

Bcl-2:Beclin1 Complex and Bh3 Mimetics

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Abstract- Autophagy is a complex, physiological process responsible for degradation and recycle of cellular components, thus ensuring cell survival during stress conditions. But during high and prolonged stress, it enhances autophagic cell death. Apoptosis is another, yet primary, programmed cell death in which the caspases execute cell death. Cancer is an uncontrolled growth of cells, involving metastasis and angiogenesis and is devoid of the mechanism of autophagy and apoptosis. Bcl-2 is an anti-apoptotic protein, known to regulate both apoptosis and autophagy. It binds to Bax/Bak (pro-apoptotic proteins), thereby downregulate their function and prevent apoptosis. Similarly, it interacts with Beclin1 (an autophagic protein), forming Bcl-2:Beclin1 complex and thereby inactivating autophagy. Since this interaction with Beclin1 is via BH3-only domain, the researchers have focused on the development of BH3 mimetics, in order to design better chemotherapeutics for cancer treatment.

Keywords- Beclin1, Bcl-2, PAS, Ulk-1, mTOR, Ambra-1, HMGB-1, Gossypol, Venetoclax.

REVIEW

Beclin1 is a mammalian homologue of yeast Atg6 (Autophagy related 6) protein (1). It is encoded by BECN1 gene and is known to assemble PAS (Pre Autophagosomal Structure) by recruiting essential Atg proteins, and forming the core complex (Beclin1+Vps34+Vps15) (2). Bcl-2 (B-cell lymphoma-2) is an anti-apoptotic protein of the Bcl-2 family of proteins. It has BH1 and BH2 domains (sometimes BH3 and BH4 too) (3) and interact with BH3-only domain of Beclin1, forming Bcl-2:Beclin1 complex. This complex results in the inhibition of PAS assembly, thus inhibiting autophagy (4).

promotes cell death and Non-protective/ cytotoxic autophagy, that may contribute to autophagic cell death (6). Beclin1 is a tumor suppressor gene. Reduced expression (by heterozygous disruption) of BECN1 gene reduces the autophagy of damaged organelles and tumorigenesis (1), whereas the over-expression of BECN1 inhibits tumorigenesis (7). Clinical studies revealed that BECN1 is lost in 40% to 75% of breast and ovarian cancers (1); and Bcl-2 expression is upregulated in 60% of the cancer patients (8). Unlike patients with -ve Bcl-2 lymphoma, the patients with +ve Bcl-2 lymphoma show poor response to chemotherapies (8).

MCF-7 (Michigan Cancer Foundation) cell line is a breast cancer cell line, derived from 69-year old lady (9). About 2/3rd of the abstracts on cancer include MCF-7, T-47D, MDA-MB-231 cell lines (10). RNA-interference downregulated Bcl-2 and induced about 50% cell death in MCF-7 cells. TUNEL assay (Terminal deoxynucleotidyl transferase dUTP Nick End Labeling) determined that apoptosis contributed for only 11% of cell death in 96 hrs, thereby supporting the fact that autophagic cell death has dominated here (11). HT-29 cell line is a human colon cancer cell line (12) that donot express detectable levels of Bcl-2. When the cell line was transfected with Bcl-2 expression vectors, starvation-induced autophagy was inhibited by disruption of Beclin1 / Vsp34 complex. Also, when the HeLa cells, that express Bcl-2, were transfected with BCL-2 siRNA vectors, they showed increased starvation-induced autophagy (13). Etoposide and Doxorubicin are the cancer treating drugs, injected directly into the veins. They enhance either apoptotic or autophagic

