TGFβ and Bim: Partners in cell death

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Introduction

Transforming growth factor β (TGFβ) is the prototype member of a large superfamily of cytokines that also includes the bone morphogenic proteins (BMPs), growth and differentiation factors (GDFs) and activins. There are three genes for TGFβ in humans and mice (TGFβ1, TGFβ2 and TGFβ3) that each encode ~25 kDa mature, processed proteins and share over 70% identity in their amino acid sequences. Despite this identity in protein structure, homozygous deletion of individual TGFβ genes reveals distinct functions for each isoform (Dünker and Krieglstein, 2000). TGFβ1 knockout mice appear normal at birth but quickly succumb to a diffuse and lethal inflammation at approximately 3 weeks. Deletion of TGFβ2 or TGFβ3 is perinatal lethal, with TGFβ2 mice demonstrating a wide range of developmental defects and TGFβ3 mice displaying abnormal pulmonary development. Both TGFβ2 and TGFβ3 knockout mice exhibit cleft palates. These observations highlight the broad involvement of TGFβ cytokines in mammalian physiology, as would be anticipated given the wide distribution of TGFβ expression and cell-responsiveness among tissues. More important to this review, however, is the idea that the clear role for TGFβ in development points to a prominent role for these cytokines in cell proliferation and apoptosis.

TGFβ signaling

Signaling by TGFβ isoforms is initiated by an oligomeric receptor complex consisting of two types of transmembrane subunits that each possesses serine/threonine kinase activity. Binding of ligand to the constitutively active type II receptor (TβRII) promotes complex formation with the type I receptor (TβRI/ALK5). TβRII then transphosphorylates TβRI/ALK5 in a juxtamembrane region rich in glycine and serine residues (GS region), leading to activation of TβRI/ALK5 and propagation of TGFβ signaling by several signaling cascades.

Signaling by TGFβ through the Smad pathway has been extensively characterized and is considered the canonical pathway (Massagué, 2008; Schmierer and Hill, 2007) (see Figure 1). Smad proteins can be divided into three functional groups. The receptor-regulated Smads (R-Smads), Smad2 and Smad3, are directly phosphorylated and activated by ALK5. Phosphorylation occurs at C-terminal Ser-Ser-X-Ser (SSXS) motifs and promotes the formation of heteromeric complexes with the common-mediator Smad, or co-Smad, Smad4. The Smad complexes translocate into the nucleus, where they regulate gene expression by directly interacting with resident DNA-binding proteins and by recruiting co-activators or co-repressors to the promoter (Ross and Hill, 2008). The inhibitory Smads (I-Smads), Smad6 and Smad7, are induced by TGFβ and thereby act as a negative feedback loop. I-Smads
prevent activation of R-Smads by either competitively inhibiting their binding to ALK5 or by recruiting Smurf family ubiquitin ligases to ALK5, leading to its degradation. Several additional proteins are thought to play a role in facilitating R-Smad activation by TGFβ receptors, such as SARA, Hgs/Hrs, and cPML. We have shown that the adaptor molecule disabled-2 (Dab2) links TGFβ receptors to Smad proteins (Hocevar et al., 2001), presumably in early endocytotic vesicles due to the known interaction of Dab2 with the clathrin adaptor molecule AP-2.

Homozygous deletion of TβRII, Smad2 and Smad4 are all embryonic lethal in mice, confirming a role for TGFβ signaling during development (Dünker and Krieglstein, 2000). Interestingly, Smad3 knockout mice are viable and fertile, although they develop colorectal adenocarcinomas as adults. A role for canonical TGFβ signaling in cancer is further demonstrated by studies showing that TβRII, TβRI/ALK5, Smad2 and Smad4 are all inactivated by mutations or allelic loss of heterozygosity in various tumors (Bierie and Moses, 2006).

