Cancer Drug Discovery and Development

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Handbook of Anticancer Pharmacokinetics and Pharmacodynamics

Second Edition

Humana Press

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Preface

The *Handbook of Anticancer Pharmacokinetics and Pharmacodynamics* was designed with the objective of having a single reference on clinical pharmacology to serve as a guide to drug development with a focus on cancer therapy. The first edition of the handbook was organized to closely follow a logical flow of events of the drug development plan from identification of cancer-specific targets to preclinical testing to clinical trial design and all phases of clinical trials.

In this thoroughly updated and expanded second edition, we embarked on an even more comprehensive approach to adapt to the ever-changing drug development landscape, highlighting the recent changes involved in shifting the paradigm of the process over the last decade. The outline and objective of the handbook remain focused on a roadmap for moving an agent toward NDA submission. We have incorporated in this revised second edition new material on phase 0 trials in oncology, organ dysfunction trials, drug formulations, and their impact on anticancer drug pharmacokinetics/pharmacokinetics including strategies to improve drug delivery, pharmacogenomics and cancer therapy, high-throughput platforms in drug metabolism and transport pharmacogenetics, imaging in drug development, and nanotechnology in cancer. Together these chapters provide for a comprehensive overview of anticancer drug development.

Advances in understanding the molecular basis of cancer are critical for oncology drug researchers to translate molecular targets into new therapies. As the search for cancer-specific targets continues over the next decade, advances in drug discovery and development efforts are underway, and a practical guide detailing the underlying principles of these processes will be invaluable to cancer researchers. We hope to achieve this with an indispensable reference that should be of interest to both the clinical pharmacologist and the pharmaceutical scientist.

We would like to thank all of the authors for their thoughtful and thorough contributions. Our task of compiling this book was made easy by their high-quality efforts. We continue to be conscious of our patients who keep us focused on the goal of finding treatments and cures for all types of cancers.

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Molecular Targets

Christina M. Annunziata and Phillip A. Dennis

Abstract The optimal targeting of cancer requires not only the selection of the target but also the identification of the patients whose cancer depends on the targeted pathway. The objective of this chapter is to give an overview of molecular targets in cancer therapeutics. Targets have been categorized as either established or novel types. Established targets include those against which most currently licensed anticancer drugs were developed and include DNA, microtubules, and nuclear hormone receptors. Novel targets are those under current preclinical and clinical investigation. The section on novel targets emphasizes the relationships of the novel targets to the biological traits of cancer.

Keywords Drug targets • Signaling pathways • Cancer treatment

1 Introduction

Cancer is a major cause of mortality throughout the world. There are an estimated 10.9 million new cases and 6.7 million deaths from cancer worldwide [1]. Cancer cells differ from normal cells by the following hallmark traits [2]: (a) ability to proliferate due to self-sufficiency in growth signals, (b) insensitivity to growth inhibitory signals, (c) evasion of apoptosis and senescence, (d) limitless replication potential, (e) sustained angiogenesis, and (f) potential to invade tissue and metastasize. Although each of these traits may be targeted for drug development, two additional areas are important: chemoprevention and modulation of resistance (Fig. 1).

Over 100 anticancer drugs have been approved by the US Food and Drug Administration (FDA) since the use of mustine to treat a patient with acute lymphoblastic leukemia in 1943 [3]. Although the development of new anticancer drugs has lead to cure of some patients with rare cancers such as childhood leukemia and testicular cancer, conventional drug development has provided only incremental improvements in survival for the majority of cancers.

Traditionally, anticancer drug development has focused on DNA as a target, based on the fact that a high turnover of nucleic acids in cancer cells during DNA replication and cellular proliferation will provide a therapeutic margin. The molecular biology revolution, epitomized with the sequencing of

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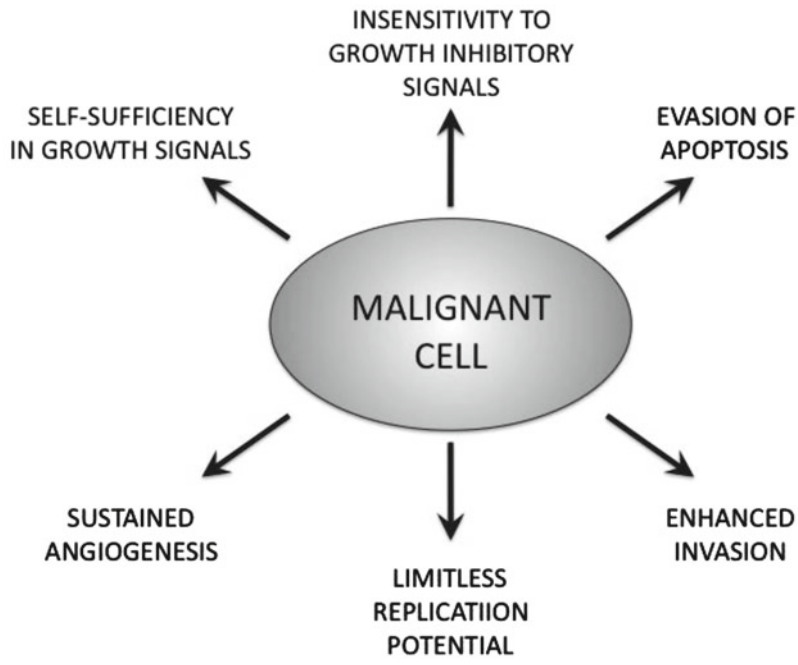

Fig. 1 Characteristic hallmarks of a malignant cell are targeted for anticancer drug development

the human genome and individual cancer genomes, has increased our understanding of cancer at a basic level. More recent efforts in drug development build on this improved understanding of the molecular basis of cancer and use this information to identify and validate new targets for rational drug development [4].

Criteria for target identification and validation include:

- Evidence of pathological deregulation, including mutation leading to constitutive activity of an oncogene or loss of a critical tumor suppressor gene.
- Creation of a malignant phenotype by mutation or increasing or decreasing expression of proposed target.
- Reversal of a malignant process by correcting the genetic abnormality by gene knockout, RNA interference, or transfection.
- Evidence of adverse clinical outcome correlating with target deregulation.

