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Targeting the Regulatory Machinery of BIM for Cancer Therapy

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Abstract

BIM represents a BH3-only proapoptotic member of the BCL-2 family of apoptotic regulatory proteins. Recent evidence suggests that in addition to its involvement in normal homeostasis, BIM plays a critical role in tumor cell biology, including the regulation of tumorigenesis through activities as a tumor suppressor, tumor metastasis, and tumor cell survival. Consequently, BIM has become the focus of intense interest as a potential target for cancer chemotherapy. The control of BIM expression is complex, and involves multiple factors, including epigenetic events (i.e., promoter acetylation or methylation, miRNA), transcription factors, posttranscriptional regulation, and posttranslational modifications, most notably phosphorylation. Significantly, the expression of BIM by tumor cells has been shown to play an important role in determining the response of transformed cells to not only conventional cytotoxic agents, but also to a broad array of targeted agents that interrupt cell signaling and survival pathways. Furthermore, modifications in BIM expression may be exploited to improve the therapeutic activity and potentially the selectivity of such agents. It is likely that evolving insights into the factors that regulate BIM expression will ultimately lead to novel BIM-based therapeutic strategies in the future.

Keywords

BIM; regulation; BCL-2; apoptosis; transcription; translation

I. INTRODUCTION

Programmed cell death (apoptosis) is a well-conserved genetic pathway with basic tenets that appear to be common to all metazoans. Defective control of this pathway is implicated in disorders ranging from cancer to autoimmune diseases to degenerative syndromes. Specific defects in the capacity to undergo apoptosis, or in the upstream signal transduction pathways, not only provide cancer cells with an intrinsic survival advantage, but may also confer inherent resistance to chemotherapeutic drugs. Tumor cell death induced by chemotherapy or lack of appropriate cellular survival signals is mediated by the intrinsic apoptotic pathway. The integrity of the outer mitochondrial membrane is regulated by the balance of pro- and antiapoptotic members of the BCL-2 family proteins.^{1,2} The BCL-2 family is subdivided into three main groups based on regions of BCL-2 homology (BH) and function: multidomain antiapoptotic (e.g., BCL-2, MCL-1, BCL-X_L), multidomain proapoptotic (e.g., BAX, BAK), and BH3-only proapoptotic (e.g., BAD, BID, BIM,

PUMA). It is well appreciated that activation of BH3-only proteins by apoptotic stimuli initiates mitochondria-dependent cell death pathway. BH3-only proteins cause cytochrome c release by activating BAX and/or BAK, and the antiapoptotic BCL-2 family of proteins prevents this process. BIM (BCL-2-interacting mediator of cell death) was identified as a BH3-only protein that induces apoptosis and is antagonized by antiapoptotic BCL-2 family members.³ It is now widely recognized that BIM plays a critical role in cellular responses to conventional and targeted chemotherapies.

II. ROLE OF BIM IN NORMAL TISSUE HOMEOSTASIS

The role of BIM in physiological contexts has largely been established by gene targeting experiments in mice. Studies of *Bim*-knockout mice (*Bim*^{-/-} mice) indicate important roles in hematopoietic cell homeostasis. *Bim*^{-/-} mice show splenomegaly and lymphadenopathy because of accumulation of excess lymphoid and myeloid cells.⁴ BIM is an important effector of cell death in lymphocytes induced by a variety of signals including growth factor withdrawal, deregulated Ca²⁺ flux, or glucocorticoid treatment. BIM is required for the deletion of autoreactive T and B cells,^{5,6} the death of germinal center-derived memory B cells and antibody-forming cells,⁷ and the regulation of naive and memory T cell homeostasis.⁸ Furthermore, apoptosis of activated T and B cells and the proper termination of immune responses are largely dependent on BIM. For example, *Bim*^{-/-} mice are incapable of terminating CD8-driven immune responses after clearing a viral challenge,⁹ and superantigen-induced T cell death is substantially impaired in *Bim*^{-/-} mice,¹⁰ whereas both functions do not require death receptor signaling through CD95.

