Arch. Immunol. Ther. Exp. (2011) 59:277–287
DOI 10.1007/s00005-011-0126-1

REVIEW

Bim: Guardian of Tissue Homeostasis and Critical Regulator of the Immune System, Tumorigenesis and Bone Biology

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Received: 28 September 2010 / Accepted: 11 January 2011 / Published online: 3 June 2011

Abstract One of the most important roles of apoptosis is the maintenance of tissue homeostasis. Impairment of apoptosis leads to a number of pathological conditions. In response to apoptotic signals, various proteins are activated in a pathway and signal-specific manner. Recently, the pro-apoptotic molecule Bim has attracted increasing attention as a pivotal regulator of tissue homeostasis. The Bim expression level is strictly controlled in both transcriptional and post-transcriptional levels. This control is dependent on cell, tissue and apoptotic stimuli. The phenotype of Bim-deficient mice is a systemic lupus erythematosus-like autoimmune disease with an abnormal accumulation of hematopoietic cells. Bim is thus a critical regulator of hematopoietic cells and immune system. Further studies have revealed the critical roles of Bim in various normal and pathological conditions, including bone homeostasis and tumorigenesis. The current understanding of Bim signaling and roles in the maintenance of tissue homeostasis is reviewed in this paper, focusing on the immune system, bone biology and tumorigenesis to illustrate the diversified role of Bim.

Keywords Bim · Apoptosis · Tissue homeostasis · Immune system · Tumorigenesis · Bone biology

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Introduction

Programmed cell death has become one of the most intensively researched fields in all of biology. The first report of "physiological cell death" during vertebrate development was described by Carl Vogt in 1842. Since then, the study of one particular form of programmed cell death, known as "apoptosis", has progressed remarkably, among the other forms of cell death, including necrosis and autophagy (Meier and Vousden 2007). The important role of apoptosis as a guardian of tissue homeostasis has been the subject of many reviews (Akiyama et al. 2009; Bouillet and O'Reilly 2009; Giam et al. 2008; Lomonosova and Chinnadurai 2008; Strasser 2005). Defects in apoptosis regulation can promote various pathological conditions, including tumorigenesis, autoimmune diseases, neurodegenerative disorders and developmental abnormalities (Akiyama et al. 2003; Baehrecke 2002; Naoi et al. 2009). In response to apoptotic signals, various proteins are activated in a pathway-specific manner, and the classical caspase activation chain reaction is set in motion (Akiyama et al. 2009). This review provides certain glimpses of the research into Bim and illustrates a wide variety of the important roles of Bim among the large number of apoptosis-related proteins. First, it summarizes the signaling cascades of apoptosis. Subsequently, the Bim regulation system is discussed. Bim is a critical regulator of tissue homeostasis in various situations. Clearly, it is beyond the scope of this short review to attempt to encompass the entire roles and regulation systems of Bim. This review thus focuses on three specific areas: the immune system, bone biology and tumorigenesis. Although these systems appear quite different superficially, all of them are controlled and related to each other by Bim. Thus, to obtain an understanding of Bim would open the door to as yet undiscovered aspects of tissue homeostasis.

Apoptosis Signaling Cascade

Programmed cell death comprises three main types of cell death: apoptosis, necrosis and autophagy. Apoptosis is a genetically programmed process, which eliminates unwanted cells. Dying cells exhibit characteristic morphological changes such as cell shrinkage, chromatin condensation and fragmentation followed by ingestion by the phagocytes (phagocytosis) that degrade apoptotic cell fragments (Baehrecke 2002; Danial and Korsmeyer 2004). In apoptotic cells, various enzymes are activated in a pathway-specific manner, and the classical caspase activation chain reaction is set in motion in response to apoptotic signals (Hengartner 2000).

Mammals generally have two distinct apoptosis signaling pathways, the intrinsic and the extrinsic pathway. In the intrinsic pathway, death signals include a range of extracellular and developmental signals which invoke mitochondrial outer membrane permeabilization, leading to the activation of caspases and the apoptosome. The extracellular signals include irradiation, growth factor deprivation and various chemotherapeutic agents. The B cell lymphoma-2 (Bcl-2) family of proteins constitutes a critical intracellular checkpoint in this pathway. The Bcl-2 family of proteins can be classified into one anti-apoptotic group and two pro-apoptotic groups. The anti-apoptotic members include Bcl-2, Bcl-xL, BCL-w, myeloid cell leukemia sequence 1 (Mcl1), A1, Bcl-2 homolog of ovary/death inducer binding to vBcl-2 and Apaf-1 (BOO/Diva). They share a close similarity within three or four Bcl-2 homology (BH) domains and prevent the effector group of the pro-apoptotic Bcl-2 family of proteins, including Bcl-2-associated X protein (Bax) and Bcl-2 antagonist/killer (Bak), from inducing the mitochondrial outer membrane permeabilization and subsequent caspase activation (Bouillet and O'Reilly 2009). Bim is categorized in a third group, i.e., the Bcl-2-homology domain 3-only (BH3-only) proteins. BH3-only proteins sense cell death signals and release the inhibition imposed on Bax and Bak by the pro-survival proteins. They include Bcl-2-antagonist of cell death (Bad), Bcl-2-interacting killer, BH3-interacting domain death agonist (Bid), harakiri, NADPH oxidase activator 1, p53-upregulated modulator of apoptosis (Puma) and Bcl-2-modifying factor (Bmf) (Akiyama et al. 2003; Bouillet and O'Reilly 2009).