The emergence of high-throughput "-omics" screening and discovery methods will add to the already large number of targets that are a focus for new anticancer drug development [5]. Once a target has been validated, it can be channeled into the drug discovery process that includes screening, lead identification, lead optimization, preclinical toxicology, and clinical trials [6]. Better understanding of the biology and molecular pathology of cancer, coupled to improvements in innovative technologies, is crucial to every step of the drug development process. High-throughput screening, combinatorial chemistry, and the input of structural biology play important roles in lead identification and optimization.

Previously, pharmacokinetic, efficacy and toxicology profiles were the most important criteria as to whether a compound would be a viable candidate for clinical development. With identification and validation of new molecular targets, rational drug design now provides greater opportunity for improving the therapeutic indices of new drugs. Moreover, pharmacokinetic–pharmacodynamic relationships are playing an increasingly important role in the development and use of new

anticancer drugs. A thorough understanding of a drug's pharmacokinetics and pharmacodynamics is dependent upon detailed knowledge of the drug's mechanism and an understanding of the molecular targets on which they act.

Cancer chemotherapy has reached a fascinating stage, in which new molecular therapeutics are being tested individually and in combination with traditional cancer drugs such as the hormonal and cytotoxic agents. It is hoped that agents targeted to the molecular pathology of cancer may minimize the use and maximize the benefit of cytotoxic drugs. A future in which patients will be prescribed personalized mechanism-based anticancer drugs targeted to their individual molecular and genomic profiles is becoming a reality.

The optimal targeting of cancer requires not only the selection of the target but also the identification of the patients whose cancer depends on the targeted pathway. Concurrent biomarker development to identify predictors of response will therefore become essential to the approval process for these new agents and could further refine the use of approved agents [7].

The objective of this chapter is to give an overview of molecular targets in cancer therapeutics. Selected examples and a detailed listing of literature references are included. Targets have been categorized as either established or novel types. Established targets include those against which most currently licensed anticancer drugs were developed and include DNA, microtubules, and nuclear hormone receptors. Novel targets are those under current preclinical and clinical investigation. They include the products of oncogenes and the genes responsible for the multistep transformation of normal cells into cancer cells, such as receptors and receptor tyrosine kinases, as well as new approaches to established targets, such as microtubules and DNA repair. The section on novel targets emphasizes the relationships of the novel targets to the biological traits of cancer as described earlier in this section.

2 Established Molecular Targets

2.1 DNA

DNA is one the most successfully exploited targets for anticancer drug development. The use of mustine to treat leukemia in 1943 predated the discovery of the double helical structure of DNA by Watson and Crick in 1953. However, some of the early attempts at rational drug design led to highly effective drugs such as 5-flurouracil (5-FU). With further understanding of the structure and function of DNA and molecules that regulate it, there may be new targets within and around DNA that can be further exploited for anticancer drug development (Fig. 2).

2.1.1 Nucleotides

The bases of DNA—adenine, guanine, cytosine, and thymine—are heterocyclic rings that make up the genetic code. Adenine and guanine are purines, while cytosine and thymine are pyrimidines. Adenine normally interacts non-covalently with thymine via two hydrogen bonds, whereas guanine forms three hydrogen bonds with cytosine. Methylating agents (e.g., temozolomide) add a methyl group to the O6 position of guanine bases, thus causing mis-pairing of guanine to thymine. Alkylating agents such as melphalan have an active moiety that bind directly to the DNA bases, particularly guanine. Nitrogen mustards are chlorethylating agents that permanently alkylate the N7 position of guanine residues, thus causing interstrand cross-links in the DNA and preventing proper replication of the DNA [8, 9]. Platinum compounds react with the N7 position of guanine to form both monofunctional and bifunctional DNA adducts. Although covalent adduct formation is the mechanism of cytotoxicity

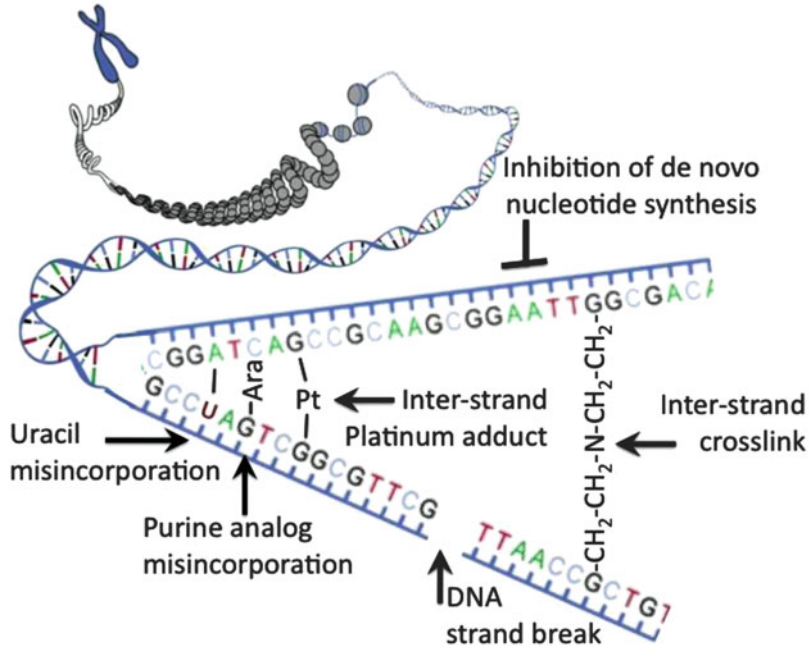

Fig. 2 DNA is a target for anticancer agents

of all DNA-modifying antitumor agents, these drugs exhibit widely different potency, toxicity, and tissue specificity. These differences can be attributed to structural features that affect the drugs' pharmacokinetics and biodistribution.

2.1.2 Purine and Pyrimidine Incorporation

Native purine and pyrimidine nucleotides are natural targets for rational drug design. Pyrimidines such as cytosine are incorporated into DNA as deoxycytidine triphosphate (dCTP), and competitive inhibition of this incorporation by Ara-C triphosphate (Ara-CTP) causes deregulation and inhibition of a wide range of enzymes including DNA polymerase and ribonucleotide reductase [10, 11].