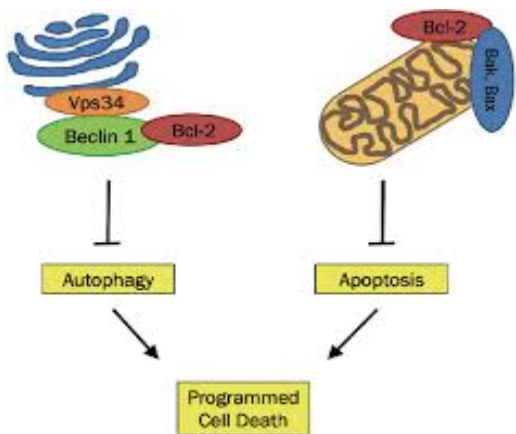


Figure 1. Bcl-2 binds to Beclin1, prevents assembly of PAS and inhibits autophagy. On the other hand, when it binds to Bak/Bax, it prevents apoptosis (5). Tumor biology reveals the dual functions of autophagy: Protective autophagy, where autophagy activation

cell death. With Bcl-2 inhibited via siRNA knockdown in breast cancer cells, study reveals that Beclin1 has shown increased expression (14).

Studies have revealed that cellular localization plays a great role in determining inhibition of autophagy by Bcl-2. The Bcl-2: Beclin1 complex is formed at both Endoplasmic reticulum and Mitochondria, but the one at ER only inhibits autophagy (13). Bcl-2 is known to first form complex with NAF-1 (Nutrient- deprivation autophagy factor-1) at ER. This binding of Bcl-2 with NAF-1 or any other regulatory protein is independent of BH3 domain. Then NAF-1 binded Bcl-2 interacts with Beclin1 and inhibits Beclin1 mediated autophagy (15).

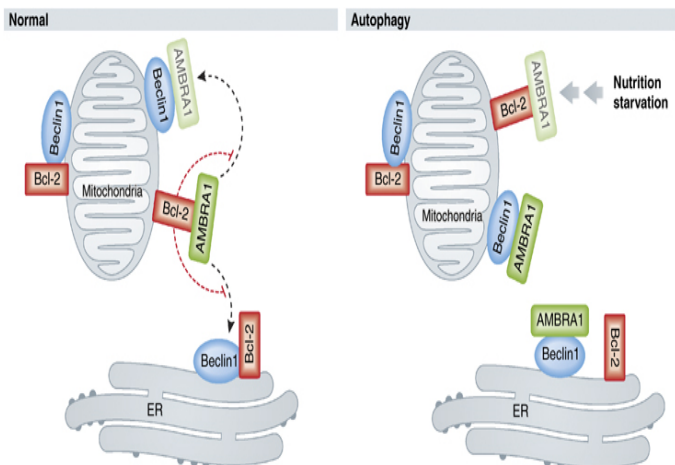
AMBRA-1 (Activating Molecule in Beclin-1 Regulated Autophagy) interacts with both Beclin1 and Bcl-2. Its interaction with Bcl-2 occurs at mitochondria and is downregulated during autophagy (16). At ER, AMBRA-1 interacts with Beclin-1 (17). Under growing conditions, mTOR (mammalian Target Of Rapamycin) phosphorylates AMBRA-1 and under stress, mTOR gets inactivated, thereby de-phosphorylating AMBRA-1. AMBRA-1 then binds to TRAF-6 (TNF receptor associated factor 6) and activates ULK-1 (18). ULK-1 (unc-51 like kinase; unc is mammalian homologue of yeast Atg-1) and PI3K-III Kinase (Class III Phosphoinositide-3 kinase) are the major protein complexes in mammalian cells, responsible for autophagy initiation and autophagosome structure (19). Autophagic stimuli (especially aminoacid withdrawal) thus allow ULK-1 to phosphorylate AMBRA-1 and dissociate it from Dynein Motor complex. Then AMBRA-1 translocates to ER, where it binds to Beclin1 and induces autophagy, by forming autophagy initiation complex (17).

Figure 2. Under normal conditions (left), AMBRA-1 appears to act as a negative regulator of autophagy. It binds to Beclin1 and bcl-2 at mitochondria. And during starvation (right), it disrupts Bcl-2/Beclin1 complex by binding to Beclin1 and thus, mediates autophagy induction (20).

