

## MOLECULE PAGE

# BECLIN 1

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Version 1.0, December 12, 2012 [DOI: 10.3998/panc.2012.16]

**Gene Symbol:** [BECN1](#)

**Other Names:** ATG6, Vps30

### 1. General Function

Beclin 1/ATG6/Vps30 (UniProtKB/Swiss-Prot Q14457) is a 450 amino-acids length protein, with three domains; BH3 (aminoacid 114 to 123), Coiled Coil domain (CCD; aminoacid 144 to 269) and the Evolutionarily Conserved Domain (ECD; aminoacid 244 to 337). BH3 proteins are part of the Bcl-2 family; they are pro-apoptotic damage sensors that play an important role in protecting against cancer (1). The BH3-only domain of Beclin 1 can interact with Bcl-2 and Bcl-X<sub>L</sub> (2;3). Both cellular and viral Bcl-2 (vBcl-2), or more specifically ER-targeted Bcl-2, inhibit Beclin 1-dependent autophagy by interfering with the Beclin 1-PtdIns 3-kinase interaction (PI3K) and the Beclin 1-associated PI3K activity (3,4). The interaction between Bcl-2 and Beclin 1 is greatly reduced upon starvation, which suggests that the dissociation of Bcl-2 from Beclin1 is important for activating autophagy. We demonstrated that, VMP1 (Vacuole Membrane Protein 1) displaces

Bcl-2 from Beclin 1, partitioning Beclin 1 to the autophagic pathway (Molejon et al., Sci Rep. 2012; *In press*).

The ECD is essential for Beclin 1 mediated autophagy and to inhibit tumorigenesis. Beclin 1 also contains a short leucine-rich amino acid sequence that is responsible for its efficient nuclear export signal (NES) (5). Mutations of the Beclin 1 NES interfere with its ability to promote nutrient deprivation-induced autophagy and suppress tumorigenesis. PI3KC3/Vps34 interacts with the ECD and CCD domains, while the activating molecule in Beclin 1-regulated autophagy (Ambra1)/UV radiation resistance-associated gene (UVRAG)/Atg14L interact with the CCD domain. The CCD, a universal oligomerization domain, mediates Beclin 1 self-interaction and dimer formation *in vivo* and *in vitro*. The amino terminus binds less effectively than the CCD to contribute to Beclin 1 self-assembly.



**Figure 1.** Schematic representations of domains of human Beclin 1 and the Bcl-2-Beclin 1-PtdIns 3-kinase-UVRAG multiprotein complex. Beclin 1 has a BH3 domain (amino acids 114-123), a central coiled-coil domain (CCD, amino acids 144-269) and an evolutionarily conserved domain (ECD, amino acids 244-337). VMP1 and Bcl-2 interacts with the BH3 domain of Beclin 1, UVRAG with the CCD, and the class III PtdIns 3-kinase with the ECD and CCD.

Beclin 1, through its BH3 domain, binds to VMP1-coxyl-terminus domain (named: Autophagy related domain (AtgD)) promoting autophagy (6; Molejon, *Sci Rep.* 2012; *In press*). This interaction promotes Beclin1-hVps34 complex, which is responsible for autophagic vesicle nucleation in mammals (7,8). Also, Beclin1-BH3 interacts with Bcl-2 and this interaction leads to inhibition of autophagy by interfering with the formation and activity of the autophagy promoter complex, Beclin 1-hVps34 (4). Mutations impairing VMP1-Beclin1 interaction lead to inhibition of the autophagic process in pancreatic human cells (Molejon et al., *Sci Rep.* 2012; *In press*).

#### **a. Beclin 1 is a mammalian tumor suppressor**

The autophagy function of Atg6/Beclin 1 is highly conserved throughout eukaryotic evolution. In addition to Beclin 1's role in autophagy, genetic knockdown or knockout studies of *beclin 1* have demonstrated its role as a tumor suppressor gene. In humans, monoallelic deletions of *beclin 1* are frequently observed in sporadic breast, ovarian and prostate carcinoma (9).

Bcl-2 is an anti-apoptotic protein (10), and Beclin 1 was first identified as a Bcl-2-interacting protein in a yeast two-hybrid screen (8,11). The *beclin 1* gene is monoallelically deleted in up to 75% of ovarian, 50% of breast and 40% of prostate cancers (12). Decreased expression of Beclin1 is also observed in other types of cancers including human brain tumors (13) and cervical cell

carcinoma (14). Expression of Beclin 1 in human MCF7 breast carcinoma cells promotes autophagy and inhibits *in vitro* clonogenicity and tumorigenesis in nude mice (7). *Beclin 1* is a haploinsufficient tumor suppressor gene in mice (15, 16), sharing 98% identity with human *beclin 1*. Homozygous *beclin 1*<sup>-/-</sup> mice die in early embryogenesis, and heterozygous *beclin 1*<sup>+/-</sup> mice have reduced autophagy levels and increased incidence of spontaneous tumors, which, together with the *in vitro* data, establishes a clear role for autophagy, and Beclin 1, in tumor suppression.