In addition to the canonical Smad pathway, TGFβ has been shown to activate extracellular signal regulated kinase (ERK), Jun N-terminal kinase (JNK), p38 mitogen activated protein kinase (p38), and PI-3K/AKT (Derynck and Zhang, 2003; Moustakas and Heldin, 2005). The TGFβ responses regulated by these kinases are varied, and there are reports suggesting that members of the Rho family of small GTPases may directly couple activated TGFβ receptors to these signaling pathways or that activation of these pathways may be indirect, possibly resulting from Smad-dependent transcriptional responses.

Figure 1: The canonical Smad signaling pathway.
Small GTPases also play a role in canonical TGFβ signaling, as Rab GTPases regulate TGFβ receptor internalization and Ran GTPases regulate Smad trafficking through nuclear pores (Kardassis et al., 2009).

**TGFβ-induced apoptosis**

TGFβ exerts both pro-apoptotic and anti-apoptotic effects, depending on the cell type and cellular context. This duel nature of TGFβ action is also evident during cancer formation, wherein the growth inhibitory/tumor suppressive effect of TGFβ on normal epithelial cells switches to a growth stimulatory/tumor promoting effect on cancer cells (Massagué, 2008). Pro-apoptotic responses to TGFβ have been demonstrated in a wide variety of cells, but mostly involve hepatocyte and hepatoma cell lines, B cells and lymphomas and prostate epithelium (Lee and Bae, 2002; Pardali and Moustakas, 2007). The reported mechanisms mediating the pro-apoptotic effects of TGFβ are equally diverse and appear to be cell type dependent. **Daxx**, a Fas-receptor associated protein that activates the JNK pathway, interacts directly with TβRII and couples TGFβ signaling to the apoptotic machinery in AML12 hepatocytes (Perlman et al., 2001). ARTS (Apoptotic protein in the TGFβ signaling pathway), a septin-like protein, promotes apoptosis in prostate epithelial cells by binding to and neutralizing the anti-apoptotic protein XIAP (X-linked inhibitor of apoptosis) (Larisch et al., 2000). Studies have also demonstrated the Smad-dependence of additional mediators of TGFβ-induced apoptosis. In Hep3B hepatoma cells, TGFβ has been shown to induce Smad-dependent expression of the death-associated protein kinase (DAP-kinase), a calcium/calmodulin-regulated serine/threonine kinase previously implicated in several apoptotic responses (Jang et al., 2002). Functional cooperation between Smads and Activator Protein-1 (AP-1) mediates TRAIL induction (Tumor necrosis factor-related apoptosis-inducing ligand) and TGFβ-induced apoptosis in hepatoma cells (Herzer et al., 2008). The immediate early gene Gadd45b (Growth arrest and differentiation-dependent) is also Smad-dependent and mediates TGFβ-induced p38 activation and apoptosis in AML12 hepatocytes (Yoo et al., 2003). Interestingly, a Smad-independent pathway of TGFβ-induced p38 activation and apoptosis was originally demonstrated in mouse NMuMG mammary epithelial cells (Yu et al., 2002), and expanded upon recently in AML12 hepatocytes (Sorrentino et al., 2008, Yamashita et al., 2008). It was shown that TRAF6, an E3 ubiquitin ligase, binds to a consensus motif in TβRI. TGFβ-induced oligomerization of TβRII promoted transient auto-ubiquitination of TRAF6 via K63 linkages and its subsequent association with and activation of **TAK1** (TGFβ activated kinase 1), an upstream effector of the p38 and JNK signaling pathways.