BNIP3 (Bcl-2/ adenovirus E1B 19KDa protein-interacting protein 3) is a pro-apoptotic protein with BH3 domain (21). It interacts with Bcl-2 and disrupts Bcl-2:Beclin1 complex and induces autophagy (22). DAPK-1 (Death-Associated Protein Kinase-1) phosphorylates BH3 domain of Beclin1, by which Beclin1 is unable to form complex with Bcl-2 and thus induces autophagy (23). HMGB1 (High Mobility Group Box 1 protein) is a group of chromatin proteins like histones. They belong to the DAMP (Damage-Associated Molecular Pattern) group that initiate or perpetuate a non-infectious but inflammatory response (24). HMGB1 interact with nucleosomes, transcription factors and histones (25). Normally, it competes with Bcl-2 for interaction with Beclin1 and orients Beclin1 to autophagosome (26). During stress, it is regulated by transcription factors and microRNAs (miRNAs). It is the direct target for miR34A. miR34A, miR22 and miR-let-7f-1 inhibit HMGB1 and thus, inhibit autophagy in retinoblastoma, osteosarcoma and medulla blastoma cells respectively (27).

Another regulatory mechanism by JNK-1 occurs during stress. JNK-1 (c-Jun N-terminal Kinases) belong to the MAPK (Mitogen Activated Protein Kinase) family (28). JNK-1 phosphorylates c-Jun and subsequently Bcl-2, and leads to either apoptosis and autophagy (29).

Caspase 8, recruited by death receptors, act by either activating downstream caspases or by cleaving BH3-Bcl-2 interacting protein, thereby triggering the cytochrome C release and thus, causing apoptosis (30). It cleaves Atg6/Beclin1 protein and this cleaved Beclin1 remains cytosolic and unfunctional (31). But studies have revealed that in IL-3 (Interleukin-3) deprived cells, the C-terminus of cleaved Beclin1 triggers the release of apoptotic factors from mitochondria, thus causing apoptosis (32).



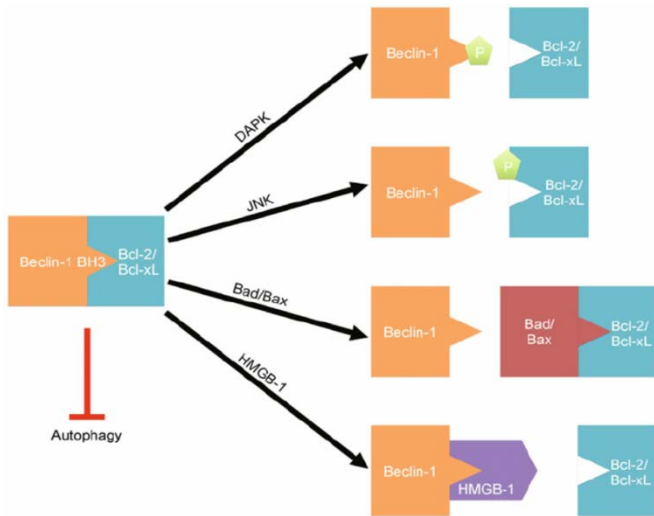


Figure 3. Beclin1 is bound by Bcl-2/Bcl-xL under normal conditions, thereby preventing autophagy. But during stress, several regulatory mechanisms disrupt this complex to promote autophagy induction. These mechanisms include phosphorylation of BH3-only domain of Beclin1 by DAPK, JNK-mediated phosphorylation of Bcl-2, competition of Bad/Bax with Beclin1 for Bcl-2/Bcl-xL, and binding of DAMP protein molecule HMGB1 to Beclin1 (33).

These regulatory mechanisms reveal the role of Bcl-2:Beclin1 complex interaction and their dissociation. The possible way to disrupt this complex is via BH3 mimetics, that can bind to Bcl-2, thereby allowing Beclin1 to induce autophagic cell death (34, 35).

