ABSTRACT

Globally, tremendous resources are being devoted in prevention, diagnosis, and treatment of cancer. Discovery and development of anticancer agents are the key focus of several pharmaceutical companies as well as nonprofit government and non-government organizations. This review will highlight the unique anticancer drug targets for future application of structure based drug discovery and development strategies.

KEYWORDS: Cancer, Anticancer drug targets. Structure based drug discovery

INTRODUCTION

Cancer is one of the major causes of death all over the world. Current estimates from the American Cancer Society and from the International Union Against Cancer indicate that 12 million cases of cancer were diagnosed last year, with 7 million deaths worldwide; these numbers are expected to double by 2030 (27 million cases with 17 million deaths). Interest in anticancer drug discovery and development has grown during the past century as infectious diseases have increasingly been controlled as the result of improved chemotherapeutic interventions. The aim of this article is to review some selected potential anticancer protein drug targets involved in carcinogenesis, gaining novel initiative to design new drug candidate to generate anticancer drugs against different types of cancers.
Literature Review

Epidermal Growth Factor (EGF)

Epidermal growth factor (EGF) was first described by Cohen as a peptide which stimulated eyelid opening and tooth eruption in newborn mice. Its ability to stimulate or inhibit the proliferation and/or differentiation of a wide variety of cells was recognized later. Recently, the autocrine mechanism of EGF/epidermal growth factor receptor (EGFR) has been found to be correlated with tumor invasion and prognosis in colon, gastric, and lung carcinomas. Hematogenous metastasis is one of the main characteristics of choriocarcinoma, suggesting that a growth mechanism such as EGF/EGFR autocrine function may play an important role in proliferation and invasion of choriocarcinoma.\(^1\) The X-ray crystal structure was obtained from the Brookhoven Protein Data Bank (http://www.rcsb.org/pdb) (PDB.ID: 1M17).

Vascular Endothelial Growth Factor (VEGF)

Angiogenesis, the formation of new blood vessels sprouting from the pre-existing vasculature, is critical for the development and subsequent growth of human tumors and is a prerequisite for the formation of metastases. Various proangiogenic factors secreted by tumor cells and/or host factors stimulate endothelial cells to proliferate and to form new, qualitatively poor and often leaky new blood vessels. As few as 60–200 tumor cells can initiate the process of angiogenesis. Although various proangiogenic factors such as basic fibroblast growth factor (bFGF) and platelet derived growth factor (PDGF) are involved, the VEGF family, and especially isoform VEGF-165, is the predominant proangiogenic factor. VEGF exerts its activity through binding to several high-affinity transmembrane endothelial cell receptors, most notably VEGF receptors (VEGFR) types 1 and 2 (VEGFR-1 or Flt-1 and VEGFR-2 or KDR/Flk-1). Binding of VEGF to these receptors leads to intracellular receptor phosphorylation which initiates various intracellular downstream receptor pathways leading to endothelial cell proliferation and blood vessel formation. VEGF binding to VEGFR-3 expressed on the lymphatic endothelium initiates lymphangiogenesis. Angiogenesis is a pivotal target for the development of a totally new class of inhibitory agents. With regard to mechanisms to inhibit VEGF induced angiogenesis, both VEGF and VEGFR neutralizing antibodies and small molecule VEGFR tyrosine kinase inhibitors have been developed.\(^2\)–\(^5\) The X-ray crystal structure was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (PDB.ID: 3C7Q).
Cyclin-Dependent Kinase-2 (CDK-2)
Cyclin-dependent kinases are the key regulators of cell-cycle transitions. In mammalian cells, Cdk2, Cdk4, Cdk6 and associated cyclins control the G1 to S phase transition. Because proper regulation of this transition is critical for an organism’s survival, these protein kinases are exquisitely regulated at different mechanistic levels and in response to a large variety of intrinsic and extrinsic signals. Cyclin-dependent kinase 2 (CDK2) in complex with cyclins E and/or A is a key cell cycle regulator and continues to be an attractive target for the discovery of new anti-tumor agents. In particular, inhibitors of CDK-2/cyclin A/E have already progressed into clinical trials with encouraging early results.\cite{6,7} The X-ray crystal structure was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (PDB.ID: 3PY1).