Enzymes important to de novo purine synthesis such as phosphoribosyl pyrophosphate amidotransferase are inhibited by monophosphate derivatives of 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG). Misincorporation of ribonucleotide and deoxyribonucleotide metabolites of thiopurines can cause DNA strand breaks [12]. Cytosine arabinoside [13] and gemcitabine [14] are good examples of pyrimidine analogs used successfully in the clinic, whereas 6-MP [15] and 6-TG [16] are purine analogs with more limited application.

2.1.3 Dihydrofolate Reductase and Thymidylate Synthase

Thymidylate synthase (TS) is essential for the production of dTTP, and inhibition of TS leads to depletion of dTTP as well as increased levels of dUMP, which when phosphorylated leads to the misincorporation of dUTP into DNA causing DNA damage by uracil DNA glycosylase [17]. Dihydrofolate reductase (DHFR) is important in maintaining the reduced folate pool, which in turn is essential for the conversion of dUMP to dUTP by TS. Reduced folate pools are important for de novo

purine biosynthesis [18]. Both TS and DHFR are important targets and offer a degree of therapeutic selectivity. Both TS and DHFR are important for ongoing DNA replication and repair; thus, malignant cells can be more susceptible due to their rapid multiplication. Methotrexate is a commonly used anticancer agent that inhibits DHFR. On the other hand, 5-fluorouracil inhibits the TS enzyme in the folate synthesis pathway. Unfortunately, cancer cells can be selected to overexpress TS [19] and DHFR [20] as a mechanism to develop resistance to TS inhibitors such as 5-FU and methotrexate, respectively. Pemetrexed is a multi-targeted antifolate agent that blocks both of these enzymes as well as GARFT [21]. It may also have a secondary effect on inhibition of the AICART pathway of folate synthesis that can lead to subsequent inhibition of mTOR [22]. Pemetrexed is FDA approved for use in non-squamous NSCLC and in patients with pleural mesothelioma who are not surgical candidates.

2.1.4 Topoisomerase I and II

Eukaryotic DNA has a complex structure and is frequently supercoiled, knotted, or interlinked. Topoisomerase I and II are enzymes that modify the topological state of DNA by first cleaving the phosphodiester backbone of the DNA so as to allow the passage of another single- or double-stranded DNA, following which the topoisomerase reseals the strand, thus relieving DNA torsional strain [23, 24]. Relaxing the tertiary structure of DNA is essential for transcription, replication, and repair of DNA.

Two topoisomerases are current targets for anticancer drugs. Topoisomerase I causes single-stranded DNA breaks and is not ATP dependent, whereas topoisomerase II causes double-stranded DNA breaks and is ATP and Mg2+ dependent. Both topoisomerase I and II can be overexpressed in cancer, confirming their importance as valid targets. Successfully used topoisomerase I inhibitors include topotecan and irinotecan, whereas etoposide and doxorubicin are examples of topoisomerase II inhibitors that are used in the clinic [23, 24].

2.2 Microtubules

Microtubules are components of the mitotic spindles that are essential for dividing the replicated DNA into separate daughter chromatids during cell division. The microtubules are in dynamic equilibrium with the pool of soluble tubulin dimers present in the cell. There is a constant flux between incorporation of free dimers into the polymerized structures and release of the dimers into the soluble tubulin pool [25]. Although tubulin and microtubules are present in normal as well as tumor tissue, their fundamental involvement in cell division makes them important targets for anticancer drug development. Vinca alkaloids bind to distinct high-affinity binding sites on tubulin, causing alteration of microtubular dynamics at the ends of the mitotic spindle [26]. Clinically used vinca alkaloids include vincristine, vinblastine, and vinorelbine [27].

Taxanes bind mainly to polymerized tubulin. They decrease the lag time and shift the dynamic equilibrium between tubulin dimers and microtubules toward polymerization, thus stabilizing the microtubules [28]. Clinically, examples of drugs known to act as microtubule stabilizers include paclitaxel and docetaxel [29].

2.3 Nuclear Hormone Receptors

Hormones are known to influence a wide variety of malignancies. Most are steroids that enter the cell and bind receptors in the cytoplasm. Binding of the steroid hormone to its intracellular receptor then

allows the complex to translocate to the nucleus and activate intranuclear transcription factors. Examples of clinically relevant hormone receptors include those for estrogen, androgen, glucocorticoid, and retinoic acid.

2.3.1 Estrogen Receptors

Intranuclear estrogen receptors (ERs) have transactivation domains (AF-1 and AF-2) that when activated lead to transcription [30]. ER binds to specific DNA consensus sequences and also has considerable cross talk with the insulin-like growth factors and the oncogenic transcription factors c-Fos and c-Jun [31, 32]. Modulating ER by selective estrogen receptor modulators (SERMs) is standard treatment for breast cancers that express ER. Tamoxifen and raloxifene are SERMs that are also used in the preventative setting for reducing the incidence of contralateral breast cancer. Steroidal antiestrogens such as anastrozole and exemestane inhibit the conversion of estrogen to its active form, estradiol, by inhibiting the aromatase enzyme [33]. These drugs are approved for use in ER-positive breast cancers in postmenopausal women, where the majority of estrogen is formed by this peripheral conversion of the hormone, rather than secretion from the ovaries.

2.3.2 Androgen Receptors

Androgen receptors (ARs) bind both testosterone and the more potent dihydrotestosterone, which is formed by the action of the enzyme 5-alpha reductase [34]. Prostate carcinomas are heavily dependent on androgenic stimulation for growth and evasion of apoptosis, even when the receptor itself is expressed at seemingly normal levels [35]. Mutations of AR hormone binding domain, amplification of the AR gene, or alteration of AR coactivators lead to increased sensitivity to physiologic levels of androgens. Inhibition of AR signaling can be achieved clinically via reduction of testosterone (medical or surgical castration) or blocking the binding of testosterone to AR by agents such as flutamide and bicalutamide [36]. Constitutive cell-autonomous activation of the AR signaling pathway may allow escape from hormone dependence and resistance to antiandrogen therapy [37].

