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BCL2L11/BIM

A novel molecular link between autophagy and apoptosis

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In response to toxic stimuli, BCL2L11 (also known as BIM), a BH3-only protein, is released from its interaction with dynein light chain 1 (DYNLL1 also known as LC8) and can induce apoptosis by inactivating anti-apoptotic BCL2 proteins and by activating BAX-BAK1. Recently, we discovered that BCL2L11 interacts with BECN1 (Beclin 1), and that this interaction is facilitated by DYNLL1. BCL2L11 recruits BECN1 to microtubules by bridging BECN1 and DYNLL1, thereby inhibiting autophagy. In starvation conditions, BCL2L11 is phosphorylated by MAPK8/JNK and this phosphorylation abolishes the BCL2L11-DYNLL1 interaction, allowing dissociation of BCL2L11 and BECN1, thereby ameliorating autophagy inhibition. This finding demonstrates a novel function of BIM beyond its roles in apoptosis, highlighting the crosstalk between autophagy and apoptosis, and suggests that BCL2L11's dual effects in inhibiting autophagy and promoting apoptosis may have important roles in disease pathogenesis.

Keywords: BIM, autophagy, apoptosis, BH-3 domain, BECN1

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Macroautophagy (hereafter autophagy), is a bulk degradation system that mediates clearance of long-lived cytoplasmic proteins, certain pathogens and damaged organelles. Autophagosome formation begins with a nucleation step, in which membranes first form phagophore assembly sites, which then expand to form completed autophagosomes. After autophagosomes fuse with lysosomes, the contents of the autophagosomes are degraded. Autophagy is thought to have prosurvival functions and can protect cells from proapoptotic stimuli.

Apoptosis is a form of programmed cell death mediated by caspase activation. Apoptosis can be induced by intrinsic and extrinsic pathways, based on the upstream stimuli that can activate the process. The intrinsic, mitochondrion-dependent, pathway is regulated by BCL2-family proteins. In response to pro-apoptotic insults, BAX and BAK1 activate caspases to induce apoptosis. BH3-only proteins appear to kill cells by binding to BCL2 proteins to inhibit their abilities to restrain BAX-BAK1, or by binding and activating BAX-BAK1. As a BH3-only protein, BCL2L11 (also called BIM) can inhibit BCL2 proteins and activate BAX-BAK1 proteins. In healthy cells, BCL2L11 is sequestered by dynein light chain 1 to microtubules and remains inactive. In response to death stimuli, BCL2L11 is phosphorylated and released from DYNLL1 and can induce apoptosis.

Recently, we showed that BCL2L11/BIM inhibits autophagy independent of its proapoptotic function. BCL2L11 exists in three different splicing isoforms, namely BimEL (BCL2L11/BIM extra long, the major isoform), BimL (BCL2L11/BIM long), and BimS (BCL2L11/BIM short). We found that BimEL and BimL interact with BECN1, but only a very weak interaction is seen with BimS. Since BECN1 is a key autophagy protein, we tested if BCL2L11 regulated autophagy. Since overexpression of wild-type BCL2L11 may induce caspase activation, which will impair autophagy, we generated a BCL2L11 mutant, BCL2L11 L152E F159E (BimEE) that does not have apoptotic activity. Overexpression of BimEE or wild-type BCL2L11 inhibits

autophagosome formation and enhances the accumulation of autophagic substrates. This is associated with decreased numbers of PtdIns3P-associated vesicles, a phenotype regulated by BECN1 via its ability to stimulate the class 3 PtdIns3 kinase, PIK3C3 (also called VPS34). Consistent with these data, genetic knockdown of *Bcl2l11* enhances autophagosome formation and decreases autophagic substrate levels, and this is associated with an increase in the abundance of PtdIns3P-associated vesicles. We confirmed the inhibitory effects of BCL2L11 on autophagy in *Bcl2l11* knockout mice. These data suggest BCL2L11 inhibits autophagosome formation.

The effects of BCL2L11 on autophagy appear to be BECN1-dependent, since *Becn1* knockdown abolishes the effects of *Bcl2l11* knockdown on autophagy. Interestingly, we revealed that starvation dramatically reduces the BCL2L11-BECN1 interaction by phosphorylating BCL2L11 at threonine 116 (T116). We found that mutations in BCL2L11 in the DYNLL1-binding domain that reduce the strength of the DYNLL1-BCL2L11 binding also impair the BCL2L11-BECN1 interaction, even though these mutated residues are outside the domain critical for BECN1 binding. With in vitro binding and DYNLL1 knockdown assays, we concluded that DYNLL1-BCL2L11-BECN1 forms a complex and that DYNLL1 facilitates the BCL2L11-BECN1 interaction. This effect is likely due to DYNLL1 altering the conformation of BCL2L11, thereby enhancing its ability to interact with BECN1. Phosphorylation of BCL2L11 at T116 disrupts the DYNLL1-BCL2L11 interaction, thereby weakening the BCL2L11-BECN1 interaction.

These findings eventually led us to discover that BCL2L11 inhibits autophagy by sequestering BECN1 to DYNLL1 on microtubules away from the presumed site of BECN1 autophagic activity at the ER. In normal conditions, DYNLL1-bound BCL2L11 inhibits autophagosome formation by recruiting much of the BECN1 complex to microtubules. However, in stress conditions, BCL2L11 is phosphorylated and freed from DYNLL1, allowing BECN1 to be released from microtubules and moved to the ER.

Phylogenetic analyses suggest that different BH3-only proteins may have acquired BH3 motifs by convergent evolution and may have biological functions in addition to apoptosis regulation. BCL2 binds BECN1 and inhibits autophagy, whereas the pro-apoptotic BH3-only proteins, BAD and BNIP3, disrupt the interaction between BCL2 and BECN1 to enhance autophagosome formation. In contrast, our data reveal that BCL2L11, another BH3-only protein, interacts with BECN1 (which is a BH3-only protein) and inhibits autophagy. BCL2L11 is the first identified molecule possessing both anti-autophagy and pro-apoptotic effects, suggesting that the dual effects of BCL2L11 may be important for its critical roles in development and disease pathogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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