In the extrinsic pathway, apoptosis is initiated by the stimulation of death receptors. They are members of the tumor necrosis factor receptor family, such as Fas, which have an intracellular death domain. The engagement of the death receptor by its ligand activates pro-caspase 8 to caspase 8 by means of the adaptor protein Fas-associated death domain via homologous domain interaction. Caspase 8 activates the pro-caspases 3, 6 and 7, which execute cell death through the degradation of vital cellular substrates. The extrinsic pathway engages the intrinsic pathway in certain cell types. Pro-apoptotic Bcl-2 family member Bid is activated by caspase 8. Active Bid amplifies the apoptosis cascades. Recently, the cooperation between Fas and Bim in the shutdown of T cell responses has entered the spotlight. This topic is discussed in the following chapter.

Apoptosis is not limited to the intrinsic and extrinsic pathways. Cellular stress induces the accumulation of unfolded proteins in the endoplasmic reticulum (ER). Cellular stress inducers include hypoxia, oxidative injury, protein inclusion bodies, hypoglycemia, high-fat diets, viral infections and cytotoxic drugs. The accumulation of unfolded proteins triggers an evolutionarily conserved series of signal transduction events, including apoptosis (Kim et al. 2008). Bim is essential for ER stress-induced apoptosis in a diverse range of cell types, including mouse embryonic fibroblasts, mouse thymocytes, MCF-7 and Vero African green monkey kidney epithelial cells. ER stress activates Bim through transcriptional and post-transcriptional pathways, CHOP-C/EBPa-mediated direct transcriptional induction and phosphatase 2A-mediated dephosphorylation, which inhibits the ubiquitination and subsequent proteasomal degradation of Bim (Puthalakath et al. 2007). An ensuing study reported that the protective effects of nerve growth factor in a PC12 neuronal derivative cell line against ER stress-induced apoptosis were derived from the prevention of Bim induction and subsequent mitochondrial translocation of Bax (Szegezdi et al. 2008).

The Discovery of BIM

Bim was identified by two groups independently. O'Connor et al. (1998) identified Bim as a Bcl-2- binding protein by the screening of a λ-phage expression library from a T cell lymphoma. Hsu et al. (1998) identified it as a Mcl1-binding protein by the screening of a yeast two-hybrid library from ovarian tissue. They named this protein Bcl-2-related ovarian death gene. To date, alternative splicing reportedly generates at least 19 Bim isoforms, including BimS, BimL, and BimEL, which have distinct sizes and pro-apoptotic activities (Liu et al. 2002, 2007; O'Connor et al. 1998). O'Connor et al. (1998) displayed that the apoptosis inducing ability of these Bim isoforms were different from each other. When the transfectants with Bim isoforms were deprived of interleukin (IL)-3 or subjected to γ-irradiation, it became apparent that BimS antagonized Bcl-2 more effectively than BimL or BimEL (O'Connor et al. 1998). The following studies revealed the existence of a lot of Bim isoforms in sequence. A nomenclature for the Bim isoforms based on domain structure has been

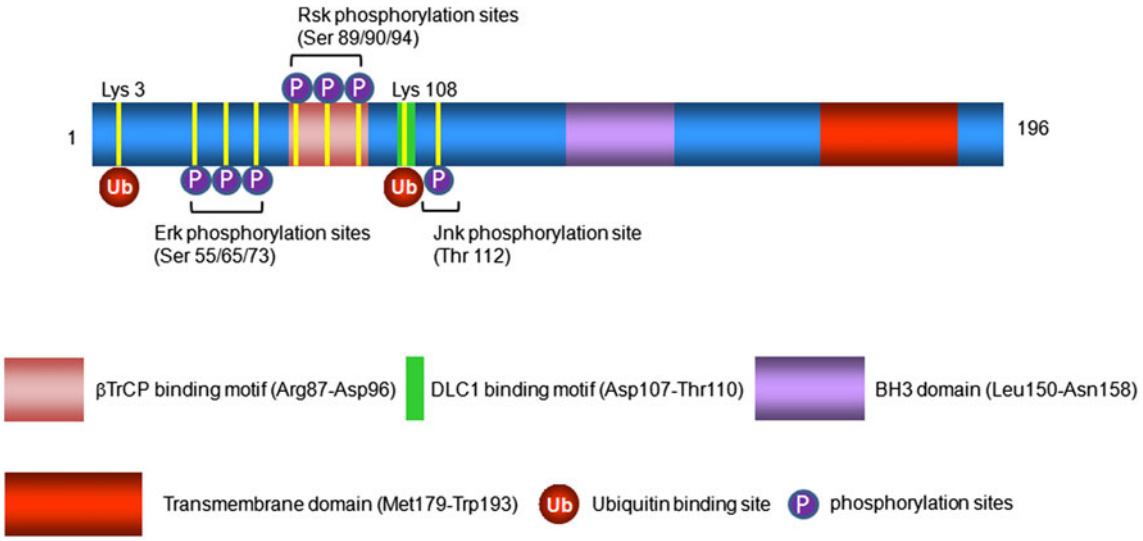

Fig. 1 Schematic representation of the structural domains of the longest isoform BimEL. Murine BimEL is composed of 196 amino acids and contains a BH3 domain. There are two ubiquitination sites, three ribosomal protein S6 kinase (RSK) phosphorylation sites, one βTrCP binding motif, three extracellular signal-regulated kinase (ERK) phosphorylation sites, one Jun amino-terminal kinase (JNK) phosphorylation site, and one transmembrane domain