Beclin 1 levels appear to be one of the critical factors that affect the induction of autophagy. As indicated above, *beclin 1* is haploinsufficient, and various cancer cells show decreased levels of Beclin 1 (17). The anti-cancer drug tamoxifen may work in part by increasing expression of this protein (18). More evidence also supports the importance of Beclin 1 levels in autophagy regulation. For example, ceramide is thought to play a role in apoptosis (19, 20). Altered levels of Beclin 1 are also seen in situations other than cancer that involve autophagy; high levels of Beclin 1 are associated with neurons at the site of traumatic brain injury (21), whereas inhibition of Beclin 1 expression protects against cell death due to ischemia/reperfusion (22). Finally, a recent elegant work showed that Akt-mediated phosphorylation of Beclin 1 functions in autophagy inhibition and oncogenesis (23).

### ***b. Induction of autophagy by Beclin 1- PI3K Complex***

Although more Beclin 1 binding proteins and complexes are being identified in mammals, each of the individual complexes seems to recycle various elements derived from other cellular processes. Beclin 1 (Atg6/Vps30), PI3KC3/Vps34 and Vps15 have been predicted to regulate autophagy in a similar manner to yeast. Phosphatidylinositol 3-phosphate (PI3P) is essential for canonical autophagosome formation. Vacuolar protein sorting protein 34 (Vps34) and class III PI3K (hVps34) produce PI3P for autophagy in yeast and mammals, respectively. In yeast, Vps34 forms two distinct protein complexes: Complex I consist of Atg14, Atg6/Vps30, Vps15, and Vps34, whereas complex II is composed of Vps38, Atg6/Vps30, Vps15, and Vps34 (8). Only complex I functions in autophagy, whereas complex II is required for vacuolar protein sorting. The two PtdIns 3-kinase complexes contain three common subunits: the PtdIns 3-kinase enzyme Vps34, the regulatory protein Vps15 and Atg6/Vps30. In addition, each complex has a specific factor, Atg14 for complex I and Vps38 for complex II. Atg14 directs complex I to the phagophore assembly site, also termed the pre-autophagosomal structure (PAS). An autophagosome formation-specific PI3K complex has also been identified in mammals recently. It consists of Atg14L (also known as Atg14 and Barkor), Beclin 1 (Atg6 homolog), hVps15, and hVps34 (24-26). Mammals have at least two other stable hVps34 complexes, which play roles in endosomal transport and autophagosome-lysosome fusion by including UVRAG (UV irradiation resistance-associated gene) and Rubicon (Run domain protein as Beclin 1 interacting and cysteine-rich containing) as subunits instead of Atg14L (one complex contains UVRAG alone, whereas the other contains both UVRAG and the negative regulator Rubicon) (25; 26). Mammalian cells also possess other Beclin 1 binding proteins including Bcl-2, AMBRA1, Bif-1

(also known as Endophilin B1), and the pancreatitis associated transmembrane protein VMP1 (6,27; Molejon et al., *Sci Rep.* 2012; *In press*). In addition to PI3K, phosphatidylinositol 3-phosphatases including myotubularin-related phosphatase 3 (MTMR3) and Jumpy (MTMR14) are implicated in autophagy (28, 29). They negatively regulate autophagosome formation and autophagosome size. Therefore, the balance between PI3K and phosphatidylinositol 3-phosphatase determines autophagy initiation mediating local PI3P levels.

### ***c. Inhibition of autophagy by Beclin 1–Bcl-2/Bcl-X<sub>L</sub> complexes***

Mutations of either the BH3-only domain within Beclin 1, or the BH3 receptor domain within Bcl-2 or Bcl-X<sub>L</sub>, disrupted the Beclin 1–Bcl-2 complex, resulting in the stimulation of autophagy. Bcl-2 or Bcl-X<sub>L</sub> reduces the pro-autophagic activity of Beclin 1 (2, 4). Interestingly, ER-localized Bcl-2, but not mitochondrial-localized Bcl-2, inhibits autophagy (30), which is consistent with the older notion that ER-associated class III PI3K activity may be crucial in the nucleation of autophagosome formation.

