ROLE OF BIM-TARGETED THERAPY IN CANCER TREATMENT

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ABSTRACT

In the past few years, the pro-apoptotic molecule Bim has attracted increasing attention as a plausible target for tumor therapy. A variety of normal and pathological systems regulated by Bim, dependent on cell type, apoptotic stimulation, and chemotherapeutic agents, have been documented. Bim promotes anoikis of many tumor cells, such as lung cancer, breast cancer, osteosarcoma, and melanoma. Various chemotherapeutic agents use Bim as a mediating executioner of cell death. Hence, Bim suppression supports metastasis and chemoresistance. Imatinib, gefitinib, bortezomib, and Bim protein itself are spotlighted as current and future Bim-targeting therapeutic agents. The potential benefits of Bim-targeted therapies are selectivity of treatment for tumor cells and reduction in tumor-associated phenomena such as chemoresistance and metastasis. Thus, Bim-targeting therapies may provide more effective and unique tumor management modalities in future. This review article discusses all these issues.

Key words: Apoptosis, BIM, Cancer

INTRODUCTION

Apoptosis, or programmed cell death, is a major control mechanism by which cells die if DNA damage is not repaired[1].Apoptosis is also important in controlling cell number and proliferation as part of normal development. It is also essential for the elimination of self-reactive lymphocytes and for removing lymphocytes with nonproductive rearrangements of the antigen receptor gene. Some types of cancers, such as B-cell chronic lymphocytic leukemia (CLL), follicular lymphoma[2]and tumors infected by human T-cell leukemia/lymphoma virus-1,[3]are characterized by defects in apoptosis leading to immortal clones of cells. Other malignancies have defects in the apoptotic regulatory pathways such as p53, the nuclear factor kappa B (NFκB), or phosphatidylinositol 3-Kinase (PI3K)/Akt leading to defects in apoptosis[4].

Genes, the functional unit of heredity, are specific sequences bases that encode instructions to make proteins. Although genes get a lot of attentions, it is the proteins that perform most life functions. When genes are altered, encoded proteins are unable to carry out their normal functions, resulting in genetic disorders. Gene therapy (use of genes as medicines) is basically to correct defective genes responsible for genetic disorder by one of the following approaches[5,6]

❖ A normal gene could be inserted into a nonspecific locationwithin the genome to replace the Nonfunctional gene (mostcommon)

❖ An abnormal gene could be swapped for a normal genehomologous recombination

❖ An abnormal gene could be repaired through selectivereverse mutation

❖ Regulation (degree to which a gene is turned on or off) ofa particular gene could be alteredand many researches remain to be performed before thetreatment will realize its potential. Majority of the gene therapytrials are being conducted in United States and Europe, withonly a modest number in other countries including Australia.Scope of this approach is broad with potential in treatment ofdiseases caused by single gene recessive disorders (like cysticfibrosis, hemophilia, muscular dystrophy, sickle cell anemiaetc), acquired genetic diseases such as cancer and certain viralinfections like AIDS[7,8].

Other gene therapy projects are targeted at conditions suchas heart disease, diabetes mellitus, arthritis andAlzheimer'sdisease, all of which involve genetic susceptibility to illness[9].

Historical Perspectives:

Since the earliest days of plant and animal domestication, about 10,000 years ago, humans have understood that characteristics traits of parents could be transmitted to theiroffspring. The first to speculate about how this process workedwere ancient Greek scholars, and some of their theories remainedin favor for several centuries. The scientific study of geneticsbegan in 1850s, when Austrian monk Gregor Mendel, in aseries of experiments with green peas, described the patternof inheritance, observing that traits were inherited as separateunits we know as genes. Mendel's work formed the foundationfor later scientific achievements that heralded the era of modern genetics. But little was known about the physical nature of genesuntil 1950s, when American biochemist James Watson and Britishbiophysicist Francis Crick developed their revolutionary modelof double stranded DNA helix. Another key breakthroughcame in the early 1970s,

when researchers discovered aseries of enzymes that made it possible to snip apart genes atpredetermined site along a molecule of DNA and glue them backtogether in a reproducible manner. Those genetic advances setthe stage for the emergence of genetic engineering, which hasproduced new drugs and antibodies and enabled scientists tocontemplate gene therapy. A few years after the isolation of genesfrom DNA, gene therapy was discovered in 1980s[10].