2.3.3 Glucocorticoid Receptors

Intracellular glucocorticoid receptors (GRs) are members of the steroid hormone superfamily and have both a hormone binding site and a DNA-binding domain. Activation and transcription of GR-dependent genes inhibits the growth of acute lymphoblastic leukemia (ALL) cells, whereas mutations in GR have been shown to confer resistance to dexamethasone [38]. Glucocorticoids are part of standard chemotherapy regimens in lymphoid malignancies such as ALL, Hodgkin and non-Hodgkin lymphomas, and multiple myeloma.

2.3.4 Retinoic Acid Receptors

Retinoic acid receptors (RARs) are also members of the nuclear steroid receptor superfamily. In acute promyelocytic leukemia (APL), a t(15;17) translocation fuses RAR-α, to a nuclear matrix protein PML (promyelocytic leukemia protein). The oncogenic fusion leads to constitutive expression of the PML protein, preventing differentiation and leading to a leukemic phenotype. Exposure of the cells to retinoic acid causes degradation of the PML–RAR fusion product, thus forcing the cells to terminally differentiate. Therefore, administration of all-*trans* retinoic acid (ATRA) effectively induces remissions in patients with APL [38].

2.4 Signal Transduction

2.4.1 Receptor Tyrosine Kinases

Receptors with tyrosine kinase activity are important targets for cancer. They may be overexpressed, mutated, or lie upstream of other signal transduction defects. Inhibiting the tyrosine kinase activity of key receptors has been successfully achieved using monoclonal antibodies and small molecule kinase inhibitors. Both strategies have been used against the epidermal growth factor receptor (EGFR). Cetuximab is a monoclonal antibody approved for head and neck squamous cell carcinoma and colon cancer that binds to EGFR and competitively blocks EGF from binding and stimulating its receptor [39, 40]. Erlotinib is a small molecule that specifically blocks the tyrosine kinase activity of the EGFR and is approved for use in non-small cell lung cancer and pancreatic carcinoma [39].

HER2 is a member of the EGFR family that has no known ligand, but when overexpressed, it signals by forming homodimers or heterodimers with other EGFR family members [41]. Trastuzumab is a monoclonal antibody licensed for treatment of HER2-positive breast cancer and gastric cancer. The antibody functions by preventing signaling through the dimerized receptors and/or by promoting antibody-dependent cellular cytotoxicity.

Proliferating tumor masses depend on new blood vessel formation in order to sustain growth. Thus, angiogenesis has been a promising target in several tumor types. Bevacizumab is a monoclonal antibody that inhibits angiogenesis by blocking vascular endothelial growth factor (VEGF) from binding to its receptor [42]. It is approved for use in glioblastoma multiforme, colorectal cancer, non-small cell lung cancer, and renal cell carcinoma.

2.4.2 Cytoplasmic Signaling Proteins

Imatinib is the first agent specifically designed to inhibit to an oncogenic intracellular kinase. In chronic myelogenous leukemia (CML), the (9:22) translocation results in the fusion protein BCR–ABL. The constitutively expressed and active kinase drives proliferation and survival through Ras, phosphatidylinositol 3-kinase (PI3 kinase), and Crkl pathways [43]. Imatinib is a potent inhibitor of BCR–ABL and achieves hematologic responses of 95 %, 53 %, and 29 % in chronic, accelerated, and blast phases of CML, respectively, which provided a basis for licensure [44, 45].

Sorafenib takes a broader approach to "targeted" therapy. This drug was developed to inhibit RAF kinase activity but also has activity against several other tyrosine kinases including VEGF receptor 2 (VEGFR2) [46]. Sorafenib is approved for use in renal cell carcinoma (RCC) as well as hepatocellular carcinoma (HCC). Its clinical utility in RCC most likely is a result of its activity against VEGFR2 [47]. In HCC, however, its efficacy is more likely due to its anti-RAF activity [48].

The mammalian target of rapamycin (mTOR) is a protein kinase that phosphorylates the initiation factor 4E binding protein (4E-BP1); this in turn binds eIF-4E, which is important for the translation of cyclin D1 mRNA. This crucial link to the cell cycle control pathway and the fact that it is downstream of the PI3 kinase–Akt pathway make mTOR an interesting target [49]. Rapamycin binds to the immunophilin FKBP12 that inactivates mTOR. This agent is approved for controlling solid organ transplant rejection due to its effects on T cell function. Rapamycin analogs temsirolimus and everolimus, however, are approved for RCC [50].

2.5 Chromatin Modulation

Histone deacetylases (HDACs) modulate chromatin structure and control other cellular functions. Four different classes of HDACs have been identified [51]. Studies in yeast in which specific HDACs

were deleted indicate that Rpd3, Sir2, and Hda1 have distinct functions in cell cycle progression, amino acid synthesis, and carbohydrate transport [52]. HDACs also have nonhistone targets and can regulate the deacetylation of p53 and E2F [53]. The binding of HDAC with the PML-RAR translocation product leads to inhibition of differentiation in the M3 subtype of AML. The treatment of such patients with retinoic acid, as mentioned previously, results in the displacement of HDACs and allows ligand-dependent coactivators such as SRC-1 to bind and activate transcription, which leads to reactivation of the differentiation process [54]. Two HDAC inhibitors, vorinostat and romidepsin, are approved for clinical use in cutaneous T cell lymphoma.

2.6 Protein Folding and Degradation

Rapid and irreversible proteasomal protein degradation is the key to the activation or repression of many cellular processes. The primary component of the protein degradation pathway in the cell is the 26S proteasome, which is where proteins marked with polyubiquitin chains are degraded [55]. The proteins are denatured to short (3- to 22-residue) polypeptides while the ubiquitin chain is recycled [56]. Proteins with tumor suppressor functions such as p27 (that inhibits CDK 2, 4, and 6) and IκB (that inhibits NF-κB) are degraded by the ubiquitin proteasome pathway. Proteasomal inhibition decreases cellular proliferation in vitro and in vivo in several cancer model systems [57]. The proteasome inhibitor bortezomib is approved for use in multiple myeloma and mantle cell lymphoma [58].

