Drug Targets for Cancer Treatment: An Overview

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Abstract
Cancer is one of the major cause of death worldwide. Malignant cells display metabolic changes, when compared to normal cells, because of both genetic and epigenetic alterations. Number of drugs being used for the cancer treatment follows different mechanisms of action. Therapeutic strategies include targeting of drugs at specific genes or proteins/enzymes found in cancer cells or the internal tissue environment which contributes to growth and survival of these cells. Targeted therapy is often used along with chemotherapy and other treatments to restrict the growth and spread of cancer cells. During the past few decades, targeted therapy has emerged as a promising approach for the development of selective anticancer agents. There is a class of targeted therapy drugs called angiogenesis inhibitors which focus on blocking the development of new blood vessels in tumor tissues. In addition, anticancer drugs also include DNA intercalators, DNA synthesis inhibitors, transcription regulators, enzyme inhibitors etc. This review focuses on major classes of anticancer drug targets and their therapeutic importance.

Keywords: Anticancer drug targets; Angiogenesis; Gene regulation; Enzyme; Microtubules

Introduction
Cancer is the second leading cause of death in Europe and America. Tremendous resources are being invested all around the world for developing preventive, diagnostic, and therapeutic strategies for cancer [1]. Several pharmaceutical companies and government/non-government organizations are involved in the discovery and development of anticancer agents [2]. Identification of novel cytotoxic compounds has led to the development of anticancer therapies for several decades. Boom of knowledge in molecular sciences, genomics and proteomics has also helped in creating new potential drug targets. This has changed the paradigms of anticancer drug discovery toward molecularly targeted therapeutics. There are unique challenges and opportunities in discovery of anticancer drug delivery which might reflect at each stage of the drug development process [3]. Cancer is primarily a disease of uncontrolled cell division, thus identification of anti-proliferative compounds and their effects on regression of tumor size are the main aims for therapeutic discovery. For this purpose murine models of cancer were developed and several clinically important anticancer compounds were identified [1]. Differentiated result outputs among fast growing and slow growing tumors led investigators to modify the screening protocols to include a variety of cell lines and tumor types. The rationale that cancer cells are more likely to be replicating than normal cells makes the basis for targeting cell division process by most of the chemotherapeutics. Unfortunately significant toxicity is associated with chemotherapeutics as they lack specific action [1-3].

Double-helical DNA consists of two complementary strands running anti-parallel having sugar-phosphate poly-deoxyribonucleotide backbone associated with specific hydrogen bonding between nucleotide bases [4]. In a given DNA sequence difference in chemical feature of the molecular surfaces in either groove forms the basis for molecular recognition by small molecules and proteins. B-form of the DNA i.e. biologically relevant double helix is characterized by a shallow wide major groove and a deep narrow minor groove [5]. DNA replication, transcription and protein synthesis are the major steps in cell growth and division. Being carrier of genetic information as well as central to tumorigenesis and pathogenesis, DNA is a major target for drug development. There is always a challenge for drug to achieve maximum specific DNA binding affinity. The other thing that needs consideration is that drug should not affect cellular and nuclear transport activity of the normal cells. Some of the most effective anticancer agents that target DNA are known to produce significant survival rate in cancer patients when used in combination with drugs having different mechanisms of action [6]. Besides DNA, RNA, enzymes and other proteins also contributes as major targets for anticancer drug development [7]. Structures of some anticancer drugs are depicted in Figure 1. In this review we have tried to discuss some molecular aspects of anticancer drug mechanisms.

Angiogenesis Inhibitors
Angiogenesis (AG) is the process by which tumour develops new blood supply (neovascularisation) for the growth and metastasis. Small tumours can obtain oxygen and nutrients by diffusion but as they become enlarged they need to develop new blood vessels for the fulfillment of required nutrients for growth, invasion and metastasis. Different anti- and pro-angiogenic factors are involved in the development of blood vessels in a complex equilibrium [8]. In physiological processes such as wound healing this equilibrium may go in favor of angiogenesis by inflammation or hypoxia. But on the other hand it may be the part of the pathological process in cancer or other chronic inflammatory diseases. Vascular endothelial growth factor (VEGF), angiogenin, transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF) are some pro-angiogenic factors that are released in tumor associated angiogenesis which in turn induces the proliferation, migration and invasion of endothelial cells in new vascular structures [8]. Platelet derived growth factor receptor and cell adhesion molecules (e.g., integrins) play important role in the process of angiogenesis. Oxygen deprivation, oncogenic mutations, inflammation and mechanical stress are the stimulus that initiates growth of new vessels in tumor (angiogenic switch). This leads to vascularisation

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interaction with VEGFR-2 phosphorylation site. For last three decades AG has been taken as an appealing target for anticancer drugs [9]. Till know about thirty AG inhibitors are in clinical trials and some of them have been approved for the treatment of malignancy. AG inhibitors play role as cytostatic rather than cytotoxic drugs. The anti-angiogenic drugs have capability to reduce the production of pro-angiogenic factors as well as their binding efficacy to respective receptors which results into their blockage of action [8,9].