proposed. This nomenclature system divides the 18 Bim isoforms into six groups: BimS, BimL, BimEL, BimD, BimDd and BimEDd (Adachi et al. 2005). Recently, a novel BimS group member, BimSs3, was reported (Liu et al. 2007). Interestingly, BimSs3 mainly localizes to the nucleus, although it can induce apoptosis via mitochondria depolarization (Liu et al. 2007). Each isoform was classified and named by the domain structure of the isoforms. For example, BimD has no BH3 domain. The isoforms lacking the BH3 domain and/or DLC-binding domain probably act as decoys of pro-apoptotic Bim products. These facts should suggest the existence of a novel regulatory mechanism for Bim. However, the cell or signal specificity of each isoform is yet unknown. The longest isoform, murine BimEL, is composed of 196 amino acids and contains not only the BH3 domain, but also two ubiquitination sites, three ribosomal protein S6 kinase (RSK) phosphorylation sites, one βTrCP binding motif, three extracellular signal-regulated kinase (ERK) phosphorylation sites, one Jun amino-terminal kinase (JNK) phosphorylation site and one transmembrane domain (Fig. 1).

The BIM-Regulatory System

The pro-apoptotic activity of Bim is controlled both transcriptionally and post-transcriptionally in a cell and tissue-specific manner (Fig. 2). The forkhead-like transcription factor FOXO3a (forkhead box O3a) and tumor suppressor gene Runx3 (runt-related protein 3) are key transcriptional regulators of Bim. Some apoptotic stimuli, including cytokine withdrawal or paclitaxel treatment, upregulate the Bim mRNA level via activation of FOXO3a in primary osteoblasts, hepatocytes, neurons and several types of cancer cells (Barreyro et al. 2007; Kawamura et al. 2007; Strasser 2005; Sunters et al. 2003). Paclitaxel is a cancer therapeutic agent, which interacts with cellular microtubules (Sunters et al. 2003). Paclitaxel sensitivity is controlled by the FOXO3a and Bim expression levels (Olsen 2005; Sunters et al. 2003). The basal level of FOXO3a expression in MCF-7, a paclitaxel-sensitive breast cancer cell line, is quite high. Paclitaxel treatment upregulates the expression level of the Bim protein without influencing the levels of other Bcl-2 family members. However, paclitaxel does not increase the Bim expression level in MDA-MB231 cells, which express low levels of FOXO3a and Bim. Paclitaxel sensitivity correlates with the FOXO3a and Bim levels in non-small cell lung cancer (NSCLC) cell lines as well (Li et al. 2005).

Brain-derived neurotrophic factor (BDNF) is a useful biomarker of poor prognosis in neuroblastoma patients. BDNF provides chemoresistance to neuroblastoma via activation of mitogen-activated protein kinase (MAPK) and the phosphatidylinositide 3-kinase (PI3K) pathways. BDNF inhibits paclitaxel-induced cell death by suppressing Bim expression. BDNF suppresses FOXO3a activity through phosphorylation by the PI3K/AKT pathway (Li et al. 2007).

RUNX3 deficiency results in gastric mucosa hyperplasia. Approximately, one half of human gastric cancer cells express significantly low levels of RUNX3 (Li et al. 2002). RUNX3 upregulates Bim transcription by regulating FOXO3a levels and induces transforming growth factor (TGF)-β-associated apoptosis (Yano et al. 2006).