Process of Gene Therapy:

Approach:

The process of gene therapy remains complex and manytechniques need further developments. Thechallenge ofdeveloping successful gene therapy for any specific condition isconsiderable. The condition in question must be well understood,the undying faulty gene must be identified and a working copyof the gene involved must be available. Specific cells in thebody requiring treatment must be identified and are accessible.A means of efficiently delivering working copies of the gene tothe cells must be available. Moreover, diseases and their strictgenetic link need to be understood thoroughly.

Types of gene therapy:

There are 2 types of gene therapies.

1. **Germ line gene therapy:** where germ cells (sperm or egg) aremodified by the introduction of functional genes, whichare integrated into their genome[11]. Therefore, changes due totherapy would be heritable and would be passed on to latergeneration. Theoretically, this approach should be highlyeffective in counteracting genetic disease and hereditarydisorders. But at present many jurisdictions, a variety oftechnical difficulties and ethical reasons make it unlikelythat germ line therapy would be tried in human beings innear future.

2. **Somatic gene therapy:** where therapeutic genes are transferredinto the somatic cells of a patient. Any modifications andeffects will be restricted to the individual patient only andwill not be inherited by the patients offspring or any latergeneration[12].

The important role of "apoptosis" in tumorigenesis and tumor treatment has been the subject of various reviews[13].The completion of tumorigenesis requires a multistep process whereby disturbing physiologic and genetically mediated programs mandates unceasing proliferation and growth[14].Thus, it is essential that the acquisition of these characters protects cells from being channeled into the normal genetic and programmed cell death system-apoptosis. In addition, metastatic ability and chemotherapy resistance require the abrogation of apoptosis [13,15,16].Experimental evidence shows that a single oncogene can be critical to tumorigenesis or that tumors become addicted to the oncogene for their tumorigenesis[14].The latter is an "oncogene addiction" hypothesis. Naturally, these addicted genes pose as suitable targets for tumor control. Bim is one such possible candidate of an addicted oncogene.

This review focuses on the normal Bim regulatory system at first. Subsequently, it discusses the role of Bim suppression in tumorigenesis, focusing on the areas of tumor metastasis and chemotherapy. Some

current chemotherapeutic agents, especially molecular-targeting agents, use Bim as an executioner. These agents could be classified as "primitive" Bim-targeting agents. The diversity of Bim-regulatory systems depends on cell type, stimulus, and pathological events, such as metastasis and chemoresistance.

Thus, proper control of Bim expression provides tumor-selective therapeutic effects. This is a major possible advantage for Bim-targeted therapies, which use not only Bim induced by agents but also the Bim protein itself. Modified Bim protein is also a possible candidate for targeted oncological therapy in the future. Although one of the disadvantages of Bim-targeted therapy is broad and strong apoptosis-inducing ability, this in turn can be exploited for therapeutical outcomes. Finally, the review concludes with a discussion on the advantages and disadvantages of Bim-targeted therapies.

The Role of Bim as a Guardian of Tissue Homeostasis:

In response to apoptotic signals, various enzymes are activated in a pathway-specific manner and the classical caspase activation chain reaction is set in motion[17].Mammals have mainly two distinct apoptosis signaling pathways, the death receptor pathway and the mitochondrial pathway. In the mitochondrial pathway, the B-cell lymphoma-2 (Bcl-2)-family of proteins have a crucial role. The Bcl-2 family comprises three subfamilies, namely, an anti-apoptotic family, pro-apoptotic multi-domain family, and pro-apoptotic BH3-only protein family. The Bcl-2-homology domain 3 only (BH3-only) proteins share only the short BH3 domain with members of the BCL-2 family. BH3-only proteins are strictly regulated through both transcription and post-transcription mechanisms[18].They are essential for initiation of various physiological apoptotic situations, including developmentally programmed cell death and stress-induced apoptosis[19]. So far, at least eight BH3-only proteins have been discovered in mice and humans. Bim is one of these BH3-only proteins. Bim upregulation triggers cytochrome c release from mitochondria, which consequentially induces a chain reaction that entails the formation of the apoptosome and the activation of its effector, caspase-9. Thus, Bim upregulation induces apoptosis.