**Bim and TGFβ**

Most of the above studies outline signaling pathways and mediators that are proximal to TGFβ receptor activation. Studies of more distal events in TGFβ-induced apoptosis focused on the intrinsic pathway of cell death. This pathway is regulated by the interplay of the Bcl-2 (B-cell CLL/Lymphoma 2) family of proteins at the mitochondria (Hotchkiss et al., 2009). There are three functionally-distinct groups within the Bcl-2 family (see Figure 2), but all share at least one of four Bcl-2-Homology (BH) domains that allows hetero-dimerization among Bcl-2 family members. The Bcl-2-like proteins promote survival, whereas the Bax-like proteins and ‘BH3-only’ proteins (BOP) promote cell death. The BOP are the sensors of apoptosis while the Bax-like proteins are its executioners. Following an apoptotic stimulus, BOP initiate the mitochondrial cell death pathway by either directly activating Bax-like proteins (direct activation model) or by binding to pro-survival Bcl-2 family members and thereby releasing Bax-like proteins (indirect activation model) (Adams and Cory, 2007; Galonek and Hardwick, 2006). Bax proteins then oligomerize to form channels and induce mitochondrial outer membrane permeabilization (MOMP), leading to the release of apoptogenic molecules such as cytochrome c that are needed for caspase activation and cell death.

Given the crucial role of the Bcl-2 family in regulating cell survival, it was not surprising that these proteins were also implicated in TGFβ-induced apoptosis. Early studies in the WEHI 231 B lymphocyte cell line demonstrated that stable overexpression of the pro-survival protein Bcl-XL abrogated TGFβ-mediated apoptosis (Arsura et al., 1996, Chipuk et al., 2001). In FaO rat hepatoma cells, TGFβ does
not affect the expression levels of Bcl-2 family members but did induce the caspase-dependent cleavage of BAD, a pro-apoptotic BOP family member (Kim et al., 2002). Overexpression of Smad3 in these cells was shown to promote caspase-3-mediated cleavage of BAD and apoptosis, whereas antisense Smad3 cDNA blocked TGFβ-mediated apoptosis and BAD cleavage.

Figure 2: The three functional groups of the Bcl-2 family.

We were the first to demonstrate that TGFβ induces the expression of the BOP protein Bim (Bcl-2-interacting mediator of cell death) in two different B-cell lines, WEHI 231 and Ba/F3 (Wildey et al., 2003). The induction of Bim was greater in Smad3-overexpressing B-lymphocytes compared to wild type cells, and was not observed in lymphocytes overexpressing dominant-negative Smad3. TGFβ promoted mitochondrial Bim accumulation, heterodimerization with Bcl-2 and loss of the mitochondrial membrane potential. These observations are consistent with the role of Bim as an activator of the intrinsic pathway of apoptosis. We further demonstrated that stimulation of the pro-survival CD40 receptor was capable of inhibiting TGFβ-mediated Bim expression and cell death in WEHI 231 cells concomitant with its rescue of the cells from apoptosis. Therefore, Bim appeared to be a convergence point of cell fate determination in B lymphocytes. Our study was also the first demonstration that addition, and not withdrawal, of a cytokine results in enhanced Bim expression. Previous studies documented a clear role for Bim in mediating growth factor withdrawal or stress-induced apoptosis. Withdrawal of IL-2 (Stahl et al., 2001) or IL-3 (Shinjyo et al., 2001) from cytokine-dependent hematopoietic cells resulted in an upregulation of Bim expression with an associated induction of apoptosis. Similar results were obtained when NGF was withdrawn from cultured neuronal cells (Putcha et al., 2001; Whitfield et al., 2001).