DNA Intercalators and Groove Binding Agents

Intercalation and groove binding are the major mechanisms underlying drug-DNA interaction. Insertion of a planar molecule between DNA base pairs is known as intercalation which results and expression of pro-angiogenic factors in tumor [8]. Some of the angiogenesis inhibitors and their mode of action are shown in Table 1.

VEGF signaling through its receptor tyrosine kinase is the major inducer of angiogenesis. VEGFR-1, 2, and 3 are the three receptor tyrosine kinases of VEGFR family which mediate the angiogenic effect [9]. In endothelial cells stimulation of VEGFRs, other tyrosine kinases, G-proteins and serine/threonine kinases cause massive activation of signaling pathways. Src homology 2 (SH2) and b-cell (Shb) protein act as adapter molecules in VEGFR mediated signaling in angiogenesis. Endothelial cell migration, proliferation, and survival are the important processes involved in angiogenesis. These event takes place by the activation of PI3K (phosphatidylinositol 3-kinase) and Akt/PKB (serine threonine kinase/protein kinase B), by virtue of Shb protein interaction with VEGFR-2 phosphorylation site. For last three decades AG has been taken as an appealing target for anticancer drugs [9]. Till know about thirty AG inhibitors are in clinical trials and some of them have been approved for the treatment of malignancy. AG inhibitors play role as cytostatic rather than cytotoxic drugs. The anti-angiogenic drugs have capability to reduce the production of pro-angiogenic factors as well as their binding efficacy to respective receptors which results into their blockage of action [8,9].
Table 2 shows some more examples of DNA intercalators used as both DNA crosslinkers as well as groove-binding molecules [24]. Bifunctional binders have proven clinical utility both as anticancer and antibacterial agents. This idea got support from the observation of differential expression of RNR in cancer cells compared to normal cells [36,37]. Aberrant replication forks, activation of S-phase checkpoint, and cell-cycle arrest are some key goals that might be achieved by targeted inhibition of RNR [37]. RNR is expressed at relatively low level in normal cells while in cancer cells its expression level is very high for maintaining high dNTP pools required for DNA synthesis and proliferation. Using a structure and mechanism based approach scientists have designed and developed novel classes of RNR inhibitors with potential clinical use. Recently COH29, an RNR inhibitor was discovered that showed activity in tissue culture and human tumor xenografts in mice [38]. S-phase arrest was observed in cell cultures treated with COH29 which is consistent with inhibition of RNR and its established role of catalyzing the rate-limiting step in dNTP synthesis and therefore DNA synthesis [39,40]. Novel binding pocket in RNR have been identified which is located on such a position that makes it potentially capable of multiple functional and biologically relevant effects. Gemcitabine (2\',2\'-difluoro-2\'-deoxycytidine, dFdC) is metabolized intracellularly to 5\'-diphosphate (dFdCDP). It is another potent inhibitor of ribonucleotide reductase and a very promising anticancer drug [41]. Several DNA synthesis inhibitors have been enumerated along with their mode of action in Table 3.

### DNA Synthesis Inhibitors

It is well established that without purines, pyrimidines, serine, and methionine the de novo synthesis of DNA in mammalian cells can not be possible. Folate is a family of B9 vitamins that are essential to mammalian cells. Folic acid is not a naturally occurring folate, it is composed of a pteridine ring, para-aminobenzoic acid (pABA) and glutamate [33]. In cells folic acid undergoes reduction process mediated by dihydrofolate reductase (DHFR) which ultimately leads to production of folate polyglutamates. These polyglutamates serve as one-carbon donors in de novo synthesis of purines, thymidylate and glutamate [33]. In cells folic acid is metabolized intracellularly to 5\'-diphosphate (dFdCDP). It is another potent inhibitor of ribonucleotide reductase and a very promising anticancer drug [41]. Several DNA synthesis inhibitors have been enumerated along with their mode of action in Table 3.