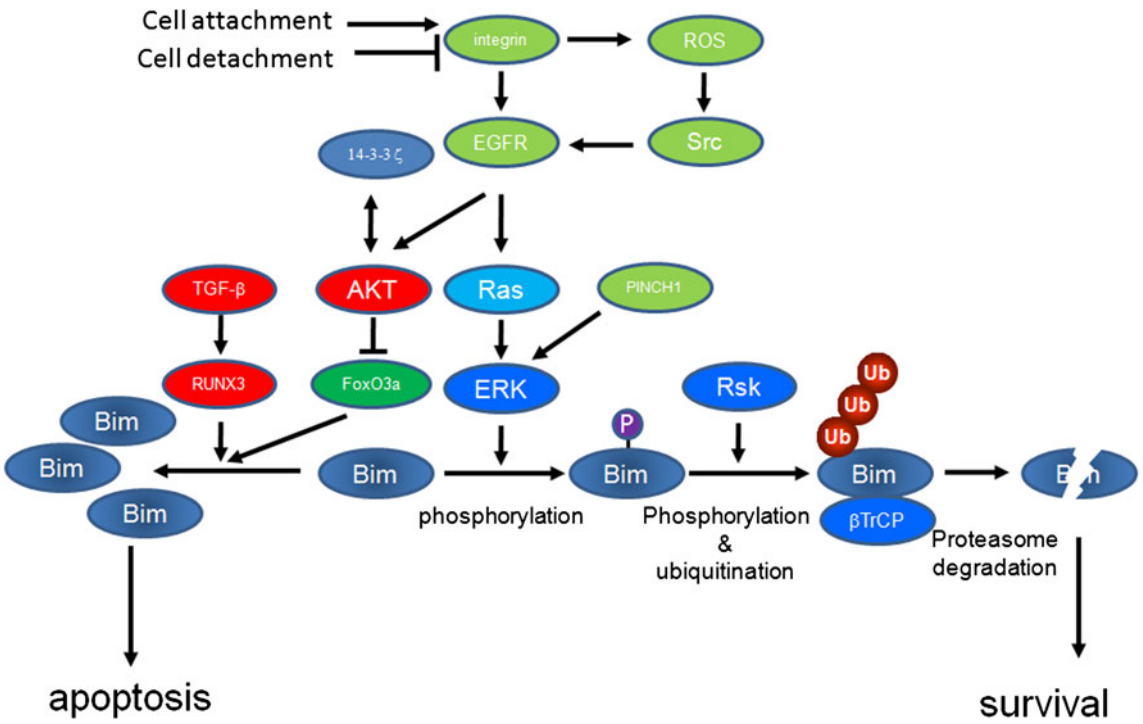

Fig. 2 The regulatory system of Bim. Bim is controlled both transcriptionally and post-transcriptionally in a cell and tissue-specific manner

TGF-β regulates apoptosis signals not only transcriptionally through the upregulation of Bim expression, but also posttranscriptionally through increasing the phosphorylation of Bim by ERK. ERK-mediated phosphorylation induces ubiquitination and subsequent proteasomal degradation of Bim (Hübner et al. 2008; Strasser 2005). TGF-β induces the MAPK phosphatase (MKP)2 expression through SMAD3. MKP2 abrogates ERK activity and ERK-induced Bim ubiquitination, thus leading to the increase in Bim levels (Ramesh et al. 2008).

Growth factor-mediated stimulation also activates ERK and downregulates Bim in a post-translational manner. ERK-dependent phosphorylation on Ser69 in BimEL promotes degradation of BimEL (Ley et al. 2004; Luciano et al. 2003).

Distinct E3 ligase complexes, which recognize specific proteins and signals, determine the specificity of ubiquitination-proteasome degradation. Previously, several enzymes were reported as possible candidates for Bim E3 ligase. At first, involvement of c-Cbl in Bim ubiquitin–proteasome degradation was suggested, although a following study reported a negative result (Akiyama et al. 2003; Wiggins et al. 2007). Another previous candidate was the 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 the paclitaxel resistance of the MDA-MB468 and MCF7 breast cancer cell lines in vitro and in vivo (Zhang et al. 2008). This report suggests that RACK1 contributes to the paclitaxel resistance through the promotion of Bim degradation. However, this finding has been contradicted by other reports. Cullin1, but not Cullin2, coimmunoprecipitated endogenous BimEL in the presence of PMA, an activator of the ERK pathway. SCF (Skp1-Cullin1-F-box protein) ubiquitin ligase complexes often target phosphorylated proteins. ERK1/2-mediated phosphorylation of BimEL on Ser69 induces ribosomal protein S6 kinase RSK 1/2 mediated phosphorylation of BimEL on Ser93, Ser94 and Ser98. These phosphorylation events provide binding sites to the F-box protein β-transducin repeat containing protein (βTrCP) and facilitate proteasomal degradation of Bim via βTrCP (Dehan et al. 2009). The silencing of either βTrCP or RSK1/2 resulted in BimEL-mediated apoptosis even in NSCLC cells, which are resistant to gefitinib, an epidermal growth factor receptor (EGFR) inhibitor currently in clinical use.

In contrast, the role of JNK for Bim is still unclear. In the T cell acute lymphoblastic leukemia cell line Sup-T, JNK-mediated phosphorylation of Bim promotes proteasomal Bim degradation (Leung et al. 2008). However, several studies have supported the pro-apoptotic activity of JNK-mediated phosphorylation of Bim. JNK-mediated phosphorylation decreases the binding of Bim to the anti-apoptotic protein Bcl-2 and activates Bim as a result (Hübner et al. 2008). Preventing the phosphorylation of Bim by JNK in vivo impaired negative selection, because

of the impaired apoptotic activity of mutated Bim (Hübner et al. 2008). Recently, mitogen-activated protein kinase kinase kinase kinase (MAP4K) 3 was reported to be implicated as a novel apoptosis inducer in JNK and mammalian target of rapamycin (mTOR) signaling. MAP4K3-mediated Bim phosphorylation via JNK promotes the oligomerization of Bim, and Bax induces subsequent apoptosis in coordination with Puma and Bad (Lam et al. 2009). Hence, JNK-mediated phosphorylation of Bim may facilitate the pro-apoptotic efficacy of Bim.