The induction of Bim during TGFβ-induced apoptosis has been demonstrated more recently in the gastric epithelial cell line SNU16 (Ohgushi et al., 2005), AML12 hepatocytes and NMuMG mammary epithelial cells (Ramjaun et al., 2007) and Hep3B hepatoma cells (Yu et al., 2008). We (Wildey and Howe, 2009) and others (Ramjaun et al., 2007, Yu et al., 2008) have demonstrated significant inhibition of TGFβ-induced apoptosis in hepatic cells by RNAi-mediated knockdown of Bim expression, confirming a central role for Bim in mediating TGFβ-induced apoptosis. Furthermore, RNA interference of Smad3 and Smad4 expression, but not Smad2, inhibited both TGFβ-induced Bim protein and mRNA expression, confirming a role for the canonical Smad signaling pathway in mediating TGFβ-induced apoptosis. This idea was supported by experiments showing that Smad7 overexpression inhibited both TGFβ-induced Bim expression and apoptosis in gastric epithelial cells. Interestingly, Bim expression and apoptosis was also reduced by RNAi-mediated knockdown of p38 in AML12 hepatocytes and NMuMG mammary epithelial cells, supporting the results of the studies described above for a role of this MAPK pathway in TGFβ-induced apoptosis.
A prominent role for Bim as a mediator of apoptosis is gaining in importance (Kim et al., 2006). Unlike most BOP, Bim can interact with all pro-survival Bcl-2 proteins with high affinity, and is one of the few BH3-only proteins that can directly activate Bax and Bak, the executioners of the mitochondrial apoptotic pathway. The diversity of physiological processes that utilize Bim as an apoptotic mediator can be appreciated from the array of pathological phenotypes observed in homozygous Bim knockout mice. The original study using Bim knockout mice indicated that Bim was required for BCR-induced apoptosis in immature and mature B cells, and for the negative selection of autoreactive B cells (Bouillet et al., 1999; Strasser, 2005). These mice succumb to autoimmune kidney disease. Bim knockout mice have also been used to demonstrate a role for Bim in T cell death (Hughes et al., 2008) and fatal hepatitis mediated by TNE (Tumor necrosis factor) (Kaufmann et al., 2009). Similar studies indicate that Bim-mediated apoptosis may be involved in ductal morphogenesis during mammary development (Mailleux et al., 2007), programmed death in memory B cells (Fischer et al., 2007) and gastric epithelial cell proliferation (Ohgushi et al., 2005). The diversity of phenotypes observed in Bim knockout mice, from autoimmune disease to developmental abnormalities, makes it reasonable to suggest that Bim may play a critical role in the inflammatory and developmental phenotypes observed in the Tgfβ knockout mice.

**Pathways of Bim regulation**

Studies of BH3-only proteins have demonstrated that their apoptotic function may be regulated by several different mechanisms including subcellular localization, proteolytic cleavage, phosphorylation and transcription (Puthalakath and Strasser, 2002). Remarkably, all of these cellular control mechanisms apply to Bim regulation and TGFβ has been shown to utilize all of them. There are three major isoforms of Bim (BimEL, BimL, and BimS) that are generated by alternative splicing of a single transcript (Bouillet et al., 2001; O'Connor et al., 1998) (see Figure 3). BimEL is the predominant isoform observed in cells. BimEL and BimL are bound and sequestered to the microtubular motor complex via a dynein light chain binding domain, or 'DKS' domain, that is absent from BimS (the lack of sequestration of BimS is thought to be the reason for its greater apoptotic potency). Following an apoptotic stimulus, the release of BimEL and BimL from microtubules allows their interaction with pro-survival Bcl-2 family members at the mitochondria, resulting in Bax activation (Puthalakath et al., 1999). Two studies have demonstrated that the association of Bim with the cytoskeleton is regulated. First, JNK activation in response to UV radiation causes Bim phosphorylation at a threonine residue (T116) near the 'DKS' domain, leading to the release of Bim from the dynein motor complex and activation of Bax (Lei and Davis, 2003). Second, induction of the stress protein Gadd45a disrupts cytoskeletal stability, leading to Bim accumulation at the mitochondria and the release of cytochrome c (Tong et al., 2005).