3 New Approaches to Established Targets

3.1 DNA Damage

The use of PARP inhibitors is an exciting and novel approach to exploit DNA damage in cancer cells. More than half a dozen highly potent and specific PARP inhibitors are currently undergoing clinical development in cancer populations. PARP (poly-ADP ribose polymerase) is a highly abundant nuclear protein that is activated when DNA is damaged [59]. The action of PARP1 is essential for repair of single-stranded DNA breaks, predominantly through the BER mechanism [59]. PARP also contributes to repair double-stranded breaks through NEHJ, which is further impaired when PARP activity is inhibited [60]. Small molecule inhibitors of PARP activity began development as sensitizers to DNA-damaging chemotherapy or ionizing radiation [61]. However, it was soon discovered that cells otherwise deficient in DNA repair pathways were strikingly more sensitive to PARP inhibition. For example, BRCA-deficient cells are 1,000-fold more sensitive to single-agent PARP inhibition than are wild-type BRCA1 and BRCA2 cells [62, 63]. BRCA1 and BRCA2 regulate repair of damaged DNA through HR [64]. In addition to BRCA1 and BRCA2 germline mutations, other inherited molecular defects may prevent effective HR DNA repair in cancer. Irreparable DNA damage triggers apoptotic cell death in cells with or without intact p53 [65]. Therefore, inhibiting PARP has been undertaken as a strategy to selectively kill cells with dysfunctional HR [66].

3.2 Microtubules

Epothilones are compounds derived from the myxobacterium Sorangium cellulosum that stabilize microtubules by interacting with the taxane-binding site. The epothilones improve upon

first-generation taxanes by overcoming multidrug resistance mediated by p-glycoprotein [67]. Ixabepilone is the first macrolide epothilone approved for clinical use in metastatic breast cancer [68]. Microtubule-stabilizing agents that bind to other sites are also under investigation, including vinflunine (vinca alkaloid binding site) and combretastatins (colchicine binding site) in order to improve on safety, tolerability, and efficacy.

3.3 Growth Factor Receptor Signaling and Tyrosine Kinase Inhibition

Resistance to first-generation targeted therapies is a common occurrence, and identification of these mechanisms of resistance has led to the development of second-generation targeted agents. In the case of tyrosine kinase inhibitors, cancer cells may develop a mutation in the ATP-binding pocket that lowers the affinity of the kinase for the drug and/or increases affinity for ATP. In CML, resistance to imatinib evolves in up to half of the patients, most often due to mutations in the BCR–ABL kinase. Dasatinib, a second-generation inhibitor, has a 350-fold greater potency against the unmutated ABL kinase and has activity against most of the imatinib-resistant BCR–ABL mutants, except the gatekeeper mutation T351I [69].

Tyrosine kinase inhibitors were originally developed as competitive inhibitors of the ATP-binding site. More recently, irreversible inhibitors have been developed to improve on the potency and sustainability of inhibition and to overcome acquired resistance to reversible TKIs such as erlotinib. For example, the EGFR T790M mutation is responsible for acquired resistance to erlotinib in many NSCLC patients who initially responded to erlotinib. Neratinib is an irreversible TKI that binds covalently to the ATP-binding cleft of EGFR and may show activity in cells with the T790M mutation due to its inability to be displaced by ATP [70]. Most irreversible TKIs also target other EGFR family members including HER2 and are therefore under clinical investigation in both lung cancer and breast cancer.

Resistance to trastuzumab develops in HER2-positive breast cancers. One mechanism for resistance is deletion of the extracellular domain of the HER2 molecule. This mutation prevents binding of the mAb trastuzumab but retains intracellular tyrosine kinase activity. Lapatinib is a dual inhibitor of both EGFR1 and HER2 that targets this intracellular kinase activity of the truncated HER2 protein. Lapatinib is approved for the treatment of trastuzumab-resistant HER2-expressing metastatic breast cancer [71].

3.4 Antiandrogens: MDV3100

MDV3100 is a novel antiandrogen that has three distinct mechanisms of action. It blocks testosterone from binding to its receptor, inhibits movement of the receptor to the nucleus, and prevents the binding of the complex to DNA [72]. This agent achieves clinical responses in over 50 % of patients in phase 1 and 2 trials and is currently under phase 3 investigation for the treatment of metastatic prostate cancer [73].

3.5 Tumor Blood Vessels

Vascular disrupting agents—drugs targeting existing endothelial cells—are classified as antivascular rather than antiangiogenic. Two classes of vascular disrupting agents (VDAs) are currently in

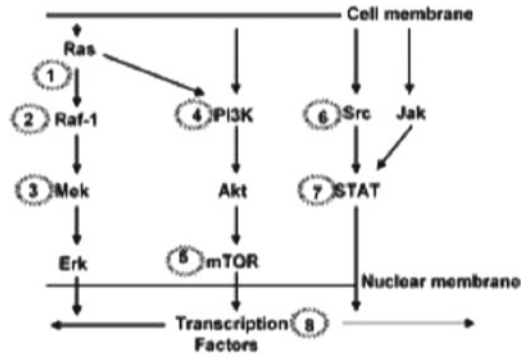

Fig. 3 Signal transduction pathways and nonreceptor sites targeted for anticancer drug development: (1) Ras prenylation, (2) RAF1, (3) MEK, (4) P13 kinase, (5) mTOR, (6) SRC, (7) STAT, (8) transcription factors

development [74]. The first class targets microtubules, and those binding to the vinca alkaloid domain appear to be more selective to endothelial cells. Drugs such as combretastatin A and its analogs destabilize vasculature, resulting in the rapid destruction of blood vessels [75]. The second class of VDA includes flavonoid compounds derived from flavone acetic acid (FAA) [74]. These agents appear to induce apoptosis in endothelial cells, via a TNF-related mechanism. The drug vadimezan (ASA404) is currently in phase 3 clinical trials in combination with carboplatin and paclitaxel for second-line treatment of NSCLC.