A mechanistic scheme of how Bim activation can act to promote apoptosis. Cytokine withdrawal, chemotherapeutic agents, and cell detachment trigger apoptosis by upregulation of Bim. Bim upregulation leads to disruption of the outer mitochondrial membrane, resulting in release of cytochrome c. Released cytochrome c promotes apoptosome-mediated caspase-9 activation. The apoptosome is composed of Apaf1 (apoptotic-protease-activating factor 1) and released cytochrome c. Once activated, caspase-9 cleaves executioner caspase-3,-6, and -7 and leads to apoptosis in the stimulated cell.

Bim was independently identified by two groups as a Bcl-2- or Mcl1-binding protein, by screening of a λ-phage expression library (from a T-cell lymphoma) and by screening of a yeast two-hybrid library (from ovarian tissue), respectively. Alternative splicing generates at least three Bim isoforms, including Bim_S, Bim_L, and Bim_{EL}, which differ in their proapoptotic activity. Bim_{EL} is composed of 196 amino acids, and contains not only the BH3 domain but also two ubiquitination sites, three ERK phosphorylation sites, one JNK phosphorylation site, and one transmembrane domain. In vitro, Bim is essential for apoptosis of various cell types, including lymphocytes, osteoclasts, osteoblasts, mast cells, epithelial cells, endothelial cells, and

neurons[18-20]. Bim is a critical regulator for the development and normal responses of various immune systems. Bim deficiency causes abnormal accumulation of lymphoid and myeloid cells. The phenotype of Bim$^{-/-}$ mice is fatal systemic lupus erythematosus-like autoimmune disease. In the area of tissue development and homeostasis, Bim plays a key role in skeletal homeostasis, spermatogenesis, and mammary gland formation[21-23].

The Bim Regulatory System:

The pro-apoptotic activity of Bim is controlled by transcriptional and post-transcriptional systems. The forkhead-like transcription factor FOXO3a (forkhead box O3a) and tumor suppressor gene Runx3 (runt-related protein 3) are key transcriptional regulators of Bim. Cytokine withdrawal or other apoptotic stimuli cause the upregulation of Bim mRNA level through activation of FOXO3a in primary osteoblast, hepatocyte, neurons, and paclitaxel-treated cancer cells[19,20,24,25].

Paclitaxel is used for node-positive early-stage breast cancer, metastatic breast cancer, and advanced ovarian cancer, interacting with cellular microtubules[25,26]. Paclitaxel up-regulates the Bim EL expression level via increasing FOXO3a expression. MCF-7, a paclitaxel-sensitive breast cancer cell line, expresses high basal levels of FOXO3a. Paclitaxel treatment increases Bim protein dramatically without affecting the levels of other Bcl-2 family members. Thus, only the Bim expression level correlated with apoptosis induction. However this was not observed in MDA-MB231 cells, which expressed low levels of FOXO3a and Bim. Therefore, paclitaxel sensitivity is controlled by FOXO3a and Bim expression levels[25].

Paclitaxel sensitivity correlates with non-small cell lung cancer (NSCLC) cell lines as well[27]. Brain-derived neurotrophic factor (BDNF) is a biomarker of poor prognosis of neuroblastoma patients. BDNF provides chemoresistance to neuroblastoma via activation of MAPK and PI3K pathways. Interestingly, Bim was involved in paclitaxel but not etoposide- or cisplatin-induced neuroblastoma apoptosis. Paclitaxel-induced cell death via Bim expression is abrogated by BDNF. BDNF activates the PI3K/AKT pathway and suppresses FOXO3a activity[28]. Thus, paclitaxel is a Bim-targeting agent. Other drugs are discussed below.