Recently, Bim has come into the spotlight as a target of the microRNA (miRNA) silencing pathway in embryonic stem (ES) cells. miRNA silencing is a finely tuned adjustment system of protein expression, with mRNA degradation or translational repression in a sequence-specific manner. It is a highly conserved system of gene regulation in almost all eukaryotic organisms. It critically regulates gene expression in both developmental stages and disease conditions (Spruce et al. 2010; Su et al. 2009). In the first step, primary miRNAs are processed within the nucleus into pre-miRNAs. Pre-miRNAs are cleaved by Dicer to produce miRNA–miRNA (miRNAs) complement strand duplexes after their exportation from the nuclear pores. It is estimated that miRNAs may regulate as many as one-third of all human genes and miRNAs have been shown to regulate diverse biological processes. The association of these small RNAs with Argonaute (Ago) proteins forms the core effector complexes, known as RNA-induced silencing complexes (RISCs). Small RNAs guide RISCs to complementary target sequences to mediate different silencing effects (Su et al. 2009). Knockout of Dicer leads to severe growth retardation and embryonic death by 7.5 days post-coitum (Bernstein et al. 2003). The miRNAs are an important regulator of the self-renewal and multi-potency of the cells within the extraembryonic tissues of the trophectoderm and visceral endoderm. In addition, miRNAs are involved in controlling cell survival in epiblasts. Indeed, Bim was significantly upregulated in the Dicer-deficient epiblasts compared with controls. Dicer-deficient epiblasts easily enter into apoptosis and developmental delay. Intriguingly, Dicer deficiency exhibits little effect on the initiation of gastrulation (Spruce et al. 2010). Bim mRNA is a target of Ago as well. Murine ES cells lacking Ago1-4 are completely defective in miRNA silencing and undergo apoptosis through an upregulation of Bim. Constitutively active AKT or the introduction of any single Ago into Ago-deficient cells inhibits Bim expression and partially rescues the growth defect in Ago-deficient ES cells (Su et al. 2009). Thus, Bim mRNA control effected by miRNA regulation is critical in the primary development of embryonic cells.

Furthermore, an intriguing report about Bim regulation was recently reported: the control of Bim by viruses for the carrying out of virus infection and replication. Human herpesvirus (HHV)-8 induces various pathological conditions, including Kaposi's sarcoma, an endothelial cell disease, and B cell malignancies with primary effusion lymphoma and multicentric Castleman's disease. The protection system from viral infection is apoptosis of the infected cells. A viral protein, viral interferon regulatory factor (vIRF)-1, associates with Bim and induces the nuclear sequestration of Bim to avoid cellular apoptosis during infection and thus promotes viral replication. HHV-8 utilizes vIRF-1 to inactivate Bim. The association of vIRF-1 with Bim promotes the nuclear translocation of the complex and separates Bim from the mitochondrial membrane (Choi and Nicholas 2010). This in contrast suggests that Bim is a guardian against viral infection.

The molecular biological studies are ahead of clinical studies in the area of the relationships between Bim and tumorigenesis. However, clinical studies have rapidly followed molecular biological studies and are evidencing the importance of Bim in tumorigenesis even in the clinical fields. The 14-3-3 family is a critical regulator of various cellular responses including proliferation, differentiation, cycling, apoptosis and tumorigenesis. 14-3-3ζ is a member of the 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 provides NSCLC cells anchorage-independence or anoikis resistance ability by Bim downregulation (Li et al. 2008). In vitro studies revealed that ERK activation by B-Raf and N-Ras, commonly activated in human cutaneous melanoma, provided melanoma cells with resistance to anoikis via Bim suppression(Boisvert-Adamo et al. 2008; Goldstein et al. 2009). The resistance to anoikis is indispensible for tumor metastasis, which is one of the critical determinants of prognosis. The following clinicopathological study demonstrated that reduced Bim expression was significantly correlated with poor prognosis of human cutaneous malignant melanoma (Dai et al. 2008). Similar data have been reported in colon cancer and oral squamous cell carcinoma (OSCC) (Coutinho-Camillo et al. 2010; Sinicrope et al. 2008). Elevated expression level of Bim in stage II and III colon cancer specimen with immunohistochemical analysis indicated more favorable overall survival and disease-free survival (Sinicrope et al. 2008). In OSCC, immunohistochemical study revealed that increased expression of Bim was associated with the presence of perineural infiltration and moderately to well-differentiated tumors (Coutinho-Camillo et al. 2010). Further studies are required to apply the contributions of Bim researches in molecular biological setting to a clinical setting, especially to tumor treatment.