3.6 PI3K/Akt/mTOR

3.6.1 PI3 Kinase

PI3 kinase is a lipid kinase activated by Ras and a number of receptor tyrosine kinases including PDGFR and EGFR [76, 77] (Fig. 3). Downstream of PI3 kinase, Akt and mTOR are important kinases that are critical for cell proliferation [78]. The tumor suppressor gene PTEN (phosphate and tensin homolog deleted from chromosome 10) dephosphorylates and inactivates phosphatidylinositol 3,4,5-triphosphate (PIP-3), which is the lipid product formed by the activation of PI3 kinase [79]. PI3K is an attractive target for anticancer drug development based on several features: genes encoding PI3 kinases are amplified in certain cancers, PI3 kinase lies downstream of receptor tyrosine kinases that are overexpressed or mutated in cancer, PI3K is upstream of known oncoproteins such as PKB/Akt, and PTEN loss or mutation is the second most common tumor suppressor gene abnormality in human cancers [80].

3.6.2 mTOR

The mTOR pathway is a key checkpoint in the sensing of nutrients within the cell. Amino acids, glucose, and oxygen regulate the mTORC complex and upstream TSC proteins. Cancer cells appear to override the growth inhibitory signals of nutrient deprivation, perhaps by overactivation of the mTOR signaling complex. Similarly, resistance to rapamycin and analogs, which specifically inhibit mTORC1, may result from overactivation of mTORC2, or by feedback activation of Akt itself. Dual mTORC1 and mTORC2 inhibitors are under development, as are Akt inhibitors [81].

4 Novel Molecular Targets

4.1 Transcription Factor Pathways

4.1.1 NF-κB

NF-κB is an antiapoptotic transcription factor that is normally inhibited by IκB [82]. Amplification or overexpression of the NFκB gene has been seen in several hematologic malignancies and solid tumors, whereas inactivation of IκB has been demonstrated in Hodgkin's lymphoma. One approach to inhibit NF-kB activation is through blockade of the I-kappaB kinases, specifically IKK-beta. Several inhibitors of IKK-beta are in early phases of development [83].

4.1.2 JAK/STAT Pathway

Signal transducers and activators of transcription (STAT) are cytoplasmic transcription factors that are activated by growth factor receptors (interferons) and cytoplasmic tyrosine kinases such as JAK and SRC [84] (Fig. 3). STAT3 is required for the activation of v-SRC transformation, and a constitutionally active STAT3 mutation is sufficient to induce malignant transformation [85, 86]. Approaches to modulate STAT activity aim to target STAT dimerization, translocation, and DNA binding [84]. Inhibitors of JAK are also in clinical development, specifically for myeloproliferative disorders, which harbor the activating V617F mutation in JAK2 [87].

4.1.3 AP-1 Family

The activator protein-1 family (AP-1), consisting of Fos, Jun, and ATF proteins, plays an important role in development [88]. Overexpression of c-Fos has been shown to induce cartilaginous tumors, and absence of c-Fos is associated with reduced expression of matrix metalloproteinases, thus affecting angiogenesis and invasion [89]. c-Jun may transform mammalian cells but requires coexpression of other oncogenes such as Ras and SRC, but other Jun proteins may have tumor suppressor functions [90]. Better understanding of these transcription factors will help develop specific inhibitors. However, inhibition of protein–protein and protein–DNA interactions in which these proteins participate is technically challenging.

4.1.4 c-MYC

c-Myc is a prototype for oncogene activation by chromosomal translocation [91]. It is involved in several prooncogenic events such as protein synthesis, cell cycle progression by inactivation of cell cycle inhibitor p27, and activation of cyclin E and E2F [92]. Myc targets genes that regulate apoptosis such as p53 and affects cell adhesion by downregulation of LFA-1. Finally, c-Myc has been associated with a number of hematologic malignancies and solid tumors, making it an attractive target for anticancer drug development [93].

4.2 Cytoplasmic Kinases

4.2.1 SRC

SRC is a cytosolic nonreceptor tyrosine kinase that is activated by growth factor receptors and focal adhesion kinase [94]. SRC was the first identified oncogene and regulates many downstream cellular functions including proliferation, survival, adhesion, and angiogenesis. SRC kinase activity has been shown to be elevated in many types of cancer. Dasatinib, developed to treat imatinib-resistant CML, inhibits kinase activity of SRC in addition to its intended target, ABL [95]. Dasatinib is currently under clinical investigation as a SRC inhibitor in solid tumors.

4.2.2 Ras–RAF–MEK–ERK Pathway

Members of the Ras superfamily of proteins that are implicated in cancer include H-Ras, N-Ras, and K-Ras. Ras mutations are found in a variety of tumor types and have also been shown to be a marker for poor prognosis [96]. Ras undergoes prenylation, a lipid posttranscriptional modification required for proper localization to the inner surface of the plasma membrane. Some members of the Ras family require farnesylation, while others also undergo geranylgeranylation such as Rho, Rac, and cdc42, which are important to malignant transformation mediated by Ras. The enzymes that mediate farnesylation and geranylgeranylation are farnesyltransferase (FT) and geranylgeranyltransferase (GGT), respectively [97]. While H-Ras prenylation is inhibited by FT inhibitors, K-Ras is more difficult to inhibit and may require both FT and GGT inhibitors to block malignant transformation. There is increasing evidence that FT inhibitors may not act solely or even partly via Ras, since many other cellular proteins are farnesylated. Research to identify the downstream targets continues, with a potential opportunity for therapeutic intervention at the point of Rho-kinase (ROK) [98].