FOXO3a activity is suppressed through AKT-mediated phosphorylation. The deficiency of the tumor suppressor gene RUNX3 evokes gastric mucosa hyperplasia, and around one half of human gastric cancer cells do not significantly express RUNX3[29]. RUNX3 is responsible for transforming growth factor-β (TGF-β)-induced gastric epithelial cells apoptosis. RUNX3 upregulates Bim transcription with FOXO3a association and cells undergo TGF-β–induced apoptosis[30]. TGF-β induces apoptosis signals not only through upregulation of Bim mRNA but also through the post-transcriptional pathway. It upregulates Bim through its inhibition of phosphorylation by extracellular signal-regulated kinase (ERK). ERK-mediated phosphorylation targets Bim for ubiquitylation and induces proteasomaldegradation[19, 31]. TGF-β transcriptionally induces the mitogen-activated protein kinase (MAPK) phosphatase (MKP)2 through SMAD3. MKP2 abrogates ERK activity and ERK-induced Bim ubiquitination. Thus, MKP2 induced by TGF-β upregulates Bim protein levels[32].

Growth factor-mediated stimulation can also activate ERK and downregulate Bim in a post-translational manner. On the contrary, JUN amino-terminal kinase (JNK)-mediated phosphorylation causes

decreased binding of Bim to the anti-apoptotic protein Bcl2 and activates Bim as a result[31]. In the T-cell acute lymphoblastic leukemia (T-ALL) cell line Sup-T, JNK-mediated phosphorylation of Bim promotes proteasomalBimdegradation[33]. However, the role of JNK for Bim is still unclear.

Bim and Tumorigenesis:

The breakdown of tissue homeostasis leads to various pathological situations including tumor formation. Thus, Bim plays important roles for tumorigenesis and tumor treatment. Baby mouse kidney epithelial (BMK) cells transformed by E1A and dominant negative p53 (p53DD) form tumors in nude mice. The formation and growth of the tumors in nude mice are supported by Bimdeficiency[34]. These data suggest that Bim is possibly a key regulator of epithelial tumors as well. However, the absence of Bim plays a more important role for the formation of tumor metastasis and the acquisition of resistance to chemotherapy. of Bim regulation in tumorigenesis and cancer treatment. Bim expression level controls the fate of the tumor cell. There are several transcriptional and post-transcriptional regulation points for Bim. The two main direct regulation systems are the ERK and AKT pathways. ERK phosphorylates Bim and promotes Bim degradation post-transcriptionally. AKT suppresses Bim transcription factor FOXO3a. Tumor cells mainly use ERK-proteasome axis in order to escape from apoptosis induced by Bim.

The Role of Bim in Metastasis:

The acquisition of "anchorage-independence" is an indispensable step for tumor metastasis. Cells are normally dependent on anchorage and undergo apoptosis after their loss of attachment with their neighboring cells or their extracellular matrix. Therefore, apoptosis induced by cell detachment or "anoikis" is a crucial barrier against metastases. Anoikis stimuli can activate both the death receptor and mitochondrial pathways. Anoikis stimuli-activating mitochondrial pathways recruit or suppress several molecules, such as Bim, Bmf, Omi/HtrA2, Bcl-XL, and Mcl-1. Bim plays a key role in the anoikis of a variety of tumor cells, such as breast cancer, lung cancer, osteosarcoma, fibrosarcoma, and melanoma[35-37]). These tumor cells have to bypass or abrogate Bim-mediated cell death one way or another in order to metastasize.

