REVIEW

Endpoint of Cancer Treatment: Targeted Therapies

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Abstract

　　Nowadays there are several limitations in cancer treatment. One of these is the use of conventional medicines which not only target cancer cells and thus also cause high toxicity precluding effective treatment. Recent elucidation of mechanisms that cause cancer has led to discovery of novel key molecules and pathways which have have become successful targets for the treatments that eliminate only cancer cells. These so-called targeted therapies offer new hope for millions of cancer patients, as briefly reveiwed here focusing on different types of agents, like PARP, CDK, tyrosine kinase, farnysyl transferase and proteasome inhibitors, monoclonal antibodies and antiangiogenic agents.

Keywords: Cancer - cytotoxic chemotherapy - targeted therapy

Introduction

　　Cancer can be defined as a disease in which a group of abnormal cells grow uncontrollably by disregarding the normal rules of cell division. Normal cells are constantly subject to signals that dictate whether the cell should divide, differentiate into another cell or die. Cancer cells develop a degree of autonomy from these signals, resulting in uncontrolled growth and proliferation. If this proliferation is allowed to continue and spread, it can be fatal. In fact, almost 90% of cancer-related deaths are due to tumor spreading process metastasis (Hejmadi, 2005).

　　Cancer chemotherapy specifically is the treatment of cancer by chemicals that maximize the killing of neoplastic cells while minimizing the killing of most or all other cells of the host (Pitot and Loeb, 2002). Chemotherapeutic agents do not specifically target tumor cells, but rather interfere with cell division or inhibit enzymes involved DNA replication or metabolism. These drugs therefore also damage the normal dividing cells of rapidly regenerating tissues, such as those of the bone marrow, gut mucosa and hair follicles (Wu et al., 2008).

　　Chemotherapy possess many difficult problems. In order to cancer cells and rapidly dividing normal cells resemble each other selection is difficult. Cells can develop resistance to drugs, so a carefully tested small set of drugs is applied to the tumor, chosen from the several dozen currently available. These drugs must be applied with proper dosage and schedule and be supervised carefully. Illnesses can develop from treatment. Also, not all drugs can help all patients, since each person and each tumor is genetically different. Some cells treated by chemotherapy can survive and grow into drug- resistant cancers.

Treatment-related diseases can develop later. Many of the drugs that kill tumors can cause mutations that transform normal cells to cancer (Aqeilan et al., 2009). Also cancer stem cells with a small population in tumor tissue which is non-homogeneous are not effected by traditional chemotherapeutic drugs. Thus, cancer stem cells cannot be eliminated even if cancer cells die and these cancer stem cells cause cancer again (Cetin and Topcul, 2012).

　　As our understanding of the processes involved in the transformation of healthy to malignant cells grows, so too will the potential sites for new targeted agents. It remains an exciting time for the development of anticancer drugs (Wright, 2007). Targeted therapy refers to a new generation of anticancer drugs that are designed to interfere with a specific molecular target, usually a protein with a critical role in tumor growth or progression. Targeted therapy has been a very promising strategy of drug development research. Many molecular mechanims of diseases have been known to be regulated by abundance of proteins, such as receptors and hormones (Meiyanto et al., 2012).

　　This approach differs from the more empirical approach used in conventional cytotoxic chemotherapy, which has remained the mainstay of anticancer drug use over the past several decades (Sawyers, 2004).

　　In this review we discussed about novel therapeutic approaches that target critical molecules and pathways involved in cancer formation and progression.

PARP Inhibitors

　　Genetic aberrations of DNA repair enzymes are known to be common events associated with different cancer types (Alanazi et al., 2013). Environmental exposures and

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cell replication result in DNA damage that is repaired by a variety of mechanisms, including base excision repair (BER), mismatch repair (MMR), nucleotide excision repair (NER), single strand annealing (SSA), homologous recombination (HR), and nonhomologous end joining (NHEJ) (Sharova, 2005). PARP enzymes are activated in response to DNA damage induced by ionizing radiation, oxidative stress, and DNAbinding antitumor drugs (Lindahl et al., 1995; D'Amours et al., 1999).