### Transcription Regulators

In all living cells transcription is required for the growth and survival. However, tumor cells require excess levels of transcription, including ribosomal RNA and mRNA transcription by RNA polymerase I and II respectively. Mutations are responsible for the enhanced transcription in cancer cells. DNA transcription is dependent on the spatially and temporally coordinated interaction between transcriptional machinery and transcriptional regulatory components. Different transcription factors (TFs) have been reported to associate with cancer. Transcription deregulation can occur by aberrant activation, repression, temporal/spatial dyscoordination, structural changes including mutations, translocations, and fusion. Dysregulation of transcriptional and thereby post-transcriptional processes contributes to cancer initiation [51]. The TF nuclear factor (NF)-κB is a family of five reticuloendotheliosis (REL) proteins. The protein influences gene transcription that allows its translocation into the nucleus. Its inhibition sequesters the complex (NF-κB and its inhibitor IκBα) in the cytoplasm in an inactive conformation. Activation of NF-κB transcription factor may lead to IκBα degradation. NF-κB has been known to be active constitutively in several cancer types. It is associated with the regulation of cell survival, cell proliferation, invasion, metastasis and apoptosis inhibition. Thus inhibition of NF-κB transcription factor may result into retarded tumor formation [51]. Targeting of a TF might inhibit several cancer related genes, since

<table>
<thead>
<tr>
<th>Name</th>
<th>Mode of Action</th>
<th>References</th>
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<tbody>
<tr>
<td>Angiotatin K13</td>
<td>Inhibitor of endothelial cell growth and angiogenesis.</td>
<td>[10]</td>
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<tr>
<td>DL-difluoromethylornithine</td>
<td>Inhibition of ornithine decarboxylase (ODC) and blocks angiogenesis.</td>
<td>[11]</td>
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<tr>
<td>Endostatin</td>
<td>Inhibits endothelial cell proliferation; Potent inhibitor of angiogenesis and tumor growth as well.</td>
<td>[12]</td>
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<tr>
<td>Fumagillin</td>
<td>Inhibitor of endothelial cell proliferation and angiogenesis.</td>
<td>[13]</td>
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<tr>
<td>Genistein</td>
<td>Down regulates the transcription of genes involved in controlling angiogenesis.</td>
<td>[14]</td>
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<tr>
<td>Minocycline</td>
<td>Inhibits endothelial cell proliferation and angiogenesis.</td>
<td>[15]</td>
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<tr>
<td>Staurosporine</td>
<td>Blocks angiogenesis by inhibition of up regulated VEGF expression in tumor cells.</td>
<td>[16]</td>
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<tr>
<td>(±)Thalidomide</td>
<td>Inhibits biosynthesis of tumor necrosis factor α (TNFα); inhibits angiogenesis.</td>
<td>[17]</td>
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Table 1: Angiogenesis inhibitors and their mode of action as anticancer agents.
it regulates different downstream target genes. In cancer therapy, the drugs that targets TFs are less known than inhibitor molecules targeting the signal transduction. Recently novel immunotherapies have been documented against some transcription factors. For example transcription factor WT-1 and PML-RARα are the targets for the development of resistance to AIs there is need for the decreased level of estrogen. Thus in the progression and development of hormone responsive breast cancers aromatase enzyme inhibitors may have significant effects and their inhibitors (AI) can be utilized as a tool for the development of resistance to AIs. Amino glutethimide (Ist generation); formestane and vorozole (II nd generation); anastrozole, letrozole, and exemestane (III rd generation) or nonsteroidal (Type II inhibitors). Type I inhibitors binds covalently while type II binds reversibly to the aromatase enzyme. Amino glutethimide (1st generation); formestane and vorozole (2nd generation); anastrozole, letrozole, and exemestane (3rd generation) are some examples of AIs. Testolactone, a first generation AI and is approved for treatment of advanced breast cancer in the United States [67]. Due to the development of resistance to AIs there is need to develop new aromatase inhibitors that could offer less severe side-effects and increased clinical efficacy. Unwinding and rewinding of the DNA helix during various processes such as replication, repair, and chromatin remodeling, entanglement of DNA occurs. The enzyme DNA topoisomerases a nature’s tool solve the problem by performing topological transformations in DNA. They form a covalent adduct with DNA resulting into a transient DNA break through which strand passage can occur. The two types of topoisomerasies i.e., type I and type II enzymes involves a nucleophilic attack of a DNA phosphodiester
bond by a tyrosyl residue [68]. Type 1 enzyme is composed of N-terminal, core, linker and the C-terminal domains [69-71]. Different natural and synthetic molecules are known to target DNA topoisomerases represents the important class of antitumor drugs. The transference reaction involved in cleavage and religation of DNA backbone is exploited by cytoxic agents. Table 5 shows example of some enzyme modulators used as anticancer agents.