The cell detachment signal arises from the cell surface. Thus, cell surface molecules play important roles in anoikis. The cell surface glycoprotein mesothelin can activate a Bim suppression system via ERK activation in the human breast cancer cell line MDA-MB231 as well[36]. A particularly interesting new cysteine-histidine-rich protein, (PINCH)-1, a cytoplasmic component of cell-extracellular matrix adhesions, can activate not only ERK-mediated Bim suppression but also the suppression of Bimtranscriptionally[38].

In fact, several reports propose the relationship between various cell surface molecules and Bim. However, epidermal growth factor receptor (EGFR) is one of the most important cell surface molecules. In the human breast cancer cell line MCF-10A, Bim functions as a key regulator of anoikis downstream of the EGFR-ERK pathway. Cell detachment attenuates the integrin signal. The integrin signal is indispensible for the maintenance of EGFR expression. Loss of integrin suppresses EGFR expression and inhibits ERK signaling. As a result, EGFR suppression by cell detachment upregulates Bim expression. Downregulation of Bim with RNA interference (RNAi) inhibits MCF-10A anoikis. Overexpressed EGFR maintains ERK activation after cell

detachment and blocks Bim expression and anoikis[39].

Cell detachment-induced reactive oxygen species (ROS) are candidates upstream of the EGFR-ERK-Bim suppression system. Extracellular matrix-cell contact increases intracellular ROS through integrin. ROS oxidizes and activates tyrosine kinase Src. The active form of Src stimulates signaling downstream of EGFR including both AKT and ERK in a ligand-independent manner, culminating in the down-regulation of Bim[40]. Acting downstream to cell surface signals, both ERK and AKT play pivotal roles. The breast cancer cell line MDA-MB231 and HBC4 cells evade anoikis by the suppression of Bim with constitutive activation of the MEK-ERK pathway. MEK inhibitors sensitize these cell lines to anoikis by blocking proteasomal degradation of Bim_{EL}[41]. In mutant MCF-10A cells expressing ΔRaf-ER, Raf-ERK signaling is activated by 4-OHT stimulation. Mutant MCF-10A cells show Bim downregulation and anoikis resistance with 4-OHT stimulation[42].

The 14-3-3 family is a critical regulator of various cellular responses such as proliferation, differentiation, cycling, apoptosis, and tumorigenesis. 14-3-3ζ is a member of 14-3-3 family and forms a positive feedback loop with AKT. 14-3-3ζ is highly expressed in NSCLC tissue and involved in anoikis resistance of lung cancer cells. 14-3-3ζ knockdown causes the restitution of anchorage-dependence by Bim and Bad upregulation and Mcl-1 downregulation. As a result, lung cancer cells lose resistance to anoikis. Bim, but not Bad, can induce Bax activation and anoikis. Bim down-regulation with RNAi abrogates restitution of anchorage-dependence by 14-3-3ζ knockdown[43]. B-Raf and N-Ras, commonly activated in human cutaneous melanoma, provide melanoma cells with resistance to anoikis. The constitutively active mutants of N-Ras and B-Raf downregulate Bim expression levels in melanocytes or melanoma cell lines via ERK activation. Bim suppression by the constitutively active mutants of N-Ras or by RNAi inhibits melanocyte anoikis[44,45]. Tumor metastasis is one of the critical determinants of prognosis. These data suggest that the Bim expression level is inversely related to prognosis. Indeed, reduced Bim expression is significantly correlated with poor prognosis of human cutaneous malignant melanoma[46]. Currently, an increasing number of in vitro studies suggest that the ERK-Bim axis plays a pivotal role in tumor metastasis. Further analyses will reveal the role played by Bim in metastasis in patients.