Casein Kinase II (CK2)
CK2 is a highly conserved protein serine/threonine kinase that is ubiquitously distributed in eukaryotes, constitutively active and has been implicated in multiple cellular functions, as well as in tumorigenesis and transformation. CK2, formerly called casein kinase II, is a ubiquitously expressed and highly conserved cellular serine/threonine kinase that targets an S/T-x-x-D/E motif. With over 300 known cellular substrates, CK2 is implicated as functioning in a wide variety of cellular processes, including apoptosis, proliferation, and transcription, where its activity can be broadly described as pro-cell survival and pro-cell growth. While there are many cellular factors implicated in the regulation of its activity, CK2 is typically described as constitutively expressed and active. However, this regulation is important as CK2 is frequently found to be overactive in a number of pathogenic conditions, including tumorigenesis. Consequently, CK2 has emerged as a relevant therapeutic target, and diverse strategies to inhibit CK2 functions have been going in preclinical trials.\cite{8} The X-ray crystal structure was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (PDB.ID: 1RQF).

Aurora Kinase-A (AUR-A)
The Aurora kinase family consists of highly related serine/threonine kinases that are involved in the regulation of mitosis. Aurora kinases have been conserved throughout eukaryotic evolution and have evolved into three related kinases known as Aur-A, Aur-B, and Aur-C in mammalian cells. All Aurora kinases contain a variable N-terminal domain followed by a conserved catalytic domain, and a short C-terminal extension. Despite significant sequence
homology, the localization and functions of these kinases are largely distinct from one another. Aur-A localizes to centrosomes during early S phase and is involved in centrosome maturation and separation, bi-polar spindle assembly, mitotic entry, and mitotic exit. Aur-B kinase belongs to the chromosome passenger protein family. Less is known about Aur-C kinases, which are specifically expressed at high levels in the testis and show centrosomal localization from anaphase to telophase. Over expression of Aur-A has been shown to lead to centrosome amplification and aneuploidy, which usually results from incomplete cytokinesis and can be a driving force in genomic instability and tumorigenesis. Aur-A also has been implicated in the regulation of cell cycle checkpoints. Checkpoint defects may ultimately contribute to genomic instability and carcinogenesis. Collectively, this evidence indicates that Aur-A acts as an oncogene and plays an important role in cell cycle progression and carcinogenesis. Hence, the development of small molecule inhibitors of Aur-A that target the ATP binding site is emerging as a new anticancer target-based therapeutic approach.

VRAF Murine Sarcoma Viral Oncogene Homologue B1 (BRAF)

VRAF murine sarcoma viral oncogene homologue B1 (BRAF) is a serine-threonine kinase and a component of the Ras/Raf/MEK/ERK signaling pathway. This pathway normally regulates cell growth, division and differentiation, and has long been associated with human cancers due to the frequent oncogenic mutations identified in rapidly growing fibrosarcoma (RAF) family members. An activating mutation in the gene encoding BRAF is known to be responsible for 40–60% of melanomas. Most of the reported BRAF mutants involve the substitution of glutamate for valine at amino acid residue number 600. This V600E mutation constitutively activates BRAF and downstream signal transduction in the MAPK (mitogen-activated protein kinase) signaling pathway. This mutation-induced over activation of BRAF has also been identified in a variety of other human cancers such as ovarian, colorectal, and thyroid carcinomas. Therefore, the inhibition of BRAF kinase activity has been considered to be a promising therapeutic strategy for the treatment of cancer. The X-ray crystal structure was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (PDB.ID: 3PRI).