BIM in the Immune System

Bim is essential for apoptosis in various mature organ cell types, including osteoclasts, osteoblasts, mast cells, epithelial cells, endothelial cells and neurons (Akiyama et al. 2009). Above all, the relationship between Bim and immune cells cannot be overemphasized. Bim is a critical regulator of hematopoietic cells not only in vitro, but also in vivo. Bim deficiency results in an abnormal accumulation of lymphoid and myeloid cells. The phenotype of Bim-deficient mice is a systemic lupus erythematosus (SLE)-like autoimmune disease (Bouillet et al. 1999). A number of studies have demonstrated that Bim has an integral role in the development and function of the immune system compared with other Bcl-2 family of proteins. Since the importance of Bim in the immune system has been extensively described in a number of review articles, we will only briefly mention the role of Bim in T cell regulation. T cell apoptosis is critical in the maintenance of both a proper immune response and hematopoietic cell homeostasis. Undoubtedly, Bim has an essential role in several stages of T cell as well as B cell development and function. Thymocytes from Bim-deficient mice are more resistant to cell elimination during negative selection and also to many apoptotic stimuli, including IL-2 withdrawal, as well as T cell receptor (TCR) and CD3 stimulation (Bouillet et al. 1999, 2002). The analysis of the negative selection of T cells in mice demonstrated that Bim deficiency impairs the deletion of immature autoreactive thymocytes. The protective efficacy of Bim deletion against negative selection stimuli is stronger than Bcl-2 over-expression in thymocytes (Bouillet et al. 2002).

At the periphery, activated T cells must promptly die at the end of an immune response to avoid clogging the whole system with many useless cells. Furthermore, the various cytokines secreted by activated T cells would derange the immediate environment and result in an ensuing pathological autoimmune disorder. Recently, the coordinated control of T cell apoptosis by both the intrinsic and extrinsic apoptosis pathways has come into the spotlight. Fas is related to human autoimmune diseases. Fas or FasL knockout mice exhibit progressive accumulation of B and T cells along with autoimmunity (Takahashi et al. 1994; Watanabe-Fukunaga et al. 1992). The expression of the gene encoding FLIP, a naturally occurring inhibitor of the Fas death pathway, was reported to be markedly elevated in myeloid cells and T and B lymphocytes from a subset of SLE patients as compared to healthy controls. The expression of genes encoding antiapoptotic members of the Bcl-2 family, including Bcl-2A1 and Mcl1, were enhanced in myeloid cells as well (Hutcheson et al. 2008). Fas mutation causes the human autoimmune disease "autoimmune lymphoproliferative syndrome" (Rieux-Laucat et al. 1995). The mild autoimmune disease observed in a single mutation of either Bim or Fas was seriously aggravated by the concomitant loss of the other molecule (Hutcheson et al. 2008).

T cell clonal contraction after an acute viral infection with herpes simplex virus depends solely on Bim, whereas both Fas and Bim are required for the clearance of accumulated T cells during chronic infection with the recombinant γ-herpes virus MHV 68 (murine herpes virus 68) (Hughes et al. 2008). Intriguingly, the clearance of acute lymphocytic choriomeningitis virus (LCMV) infection also requires both Fas and Bim (Weant et al. 2008). This discrepancy may reflect the role of non-T cells in the immune response. In fact, conditional deletion of Fas in mouse antigen-presenting cells causes systemic autoimmunity (Stranges et al. 2007). Bim-deficient dendritic cells (DCs) displayed impaired spontaneous cell death, and stimulated more potent T cell activation in vitro and in vivo. Bim-deficient DCs induced autoantibody production after adoptive transfer. These data suggest that DC abnormality may contribute to the development of autoimmune diseases in Bim-deficient mice (Chen et al. 2007). These studies indicate that the proper termination of certain immune responses requires the appropriate elimination of these cells. The other possible explanation of this discrepancy is that the strength of the effect of these stimulants on the TCR is different. Indeed, activated T cells recognize three different epitopes of LCMV virus accumulated at different levels. This suggests that not only the species of antigens, but also the strength of the stimulant, determines the type of immune response (Weant et al. 2008).

Currently, the importance of Bim is recognized in the immune system. However, the correlation or coordinated regulation system of Bim with other apoptotic molecules is still unclear. Further studies will be needed to uncover the coordinated aspects of the effect of Bim in the immune system.

BIM in Bone Biology

Apoptosis plays a critical role in maintaining skeletal homeostasis. During such activities as skeletal maturation, adult bone turnover by modeling and remodeling processes, fracture healing and bone regeneration, apoptosis is required (Hock et al. 2001). Skeletal homeostasis is maintained by an incessant process of "bone remodeling", which is regulated through a delicate balance between bone formation and resorption or between osteoblasts and osteoclasts. Osteoblasts mainly regulate bone formation and are derived from mesenchymal stem cells. Osteoclasts are primarily responsible for bone resorption. They are

multinucleated cells stemming from monocyte–macrophage lineage precursor cells (Tanaka 2007). Hence, the apoptosis of both osteoblasts and osteoclasts is clearly important in skeletal homeostasis. In particular, the life span of osteoclasts is quite short in the absence of trophic factors such as macrophage colony-stimulating factor (M-CSF). They undergo rapid apoptosis after the completion of differentiation. We previously reported the pivotal role of Bim in osteoclast apoptosis (Akiyama et al. 2003). The activation of Bim is controlled by the ubiquitin–proteasome degradation system in osteoclasts. In the presence of M-CSF, the Bim expression level is suppressed by constitutive degradation via ERK-mediated ubiquitination. Cytokine or trophic factor deprivation induced a rapid upregulation of Bim due to the abolishment of its ubiquitination (Akiyama et al. 2003). Bim-deficient osteoclasts exhibit prolonged survival both in vitro and in vivo (Akiyama et al. 2003). The important role of Bim in osteoclasts was further confirmed by siRNA-mediated knockdown of Bim (Sugatani and Hruska 2005). This demonstrated that M-CSF recruited mTOR, but not ERK, to suppress the expression of Bim in isolated osteoclast precursors. However, we were unable to show a role of mTOR in the regulation of Bim expression in mature osteoclasts (unpublished data), suggesting that the mechanism of apoptosis in mature osteoclasts is different from that of osteoclast precursors.