**Gene Regulation**

Epigenetic alterations in DNA are potentially reversible and hence are involved in the earliest steps of malignant transformation. Interventions using epigenetically active compounds are considered as promising targets for anti-cancer therapy [82,83]. Beside these a number of challenges remain prior to any epigenetic intervention against cancer. Massive deregulation of the epigenetic machinery including DNA methylation, histone modifications and non-coding RNAs contributes to all major cancer hallmarks [84]. In eukaryotic cells acetylation and deacetylation of histones is an important event for transcriptional regulation for which histone acetyltransferase (HATs) and histone deacetylases (HDACs) are responsible, respectively [85]. Acetylation to lysine group of chromatin produces relaxation which intern allows increased transcription of the gene. On the other hand deacetylation of histones is an important event for transcriptional regulation as anticancer drug development. In eukaryotes gene regulation may be a potential target for chemotherapy [91]. P-glycoprotein (P-gp) is a transmembrane permeability glycoprotein and member of ABC super family (ATP binding cassette). It functions as a carrier mediated primary active efflux transporter and widely distributed throughout the body. P-gp is encoded by MDR1/ABCBI gene and was firstly identified in human cancer cells. It was found to be present in pancreas, elementary canal, kidney, capillary endothelial cells of blood brain barrier and in various other tissues like lungs, heart, adrenals, spleen and skeletal muscle [92]. The optimal P-gp expression is always required for its protective function as its over expression leads to multi drug resistance while toxic reactions occurs because of its low expression level [93]. In various cancers a correlation was found between increased P-gp expression and MDR1 gene mRNA transcription which shows its connection to MDR in cancer. A few novel antitumor drugs which are able to suppress P-gp expression are under development. Lanthanum, a new anticancer compound have been reported to block P-gp expression especially in MDR cancerous cells [94]. Gefitinib another compound is a selective tyrosine kinase inhibitor has capability to inhibited P-gp function and has been used in the treatment of lung cancer [95]. Some of the gene regulator and their targets are shown in Table 6.

**Microtubule Inhibitors**

Microtubules a component of cytoskeleton is composed of α and β tubulin. This heterodimer is involved in many biological process viz., cell signaling, cytokinesis, intracellular transport, maintenance of cell shape, and polarity [104]. Due to their role in mitosis they become an important target for anticancer drug development. In eukaryotes during cell division mitotic spindle is responsible for the movement of chromosomes to the opposite sides of the cell. These mitotic spindles are nothing but are composed of microtubules having tubulin as its monomer [105-107]. Molecules that interfere with microtubule assembly are known as microtubule inhibiting agents. Currently these agents are used in clinical therapy as they are able to suppress...
microtubule dynamics in fast dividing tumor cells by misdirecting the formation of a functional mitotic spindle. Due to this G2/M phase cell arrest occurs which leads to apoptosis of the tumor cells. According to their mode of action, microtubule inhibitors (MI) may be classified as stabilizing and destabilizing agents. Microtubule stabilizing agent (MSA) acts by promoting polymerization and microtubule polymer mass in cells. Taxanes (paclitaxel and docetaxel) and epothilones (e.g., Dolastatin 15) are known for their potent anticancer activity and are the most commonly prescribed antitumor agents [109]. There are limitations for the clinical use of other microtubule targeting drugs as they are known to involve in drug resistance emergence and intolerable toxicity. The researches are going on to discover and develop novel chemotherapeutic microtubule inhibitor agents having lower drug resistance and tolerable toxicity [108,110,111]. Colchicine is a branded drug for the treatment of several diseases such as Gout and Familial Mediterranean fever. Although there is a need to further characterize its effectiveness and side effects in the case of cancer treatment. 2-Amino-4-phenyl-4H-chromene-3-carboxylate a new class of novel microtubule-targeting agent with promising antitumor activity was recently developed. This molecule targets the potential colchicines binding site and induces apoptotic pathway [112-119]. Table 7 shows examples of microtubule inhibitors used as anticancer agent.

Conclusion

The modulation of angiogenesis, DNA (synthesis, transcription and translation), enzyme activity and microtubule inhibition remains an important therapeutic strategy against numerous diseases, including cancer. The current arsenal of anticancer agents targeting DNA or RNA activity is generally based upon their inhibitory activity against synthesis, transcription factors and enzymes. Besides effectiveness, vast majority of such anticancer agents exhibit lack of selectivity and are involved in drug resistance. This will limit the effectiveness of anticancer drugs. However, new therapeutic approaches that are currently being developed to circumvent these complications may definitely lead to discover novel anticancer drugs having low toxicity and resistance.

Acknowledgement

SK acknowledges Indian Council of Medical Research for providing financial support in the form of Research Associate fellowship.

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doi:10.4172/2161-0444.1000252
