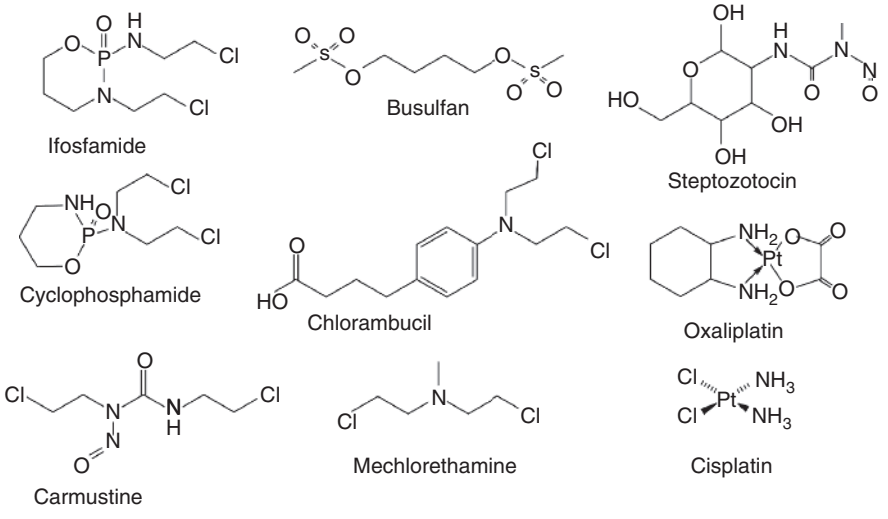


Figure 1.6 Examples for DNA alkylating anticancer drugs.

affect parts or molecules of the cell that are necessary not only for the active cell cycle but also for cell metabolism and cell survival (Figure 1.5, top). Most of these drugs induce toxic effects against both tumor and normal cells. Because most tumor cells have decreased ability to detect and to repair DNA damages caused by these drugs, more tumor cells than normal cells are killed. This decreased DNA repair ability is caused by inactivating mutations in genes that coordinate DNA repair and control DNA integrity. Examples are the tumor suppressor genes *TP53* and *MSH2*, which are mutated in more than 50% of all tumors. DNA in tumor cells with such defects is irreversibly damaged and destroyed by DNA alkylating agents such as mechlorethamine. DNA in normal cells is also damaged, though it is repaired faster and more efficiently than the DNA in tumor cells, because of the presence of intact repair systems.

1.2.4.1 DNA Alkylating Drugs

Today, many synthetic derivatives of mechlorethamine, such as the DNA-alkylating drugs cyclophosphamide, ifosfamide, and chlorambucil, are used in cancer therapy (Figure 1.6). Cyclophosphamide is an example for an inactive prodrug. It is metabolized in liver cells to the active molecule phosphoramide mustard, which forms covalent DNA interstrand bonds. Phosphoramide mustard is inactivated by oxidation by aldehyde dehydrogenases. Cells with high levels of aldehyde dehydrogenases are less sensitive against the toxic effects of phosphoramide mustard than cells with low levels of these enzymes. Because hematopoietic stem cells and stem cells in mucous membranes express high levels of aldehyde dehydrogenases, cyclophosphamide is less toxic against bone marrow and mucous membranes than other alkylating compounds.

Similar to phosphoramide mustard, the two metalorganic complexes cisplatin and oxaliplatin form DNA interstrand bonds. In addition, these two molecules are able to form covalent bonds between bases of the same strand. Such intrastrand bonds prohibit the complementary base pairing and thus block transcription and replication. Cisplatin is widely used in single drug therapies and combination therapies. More than 90% of all nonseminoma types of testicular tumors can be successfully treated by the combination therapy BEP (bleomycin, etoposide, cisplatin).