Recent studies have uncovered the upstream and downstream regulators of the ERK–Bim axis in osteoclasts. Cell division cycle 42 (Cdc42) is a member of the Rho subfamily of small GTPases, which are known to regulate the cytoskeletal organization of osteoclasts. It was recently demonstrated that M-CSF activates Cdc42, which subsequently activates various kinases including PI3K/AKT, ERK and p38. The PI3K/AKT pathways control proliferation, but not the apoptosis of osteoclasts. ERK prevents apoptosis cascade activation through Bim suppression (Ito et al. 2010). βTrCP is the most possible candidate of E3 ligase of Bim. However, the importance of the Cbl family in Bim ubiquitination cannot be ruled out. The Cbl family of proteins, including c-Cbl and Cbl-b, is a highly conserved family of adaptor proteins participating in integrin signaling, cytoskeletal organization, motility and bone resorption in osteoclasts. Cbl family proteins are considered to be involved in the stabilization of the microtubule network. Knocking down both c-Cbl and Cbl-b in osteoclasts markedly increased the levels of Bim and induced apoptosis (Purev et al. 2009). Thus, Cbl family proteins appear to play a critical role in Bim ubiquitination via cytoskeletal regulation.

In osteoclasts, the Bim expression level is controlled not only by proteasomal degradation but also by the negative feedback loop of the Bim-Caspase 3 axis. Bim degradation in osteoclasts was suppressed by a pan-caspase inhibitor, proteasome inhibitor or gene knockdown of caspase 3, and also in caspase 3-deficient osteoclasts, but not by caspase 7 knockdown. Interestingly, caspase-3-deficient osteoclasts exhibited a shorter life span than normal osteoclasts. Thus, this negative feedback loop is functional in both the control of osteoclast survival and bone-resorbing activity (Wakeyama et al. 2007a, b).

Recently, the mechanism of transcriptional regulation of Bim in osteoclasts was reported. TGF-β1 treatment induces apoptosis of human osteoclasts via Bim upregulation, which was mediated not only by ERK inactivation, but also by p38 and Smad2 activation. The Smad pathway inhibition by Smad2 siRNA abolished osteoclast apoptosis through Bim upregulation following TGF-β stimulation (Houde et al. 2009).

Interestingly, despite the pro-apoptotic function of Bim, Bim depletion impairs the bone-resorbing activity of osteoclasts. This is consistent with the in vivo observation that Bim knockout mice exhibit mild osteosclerosis, which is reminiscent of Bcl-xL/SV40 large T antigen transgenic mice (Hentunen et al. 1998). Furthermore, conditional deletion of the Bcl-x gene in osteoclasts increased the bone-resorbing activity, despite their high susceptibility to apoptosis, which is the mirror image of Bim-deficient osteoclasts (Iwasawa et al. 2009). However, impaired osteoclast apoptosis does not necessarily lead to reduced bone-resorbing activity. Osteoclasts from Bcl-2 knockout mice, Bcl-2 being an anti-apoptosis molecule, showed an impaired bone-resorbing activity compared with osteoclasts from wild-type mice. Interestingly, a single Bim allele deletion restored the impaired bone-resorbing activity of osteoclasts in Bcl-2 knockout mice (Nagase et al. 2009). These results suggest that Bcl-2 family proteins regulate both the apoptosis and activity of osteoclasts.

The apoptosis of osteoblasts also critically regulates skeletal homeostasis (Hock et al. 2001), and Bim is an important regulator of osteoblast apoptosis as well. Apoptotic stimuli, such as matrix detachment, serum withdrawal and dexamethasone, upregulate Bim expression levels in osteoblasts, leading to their apoptosis (Espina et al. 2008). Serum deprivation induces the nuclear entry of FoxO3a, which in turn transcriptionally increases Bim expression (Kawamura et al. 2007). Akt1 mediates phosphorylation of FoxO3a and prevents its nuclear localization, which suppresses the transactivation of its target gene Bim in osteoblasts. Conversely, the nuclear localization of FoxO3a is enhanced by Akt1 deficiency in osteoblasts (Kawamura et al. 2007). Bim mRNA levels were also increased in response to dexamethasone (Espina et al. 2008). Thus, the Akt1/FoxO3a/Bim axis controls the apoptosis of osteoblasts at the transcriptional level.

These reports revealed that Bim plays a pivotal role in skeletal homeostasis. Bim is not only an apoptosis inducer, but also a functional regulator of osteoclasts. Further studies are required to elucidate the precise mechanisms of the control of Bim in the maintenance of skeletal homeostasis.