Inhibitors of the poly (ADP-ribose) polymerases (PARPs) family of proteins are currently being evaluated as potential anticancer medicines at both preclinical and clinical levels. They have the peculiarity to increase the efficacy of DNA-damaging agents and to selectively target tumor cells with specific DNA repair defects (Papeo et al., 2013). In tumor cells sensitive or moderately sensitive to chemotherapy, a low or moderate dose of drug in combination with PARP inhibition may result in efficient block of DNA repair and subsequent apoptotic cell death (Nguewa et al., 2003).

Targeting Hormones and Hormone Receptors

Increased hormones or prolonged hormone exposure can be associated with increased risk of some cancers. Among the hormone dependent cancers breast cancer is the most widely studied cancer. Long-term exposure of breast tissue to estrogen plays a major role in breast tumor formation. Consequently, reproductive factors such as total numbers of pregnancies, age at first pregnancy, breastfeeding, age at first menstruation, age at menopause and hormone replacement therapy, which affect a woman's lifetime exposure to estrogen, have been shown to be strongly associated with breast cancer risk (Hebert, 2009).

Tamoxifen was the first targeted therapy for breast cancer (Jensen and Jordan, 2003). Tamoxifen was initially classified as antagonists and were developed as agents that could competitively displace estradiol from ER and inhibit its mitogenic actions in breast cancer cells (Clemens et al., 1983; Jordan et al., 1977).

Another class of targeted drugs that interfere with estrogen's ability to promote the growth of ER-positive breast cancers is called aromatase inhibitors (AIs). Aromatase inhibitors (AIs) represent a very successful targeted therapy for breast cancer in postmenopausal women (Suter and Marcum, 2007). It is involved in the conversion of androgens to estrogens. In postmenopausal women the main sites of aromatization are skin, adipose tissue and breast. Aromatase localized in breast tumor produces sufficient estrogen for its proliferation (Narashimamurthy et al., 2004).

Aromatase inhibitors block the enzyme aromatase, which is responsible for conversion of the adrenal derived precursor, androstenedione, to estrogen in tissues such as fat, muscle, and in the breast in postmenopausal women. Estrogens drive the proliferation and metastasis of estrogen receptor positive breast cancer (about 70% of the total) (Howell, 2012).

Currently, AIs that are now in clinical used and are approved by the US Food and Drug Administration (FDA) include anastrozole, letrozole, and exemestane. They are approved for postmenopausal women with hormone receptor-positive breast cancer in both the adjuvant and metastatic setting (Chumsri et al., 2011).

Monoclonal Antibodies

Monoclonal antibody (mAb) therapy is the use of monoclonal antibodies to bind specifically to target cells or antigens. This may then stimulate immune system of the patient to attack those cells or inhibit tumor growth (Guo et al., 2011)

The use of monoclonal antibodies for the therapy of cancer is one of the major contributions of tumor immunology to cancer patients. This success is built on decades on of scientific research aimed at serological characterization of cancer cells, techniques for generation optimized antibodies to tumor targets, detailed investigation of signaling pathways relevant to cancer cells, and an understanding of the complex interplay between cancer cells and the immune system (Scott et al., 2012). There are many ideal targets for monoclonal antibodies. EGFR and CD44 are novel targets among them (Duan et al., 2012).

Antibodies can destroy cancer cells by at least three mechanisms. In the first method, the antibody binds or blocks a ligand-receptor signaling pathway critical to tumor cell survival. The antibody may bind to ligand itself (i.e. vascular endothelial growth factor for bevacizumab) or the (i.e. epidermal growth factor family members for cetuximab and trastuzumab and calcium channel for rituximab). In the second method, the antibody binds to tumor cell surface and recruits host effector mechanisms including complement and antibody-dependent, cell-mediated cytotoxicity (ADCC; e.g. rituximab and alemtuzumab) or binds effector cells and overcomes tumor-mediated tolerance (e.g. CP-870, 893, ticilimumab and ipilimumab). Finally, the antibody can be chemically conjugated to a radioisotope (90Y-ibritumomab tiuxetan and 131I-totuzumab), cytotoxic compound (gemtuzumab ogozamicin), or toxin (BL22) (Posada and Frankel, 2008).