It has been suggested that a subset of the BH3-only proteins, such as BIM, BID, and PUMA, are capable of directly activating BAX and BAK unless kept in check by antiapoptotic proteins. However, evidence for direct interactions between the BH3-only proteins with BAX or BAK was lacking. Recently, new evidence of the direct physical interaction between BIM and BAX has been proposed.¹¹ Because most BH3 domain polypeptides are unstructured, stabilized alpha-helix of BCL-2 domains (SAHBs) that directly initiate BAX-mediated mitochondrial apoptosis have been developed. BIM SAHB binds BAX at an interaction site that is distinct from the canonical binding groove characteristic of antiapoptotic proteins. This interaction can induce conformational changes and oligomerization of recombinant BAX similar to that observed with endogenous BAX in apoptotic cells and trigger permeabilization of both artificial and mitochondrial membranes *in vitro*. These findings raise the possibility that a BAX activator similar to SAHBs could have therapeutic potential if a sufficiently favorable therapeutic index exists.¹²

III. MECHANISMS OF BIM REGULATION

A. Transcription Factors

Depending on external stimuli and cell types, a variety of transcription factors regulate *Bim* transcription. Transcription of *Bim* gene is normally suppressed by growth factors and cytokines. When growth factors are withdrawn, *Bim* is induced by FOXO3a transcription factor.^{13,14} In the presence of growth factors, the PI3K-AKT pathway is activated and AKT directly phosphorylates FOXO3a at three serine residues, and this allows binding to 14-3-3 proteins, thereby sequestering FOXO3a in the cytosol and preventing it from activating *Bim* transcription. BIM is also induced and contributes to neuron death in response to nerve growth factor (NGF) withdrawal. In this case, several pieces of evidence have shown *Bim* to be a transcriptional target of the JNK/c-Jun pathway in neuronal cells. For instance, dominant-negative c-Jun and a chemical inhibitor of the JNK pathway reduce *Bim* induction evoked by NGF withdrawal in neuronal cells.¹⁵⁻¹⁷

Misfolded proteins or cytotoxic drugs can kill cells through endoplasmic reticulum (ER) stress. BIM plays a critical role in ER stress-induced apoptosis in a variety of cell types both *in vitro* and *in vivo*.¹⁸ Direct transcriptional activation by CHOP-C/EBP contributes to *Bim* induction by ER stress signaling.

Treatment with chemotherapeutic drugs often induces BIM for the induction of apoptosis. Cancer cells with elevated E2F1 activity caused by enforced E2F1 expression or E1A-mediated Rb inactivation are highly susceptible to histone deacetylase (HDAC) inhibitor-induced cell death. This E2F1-mediated apoptosis proceeds through the induction of BIM. HDAC inhibition promotes the recruitment of E2F1 to the *Bim* promoter.¹⁹ In paclitaxel-sensitive breast cancer, upregulation of FOXO3a by paclitaxel results in increased levels of *Bim* mRNA and protein, leading to apoptosis in breast cancer cells and contributing to the tumor response to paclitaxel.²⁰ In Bcr-Abl positive chronic myeloid leukemia (CML), imatinib, a Bcr-Abl inhibitor, induces *Bim* transcription through dephosphorylation of FOXO3a (see below in detail).²¹

Transforming growth factor beta (TGF- β) regulates essential cellular functions such as cellular proliferation, differentiation, and apoptosis. Genes involved in the TGF- β signaling pathway are frequently altered in several types of cancers including gastric cancer, and RUNX3 appears to be an integral component of this pathway. RUNX3 is responsible for transcriptional upregulation of *Bim* in TGF- β -induced apoptosis in gastric cancer cells.²² In hepatocyte cells, TGF- β also stimulates *Bim* transcription by upregulating RUNX1 expression, which binds FOXO3a, and the two factors cooperate in the transcriptional induction of *Bim*.²³

B. Promoter Methylation and Acetylation

Epigenetic mechanisms regulating *Bim* gene expression have been reported in lymphoma and leukemia. In human B cells infected with Epstein-Barr virus (EBV), cell survival is enhanced by the inhibition of *Bim* expression.²⁴ The large CpG island located at the 5' end of *Bim* is significantly methylated in EBV-positive, but not in EBV-negative B cells. Furthermore, hypermethylation of the *Bim* promoter is observed in EBV-positive Burkitt's lymphoma.

Downregulation of BIM expression was found in a subset of patients with CML in chronic phase, and was significantly associated with a lack of optimal response to imatinib. Expression of *Bim* is mediated by promoter hypermethylation, as demonstrated by restoration of *Bim* expression after treatment of CML cells with 5-aza-2'-deoxycytidine.²⁵ Therefore, combining imatinib with a demethylating agent increases apoptosis in CML cells with low expression of BIM.