The Role of Bim in Chemotherapy:

1.   Bim plays important roles not only in tumor metastasis but also in chemotherapy .Bcr/Abl is a fusion oncogene formed by the fragment of Bcr and c-Abl, the product of the reciprocal t (q34; q22; refs)[21, 34],chromosomal translocation, the so-called Philadelphia (Ph1) chromosome. It is essential for leukemogenesis in chronic myelogenous leukemia (CML) and Ph1-positive acute lymphoblastic leukemia (ALL). Thus, Bcr/Abl was a good target candidate for novel CML or Ph1-positive ALL. Imatinib has dramatically improved clinical outcome for treatment of Bcr/Abl-positive leukemia, especially for early chronic phase CML patients. Bcr/Abl is an uncontrolled chimeric tyrosine kinase and can promote multimodal oncogenic function, including anti-apoptotic effects[47]. Bim plays a pivotal role in the ability of imatinib to induce apoptosis. Imatinib induces the mitochondrial pathway apoptosis in Bcr/Abl-expressing cells. Imatinib increases Bim and Bmf transcription and activates Bim and Bad post-transcriptionally. Bim

knockdown by RNAi abrogates imatinib-induced apoptosis in Bcr/Abl positive human leukemia cell lines[47]. These Bim accumulations are attributed to inhibition of transcriptionally FoxO3a and post-transcriptional proteasomaldegradation[48, 49]. In K562 leukemia cells, Bim-$_{EL}$ phosphorylation and proteasome degradation is promoted by the ERK signal[48] or by beta-1 integrin attachment signal without ERK activation[50]. In BaF3 Bcr/Abl-expressing cell lines, dephosphorylation of FoxO3a by imatinib promotes Bimtranscription[49].

Current Bim-targeted drugs:

Dasatinib and nilotinib have been developed for imatinib-resistant leukemia, both of which can upregulate Bim[51, 52]. The ERK pathway is important for Bim regulation by dasatinib as well. The MEK inhibitor PD184352 sensitizes the leukemia cell line K562 to dasatinibactivity[53]. Data derived from the use of the mouse DA1-3b BCR-ABL+ leukemic cell line suggest that Bim downregulation contributes to imatinib and dasatinib resistance as well. The imatinib- and dasatinib-resistant cell line secretes interleukin 3 (IL3) and is able to confer chemoresistance to nonmutated cells via a paracrine manner. IL3 activates both the MEK-ERK1/2 and the JAK2-STAT5 pathways. Both pathways can downregulate Bim, and JAK2 inhibition exhibits stronger Bim suppression. Thus, Bim, at least partially, controls the antitumor effects of imatinib and other related agents.

EGFR plays a critical role not only in anchorage-independence but also in tumorigenic features, such as uncontrollable cellular proliferation. Abnormal EGFR signaling is found in carcinomas of the lung, breast, and colon, making it a good target for chemotherapeutic agents. An EGFR-targeting agent, gefitinib, has been successful in the treatment of certain cancers, especially NSCLCs[54]. Gefitinib evaluation for breast cancer is now at the phase II trial stage. Gefitinib causes rapid Bim accumulation in NSCLC cell lines through the blockade of the MEK/ERK pathway downstream of EGFR [54].

Erlotinib is another EGFR-targeting drug to treat lung cancer and shows survival improvement of NSCLC patients. Erlotinib upregulates Bim expression in lung cancer cell lines in vitro and in mouse xenograft models[55]. Bim siRNA protects cells against apoptosis induced by both gefitinib and erlotinib. EGFR mutation is one of the adverse prognostic factors in gefitinib-treated patients with advanced lung adenocarcinoma. T790M mutation of EGFR abrogates Biminduction and subsequent apoptosis induced by gefitinib[56]. Thus, these reports suggest that Bim is a terminal target of gefitinib and erlotinib.

Bortezomib (Velcade, PS341) is a 20S proteasome inhibitor used for treatment of multiple myeloma, and is currently in clinical trials enrolling patients suffering from various types of solid tumors[57]. As mentioned above, Bim phosphorylation by ERK promotes Bim degradation through the proteasome-ubiquitination system. Therefore, bortezomib can be classified as a Bim-targeted tumor therapy agent. Although bortezomib activates Bim in order to terminate multiple myeloma, the activation system is not a direct inhibition of proteasome degradation of Bim as expected. Bortezomib upregulates the BH3 domain-only protein Noxa, not Bim. Accumulated Noxa dissociates Bim from Mcl-1, a member of the anti-apoptotic Bcl2 family. Dissociated Bim can activate the Bax/Bak complex and channel cells into apoptosis[58]. The

function of bortezomib as a Bim-targeting agent is basically as a sensitizer of other agents rather than as a direct tumor killer.