Tyrosine Kinase Inhibitors

Tyrosine kinases are important mediators of signal transduction process, leading to cell proliferation, differentiation, migration, metabolism and programmed cell death (Paul and Mukhopadhyay, 2004). Tyrosine kinases are enzymes that catalyze the transfer of the phosphate group from adenosine triphosphate to target proteins Arora and Scholar, 2005). Aberrant activation of tyrosine kinases, owing to mutation or overexpression, is sufficient for them to become transforming in cellular and animal models. Mutations affecting the epidermal growth factor receptor (EGFR) are good predictors of clinical efficacy of EGFR tyrosine kinase inhibitors (TKI) in patients with some cancers (Pan et al., 2013). The majority of targets are receptor protein tyrosine kinases (RPTKs), as deregulating mutations of over half of the known RPTKs have been associated with different human malignancies. Finally, and equally as important as the epidemiological and biochemical data, the prevalence of PTKs as targets is because of the fact that they are considered druggable

(Pearson et al., 2006). Although several strategies exist for targeting protein kinases, the most successful approach to date has been the use of a class of drugs termed "small molecule tyrosine kinase inhibitors" (TKIs). These typically work by blocking the ATP-binding site of kinases, essentially acting as competitive inhibitors that may be reversible or irreversible (London, 2009).

Proteasome Inhibitors

Proteasome is a multicatalytic enzyme complex (2.5MDa) containing a 20S catalytic core and two 19S regulatory complexes. Given that many proteins targeted by proteasome are involved in the regulation of important processes of carcinogenesis and cancer cell survival, such as cell cycle progression, cell proliferation, differentiation and apoptosis, inhibition of pro- teasome would lead to cell death or apoptosis (Tu et al., 2012).

Protein degradation mediates both normal cellular functioning and cellular response to chemotherapy. Multiple studies have shown that protein ubiquitination and degradation via ubiquitin-proteasome pathways regulates cell cycle progression, tumor suppression, transcription, DNA replication, inflammation, and apoptosis (Chauhan et al., 2005). Several important proteins that are regulated by the proteasome include the inhibitor of nuclear factor κB (NFκB; IκB), the tumor suppressor p53, the cyclin-dependent kinase inhibitors p21 and p27, and the proapoptotic protein Bax (Voorhees et al., 2003). The proteasome, which is an enzyme common to the entire pathway, has emerged as a promising target for cancer therapy. The proteasome inhibitor bortezomib is used to treat multiple myeloma and mantle lypmhoma. In cancer, proteasome inhibitors may inhibit the activation of the pro-apototic NF-κB or the degradation of several cell cycle regulators (Genin et al., 2010).

Several regulatory proteins, tumor suppressors, transcription factors, and oncogenes are degraded by the proteasome pathway. Proteasome inhibition can cause cellular apoptosis by affecting the levels of various short-lived proteins, resulting in inhibition of NF-κB activity, increased activity of p53 and Bax proteins, and accumulation of cyclin- dependent kinase inhibitors p27 and p21. Preclinical studies show that malignant, transformed, and proliferating cells are more susceptible to proteasome inhibition than normal cells (Rajkumar et al., 2005).

The proteasome, a multicatalytic proteinase complex, is responsible for the majority of intracellular protein degradation. Pharmacologic inhibitors of the proteasome possess *in vitro* and *in vivo* antitumor activity, and bortezomib, the first such agent to undergo clinical testing, has significant efficacy against multiple myeloma and non-Hodgkin lymphoma (NHL). Preclinical studies demonstrate that proteasome inhibition potentiates the activity of other cancer therapeutics, in part by downregulating chemoresistance pathways. Early clinical studies of bortezomib-based combinations, showing encouraging activity, support this observation. Molecular characterization of resistance to proteasome inhibitors has revealed novel therapeutic targets for sensitizing malignancies to these agents, such as the heat shock pathway (Voorhees and Orlowski, 2006).