BIM in Tumorigenesis

In tissue development and homeostasis, Bim plays a key role not only in immune system and skeletal homeostasis, but also in spermatogenesis and mammary gland formation (Coultas et al. 2005; Mailleux et al. 2007). The breakdown of tissue homeostasis leads to various pathological conditions, including tumor formation. Baby mouse kidney epithelial cells transformed by dominant negative p53 (p53DD) and E1A form tumors in nude mice. Bim deficiency supports the formation and growth of the tumors in nude mice (Tan et al. 2005), suggesting that Bim is a key suppressor of epithelial tumor formation. The absence or inactivation of Bim also plays an important role in tumor metastasis, since the acquisition of "anchorage-independence" is indispensable for tumor metastasis. Normal cell survival is dependent on anchorage and the cells undergo apoptosis when their attachment with the extracellular matrix or its neighboring cells is lost. Therefore, "anoikis", apoptosis induced by cell detachment, is a critical barrier against metastasis. Both the death receptor and mitochondrial pathways are activated in the process of anoikis. Anoikis recruits or suppresses several molecules involved in the mitochondrial pathway, such as Bim, Bmf, Omi/HtrA2, Bcl-XL and Mcl-1. Among these, Bim plays a pivotal role in the anoikis of tumor cells such as breast cancer, lung cancer, osteosarcoma, fibrosarcoma and melanoma (Simpson et al. 2008; Uehara et al. 2008; Woods et al. 2007), and these cells avoid Bim-mediated cell death to accomplish metastasis. Several cell surface proteins control Bim expression. A cell surface glycoprotein mesothelin suppresses Bim activation via the ERK pathway in the human breast cancer cell line MDA-MB231 (Uehara et al. 2008). Of particular interest is the finding that a new cysteine-histidine-rich protein (PINCH)-1, a widely expressed focal adhesion protein consisting of five LIM domains and a short residue C-terminal tail, suppresses Bim expression levels not only via ERK activation, but also through transcriptional regulation (Chen et al. 2008).

EGFR is one of the most important cell surface molecules and controls the expression of Bim. 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, which is required for the maintenance of EGFR expression, inhibits downstream ERK activation and increases Bim. The downregulation of Bim with RNA interference (RNAi) inhibits MCF-10A anoikis. Overexpression of EGFR maintains ERK activation even in detached cells and blocks Bim expression and anoikis (Reginato et al. 2003). The reactive oxygen species (ROS) induced by cell detachment may also be involved in Bim regulation. The extracellular matrix-cell contact, mediated by integrin, increases intracellular ROS, which oxidizes and activates intracellular tyrosine kinase Src. The active form of Src stimulates signaling downstream of EGFR such as AKT and ERK in a ligand-independent manner, culminating in the downregulation of Bim (Giannoni et al. 2008). Both ERK and AKT pathways play pivotal roles in the regulation of Bim downstream of EGFR. The breast cancer cell line MDA-MB231 and HBC4 cells evade anoikis by suppressing Bim through constitutive activation of the MEK–ERK pathway. MEK inhibitors sensitize these tumor cells to anoikis by blocking the proteasomal degradation of BimEL (Fukazawa et al. 2004). Mutant MCF-10A cells expressing ΔRaf-ER, in which Raf-ERK signaling is activated by 4-OHT stimulation, have low levels of Bim and exhibit anoikis resistance in the presence of 4-OHT (Marani et al. 2004). 14-3-3ζ is highly expressed in NSCLC tissue and is involved in the anoikis resistance of lung cancer cells as mentioned above. 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, induces Bax activation and anoikis. Bim downregulation with RNAi abrogates the restitution of anchorage-dependence by 14-3-3ζ knockdown (Li et al. 2008). B-Raf and N-Ras are generally activated in human cutaneous melanoma cells and provide melanoma cells with resistance to anoikis. Constitutively active mutants of N-Ras and B-Raf downregulate Bim expression levels in melanocytes or melanoma cell lines by means of ERK activation. Bim suppression by a constitutively active mutant of N-Ras or RNAi inhibits melanocyte anoikis (Boisvert-Adamo and Aplin 2008; Goldstein et al. 2009). Tumor metastasis is one of the critical determinants of prognosis. These data suggest that the expression level of Bim is inversely related to the prognosis in various malignant tumors. Indeed, reduced Bim expression is significantly correlated with a poor prognosis in human cutaneous malignant melanoma (Dai et al. 2008). An increasing number of in vitro studies suggest that the ERK–Bim axis plays a pivotal role in tumor metastasis. Thus, if there were a method of controlling Bim, it might be possible to suppress tumor metastasis effectively.

Conclusion

The pro-apoptotic BH3-only protein Bim is a critical regulator of tissue homeostasis. The role of Bim in various types of cells and tissues has been reviewed to provide a perspective on Bim. In fact, all of these cells and tissues are maintained in a healthy condition by Bim. However, it is still unclear why Bim controls apoptosis in such a wide variety of cell and tissue types.

Bim is a critical target for the development of novel treatment strategies in clinical medicine. The control of Bim would have potential impact on multiple organ and tissue environments. The potency of a multi-targeting strategy in clinical medicine could lead to the treatment of diseases, which are intractable when treated with traditional strategies (Akiyama et al. 2008). For example, Bim can serve as a therapeutic target in cancers, metabolic bone diseases and autoimmune diseases. The understanding of the regulatory mechanisms of Bim is thus crucial, not only for clinical applications, but also for obtaining an understanding of the whole body system.

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