Bortezomib sensitizes prostate cancer cells to apoptosis through the extrinsic pathway by the death receptor ligand TRAIL. Downstream of TRAIL, Bim and Bik activation by bortezomib is important for apoptosis. Downregulation of Bim and Bik by RNAi impairs TRAIL-induced apoptosis. Interestingly, the TRAIL-induced apoptosis of breast cancer cell lines is not amplified by bortezomib[59]. In the human prostate cancer cell lines PC3 and LNCaP-Pro5, apoptosis induced by bortezomib is not affected by Bimdeficiency[60]. However, bortezomib can abrogate paclitaxel-resistance induced by H-Ras. H-Ras promotes Bim phosphorylation and proteasome degradation of Bim upregulated by paclitaxel. Transformed BMK cells are sensitive to paclitaxel in vitro and in vivo, although Bim deficiency cancels this sensitivity. Bortezomib can abrogate H-Ras-promoted paclitaxel resistance[34]. Thus, bortezomib can select cell types and enhance chemotherapeutic agents through Bim activation.

Future Directions in Developing Bim-Targeted Tumor Treatments:

As a future direction, first we discuss the prospect of Bim protein transduction. Various tumors are immortalized and transformed with overexpression of anti-apoptotic Bcl-2 family proteins. Recently, plenty of BH3 mimetics have been developed and some of them show successful outcomes both in vitro and in clinical trials. One of the most potent BH3 mimetics so far is ABT-737. ABT-737 shows tumor suppression efficacy against small-cell lung carcinoma and various types of lymphoma and leukemia. However, both Mcl-1 and A1 overexpression cause ABT-737 resistance because the affinity of ABT-737 to Mcl-1 is weak. Mcl-1 is expressed in various tumors, so more work has to be done to increase coverage.

Bim can abrogate the functions of all of the anti-apoptotic Bcl-2 family proteins including Mcl-1 and A1[61]. Interestingly, in some tumors, Bim expression is inversely correlated to Mcl-1 expression[37, 43]. Thus, Bim protein injection into cells is a potent candidate of Bim-targeted tumor treatment, especially for tumors resistant to BH3 mimetics. Actually, it is reported that the TAT-Bim fusion protein can induce apoptosis of several cell lines, lymphoma, melanoma, and pancreatic cancer. TAT motif is a peptide transduction domain derived from the HIV-1. Thus, the modified Bim protein is a potent candidate of antitumor agent, although rigorous in vivo testing in clinically relevant models of neoplasia is required and forms the necessary next step in development in this area.

Bim downregulation is crucial not only for chemotherapy resistance but also for metastasis. The effects of some Bim-targeted agents can be restricted to the restitution of anchorage-dependence or chemotherapy sensitivity. This restriction can reduce side effects and provide possible new cocktail regimens of chemotherapeutic agents. On the other hand, the strong pro-apoptotic ability of Bim can abrogate all of the anti-apoptotic Bcl-2 molecules. This ability can make Bim-targeted agents act only as strong cytotoxins without tumor selectivity, and is a significant disadvantage.

Nevertheless, tumor-selective kill is a distinct possibility with Bim-targeted therapies. The diversity of Bim regulatory systems provides researchers tumor-selective Bim-targeted agents. Accordingly, correction

of abnormal Bim-regulation systems abrogates tumorigenesis, metastatic ability, and chemoresistance. As previously explained above, Bim down-regulation is mainly promoted by ERK phosphorylation and subsequent proteasomal degradation, although Bim is rigorously regulated by various transcriptional and post-transcriptional systems in normal mammalian cells. Thus, a restitution of the ubiquitination-proteasome Bim degradation system is the key to furnishing tumor selectivity. Furthermore, proteasome inhibitors such as bortezomib cannot target all Bim-downregulated tumors. This phenomenon suggests that the Bim proteasome degradation system is a cell-specific target. However, the search for proper targets in the ubiquitin-proteasome system is relatively young, and much more work needs to be done to furnish better options for therapy.