CDK Inhibitors

Cyclin-dependent kinases (CDKs) are core components of the cell cycle machinery that govern the transition between phases during cell cycle progression (Diaz-Padilla et al., 2009). In contrast to healthy cells, tumor cells are unable to stop at predetermined points of the cell cycle because of loss of checkpoint integrity. This can be due to inactivation of critical CDKIs, or to overexpression of cyclins (Schwartz and Shah, 2005) and deregulated CDK activity represents a hallmark of malignancy (Diaz-Padilla et al., 2009). Therefore CDKs represent an interesting therapeutic target, and their pharmacological inhibitors have been proposed for cancer treatment (Canavese et al., 2012). Thus, targeting CDKs would recapitulate cell cycle checkpoints that would necessarily limit ability of a tumor cell to cycle, and this may then facilitate the induction of apoptosis (Schwartz and Shah, 2005). Within the CDK group of kinases, CDK inhibitors fall into three classes: those that are not selective for any specific CDK [e.g. deschloroflavopiridol, flavopiridol, oxindole 16 (compound 3) and oxindole 91], those that inhibit CDK1, 2, 5 (and possibly CDK9) [e.g. olomoucine, (R)-roscovitine, purvalanol B, aminopurvalanol (NG97), hymenialdisine, indirubin-3'-monoxime, indirubin-5-sulfonate, SU9516 and alsterpaullone], and those that are selective for CDK4,6 (e.g. fascaplysin, Compound 66, PD0183812, Compound 26a, Compound 15b and CINK4). No inhibitor that is selective for a single CDK has been discovered. This is probably due to the conservation of the amino acids lining the CDK ATP-binding pocket (Knockaert et al., 2002).

Raf Kinase Inhibitors

Raf is a pivotal downstream mediator of growth factor signaling, and exerts its effects either by MEK/ERK activation or independently of MEK/ERK. The binding of growth factors, such as TGF-α, EGF, VEGF and PDGF-β, to their cognate receptors (EGFR, VEGFR-2/-3 and PDGFR-β) on the cell surface activates ubiquitous RAF/MEK/ERK signaling pathway to regulate proliferation, differentiation, survival, angiogenesis, adhesion and mobility (Gollob et al., 2006).

Several strategies have been developed that specifically target Raf kinase. These include inhibitors of Raf kinase activity such as BAY 43-9006, antisense oligonucleotides such as ISIS 5132 and LeRafAON, Raf destabilizers such as geldanamycin, and Ras-Raf interaction inhibitors such as MCP-1. Of these, only BAY 43-9006 has shown activity against B-Raf (Sridhar et al., 2005).

Antiangiogenic Agents

Increasing tumor cell numbers can also be achieved by facilitating the supply of nutrients, an indispensable process for tumor growth: angiogenesis. This is a physiological process involving the growth of new blood

vessels from preexisting vessels. Tumors beginning to develop cannot grow greater than 2 mm in diameter unless they are ensured access to the circularity system (Aqeilan et al., 2009). In order to block tumor growth and metastasis formation, a number of inhibitors targeting the tumor vasculature have been identified in in vitro and in vivo anti-angiogenesis studies. Anti-angiogenic therapeutic drugs may act by inhibiting synthesis of angiogenic proteins by cancer cells, neutralizing the angiogenic proteins, inhibiting the receptors of endothelia for angiogenic proteins, or directly inducing endothelial cell apoptosis (Wu et al., 2008). Vascular endothelial growth factor (VEGF) is a proangiogenic factor known to play a central role in tumor angiogenesis and has, therefore, emerged as a promising target for therapeutic intervention (Hurwitz, 2004). Growth factors of the VEGF family exert their biological effect via interaction with receptors located on endothelial cell membranes. Three receptors have been identified that bind different VEGF growth factors: VEGFR1 (FLT1), VEGFR2 (Flk1/KDR), and VEGFR3 (FLT4) (Karamysheva, 2008). Several potential anti-VEGF strategies are currently under investigation. The best studied of these approaches include inhibition of VEGF and VEGF receptor activity with monoclonal antibodies and inhibition of receptor signaling with tyrosine kinase inhibitors (Hurwitz, 2004).