Gossypol, a natural phenol derived from the cotton plant (genus *Gossypium*), is believed to have pro-apoptotic properties and act as a natural BH3 mimetic (36). Studies using apoptotic inhibitor, Z-VAD-FMK (Carbobenzoxy-valyl-alanyl-aspartyl-[o-methyl]-fluoromethylketone) and autophagy inhibitor, 3-MA (3-methyladenine) have shown that prostate cancers with high levels of Bcl-2 can be treated by (-)-gossypol with 60% of autophagic cell death, whereas the prostate cancer with low levels of Bcl-2 can undergo 80% apoptotic cell death (37, 38). It induces JNK signaling, phosphorylates Bcl-2, disrupts Bcl-2:Beclin1 complex and induces autophagy (5). But due to stoichiometric abundances of Bcl-2 during its overexpression, the effect of (-)-gossypol decreases and results in decreased autophagy (37).

Although autophagy is regulated by BH3 mimetics, they also activate BAX/BAK pathways. BH3 mimetics interact directly with BAX and/or BAK, induce their oligomerization and activation, causing apoptosis (39).

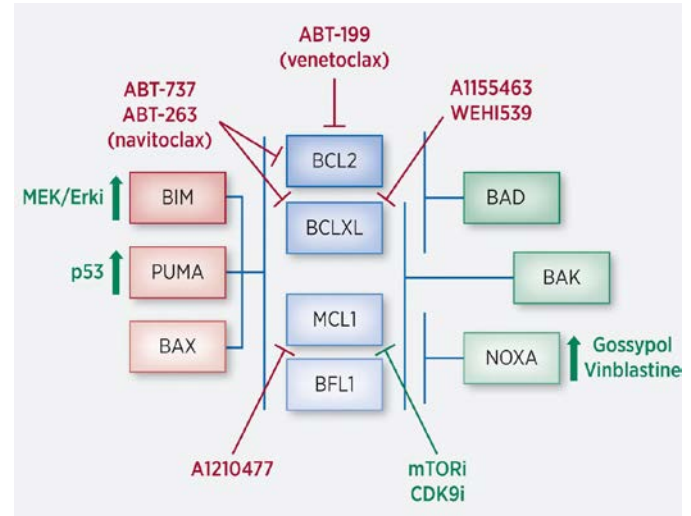


Figure 4. The interactions among pro- and anti-apoptotic proteins, and BH3 mimetics. Centre (blue) represents the anti-apoptotic proteins, left (red) represents pro-apoptotic proteins that can bind to any of the anti-apoptotic proteins and right (green) represents pro-apoptotic proteins with more restrictive binding capacity. Red text represents the BH3 mimetics and their action, while green text tells about the modifiers of pro-apoptotic proteins (40).

ABT-737 is the first compound, discovered as bonafide BH3 mimetic (41). It exhibits high affinity for Bcl-2, Bcl-xL, Bcl-w and low affinity for Mcl-1, and acts in a Bax/Bak dependent manner (42, 43), acting as a Bcl-2/Bcl-xL antagonist (44). Navitoclax (ABT-263) has its binding profile similar to ABT-737 and disrupts interaction involving Bcl-2 and Bcl-xL. Therefore, it acts by causing Bax/Bak dependent apoptosis or Beclin1 mediated autophagy (45). Similarly, Venetoclax and WEHI-539 inhibits Bcl-xL (46), whereas A-1210477 inhibits Mcl-1 (47).

Recently, Fluorescence polarization-based high-throughput screening (FP-based HTS assay) of about 50,316 small compounds have classified Beclin1 mimetics too as novel autophagy inducers (48).

CONCLUSION

Many BH3 mimetics and recently, Beclin1 mimetics have been developed. They are effective in either inducing autophagy or apoptosis. The cellular localization of interactions contribute to the mimetics development, as the interaction of Bcl-2 with BH3-only domain at ER is more effective than at mitochondria. The working of anti-cancer drugs/ BH3 mimetics/ Beclin1 mimetics is dependent on

the Bcl-2 expression; its overexpression decreases the effect of these drugs. Since different cancers involve varying anti-apoptotic Bcl-2 expression, different drugs can be designed and developed accordingly and can be taken into trial.

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