Glucocorticoids play a critical role in the therapy of lymphoid malignancies, including acute lymphoblastic leukemia (ALL). Glucocorticoid resistance in ALL is consistently associated with failure to upregulate BIM expression after dexamethasone exposure. No consistent changes in *Bim* CpG island methylation is observed; however, glucocorticoid resistance significantly correlates with decreased histone H3 acetylation. Moreover, the HDAC inhibitor vorinostat relieves BIM repression and exerts synergistic antileukemic efficacy with dexamethasone.²⁶ These findings provide a novel therapeutic strategy to reverse glucocorticoid resistance.

C. Posttranscriptional Regulation

Cytokines contribute to blood cell survival by negatively regulating steady state levels of *Bim* mRNA. *Bim* mRNA stability is regulated by heat shock cognate protein 70 (Hsc70), which binds to AU-rich elements in the 3'-untranslated region and enhances its stability on

cytokine deprivation.²⁷ The RNA-binding efficiency of Hsc70 is regulated by cochaperones such as Bag-4 and HIP, which by themselves are regulated by cytokine-activated Ras signaling. Thus, exposure of cells to cytokines ultimately leads to destabilization of *Bim* mRNA and promotion of cell survival.

Micro-RNAs (miRs) of the miR-17-92 cluster have been reported to repress *Bim* expression. Transgenic expression of this cluster in mice led to lymphoproliferative disease with autoimmune pathology and premature death of these animals,²⁸ resembling features observed in *Bim* knockout mice. In contrast, absence of miR-17-92 in mice leads to increased levels of BIM and inhibits B cell development at the pro-B to pre-B transition.²⁹ It has been recently demonstrated that glucocorticoids repress the expression of miR-17-92, which results in elevated BIM protein expression as a mechanism by which glucocorticoids induce BIM.³⁰

D. Posttranslational Modification (Phosphorylation)

Members of the MAP kinase family, in particular JNK and ERK, have been found to exert opposing effects on BIM protein and function on phosphorylation. ERK-mediated serine (Ser59/69/77) phosphorylation in human BIM_{EL} (Ser55/65/73 in mouse BIM_{EL}) on growth factor-mediated activation of the Ras/RAF/MEK/ERK signaling cascade causes destabilization of BIM_{EL} by ubiquitination and subsequent proteasomal degradation, and promotes cell survival.³¹⁻³⁴ Thus, the identification of E3 ubiquitin ligase responsible for polyubiquitination of BIM_{EL} is an important issue to understand the regulation of BIM protein stability. CBL, a RING finger protein, was originally proposed as an E3 ligase in osteoclast.³⁵ However, substrates of CBL are almost invariably phosphotyrosine-containing proteins, and to date the only pathway to play a role in BIM_{EL} degradation results in its serine phosphorylation. Subsequently, studies using fibroblasts and epithelial cells have failed to demonstrate any role for CBL by showing that BIM_{EL} is not phosphorylated on tyrosine, CBL and BIM fail to interact, and ERK1/2-driven BIM_{EL} turnover proceeds normally in cells lacking CBL.^{36,37} These studies suggest CBL may play a role in an indirect manner.

In a separate study, RACK1 forms a complex with dynein light chain 1 (DLC1) and BIM_{EL} on paclitaxel treatment.³⁸ RACK1, DLC1, and CIS mediated the degradation of BIM_{EL} through the ElonginB/C-Cullin2-CIS ubiquitin ligase complex. Downregulation of RACK1 or DLC1 by RNAi resulted in BIM_{EL} accumulation. However, a recent study failed to reproduce the Cullin2-BIM_{EL} interaction, but rather demonstrated coimmunoprecipitation of BIM_{EL} with Cullin1, suggesting that BIM_{EL} degradation occurs via a Skp-Cullin-F-box (SCF) E3 ligase.

A subsequent study demonstrated that ribosomal protein S6 kinase (RSK), activated downstream of ERK1/2, phosphorylates BIM_{EL}, which provides a binding site for the F-box proteins TrCP.³⁹ ERK1/2 can phosphorylate BIM_{EL} at Ser59/69/77, but Ser69 appears primarily to contribute to BIM_{EL} turnover. This study argued that ERK1/2-dependent phosphorylation of BIM_{EL} at Ser69 facilitates optimal phosphorylation by RSK at Ser93/94/98, and that this motif then serves as the binding site for TrCP. This model may explain why mutation of a single ERK1/2 phosphorylation site, Ser69, causes loss of phosphorylation at two additional sites in cells.