Matrix Metalloproteinase Inhibitors

Metastasis, which causes 90% of cancer deaths, makes surgery and radiation far less effective, because these treatments are local (Pardee, 2009).

The MMPs are a family of zinc-dependent neutral endopeptidases that are collectively capable of degrading essentially all of the components of the extracellular matrix. The human MMP gene family consists of at least 18 structurally related members that fall into five classes according to their primary structure and substrate specificity: collagenases (MMP-1, MMP-8, and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-7, MMP-10, MMP-11, and MMP-12), membrane type (MT)-MMPs (MT1-MMP, MT2- MMP, MT3-MMP, and MT4-MMP), and nonclassified MMPs (Hidalgo and Gail Eckhardt, 2001). MMPs degrade components of extracellular matrix (ECM), facilitating angiogenesis, tumor cell invasion, and metastasis. MMPs modulate the interactions between tumor cells by cleaving E-cadherin, and between tumor cells and ECM by processing integrins, which also enhances the invasiveness of tumor cells. MMPs also process and activate signaling molecules, including growth factors and cytokines, making these factors more accessible to target cells by either liberating them from the ECM [eg, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF)] and inhibitory complexes (eg, transforming growth factor-β), or by shedding them from cell surface (eg, heparin-binding epidermal growth factor) (Roy et al., 2009).The catalytic activity of the MMPs is regulated at multiple levels including transcription, secretion, activation, and

inhibition. Matrix metalloproteinase (MMP)-9 is an endopeptidase that digests basement membrane type IV collagen, therefore being possibly related to tumor progression (Gao et al., 2013). The last is accomplished by members of the TIMP family, which presently includes four proteins: TIMP-1, TIMP-2, TIMP-3, and TIMP-4 (Massova et al., 1998).

Farnesyl Transferase Inhibitors

Ras is synthesized as a biologically inactive cytosolic propeptide (Pro-Ras) and is localized to the inner surface of the plasma membranes only after it has undergone a series of closely linked posttranslational modifications at the C-terminus, thereby increasing its hydrophobicity and facilitating its association with the plasma membrane. The first and most critical step, farnesylation, adds a 15-carbon farnesyl isoprenoid group to H-, K-, and N-Ras and is catalyzed by protein farnesyltransferase (FTase) (Rowinsky et al., 1999).

The Ras family of proto-oncogenes are upstream mediators of several essential cellular signal transduction pathways and, as such, provide a rational target for the treatment of malignancies (O'Regan and Khuri, 2004). Inhibitors of the enzyme farnesyl protein transferase prevent a key step in the post-translational processing of the RAS protein, and were developed initially as a therapeutic strategy to inhibit cell signalling in RAS-transformed cells (Johnston, 2001). FTI comprise a novel class of antineoplastic agents recently developed to inhibit FTase with the downstream effect of preventing the proper functioning of the Ras protein, which is commonly abnormally active in cancer (Agrawal and Somani, 2011).

Protein Kinase Inhibitors

Kinases transfer phosphoryl groups onto target proteins, altering their activity as a result. This process is called phosphorylation and is reversed by the action of phosphatases, which remove phosphoryl moieties from target proteins (Blagden and Bono, 2005) Phosphorylation of the target proteins leads to the activation of signal-transduction pathways, which play an important role in a great number of biological processes (Cheetham, 2004; Kondapalli et al., 2005).