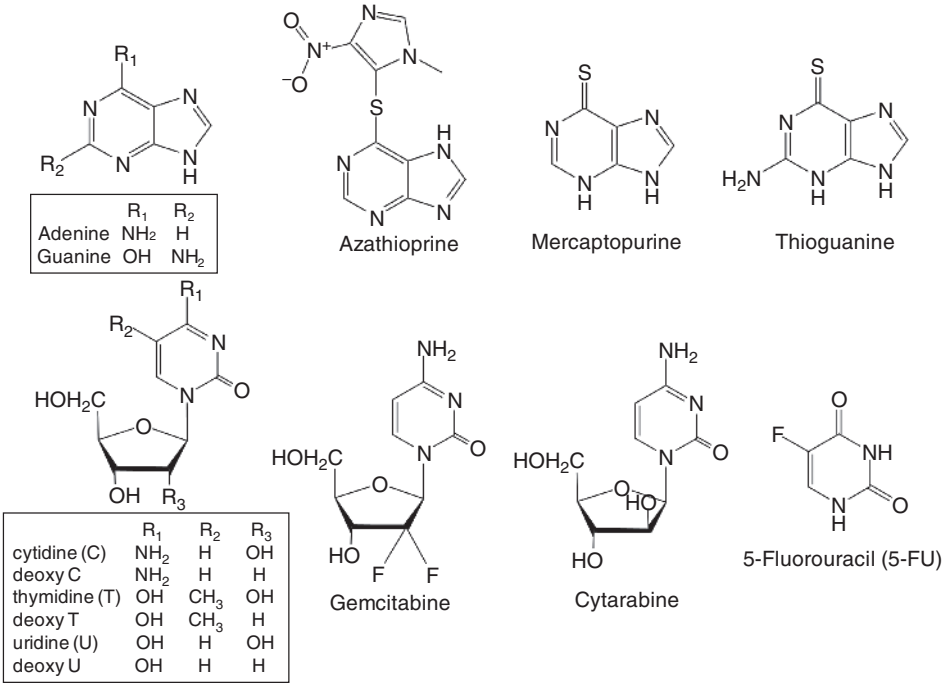
Other groups of alkylating drugs are the group of alkylsulfonates, with its main representative busulfan, and the large group of nitrosourea derivatives, including carmustine and streptozotocin. There are also some DNA-modifying antibiotics used in cancer therapy. Examples are the polypeptide dactinomycin (actinomycin D) and the large group of anthracyclines. Both are polyaromatic molecules that intercalate between neighbored base pairs in the DNA and thus inhibit the separation of the double strand into single strands. Thereby, intercalation blocks both replication and transcription. Because of its high toxicity, dactinomycin is used today against only a few cancers, such as rhabdomyosarcomas. An important representative of anthracyclines is doxorubicin (adriamycin), which is used in the therapy of Hodgkin's disease and several solid tumors.

1.2.5
Process-Blocking Drugs

With the increasing knowledge of the cellular processes that underlie biological functions, such as apoptosis and proliferation, novel potential target sites for anticancer drugs have emerged. Anticancer drugs of the second generation, which have been developed since the 1970s, interfere with RNA transcription, DNA replication, or chromosome segregation.

1.2.5.1 Drugs Blocking Synthesis of DNA and RNA

An antimetabolite is structurally related to a molecule that is a necessary substrate (metabolite) of a physiological biochemical reaction. The structural difference between the metabolite and the antimetabolite is small enough to allow the binding of the antimetabolite by the enzyme, but large enough to inhibit its metabolization in the reaction. As a consequence, the enzyme is blocked and the reaction is inhibited. The largest group of antimetabolites among the cytostatic drugs are the analogs of bases and nucleosides (Peters, Schornagel, and Milano, 1993). Typical examples are the analogs of purines (azathioprine, mercaptopurine), of pyrimidines (fluorouracil), or of pyrimidine nucleosides (cytarabine) (Figure 1.7).