The RAF family of serine threonine kinases is activated downstream of Ras [96] (Fig. 3). Sorafenib, which predominantly targets RAF1, is in clinical use for treatment of RCC and HCC [47, 48]. Mutations in the genes encoding BRAF have recently been identified in a high proportion of melanoma and in a lower proportion of colorectal and other cancers [99]. PLX4720 specifically inhibits BRAF carrying the V600E mutation. A phase I clinical trial with this agent expanded enrollment of patients with melanoma harboring the V600E mutation, which occurs in nearly 50 % of melanomas. The BRAF inhibitor achieved an overall response rate of 70 % in this subset of patients. Thus, the V600E mutation provides a molecular marker predictive of sensitivity to this drug [100]. Unfortunately, responses are short-lived, and resistance to BRAF inhibition is reflected in reactivation of ERK phosphorylation in the cancer cells. Preclinical evidence suggests that acquired mutations in MEK1 could contribute to the resistance [101]. The BRAF mutation is present in approximately 8 % of other solid tumors, yet inhibiting the mutant BRAF is ineffective if K-Ras mutations coexist. The RAF inhibitors appear to induce a conformational change in RAF that interacts with the mutant Ras-GTP and promotes signaling through unmutated RAF1 [102]. Dual inhibition of RAF and MEK is being studied preclinically to overcome this phenomenon. MEK is phosphorylated by RAF and, once activated, phosphorylates the MAP kinases ERK-1 and ERK-2 [96]. Although MEK has not been identified as an oncogene product, no other substrates apart from ERK have been identified, thus making it an important focal point for mitogenic pathways activated by RTKs and/or oncogenes. Drug development programs are actively pursuing MEK inhibitors, and several are now undergoing clinical trials [103].

4.3 Mitotic Kinases

Cancer cells undergo unrestricted cell division, thus making mitosis a logical area of anticancer drug development. During mitosis, the chromosomes are separated by microtubules on the mitotic spindle. Therefore, microtubule-targeting agents were thought to kill cancer cells by inhibiting mitosis. Microtubules are essential to many other cellular processes, however, suggesting that these traditional chemotherapeutic drugs may kill the cells by other mechanisms as well [104]. In addition, the toxicities of these drugs in nondividing cells also point to their effects on intracellular protein trafficking as well.

Aurora kinases A and B and polo-like kinase 1 (PLK1) regulate distinct points in mitosis and have been explored as potential therapeutic targets in cancer [105]. Aurora kinase A acts at the point of centrosome separation and mitotic spindle assembly. Aurora kinase B is responsible for correct chromosome alignment and triggers mitotic checkpoint delay in the setting of misaligned chromosomes in normal cells. PLK1 functions in kinetochore–microtubule interaction and completion of cytokinesis. This protein may act as a tumor suppressor during development [106] but has been identified as a potential driver of K-Ras-mediated oncogenesis [107].

Specific inhibitors of each of these kinases are under development, but their ultimate use in cancer therapy is unclear [108]. Emerging preclinical evidence suggests that combined inhibition of these kinases may be antagonistic. Patterns of cell cycle arrest upon aurora kinase or PLK inhibition suggest that specific combinations may be optimal for treating cancers. For example, aurora kinase B inhibitors might promote killing by paclitaxel since they release the mitotic arrest induced by the taxane and could then accelerate cell death. Aurora kinase A inhibitors may promote killing by DNA-damaging agents, such as the platinums, by maintaining the cell in G2 arrest since this kinase is required to restart the cell cycle after G2 arrest. PLK1 inhibitors are able to induce mitotic delay and apoptosis as single agents, but may also cooperate in cytotoxicity of DNA-damaging agents as well [105].

4.4 Epigenetic Modifications

Methylation of cytosine residues in adjacent cytosine and guanine nucleotides in DNA (CpG) is achieved by DNA methyltransferase (DNMT) [109]. Consequences of CpG methylation include silencing of a variety of tumor suppressor genes including Rb, p16, p14, BRCA1, and MLH1 [110]. Although tumors tend to exhibit global hypomethylation, there are often large areas of CpG island hypermethylation in tumors [111].

DNMT inhibitors could be used as single agents with the aim to reactivate methylation-silenced tumor suppressor genes, or in combination with conventional cytotoxics, where reactivation of genes such as those encoding the mismatch repair protein MLH1 affects the sensitivity of cancer cells to carboplatin and epirubicin [110]. Two classes of DNMT inhibitors exist. Nucleoside analogs such as 5-azacytidine inhibit DNMT activity by incorporating into replicating DNA and irreversibly binding the DNMT enzyme [112]. This drug and its analog 5-aza-2-demethoxycytidine were approved for use in myelodysplastic syndrome. The second class of DNMT inhibitors includes non-nucleoside small molecule inhibitors of the catalytic site [112]. These compounds are in early phase clinical development.

4.5 Apoptosis

Apoptosis or programmed cell death is characterized by morphological changes (shrinkage, condensation of nuclei, and loss of microvilli) [113] and the biochemical hallmark of cleavage of

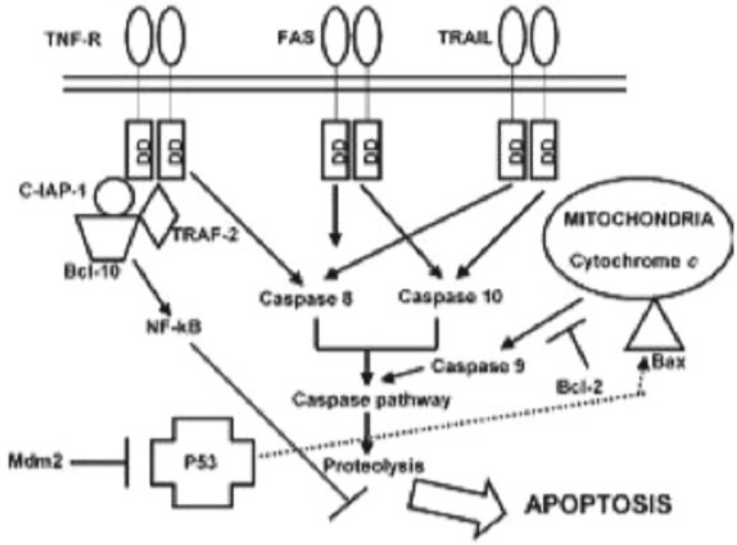

Fig. 4 Targets for apoptosis modulation

chromosomal DNA into nucleosomal units by caspases [114]. Apoptosis is governed by proapoptotic events, which include (1) death receptor signaling, (2) release of cytochrome c from the mitochondria, and (3) p53 activation. Antiapoptotic factors include (1) antiapoptotic protein Bcl-2, (2) cellular inhibitor of apoptosis 2 (c-IAP), and (3) NF-κB. Deregulation of any of these key factors can lead to inhibition of apoptosis and an inappropriate survival advantage to the affected cell [115] (Fig. 4).