It is an essential mechanism by which intracellular and extracellular signals are transmitted throughout the cell and to the nucleus. Thus, PKs play a crucial role in intracellular signalling pathways that regulate cell growth, differentiation, development, functions, and death (Shchemelinin and Šefc, 2006).

Analysis of human genome identifies 518 protein kinases (Manning et al., 2002). The first anticancer agent specifically targeted to a protein kinase was Imanitib, which acts as an inhibitor of the oncogenic kinase BCR-Abl and is active in the chronic myelogenous leukemia (Ren, 2005). Inhibition of CML cell adhesion and invasion in patients after Imatinib treatment may achieved through suppression of tumor-associated carbohydrate antigens

(TCTA) related Glycosyltransferases (Sun et al., 2013).

Glutathione-S-transferase Inhibition

Glutathione S-transferases (GST) represent a large family of Phase II detoxification enzymes widely expressed in animals and plants. These enzymes catalyse the conjugation of glutathione with some endogenous molecules and a broad range of exogenous substrates including various anticancer drugs. Due to high expression of GSTs in tumors when compared to normal tissues and their high level in plasma from cancer patients, these enzymes are considered to be cancer markers (Tew and Gaté, 2001). Elevated levels of GST in many tumor cell types have been demonstrated to limit the effectiveness of chemotherapy. Moreover, GSTs have been associated with multidrug resistance of tumor cells, and over expression of GSTs can increase susceptibility to carcinogenesis and inflammatory disease (Abdalla, 2011). GSTs have the capacity to bind and detoxify many drugs and, therefore, are possible targets for chemomodulation of drug resistance (Mukanganyama et al., 2002). They are involved in the detoxification of cells from many endogenous and xenobiotic compounds by catalysing their conjugation to the tripeptide glutathione (GSH). Many anticancer drugs are substrates for the GST and thus they can be conjugated with the GSH and efficiently extruded from the cell by specific export pumps. Selected 7-nitro-2,1,3-benzoxadiazole derivatives have been recently found very efficient inhibitors of glutathione S- transferase (GST) P1-1,5 an enzyme which displays antiapoptotic activity and is also involved in the cellular resistance to anticancer drugs (Turella et al., 2005).

Epigenetic Targets

Remodelling of chromatin between relatively 'open' and 'closed' forms has a key role in epigenetic regulation of gene expression. Such remodelling results from modifying the structure of nucleosomes - the fundamental units of chromatin - which comprise approximately two turns of DNA wound around a histone octamer (Bolden et al., 2006). Epigenetics refers to heritable modifications of DNA and associated chromatin components that influence gene expression without altering DNA coding sequence. Two key levels of aberrant epigenetic control are DNA methylation and histone acetylation. Primary regulators of these epigenetic changes include DNA methyltransferases (DNMTs) and histone deacetylases (HDACs) (Vendetti and Rudin, 2013). Consequently, epigenetic therapies aim to restore normal chromatin modification patterns through the inhibition of various components of the epigenetic machinery. Histone deacetylase and DNA methyltransferase inhibitors represent the first putative epigenetic therapies; however, these agents have pleiotropic effects and it remains unclear how they lead to therapeutic responses (Popovic and Licht, 2012).

Histone deacetylase inhibitors (HDACis) have now emerged as a powerful new class of small-molecule therapeutics acting through the regulation of the acetylation states of histone proteins (a form of epigenetic modulation) and other non-histone protein targets (Gryder et al., 2012).