In contrast, JNK-mediated phosphorylation of threonine 112 in BIM has been shown to increase its proapoptotic potential, presumably by the release of BIM from the cytoskeleton, since this phosphorylation site resides within the DLC1-binding domain, and its phosphorylation may compromise protein-protein interaction.^{40,41} In addition, p38-MAPK or JNK-mediated phosphorylation at Ser65 of BIM on trophic factor deprivation or sodium

arsenite treatment in neuronal cells has also been reported to enhance its proapoptotic potential.^{42,43}

The physiological role of these phosphorylation sites of BIM has been studied with animal models carrying three serine-to-alanine knockin mutations (BIM^{3SA}) or a threonine-to-alanine mutation (BIM^{T112A}).⁴⁴ Mice carrying these knockin mutations are viable and express protein levels of BIM comparable to those in wild-type mice, suggesting that the steady state levels and turnover of BIM do not depend on any of these phosphorylation sites.

Further analysis reveals that phosphorylation of Ser55/65/73 is mediated by ERK on restimulation of serum-deprived MEF, whereas Thr112 is targeted by JNK on UV radiation. Thr112 can also be targeted by ERK on serum stimulation, but seems dispensable for the destabilization of BIM mediated by ERK under these conditions. Consistently, BIM^{3SA}-expressing MEF are most susceptible to apoptosis triggered by serum deprivation. In contrast, BIM^{T112A} cells are equally susceptible to serum withdrawal as wild-type MEF, but thymocytes expressing BIM^{T112A} are less susceptible to the systemic effects of glucocorticoids *in vivo*. BIM^{T112A} mice also show defects in the negative selection of thymocytes. These observations support an important role for JNK-mediated Thr112 phosphorylation of BIM in developmental and glucocorticoid-triggered lymphocyte apoptosis *in vivo*. Of note, BIM^{T112A} can no longer bind to BCL-2 efficiently, but still binds to BCL-X_L or MCL-1, suggesting that this phosphorylation can facilitate apoptosis by enabling efficient neutralization of BCL-2, but not by triggering the release of BIM from the cytoskeleton, although these effects are unlikely to be mutually exclusive. However, inefficient neutralization of BCL-2 by BIM^{T112A} cannot account for reduced thymocyte-mediated killing, since CD4⁺8⁺ immature thymocytes do not express significant amounts of BCL-2. Alternatively, this phosphorylation site may inhibit the interaction of BIM with BAX at the novel interaction site.¹¹ A summary of the factors regulating BIM expression is illustrated in Fig. 1.

IV. BIM AS A TUMOR SUPPRESSOR

BIM is an important regulator of tumorigenesis functioning as a tumor suppressor. It has been demonstrated that loss of BIM facilitates Myc-induced tumorigenesis in B cells.⁴⁵ In a mouse model, inactivation of even a single allele of *Bim* accelerates Myc-induced development of tumors, particularly acute B cell leukemia.⁴⁶ None of the primary tumors from *Bim*^{+/-} *Eμ-myc* mice displays loss of the second allele of *Bim*. These findings indicate that BIM is a tumor suppressor at least in B lymphocytes and is haploinsufficient.

Bim is frequently silenced in human Burkitt's lymphoma.⁴⁷ Analyses of the methylation status of the *Bim* promoter in a panel of Burkitt's lymphoma revealed that 86% of the cell lines and 50% of patient biopsies presented a hypermethylation of the *Bim* promoter.⁴⁸ Using array-based comparative genomic hybridization, it has been shown that regions of chromosome 2q13 spanning certain exons of *Bim* presented a recurrent biallelic loss in mantle cell lymphoma.⁴⁹ In addition to its prominent role within the lymphoid compartment, BIM makes relevant contributions to the normal homeostasis of a number of tissues such as kidney and skin. In line with these observations, the loss of *Bim* mRNA and protein expression has been observed in renal cell carcinoma.⁵⁰ Thus, blocking BIM expression by gene deletion or epigenetic silencing may play a role in pathogenesis of these tumors.