Histone Deacetylase (HDAC)

The genetic code for proteins resides in the base sequences of DNA, while the expression of genes is largely regulated by the structure of chromatin (epigenetic gene regulation), which is
a complex of DNA, histones and non-histone proteins. Nucleosomes, the basic repeating units of chromatin, have a core histone octamer composed of two of each of four histones: H2A, -2B, -3 and -4 with the DNA wrapped around the core. Chromatin remodeling involves reversible post-translational modification of amino acids in the histone tails by acetylation of lysines, methylation of lysines and arginines, phosphorylation of histidines, serines and threonines, ubiquination and sumoylation of lysines and ADP-ribosylation of glutamic acid. Two groups of enzymes, histone deacetylases and histone acetyl transferases (HATs), primarily determine the pattern of histone acetylation. Acetylation and deacetylation of chromatin histone protein by histone deacetylase (HDAC) alters chromatin structure and dynamically affects transcriptional regulation. Histone acetylation induces repression of tumour suppressor gene expression. Small molecule inhibitors of HDAC (HDACI) are highly effective in up-regulating tumour suppressor gene expression, reducing tumour growth and inducing programmed cell death \textit{in vitro} and in cancer patients in phase I and II clinical trials \cite{28}. The X-ray crystal structure was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (PDB.ID: 3MAX).

**B-Cell Lymphoma-2 (Bcl-2)**

B-Cell lymphoma-2 (Bcl-2) protein is a new promising target for anticancer drugs. The proteins of the Bcl-2 family are important regulators of programmed cell death and apoptotic process. Structural studies of Bcl-2 family members have provided many important insights into their molecular mechanism of action and how members of this family interact with one another. To date, structural studies have been performed on six Bcl-2 family members encompassing both anti- (Bcl-xL, Bcl-2, KSHV-Bcl-2, Bcl-w) and pro-apoptotic (Bax, Bid) members. They all show a remarkably similar fold despite an overall divergence in amino acid sequence and function (pro-apoptotic versus anti-apoptotic). High levels of Bcl-2 are associated with most types of human cancer.\cite{29-34} The X-ray crystal structure was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (PDB.ID: 2W3L).

**Farnesyl Transferase (FT)**

Protein farnesylation catalyzed by the enzyme farnesyl protein transferase involves the addition of a 15-carbon farnesyl group to conserved amino acid residues at the carboxyl terminus of certain proteins. Protein substrates of farnesyl transferase include several G-proteins, which are critical intermediates of cell signalling and cytoskeletal organisation such as Ras, Rho, PxF and lamins A and B. Activated Ras proteins trigger a cascade of
phosphorylation events through sequential activation of the PI3 kinase/AKT pathway, which is critical for cell survival, and the Raf/Mek/Erk kinase pathway that has been implicated in cell proliferation. Ras mutations which encode for constitutively activated proteins are found in 30% of human cancers. Because farnesylation of Ras is required for its transforming and proliferative activity, the farnesyl protein transferase inhibitors were designed as anticancer agents to abrogate Ras function.\[35\] The X-ray crystal structure was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (PDB.ID: 1SA4).

Cathepsin B (CAT B)

Cathepsin B (EC 3.4.22.1) is a lysosomal cysteine protease of the papain family. It functions in intracellular protein catabolism and in certain situations may also be involved in other physiological processes, such as processing of antigens in the immune response, hormone activation and turnover. There is also evidence that cathepsin B is implicated in the pathology of chronic inflammatory diseases of airways and joints, and in cancer and pancreatitis. The presence of cathepsin B has been linked to neoplasia by two distinct lines of enquiry. Certain cancer cells secrete procathepsin B, similarly to the secretion of procathepsin B by bronchial epithelial cells (above), though in the case of cancer cells there is no evidence for extracellular activation. The presence of extracellular procathepsin B shows promise as a diagnostic marker of the development of certain neoplasms.\[36\] The X-ray crystal structure was obtained from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb) (PDB.ID: 1GMY).

Acknowledgments

One of the authors Dr. Ch. Prasad is grateful to the Principal, Vignan Institute of Pharmaceutical Technology for providing support during literature collection.

REFERENCES

    2010; 363: 809.
24. Singer, G.; Oldt, R., III; Cohen, Y.; Wang, B. G.; Sidransky, D.; Kurman, R. J.; Shih,
    Swift, K.; Matayoshi, E. D.; Zhang, H.; Thompson, C. B.; Fesik, S. W. Protein Sci.,
    83.
34. Sattler, M.; Liang, H.; Nettesheim, D.; Meadows, R. P.; Harlan, J. E.; Eberstadt, M.;
    Yoon, H. S.; Shuker, S. B.; Chang, B. S.; Minn, A. J.; Thompson, C. B.; Fesik, S. W.
    Science., 1997; 275: 983.