mTOR Inhibitors

Mammalian target of rapamycin (mTOR) is master regulator of the PI3K/Akt/mTOR pathway (Liu et al., 2012) and a crucial regulator of cell growth and proliferation and research into this area has revealed that mTOR dysregulation has a key role to play in various cancers. mTOR appears to play a central role in signaling caused by nutrients and mitogens such as growth factors to regulate translation (Advani, 2010). In a number of *in vitro* cell lines and in vivo murine xenograft models, aberrant mTOR pathway activation through oncogene stimulation or loss of tumor suppressors contributes to tumor growth, angiogenesis and metastasis. Mutations in mTOR gene that confer constitutive activation of mTOR signalling, even under nutrient starvation conditions, have been identified in a few human cancers, although not clearly linked to tumor development (Pópulo et al., 2012).The use of mTOR inhibitors, either alone or in combination with other anticancer agents, has the potential to provide anticancer activity in numerous tumor types. Cancer types in which these agents are under evaluation include neuroendocrine tumors, breast cancer, leukemia, lymphoma, hepatocellular carcinoma, gastric cancer, pancreatic cancer, sarcoma, endometrial cancer, and non-small-cell lung cancer. The results of ongoing clinical trials with mTOR inhibitors, as single agents and in combination regimens, will better define their activity in cancer (Yuan et al., 2009).

Antisense Technology

Many genes involved in cancer exert their effect by overexpression, or temporally inappropriate expression, while their gene products are structurally normal. These could all be considered as potential targets. Examples include c-fos, c-myc, N-myc, c-erbB-2 and the nucleolar antigen p120 (Carter and Lemoine, 1993). Antisense technology is a tool that is used for the inhibition of gene expression (Gupta et al., 2011). The antisense concept is to selectively bind short, modified DNA or RNA molecules to messenger RNA in cells and prevent the synthesis of the encoded protein. As anticancer agents, these molecules can be targeted against a myriad of genes involved in cell transformation, cell survival, metastasis, and angiogenesis (Kushner and Silverman, 2000). Many oligonucleotides were designed to decrease the expression of oncoproteins such as Bcl-2, c-Raf, H-Ras, c-Myc, c-Myb and XIAP. Others have focused on cell signaling molecules implicated in cancer initiation or progression, including the tumor suppressor p53 (mutant), VEGF, IGF-1R, TGF-BII, PKA, and PKCα. Still, other cancer-related molecules have been targeted including survivin, clusterin, ribonucleotide reductase, and DNA methyltransferase

(Rayburn and Zhang, 2008).

Targeting Glucose Metabolism

An outstanding biochemical characteristic of neoplastic tissues is that despite ample oxygen supply, glycolysis is the dominant pathway for adenosine 5'-triphosphate (ATP) production, a phenomenon termed "the Warburg effect" (Zheng et al., 2012). The Warburg effect, also known as aerobic glycolysis, is defined as the propensity of cancer cells to take up high levels of glucose and to secrete lactate in the presence of oxygen. Warburg's original work indicated that while glucose uptake and lactate production are greatly elevated, a cancer cell's rate of mitochondrial respiration is similar to that of normal cells (Sotgia et al., 2011). In cancer cells, the activities or expression levels of many enzymes participating in glucose metabolism are altered, those involved in glycolysis in particular. The glycolysis commonly refers to the reactions that covert glucose into pyruvate or lactate (Zheng et al., 2012). It has been hypothesized that targeting glucose metabolism may provide a selective mechanism by which to kill cancer cells. It is anticipated that understanding which metabolic enzymes are particularly critical for tumor cell proliferation and survival will identify novel therapeutic targets (Hamanaka and Chandel, 2012). Many proteins within the glycolytic pathway have been implicated in cancer based on overexpression, knockdown or inhibition studies. Glycolytic targets associated with cancer include the glucose transporter proteins, hexokinase-2, PFK2 isoforms and the pyruvate kinase isoform PKM2 (Jones and Schulze, 2012).

Targeting Cancer Stem Cells

Tumors are masses containing heterogeneous populations of cells with different biological characteristics (Reya et al., 2001). Research indicates that a small population of cancer cells is highly tumorigenic, endowed with the capacity for self-renewal, and has the ability to differentiate into cells that constitute the bulk of tumors. These cells are considered the "drivers" of the tumorigenic process in some tumor types, and have been named cancer stem cells (CSC) (Satpute et al., 2013).