	R_1	R_2
Adenine	NH_2	H
Guanine	OH	NH_2

	R_1	R_2	R_3
cytidine (C)	NH_2	H	OH
deoxy C	NH_2	H	H
thymidine (T)	OH	CH_3	OH
deoxy T	OH	CH_3	H
uridine (U)	OH	H	OH
deoxy U	OH	H	H

Figure 1.7 Examples for purine analogs (upper row), pyrimidine analogs, and pyrimidine nucleoside analogs (lower row). Residues of the physiological nucleobases and nucleosides are shown in the boxes. (Wagener and Müller, 2009), with permission.

Analogs of nucleosides are able to inhibit cell proliferation and tumor growth via several different mechanisms. First, some of these molecules compete with physiological precursor molecules and block biochemical pathways that lead to the synthesis of the DNA and RNA monomers. Some analogs are converted into the triphosphate form of the corresponding nucleotide analog. As such they can bind to DNA or RNA polymerases and inhibit the polymerization reaction by competing with the physiological substrates. Alternatively, they are incorporated by the polymerases into the growing DNA or RNA strands and block their further elongation.

1.2.5.2 Drugs Blocking the Synthesis of DNA and RNA Precursor Molecules

A common mechanism of base analogs is the inhibition of enzymes that catalyze the generation of nucleosides, the precursor molecules of the building blocks of DNA and RNA. Fluorouracil (5-FU) is an important anticancer drug used against tumors of the stomach and the colon. Fluorouracil is metabolized to 5-FdUMP, which blocks irreversibly the enzyme thymidylate synthase. This enzyme produces dTMP, which is a precursor molecule of dTTP, a substrate of the DNA polymerase (Figure 1.8).

Cytarabine was the first nucleoside of the analogs with an altered sugar component. This cytidine analog carries arabinose instead of ribose or deoxyribose (Figure 1.7). It is incorporated into the growing DNA strand instead of deoxycytidine. The following repair reactions lead to strand breaks and misincorporations. Cytarabine is used against several leukemias in adults.

Methotrexate (amethopterin), abbreviated MTX, is another standard drug working as an antimetabolite (Figure 1.9). As an antagonist of folic acid, MTX

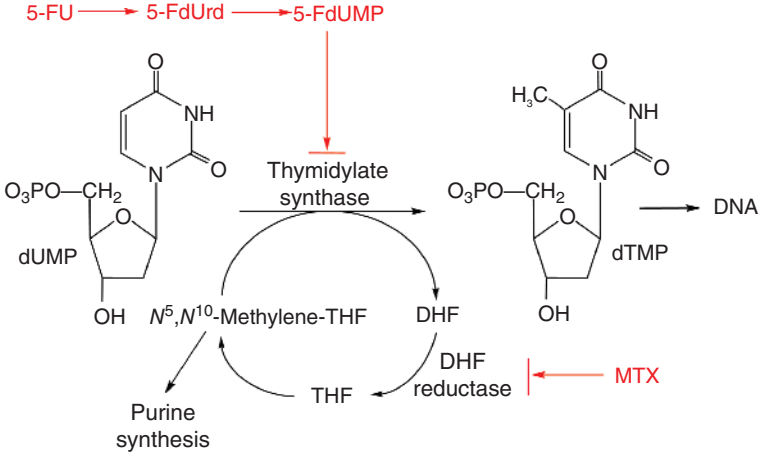


Figure 1.8 Effects of fluorouracil (5-FU) and methotrexate (MTX). 5-FU is metabolized to 5-FdUMP, which blocks irreversibly the enzyme thymidylate synthase. MTX blocks DHF reductase, which delivers the cofactor THF for purine and thymidylate synthesis. DHF, dihydrofolate and THF, tetrahydrofolate. (Wagener and Müller, 2009), with permission.