![Figure 3: Bim isoforms. The pink box designates the 'EL' domain unique to BimEL that contains the critical serine (S69) phosphorylated by ERK. The blue box designates the 'L' domain present in BimEL and BimS that contains the threonine (T116) phosphorylated by JNK. The caspase-cleaved aspartate (D13) is common to all isoforms. All numbering is for human Bim protein.](image-url)
The apoptotic function of Bim has also been shown to be regulated by caspases. Caspase activity stimulated by staurosporine, TNF-α, UV radiation or taxol cleaves BimEL in Jurkat T cells at the N-terminal Asp-13 residue common to all Bim isoforms (Chen and Zhou, 2004). Interestingly, caspase-cleaved BimEL exhibited increased binding to Bcl-2 and therefore increased apoptotic potency relative to full-length BimEL. Recently, a role for TGFβ in both caspase activation and Bim relocation has been demonstrated in Burkitt’s lymphoma cells (Clybouw et al., 2008; Schrantz et al., 2001). TGFβ triggered a p38-dependent activation of caspase 8, which cleaved the pro-survival protein Mcl-1. While this paradoxically led to a transfer of Bim from the mitochondria to the cytoskeleton, it was speculated that a transient increase in free Bim protein may be enough to activate the mitochondrial cell death pathway.

Cytokine-regulated phosphorylation of BimEL, which triggers its ubiquitin-mediated degradation through the proteasomal pathway, has recently emerged as a key regulatory mechanism controlling the apoptotic function of Bim. Initial reports of Bim phosphorylation relied on shifts in the mobility of Bim during electrophoresis (Shinjyo et al., 2001). In this way, phosphorylated forms of BimEL were demonstrated in many cell types in response to a wide array of pro-survival cytokines. Many of these studies also demonstrated that Bim phosphorylation could be blocked by the MEK/ERK pathway inhibitors PD98059 or U0126 (Biswas and Greene, 2002; Marani et al., 2004; Weston et al., 2003). Subsequent studies using Bim mutants with serine to alanine substitutions revealed that multiple ERK phosphorylation sites existed within Bim, all located within the ‘EL’ and ‘L’ domains (Harada et al., 2004; Luciano et al., 2003; Marani et al., 2004). Despite evidence that ERK phosphorylates Bim at multiple sites, two studies have demonstrated that a single mutation in Gst-BimEL fusion proteins at Ser69 of murine BimEL, or the equivalent Ser69 of human BimEL, is sufficient to completely abrogate phosphorylation by recombinant ERK in an in vitro kinase assay (Ley et al., 2003; Luciano et al., 2003). In addition, it was demonstrated that purified ERK could phosphorylate only BimEL, and not Bim, or Bims. Thus Ser69 in human BimEL is now generally regarded as the key regulatory site of ERK phosphorylation.

Bim phosphorylation was often shown to be associated with a time-dependent loss in total Bim protein. The loss of Bim protein was blocked by lactacystin and MG132 (Ley et al., 2003; Luciano et al., 2003; Marani et al., 2003; Weston et al., 2003), implicating the proteasomal pathway in Bim degradation. Furthermore, multiple ubiquitinated forms of Bim protein could be demonstrated in the presence of proteasomal inhibitors (Akiyama et al., 2003; Ley et al., 2003). A clear link between Bim phosphorylation and its subsequent ubiquitination and degradation was established using Bim phosphorylation mutants. Mutation of Ser69 in human BimEL to alanine was sufficient to abrogate ERK phosphorylation concomitant Bim degradation. Furthermore, mutation of this serine residue, either by itself or in combination with other ERK phosphorylation sites, dramatically increases the apoptotic potency of BimEL (Harada et al., 2004; Hübner et al., 2008). Therefore, regulation of the phosphorylation status of Ser69 in human BimEL might represent an important convergence point for multiple signaling pathways in the determination of cell fate.