Cancer stem cells (CSCs) possess several characteristics including self-renewal, pluripotency and tumorigenicity and constitute a rare population in a tumor mass. Because conventional cancer therapies cannot kill CSCs, these cells are responsible for tumor relapse and metastasis. Currently, with advances in the identification of CSCs, the importance of these cells is increasing in the field of cancer diagnosis and prognosis. In addition, clarifying the mechanisms responsible for the maintenance of CSCs properties led to the development of CSC-targeted therapies (Cetin and Topcul, 2012).

Extracellular signals delivered through the Hedgehog (Hh), Notch, Wnt pathways or through TGF-β and the related BMPs, or from ECM proteins and from growth factors such as hepatocyte growth factor (Met ligand) may all participate in regulating the maintenance, self-renewal, and differentiation of CSCs. Slow replication, ability to generate partially differentiated progenies (pluripotency) highly effective DNA repair, ability to eliminate xenobiotics through ABC family transporters (ABC), and expression of primitive membrane markers (CD133, Met) have been documented in many putative CSC populations isolated from tumors or cell lines. Transcription factors such as Bmi-1, Musashi, Sox2, Oct4, and others have been shown to be commonly expressed in putative CSCs and participate in controlling their phenotype (Foreman et al., 2009). Therapy that targets CSC aims to deplete the CSC pool. CSC targeting therapy could be achieved through CSC surface molecule binding, oncoprotein inhibition, CSC regulation pathway disruption, and frustration of CSC therapy resistance machinery. There is no shortage of targets for CSC-directed treatments. However, an ideal CSC targeting agent must discriminate CSC from normal stem cells. Also functional assay techniques for CSC to monitor the effectiveness of targeted treatment are critical for the CSC targeting therapy (Cheng et al., 2009).

The CSC model has opened new opportunities for cancer therapy. Traditional cancer therapies are effective against differentiated, self-limiting transit-amplifying cancer cells. The transit-amplifying cells targeted by conventional therapy are the asymmetrically descended progeny of CSC without self-renewal capacity, yet forming a much larger branch with elevated but confined proliferation capacity. These differentiated cells typically have less active DNA repair systems and greater sensitivity to chemotherapy and radiotherapy than CSC (Cheng et al., 2009).

Chemotherapy, radiotherapy, and antiangiogenic therapy are directed to the actively proliferating transit-amplifying cells of a cancer. When these therapies are discontinued, the cancer regrows from the therapy-resistant cancer stem cells. Differentiation therapy blocks the activation signals, causing maturation arrest. However, when differentiation therapy is discontinued, the cancer will reform from the cancer progenitor cells. Stem cell inhibition is directed against the signals that keep a cancer stem cell a stem cell. By blocking or reversing the stemness signals, it may be possible to force the cancer stem cell to differentiate (Sell, 2006).

The ability of retinoids to induce differentiation of teratocarcinoma cells, mentioned earlier, proves the principle that differentiation of cancer stem cells is inducible. The basic concept of differentiation therapy is that specific identifiable cell signaling pathways maintain "stemness" in cancer stem cells. If the stemness signaling pathways that regulate cancer stem cells can be modified, then the cancer stem cells should progress, becoming cancer transit-amplifying cells. As cancer transit-amplifying cells, they would be susceptible to other forms of therapy (Sell, 2009).

Conclusions

Although cytotoxic chemotherapy has an important place in cancer treatment, it poses various limitations. Targeting not only cancer cells but also healthy cells creates various adverse effects that reduce the quality of patients' life. Besides all these, most of the conventional

chemotherapy administered to patients remain palliative rather than therapeutic. To increase the quality of patients' life and of course effectiveness of the treatment, research of new generation drug continues to increase. In this context, developed targeted drugs are great hope for patients.

Elucidation of the molecular mechanisms involved in the formation and progression of cancer is the source of targeted therapies. Different types of molecules, signaling pathways, metabolic pathways and cell populations within heterogeneous tumor mass have become successful targets. Targeted therapy has brought a new perspective to the field of oncology. Medications used in this type of therapy have made great advances. Development of new drug derivatives that have reduced or completely abolished adverse effects on patients is also important.

Acknowledgements

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