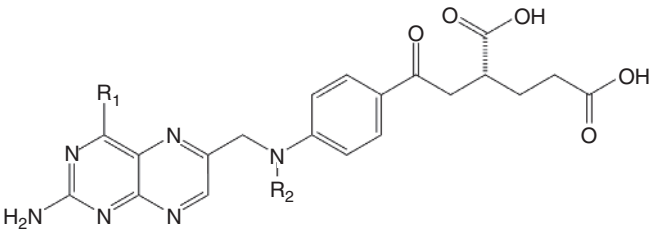


Figure 1.9 Basic structures of folic acid also called vitamin B9 ($R_1 = OH$, $R_2 = H$) and its two antagonists aminopterin ($R_1 = NH_2$, $R_2 = H$) and methotrexate ($R_1 = NH_2$, $R_2 = CH_3$).

blocks irreversibly the enzyme dihydrofolate reductase and thus prohibits the formation of tetrahydrofolate (THF) from dihydrofolate (DHF) (Figure 1.8). DHF in turn is produced from folic acid (vitamin B9). THF is the precursor molecule of N^5,N^{10}-methylene-THF, which is the coenzyme in purine and thymidylate synthesis and thus is essential for production of both DNA and RNA. In the presence of MTX and the resulting lack of thymine, the enzyme DNA polymerase incorporates uracil instead of thymine during DNA elongation. The removal of uracil by the enzyme uracil glycosylase and its replacement by thymine lead to strand breaks and misincorporations. MTX is used in single drug therapies and in combination therapies against acute lymphatic leukemia (ALL), carcinomas of the urothelium and the breast, and several other tumors.

1.2.5.3 Drugs Blocking Dynamics of Microtubules

Microtubules are intracellular protein fibers with several functions. Besides being a component of the cytoskeleton, microtubules are responsible for the equal segregation of chromosomes to the daughter cells during the mitotic anaphase. Microtubules are polymers that are built from heterodimers of one α- and one β-tubulin monomer. Dynamics of microtubules describes the regulated equilibrium between polymerization of α-/β-tubulin dimers to microtubules and depolymerization of the microtubules (Figure 1.10).

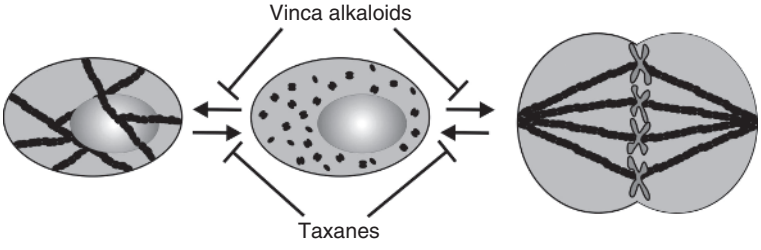


Figure 1.10 Dynamics of microtubules and the effects of vinca alkaloids and taxanes. Microtubules are components of the cytoskeleton (left) and serve as fibers for chromosome segregation during mitotic anaphase (right). Vinca alkaloids block polymerization of tubulin dimers, whereas taxanes block depolymerization of the fibers. (Wagener and Müller, 2009), with permission.