In this regard, we have recently demonstrated that the TGFβ signaling pathway regulates BimEL phosphorylation indirectly via its ability to induce the ERK phosphatase DUSP4 (MKP2) (Ramesh et al., 2008). We showed that TGFβ stimulation of BaF/3 lymphocytes or AML12 hepatocytes results in a rapid inactivation of ERK activity, thereby blocking BimEL phosphorylation and degradation. Gene array analysis allowed us to identify the MAPK phosphatase DUSP4/MKP2 as an immediate early gene induced by TGFβ in AML12 cells. Overexpression of MKP2 modulated ERK-mediated Bim phosphorylation and apoptosis in the absence of TGFβ. TGFβ-stimulated Bim induction and apoptosis was reduced in B lymphocytes derived from MKP2 knockout mice as well as in Smad3 knockout mice. These results indicated that MKP2 mediates TGFβ-dependent apoptosis by linking Smad3 to ERK activity and mitochondrial apoptotic events.

Studies on the transcriptional control of Bim have largely focused on FOXO3, a member of the forkhead family of transcriptional regulators. Multiple reports have shown that inhibition of the PI3K/Akt pathway, either by growth factor withdrawal or by using chemical inhibitors such as LY294002, leads to the activation of FOXO3 and Bim expression (Dijkers et al., 2000; Stahl et al., 2002). Inhibition of Akt activates FOXO3 because active Akt normally phosphorylates FOXO3, causing it to be retained in the cytoplasm by 14-3-3 proteins and thereby unable to activate its target genes, such as Bim (Brunet et al., 1999). Forkhead binding sites have been identified in the Bim promoter that are specific for FOXO3 and activate Bim transcription (Gilley et al., 2003). Bim has also been shown to be an
essential mediator of endoplasmic reticular (ER) stress-induced apoptosis (Puthalakath et al., 2007). Two novel mechanisms were revealed, involving Bim dephosphorylation by protein phosphatase 2A, resulting in Bim stabilization; and direct CHOP-C/EBPa-mediated transcriptional co-activation of the Bim promoter within the first intron.

Recently, we demonstrated a direct transcriptional activation of the murine Bim promoter by TGFβ in AML12 hepatocytes (Wildey and Howe, 2009). We showed that TGFβ induces Bim protein and mRNA levels, and the expression of Bim is sufficient to induce cell death. Gene array results revealed that Runx1, a member of the Runx family of transcription factors, was induced by TGFβ, and mediated activation of the murine Bim promoter at a putative forkhead binding element. Interestingly, Runx1 does not bind directly to the identified forkhead binding element but rather binds to FOXO3, which occupies this site. Knockdown of Runx1 or FOXO3 protein levels by siRNA decreased TGFβ-mediated Bim expression. Thus, our results support a mechanism in which TGFβ stimulates Bim transcription by up-regulating Runx1 expression, which binds FOXO3, and the two cooperate in the transcriptional induction of Bim. Runx proteins have also been implicated in TGFβ-mediated growth arrest and apoptosis of gastric cancer cells (Yamamura et al., 2006; Yano et al., 2006). Gastric cancer cells expressing a dominant-negative form of Runx3 or antisense Runx3 are resistant to TGFβ-induced apoptosis and, more importantly, are also deficient in TGFβ-induced Bim expression. Runx3 also directly activates the Bim promoter in gastric cancer cells, as well as in mouse embryonic fibroblasts, but at sites different from that identified in our study. Taken together, these studies suggest that Runx and FOXO3 proteins may be common co-transcriptional activators of Bim expression in many cell types. More importantly, these studies demonstrate that TGFβ ensures apoptosis via a dual approach to upregulating Bim expression. TGFβ stabilizes existing Bim proteins through its immediate induction of the ERK phosphatase DUSP4/MKP2. More long-term induction of Bim proteins is achieved through direct activation of the Bim promoter. This dual mechanism is similar to that described above for Bim induction by ER stress, although each involves a unique set of mediators.