The miR-17-92 cluster has oncogenic activity.⁵¹ Enforced expression of these miRNAs in a *Eμ-myc* transgenic mouse model of B cell lymphoma dramatically accelerates disease onset and progression.⁵² Importantly, lymphomas with enforced expression of these miRNAs lack the extensive apoptosis that usually typifies these tumors in *Eμ-myc* mice. Since haploinsufficiency for *Bim* accelerates lymphomagenesis in *Eμ-myc* transgenic mice,

downregulation of this protein by the miR-17-92 cluster therefore may contribute to the ability of these miRNAs to exacerbate disease progression in this mouse model. The miR-17-92 miRNA suppressed expression of the tumor suppressor PTEN and the proapoptotic protein BIM. This mechanism probably contributes to the lymphoproliferative disease and autoimmunity of miR-17-92-transgenic mice and contributes to lymphoma development in patients with amplifications of the miR-17-92 coding region.

V. BIM AS A TARGET IN CANCER THERAPY

In view of its importance in regulation of apoptosis, it is not surprising that BIM plays a critical role in determining the response of transformed cells to diverse cancer therapeutic agents, most notably targeted agents. Many of these agents interrupt key survival signaling pathways, and it is widely believed that the lethality of such actions is ultimately integrated at the level of pro- and antiapoptotic proteins. In this context, accumulating evidence indicates that BIM plays a particularly important role in transmitting death signals by agents that promote DNA damage. A clearer understanding of the role of BIM in targeted agent-mediated cell death takes on added significance in view of the intense interest currently focused on the rational combination of targeted agents. The latter is based on recognition that transformed cells may require, in addition to the survival benefits conferred by tumor-specific driver mutations, the presence of proteins that protect the cell from oncogenic stress. This has led to the development of so-called “orthogonal” strategies in which, in addition to interruption of the dysregulated transforming pathway, efforts are also directed at disabling cooperating stress-related pathways.^{53,54} The net effect is a form of synthetic lethality.⁵⁵ Considerable evidence now exists indicating that interventions that lead to BIM upregulation may produce a pronounced increase in lethality when coordinated with interruption of other signaling pathways.

What follows below is not intended to be an all-inclusive list, but instead a limited number of examples in which perturbations in BIM have either been shown to play a key role in the lethality of individual targeted agents, or, alternatively, contribute to synergistic interactions when combined with other agents.

A. Histone Deacetylase Inhibitors

As noted above, histone deacetylase inhibitors characteristically induce BIM upregulation in transformed cells through an E2F1-dependent mechanism.¹⁹ For example, the panhistone deacetylase inhibitor vorinostat SAHA has been shown to acetylate the promoter regions and induce upregulation of multiple BH3-only proteins, including BIM, in mantle cell lymphoma cells.⁵⁶ Histone deacetylase inhibitors have also been shown to reverse silencing of *Bim* in pediatric leukemia cells, and in so doing, to restore glucocorticoid sensitivity.²⁶ Analogously, reversal of epigenetic silencing of *Bim* by histone deacetylase inhibitors restored the sensitivity of Burkitt’s lymphoma cells to various cytotoxic agents.⁵⁷ In human leukemia cells, histone deacetylase inhibitors markedly potentiated the lethality of the BH3-mimetic ABT-737. This effect was specifically related to their capacity to induce upregulation of BIM.⁵⁸ Coadministration of histone deacetylase inhibitors with the dual Bcr/Abl kinase and aurora kinase inhibitor MK-0457 dramatically increased cell death in the endoreduplicated population of Bcr/Abl⁺ leukemia cells in association with upregulation of BIM.⁵⁹ Knockdown of BIM substantially protected cells from the lethal effects of this regimen, demonstrating a significant functional role for BIM upregulation in the antileukemic effects of this strategy. Furthermore, synergistic interactions between the histone deacetylase inhibitors belinostat or romidepsin and the proteasome inhibitor bortezomib in chronic lymphocytic leukemia cells correlated with BIM upregulation, and shRNA knockdown of BIM markedly reduced lethality.⁶⁰ Finally, the lethal effects of the histone deacetylase inhibitor vorinostat in an *Eμ-myc* B cell lymphoma model were shown

to depend functionally on expression of BIM in tumor cells.⁶¹ Collectively, these findings demonstrate that BIM plays an important functional role not only in mediating the lethal effects of histone deacetylase inhibitors in transformed cells, but also in the capacity of histone deacetylase inhibitors to potentiate the activity of multiple other targeted agents.