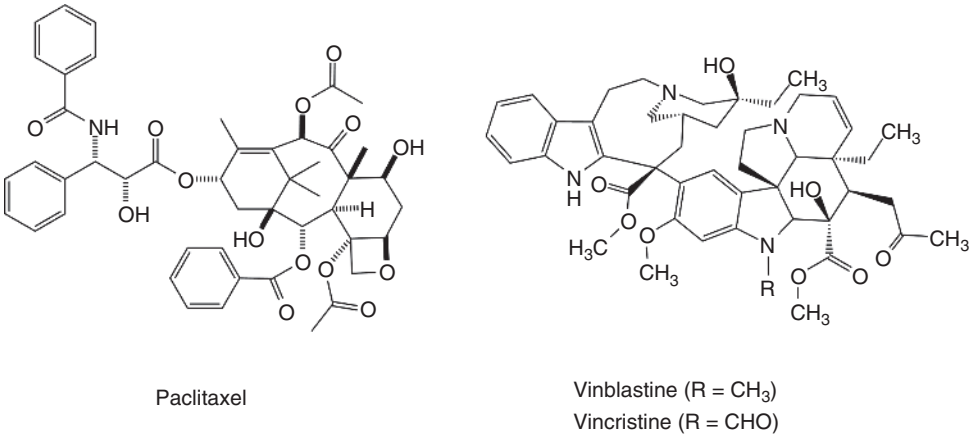


Figure 1.11 Paclitaxel and vinca alkaloids. (Wagener and Müller, 2009), with permission.

The dynamics of microtubules serves as therapeutic target of two groups of natural alkaloids. Vinca alkaloids from the Madagascar periwinkle have been used in traditional medicine against different diseases including diabetes (Figure 1.11). In systematic trials of their antidiabetic effects in the 1950s, treated laboratory animals displayed a reduced activity of the bone marrow (myelosuppression). Based on this finding, clinical trials with leukemia patients proved the antineoplastic effects of vincristine and vinblastine. Today, vincristine (Oncovin) is used in the combination therapy CHOP (cyclophosphamide, hydroxydaunorubicin, oncovin, prednisolone) for non-Hodgkin's lymphoma, as well as in the combination therapies of Hodgkin's lymphoma and ALL. Vinca alkaloids bind to the α-/β-dimer of tubulin and inhibit the polymerization of the dimers to microtubules (Figure 1.10). In addition, vincristine and vinblastine cause the detachment of the microtubules from the spindle pole. Consequently, chromosomes cannot segregate and the mitotic cell is frozen in the metaphase.

The second group of alkaloids with effects on microtubules dynamics is the taxane group from the bark of the Pacific yew tree (Taxus) with its best-known representatives docetaxel (Taxotere) and paclitaxel (Taxol) (Figure 1.11). These compounds were identified in the 1960s in a screen for new natural compounds with antineoplastic effects (Leistner, 2005). Today, the complex molecule paclitaxel is produced by semisynthesis starting from a natural precursor molecule from the European yew tree. Paclitaxel binds to the GDP-bound β-tubulin monomer in the polymerized microtubules and, in this way, leads to its stabilization. By this mechanism, the depolymerization of microtubules, which is necessary for proliferation and chromosome segregation, is blocked. Thus, taxanes shift the dynamic equilibrium of tubulin polymerization and microtubule depolymerization into the opposite direction as compared to vinca alkaloids (Figure 1.10). Paclitaxel is used in single drug and combination therapies of many solid tumors, including carcinomas of the ovary, lung (together with cisplatin), breast (together with Herceptin), and prostate.

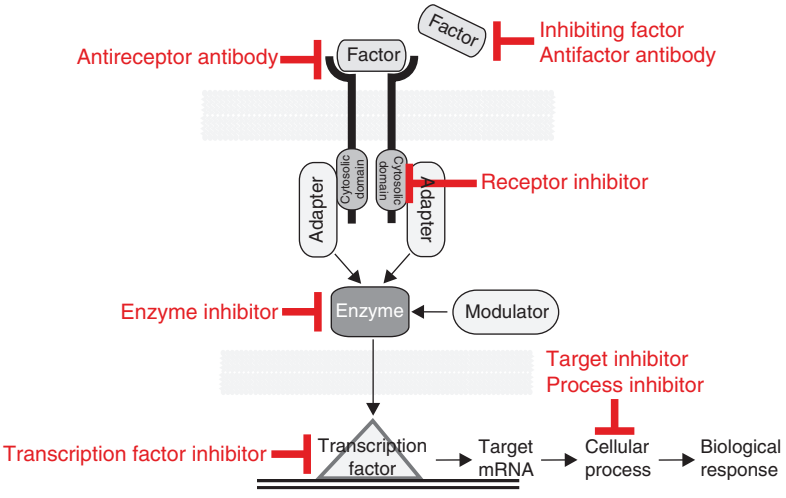


Figure 1.12 Potential drugs interfering at different levels of a general signal transduction pathway.