The specificity of ubiquitination-proteasome degradation is determined by distinct E3 ligase complexes. E3 ligase recognizes specific proteins and signals .The proteasome complex is a common and ubiquitous system, although E3 ligase-mediated degradation of Bim is unique to Bim. Thus, if a Bim E3 ligase inhibitor would be developed, the drug could yield quite unique and tumor-selective effects. However, the intricate mechanisms of E3 ligase activity on Bim has not been revealed completely, so this notion has to be treated with caution.

Scheme of Bim ubiquitination. The process of ubiquitination and proteasome degradation is an ATP-dependent chain reaction. Ubiquitin is first conjugated to E1 and the ubiquitin moiety is then transferred to E2. Finally, ubiquitin is transferred to Bim via E3. Polyubiquitin-conjugated Bim is degraded by 26S proteasome. Proteasome degradation regenerates free ubiquitin for reuse through disassembling ubiquitin chains from Bim. Ub, ubiquitin; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzymes; E3, ubiquitin ligases.

Previously, involvement of c-Cbl in Bim ubiquitin-proteasome degradation was suggested, although recently adverse data have been reported[21,62]. The most potent candidate is an ElonginB/C-Cullin2-CIS E3 ligase complex. The receptor for activated C-kinase (RACK)-1 promotes the formation and activation of this E3 ligase complex and contributes to paclitaxel resistance of MDA-MB468 and MCF7 breast cancer cell lines in vitro and in vivo[63]. This report suggests that RACK1 contributes to the paclitaxel resistance ability through the promotion of Bim degradation. Further analysis is required to reveal more precisely whether the Bim E3 ligase mechanism, RACK1, and an ElonginB/C-Cullin2-CIS E3 ligase complex are potential targets for the development of tumor-selective Bim-targeted agents.

CONCLUSIONS

Physiologically, the pro-apoptotic BH3-only protein Bim plays a key role in induction of apoptosis of various type cells and development of some organs. Recently, the knowledge pertaining to the importance of Bim in cancer is increasing. Current reports suggest that Bim downregulation is important for tumorigenesis, especially for metastatic ability. The ERK-mediated proteasomal degradation system is frequently used for Bim downregulation in order for cancer cells to obtain metastatic ability. Chemotherapeutic agents, such as

imatinib, gefitinib, and bortezomib activate Bim in order to kill tumor cells. These agents are, as it were, primitive Bim-targeted agents. Bim downregulation contributes to chemoresistance as well. A direct Bim protein transduction system is a potent candidate of tumor treatment because BH3 mimetics show successful tumor treatment data. Furthermore, Bim protein transduction may possibly overcome tumor resistance to BH3 mimetics.

A possible disadvantage of the application of drugs targeting Bim is the potential for a strong and broad range apoptosis induction. However, this point can be easily turned into an advantage, if Bim selectivity could be engineered into the candidate molecule. The potent advantage of Bim-targeting therapy is tumor cell selectivity because of the diversity of Bim controlling systems within cells. Furthermore, targeting of the Bim ubiquitination-proteasome degradation system, which is frequently used by tumor cells as an escape route from chemotherapeutic agents or anoikis, can provide tumor selectivity. Bim suppression is important not only for chemotherapy resistance but also for metastasis. The effects of some Bim-targeted agents are more a restitution of anchorage-dependence or chemotherapy sensitivity than as direct tumor killers. This potent feature may provide a new combination recipe for chemotherapeutic agents clinically. The area of Bim-targeted cancer treatment is really in an early phase of development. Further critical analysis of the Bim regulatory system is required in order to genuinely test the boundaries for such a not-so-novel concept.

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