B. Proteasome Inhibitors

The proteasome is a multiprotein complex that plays a critical role in cellular homeostasis, and is responsible for the elimination of unwanted or unfolded proteins. Proteasome inhibitors such as the boronic anhydride bortezomib (Velcade) or the irreversible proteasome inhibitor carfilzomib (PR-171) target the 20S proteasome, and in so doing, promote the accumulation of diverse proteins, leading in turn to proteotoxic stress. Because the proteasome is so ubiquitous and its functions are so universally required, it was initially felt that inhibiting proteasome function would not be a feasible antineoplastic strategy. Nevertheless, early preclinical studies suggested that proteasome inhibitors selectively targeted transformed cells compared to their normal counterparts.⁶² This phenomenon may reflect the fact that transformed cells, particularly those expressing mutant oncoproteins, may be under increased proteotoxic stress and display a greater requirement for intact systems responsible for protein disposition.⁵⁴ In transformed cells, inhibition of proteasome function can lead to upregulation of diverse proteins that can exert either pro- or antiapoptotic effects.⁶³ An example of the latter is MCL-1, which is upregulated in response to proteasome inhibitors, and which renders cells resistant to these agents.⁶⁴ On the other hand, proteasome inhibitors are known to trigger accumulation of BIM in transformed cells, and this event has been implicated in lethality.⁶⁵ As noted above, accumulation of BIM has been shown to play a significant functional role in synergistic interactions between proteasome and histone deacetylase inhibitors in human myeloid and lymphoid leukemia cells.^{60,66}

C. MEK1/2 Inhibitors

The Ras/Raf/MEK/ERK pathway is one of the most frequently dysregulated in cancer, and consequently has become a major focus of interest for pharmacologic intervention. Such approaches have involved the development of farnesyltransferase inhibitors, HMG-CoA reductase inhibitors (statins), Raf inhibitors, and MEK1/2 inhibitors.⁶⁷ MEK1/2 inhibitors, which prevent phosphorylation of ERK1/2, have numerous downstream targets, including cyclin D1, β -catenin, and the Ets transcription factors, among others. As described above, the Ras/Raf/MEK/ERK pathway plays a key role in regulating the expression and function of BIM. Of particular importance, because phosphorylation of BIM (e.g., on Ser69) by ERK1/2 promotes its proteasomal degradation,³² interruption of this process triggers BIM accumulation, which can contribute to cell death. In addition to modulating the abundance of BIM directly, inhibition of MEK1/2/ERK1/2 can also alter interactions between BIM and other BCL-2 family members. For example, activation of ERK1/2, e.g., by growth factors, promotes dissociation of BIM from MCL-1, which allows MCL-1 to exert antiapoptotic effects, and at the same time potentiates BIM turnover. Conversely, interruption of this process may potentiate BIM/MCL-1 interactions, which increase BIM accumulation as well as cell death.^{68,69}

D. BH3-Mimetics

Various BH3-mimetics have been developed (e.g., ABT-737, obatoclax, AT-101),⁷⁰ which to varying degrees interfere with the function of antiapoptotic multidomain molecules, e.g., BCL-2, BCL-X_L, and MCL-1. Some of these, such as obatoclax, act broadly and inhibit all three antiapoptotic molecules,⁶⁴ while the actions of others, such as ABT-737, are restricted to BCL-2 and BCL-X_L.⁷¹ In the case of the latter agent, numerous studies have shown that agents that downregulate MCL-1, including CDK inhibitors, sorafenib, or deubiquitinase

inhibitors, markedly potentiate ABT-737 antineoplastic activity.^{72,73} However, MCL-1 levels do not represent the sole determinant of ABT-737 activity, and studies have shown that expression of BIM plays a key role in ABT-737 lethality, i.e., in chronic lymphocytic leukemia cells.⁷⁴ Consistent with this notion, synergistic interactions between ABT-737 and HDAC inhibitors in human leukemia cells has been shown to reflect, in large part, upregulation of BIM.⁵⁸ Finally, in B-Raf-mutated tumors, BIM was shown to play a critical role in synergistic induction of cell death by MEK1/2 inhibitors and BH3-mimetics such as ABT-737.⁷⁵