1.2.6 Innovative Molecule-Interfering Drugs

The antitumor effects of most cell-killing and process-blocking drugs were identified in undirected searches by chance. In contrast, anticancer drugs of the most recent generation were the results of directed searches for molecules that specifically interfere with tumor specific proteins. During the 1990s, our insights into intracellular signal transduction increased rapidly. With the knowledge that signal transduction pathways control all cellular processes and that deregulated pathways cause tumorigenesis, many new potential targets for drug interference were identified. These findings led to the development of the new group of molecule-interfering anticancer drugs. These third-generation drugs interfere specifically with molecules involved in intracellular signal transduction (Figure 1.5). Imatinib (Gleevec), which was FDA-approved in 2001, is a paradigm for such a drug. It was purposefully developed as a selective inhibitor of the enzyme BCR-ABL1, which phosphorylates many substrates in distinct leukemic cells and promotes their proliferation (Chapter 6). Other anticancer drugs of the third generation are directed against signaling factors, extracellular or intracellular domains of receptors, transcription factors, or target proteins of signaling pathways (Figure 1.12). Examples for molecule-interfering drugs are introduced in the chapters of the corresponding signal transduction pathways.

1.2.7 Fast-Dividing Normal Cells and Slowly Dividing Tumor Cells: Side Effects and Relapse

The typical differences between the cell cycle in tumor cells versus fast-dividing normal cells or somatic stem cells are very small. Therefore, drugs targeting the cell cycle of tumor cells also affect a large group of normal cells. Consequently, such drugs induce toxic effects on normal cells. Typical side effects include hair loss, alteration of mucous membranes, nausea, vomiting, and immune suppression.

Furthermore, tumor cells that proliferate slowly, or not at all, at the time point of therapy are not affected by drugs that selectively attack the active cell cycle. These cells survive and might contribute to tumor relapse after the therapy is stopped. For this reason, slowly growing tumors, such as prostate and renal cell carcinomas, are treated with low-drug doses over a long time period. Although this low-dose therapy does not lead to complete tumor eradication, tumor growth is suppressed.

1.2.8 Drug Resistance

Knowledge of the mechanism of a cytostatic drug already facilitates understanding how a tumor cell might defy the drug effects (Lippert, Ruoff, and Volm, 2008) (Table 1.4). A common mechanism for cellular drug resistance is the export of the drug. The gene *MDR1* (Multidrug resistance protein 1) encodes for P-glycoprotein 1 (P-gp), which is a glycosylated ATP-dependent efflux pump in the plasma membrane with a broad substrate specificity. P-gp is highly expressed in normal cells of the intestinal epithelium and the liver, where it exports potentially harmful xenobiotics. A tumor cell overexpressing *MDR1* is resistant and invulnerable against many anticancer drugs.

Secondary resistance might develop during long-term therapies. This type of resistance is caused by the therapy itself. An example is the induction of the gene encoding for thymidylate synthase under therapy with 5-FU. As a consequence, the level of thymidylate synthase rises to the point that it can no longer be blocked by intracellular 5-FU.

The more specific a therapy is, the easier it is for a cell to develop resistance. When a drug attacks only one or a few targets, a tumor cell may escape by a simple single-step mechanism. A single point mutation in the fusion gene *BCR-ABL1* may lead to resistance against imatinib (Gleevec) (Chapter 6).

1.2.8.1 Drugs Circumventing Resistance

Over the past few years, several compounds that prevent or inhibit drug resistance have been developed. Two general principles to prevent resistance can be distinguished. First, the therapy can be designed in a way that lowers the likelihood of resistance. The most common and most effective way is to combine two or more therapeutic strategies that attack the tumor from different directions. An example

Table 1.4 Exemplified causes and mechanisms of resistance against anticancer drugs.