Bim degradation as a therapeutic target

The diverse array of cellular mechanisms and mediators of Bim regulation should be anticipated, given the ubiquitous nature and importance of Bim in the intrinsic cell death pathway. Similarly, it is easy to see how Bim could be a key mediator of the therapeutic actions of anti-cancer drugs. Imatinib (Gleevec) is a drug that inhibits the Bcr/Abl tyrosine kinase activity that drives uncontrolled cell proliferation in chronic myelogenous leukemia (CML) cells, thereby inducing apoptosis in these cells (Kaufmann, 2006). By inhibiting Bcr/Abl tyrosine kinase activity, imatinib also inhibits several downstream kinases, including ERK activity. Recently it has been demonstrated that Bim expression is increased during imatinib-induced apoptosis in part because of reduced Bim phosphorylation (Kuroda et al., 2006). Furthermore, it was shown that imatinib-induced apoptosis was reduced in myeloid progenitors derived from homozygous Bim knockout mice, demonstrating a key role for Bim expression in the apoptosis. Another study supported these results by showing that an optimal response to imatinib treatment in CML patients positively correlated with the level of Bim expression in their CML cells (San José-Eneriz et al., 2009). Interestingly, epigenetic downregulation of Bim expression was observed in some imatinib-resistant CML cells.

B-Raf is another upstream activator of the ERK pathway. Activating mutations in B-Raf are frequently found in solid tumors, including approximately 60% of all human melanomas. Two studies have shown that activating B-Raf mutations inhibit apoptosis in part by promoting ERK phosphorylation and inactivation of Bim (Cragg et al., 2008; Sheridan et al., 2008). Inhibitors of the ERK pathway promoted apoptosis in cells harboring B-Raf mutations and this was dependent on the dephosphorylation and upregulation of Bim. Similar inhibition of ERK activation triggered apoptosis in mitogen-stimulated T and B cells and was not observed in cells isolated from homozygous Bim knockout mice (O'Reilly et al., 2009).

The efficacy of ERK kinase inhibitors in promoting tumor cell death is enhanced by co-administration of ABT-737, a small molecule inhibitor of Bcl-2 pro-survival proteins (Cragg et al., 2008; Kuroda et al., 2006). ABT-737 is a BH3-mimetic compound, and binds with high-affinity to the hydrophobic groove of Bcl-2 pro-survival proteins and thereby displaces or prevents the binding of BH3-only proteins such as...
Bim (Deng et al., 2007; Kang and Reynolds, 2009). Thus, treatment with ABT-737 effectively increases the amount of free, non-sequestered Bim in the mitochondrial membrane to activate the apoptotic pathway. ABT-737 is particularly effective because many cancer cells avoid cell death by increasing their expression of pro-survival Bcl-2 proteins. Using a similar combinatorial drug approach, it would be of interest to determine if the efficacy of ERK kinase inhibitors could also be improved by co-administration of a small molecule stimulator of DUSP4/MKP2 ERK phosphatase activity. The development of such a drug, as yet undiscovered, would mimic the pro-apoptotic action of TGFβ, and might therefore represent another way to maintain the apoptotic potency of Bim.

Conclusion

These studies highlight the potential of Bim as an important therapeutic target for anti-cancer treatment regimens. Recently, the involvement of another kinase, Rsk, in the regulation of Bim phosphorylation and degradation has been reported (Dehan et al., 2009). It was shown that ERK phosphorylation of BimEL actually serves as a trigger for subsequent Rsk phosphorylation of BimEL at three downstream serine residues; Ser93/94/98. These Rsk-phosphorylated serine residues recruit the F-box protein β-TrCP1, a component of the Skip-Cullin-F-box ubiquitin ligase complex, to initiate Bim degradation via the proteasomal pathway. This discovery of a cooperative mechanism between two kinases to stimulate the Bim degradative pathway, as well as the identification of the specific ubiquitin ligase complex involved, allows the development of new inhibitors of Bim degradation that can used to harness the pro-apoptotic power of Bim against cancerous cell growth. Moreover, increased understanding of the Bim degradation pathway may reveal new avenues for TGFβ regulation.

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