4.5.1 Bcl-2 Family

Bcl-2 is an antiapoptotic protein that is overexpressed in a variety of malignancies [116]. Attempts to inhibit Bcl-2 by adenoviral vectors carrying a dominant negative gene or by an antisense approach have undergone clinical development with unsatisfying results [117]. More recently, specific small molecule inhibitors of Bcl-2 have been developed that directly bind to the BH3 domain of the anti-apoptotic proteins [118]. Navitoclax and its analog ABT-199 are undergoing early phase clinical development in hematologic malignancies that demonstrate Bcl-2 overexpression [119].

4.5.2 Apoptosis Inhibitor Proteins

TNF-related apoptosis inducing ligand (TRAIL) binds to the death receptors DR4 and DR5 and triggers the assembly of the death-inducing signaling complex (DISC), leading to apoptosis [120]. Synthetic TRAIL and an antibody to these death receptors have been used to target death receptors in malignant cells. Inhibitors of apoptosis, IAP1, IAP2, and XIAP block the apoptosis signaled through the DISC by inhibiting either caspase activation or directly blocking caspase activity. The secondary mitochondrial activator of caspase (SMAC) antagonizes IAP function by causing them to auto-ubiquitylate and target themselves for proteasomal degradation [121]. Small molecule mimics of SMAC are in preclinical and early clinical development.

4.6 Heat-Shock Proteins/Chaperones

Heat-shock protein 90 (HSP90) and its endoplasmic reticulum homolog GRP78 are important molecular chaperones involved in posttranslational folding of client proteins. Client proteins such as Erb-B2, BCR–ABL, SRC, RAF1, Akt/PKB, and CDK4/6 either are oncoproteins or are integral elements of signal transduction pathways that are deregulated in cancer [122]. The ability to affect multiple signal transduction pathways at different levels makes HSP90 an attractive target. The geldanamycin analog 17AAG has shown promising preclinical activity and is in clinical trials [123].

5 Targeted Chemoprevention

Given the challenges associated with inhibition of the multiple oncogenic abnormalities involved in the late stages of cancer, the concept of chemoprevention is an attractive one [124]. Chemoprevention can be primary, secondary, or tertiary. Primary prevention is aimed at healthy individuals, while secondary prevention is directed at patients with a preclinical or early stage disease. Tertiary prevention is aimed at patients who have undergone initial treatment and aims to prevent recurrence. Whereas targets for tertiary prevention have been covered under previous sections, primary and secondary prevention are discussed below.

5.1 Hormone Receptors

5.1.1 Breast Cancer

In 1998, tamoxifen was FDA approved for the prevention of breast cancer in women considered to be at high risk of the disease. Subsequently, the STAR trial (study of tamoxifen or raloxifene) resulted in the approval of raloxifene in 2007. Raloxifene was not as effective as tamoxifen in preventing invasive breast cancers but had a favorable side effect profile, with lower incidence of endometrial hyperplasia and thromboembolic events [125].

5.1.2 Prostate Cancer

Finasteride is a 5-alpha-reductase inhibitor that may prevent prostate cancer [126]. It has FDA approval for the treatment of benign prostatic hyperplasia but has not been approved for prostate cancer prevention, due to the concern for increased incidence of high-grade cancers that were detected in men taking finasteride. The value of 5-alpha-reductase inhibitors in prostate cancer prevention thus remains an area of debate.

5.2 Retinoic Acid Receptors

As described earlier, retinoic acid receptors belong to the nuclear receptor superfamily. They have been shown to influence the malignant potential of mammalian cells and are exploited in two principal ways in anticancer treatment: first to treat acute promyelocytic leukemia and second in secondary

prevention of a variety of cancers [127]. Epidemiological data suggest that geographical areas where vitamin A deficiency was endemic had an increased incidence of aerodigestive cancers [128], and underexpression of retinoic acid receptor β has been demonstrated in bronchial biopsies of chronic smokers [129]. Despite this rationale, retinoid supplementation has not been successful in large phase III cancer prevention trials. Nonetheless, retinoids are still under study as potential chemopreventive agents, either as single agents in specific populations or in combination with other agents [127].

5.3 Cycloxygenase-2

Cycloxygenase-2 (COX-2) expression has been linked to several cancers, and population-based studies showed a 40–50 % decrease in the relative risk of colorectal cancer in persons who were prolonged users of nonsteroidal anti-inflammatory drugs (NSAIDs) [130–132]. The selective COX-2 inhibitor celecoxib is FDA approved for the prevention of colon cancer in patients with familial adenomatous polyposis coli (APC) who are susceptible to developing colorectal cancer [124].

5.4 Novel Chemopreventive Agent: Metformin

Metformin is the most commonly used oral diabetes drug. Diabetics who take metformin, as opposed to other oral agents or insulin, have decreased incidence of several types of cancer [133]. This decreased cancer incidence may be related to inhibition of mTOR by metformin via activation of AMP-activated protein kinase [134]. Metformin is an effective chemopreventive agent in preclinical models of tumorigenesis that are not related to diabetes [135], which has raised the potential of metformin to be tested in humans at risk for cancer, irrespective of diabetes. Several clinical chemoprevention trials with metformin in high risk groups are planned or in progress.

6 Concluding Remarks

The development and use of new cancer therapeutics continues to progress at an exciting pace, accelerated both by the discovery of new molecular targets that have arisen from molecular oncology and genomics and also by the implementation of new discovery technologies. The rational development and application of new and established cancer therapeutics requires a thorough understanding and consideration of the mechanisms of action and molecular target involved, coupled to the principles of pharmacokinetics and pharmacodynamics. This chapter illustrates the plethora of molecular targets that are modulated by cancer drugs, from the relatively nonspecific effect on DNA to highly selective agents that are designed to attack particular loci responsible for malignant progression.

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