Principle and gene responsible for resistance	Biochemical mechanism	Acquired resistance against
Gene amplification		
MDR1 (P-glycoprotein 1)	Export of hydrophobic compounds	Dactinomycin, doxorubicin
DHFR (dihydrofolate reductase)	Excess of free dihydrofolate	Methotrexate
Increased gene expression		
MT1 (Metallothionein) genes	Complexing of metals and export	Cisplatin
GSTP1 (glutathione S-transferase P)	Complexing by glutathione and export	Several different
BCL2	Inhibition of apoptosis induced by extrinsic stimuli	Apoptosis-inducing compounds
Genes for DNA repair enzymes	Repair of DNA damages	DNA alkylating agents
TYMS (thymidylate synthase)	Replacement of blocked enzymes	5-FU
PDGFRB (PDGF receptor)	Activation of BRaf independent pathway (pathway shift)	Vemurafenib
Gene mutation		
BCR-ABL1	Insensitivity of the enzyme	Imatinib

is the combination of a cytostatic therapy with an immune therapy. The simultaneous administration of 5-FU and interferon-α represents a combination therapy of patients with progressed solid tumors (Mitchell, 2003).

The second strategy is to directly inhibit the mechanism that is responsible for resistance. Examples of resistance-inhibiting compounds are the cyclic peptide cyclosporine and its derivatives, which block the cellular export of foreign substances by the MDR1 gene product P-gp. Another example is therapy with drugs or drug derivatives that block the mutated protein that escaped the regular drug. Novel derivatives of imatinib with broader substrate specificity, such as nilotinib, are able to inhibit mutated forms of the protein BCR-ABL1.

1.3 Outlook

As in the past, anticancer therapy in the future will also likely be based on the mechanisms represented by the drug groups: cell-killing, process-blocking, molecule-interfering, DNA-repairing, and resistance-inhibiting. The fine art of

anticancer therapy is to find the right balance between attacking the entire cell and inhibiting a single molecule, while preventing resistance. From today's point of view, four trends in research for novel drugs against cancer will be followed in the future.

Identifying and targeting new specific targets. The new techniques of molecular diagnosis, including the methods of whole-genome sequencing, are providing increased information with regard to the mutation spectra and molecular events of every tumor. Based on these data, each patient can be theoretically treated by one or a few highly selective drugs. In order to reach this goal, however, many more molecule-interfering drugs need to be developed to target the growing number of proteins that are deregulated in tumor cells. The major advantage of this approach is that these drugs destroy less normal cells and are thus accompanied by fewer side effects than classical drugs.

Combating tumor heterogeneity. Unfortunately, progressed tumors are generally heterogeneous at the cellular and molecular levels. Because of the diversity and the high number of deregulated targets, it is unrealistic to attack each potential target within the heterogeneous cell population of a tumor with a specific molecule-interfering drug. Thus, more and better cell-killing and process-blocking drugs are needed in order to fight tumors successfully. The broader target spectrum of these two types of drugs has the additional advantage that the likelihood of resistance is lower.

Circumventing and fighting drug resistance. In parallel to the groups of cytostatic and cytocidal drugs, new compounds have to be developed to fight resistant tumor cells. These include mainly drugs and drug derivatives that inhibit targets and mechanisms of resistance, such as overexpressed genes or mutated proteins.

Reversing mutations. The still visionary group of DNA-repairing drugs would induce the reversion of the tumorigenic DNA mutations. This approach, involving somatic gene therapy, would be the only way to fight a tumor at its roots, to convert tumor cells back into normal cells, and to cure a tumor ultimately (Figure 1.5). A specific drug for directed DNA repair has been a dream of cancer therapists for decades. With the new techniques of the CRISPR/Cas9 technology, this dream might become reality (Sander and Joung, 2014).

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