E. Receptor Tyrosine Kinases (RTKs)

The observation that death signals mediated by inhibitors of RTKs may be integrated at the level of BCL-2 family members (e.g., BAD or BIM)⁷⁶ raises the possibility that the antitumor efficacy of such agents may be enhanced by BH3-mimetics. Indeed, in lung cancer cells, ABT-737 has been shown to enhance the activity of EGFR inhibitors toward lung cancer cells by disrupting the function of BCL-2/BCL-X_L.⁷⁷ Such findings are consistent with evidence that induction of BIM by EGFR inhibitors in lung cancer cells, as well as its mitochondrial translocation, play important functional roles in potentiation of death by ABT-737.⁷⁸ Similar results were obtained in non-small cell lung cancer cells exposed to the EGFR inhibitor gefitinib or analogous agents and ABT-737.^{79,80} BIM has also been shown to play an important functional role in the response of breast cancer cells with amplified HER2 expression to the kinase inhibitor lapatinib.⁸¹

Finally, multiple studies have shown that BIM plays a critical role in mediating the lethality of various Bcr/Abl kinase inhibitors in Bcr/Abl⁺ leukemias. Several of these suggest that Bcr/Abl regulates BIM transcription via FOXO3a, and that kinase inhibitors promote this process.^{21, 82–84} A summary of ways in which alterations in the expression of BIM may regulate the response of tumor cells to such RTK inhibitors is illustrated in Fig. 2.

F. PI3K Inhibitors

As noted above in the case of RTK inhibitors, expression of BIM predicted the response of tumor cells displaying mutant PI3K-PI3K inhibitors.⁸⁵ Interestingly, no correlations between BIM expression and responsiveness were observed in the case of standard chemotherapy, suggesting that BIM may represent a particularly important response determinant in the case of targeted therapies, most notably kinase inhibitors. In neuroblastoma cells, exposure to PI3K inhibitors such as PI103 increased the ratio of proapoptotic proteins such as BIM to antiapoptotic proteins, leading to apoptosis.⁸⁶

VI. SUMMARY AND FUTURE DIRECTIONS

Collectively, these findings indicate that BIM plays a very important role in not only tumorigenesis, but also in the behavior of transformed cells (e.g., their metastatic potential) as well as their response to chemotherapy. In addition, the bulk of the evidence indicates that BIM represents a critical chemotherapeutic response determinant, most notably in the case of targeted agents that interrupt survival signaling pathways. Because the abundance of BIM is regulated at multiple levels (e.g., epigenetic, transcriptional, translational, and posttranslational), numerous opportunities exist to increase its expression. These may include enhancing its transcription, e.g., via FOXO3a, or diminishing its degradation, e.g., by blocking its phosphorylation at ERK1/2 or AKT-dependent sites, or by interfering with proteasomal degradation. A corollary of these concepts is that it may be possible in the future to design rational combination strategies involving targeted agents based on upregulation of BIM. An example of such a strategy might involve combining an agent known to upregulate BIM (e.g., HDAC inhibitors)¹⁹ with an agent whose lethal effects are

known to be BIM dependent (e.g., Bcr/Abl kinase inhibitors²¹ or BH3-mimetics). Indeed, preclinical studies document the effectiveness of such strategies.^{58,59} It is likely that, in the near future, the effectiveness of this strategy will be able to be tested more formally in the clinic.

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FIGURE 1.

The activation of BIM by cellular stress in normal cells. The expression of BIM is regulated at multiple levels for normal homeostasis. *Bim* mRNA can be induced by the inhibition of AKT, leading to FOXO3a nuclear translocation and transcriptional activation of *Bim*. Activation of the Ras/Raf/MEK/ERK/RSK pathway leads to phosphorylation and to proteosomal degradation of BIM. Conversely, BIM can be stabilized by dephosphorylation on growth factor deprivation. Alternatively, JNK-mediated phosphorylation of BIM renders the protein more active. Once activated, BIM can neutralize antiapoptotic BCL-2 family proteins at mitochondria and/or activate BAX by direct protein-protein interaction. See details in the text.

FIGURE 2.

Tumor cell death through BIM with chemotherapy. BIM plays a critical role in determining the response of tumor cells to diverse cancer-targeted therapeutic agents. Interventions that lead to BIM upregulation produce a pronounced increase in lethality when coordinated with interruption of other signaling pathways. Representative agents for individual targeted therapeutics for (A) histone deacetylase inhibitors, (B) proteasome inhibitors, (C) MEK1/2 inhibitors, (D) BH3-mimetics, (E) receptor tyrosine kinase inhibitors are described. For details, see text.