Different mechanisms have been described to regulate dissociation of Beclin 1 and Bcl-2/Bcl-X<sub>L</sub> during autophagy in mammalian cells. These include: (A) competitive displacement of the Beclin 1 BH3 domain by other Bcl-2 family proteins. The interaction between Beclin 1 and the anti-apoptotic proteins is inhibited by tBid, Bad and BNIP3, but not by Bax and Bak (30). Moreover, the pro-apoptotic BH3-only proteins such as BNIP3, Bad, Noxa, Puma, BimEL and Bik all induce autophagy (31). (B) JNK1 or ERK-mediated phosphorylation of Bcl-2 (32; 33) or DAPK-mediated phosphorylation of Beclin 1 (34). (C) Competitive displacement of Bcl-2 by other Beclin 1-binding proteins such as HMGB1, UVRAG, Atg14L/Barkor or VMP1; this likely initiates a program of heightened anti-apoptotic state, promotes autophagy, and ultimately

protects the cell during cell stress. (D) NAF-1 (nutrient-deprivation autophagy factor-1) dysfunction. NAF-1 is a component of the inositol-1,4,5 trisphosphate (PI3P) receptor complex. NAF-1 binds Bcl-2 and this interaction is independent of a BH3 domain, but depends at least in part on its redox sensitive CDGSH iron/sulphur-binding domain (36). ROS are important signaling molecules that initiate autophagy. (E) Beclin 1 self-interaction: Beclin 1 can form large homo-oligomers, which may provide a platform for further protein-protein interactions and displacement of Bcl-2 or Bcl-XL.

## **2. Beclin 1 in Pancreas**

Beclin 1 is a VMP1 partner in pancreatic cell autophagy. VMP1-triggered autophagy is induced *in vivo* during experimental acute pancreatitis, where it is associated with Beclin1 and LC3 in the membrane of autophagosomes (6). We have reported experimental data indicating that VMP1 interacts with Beclin 1. We found the interaction of VMP1 with endogenous Beclin 1 in VMP1-expressing cells and the interaction of both endogenous proteins in rapamycin-induced autophagic cells. VMP1-Beclin 1 direct interaction was confirmed using recombinant peptides. Moreover, VMP1-Atg domain has proved to be essential for VMP1-induced autophagy, because the VMP1-defective mutant lacking its c-terminus domain -VMP1<sup>ΔAtgD</sup>- expression failed to induce LC3 recruitment (6). Our data have also shown the co-localization of transient Beclin 1 and LC3 in VMP1-induced vesicles, and this triple co-localization was abolished when cells were transfected with the defective mutant VMP1<sup>ΔAtgD</sup>. On the other hand, VMP1 expression failed to induce autophagy when it was expressed in low-Beclin 1, MCF7 cells. These findings suggest that VMP1-induced autophagy probably involves the interaction with Beclin 1. Experimental animal models have shown VMP1-Beclin 1 colocalization. VMP1-EGFP co-localized with endogenous Beclin 1 in

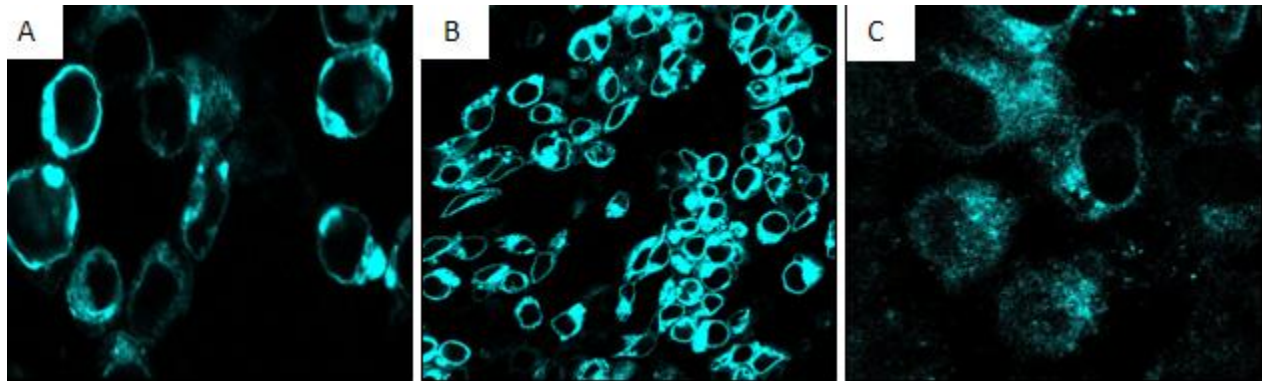
pancreas from transgenic mice, and both endogenous proteins co-localized in tissue undergoing pancreatitis-induced autophagy (6). Deletion of the suppressor domain (SD) results in the loss of distinct InsP<sub>3</sub> affinities between the isoforms and a 10-100 fold increase in InsP<sub>3</sub> affinity (19). Despite an increased InsP<sub>3</sub> binding affinity, deletion of the SD also results in the loss of channel activity, indicating that the SD is required for inducing InsP<sub>3</sub>R activation and Ca<sup>2+</sup> release. Based on hydropathy plots, the TMD is similar in structure to that of RyRs and voltage gated K<sup>+</sup>, Na<sup>+</sup> and Ca<sup>2+</sup> channels and constitutes 6 putative transmembrane regions (TM1-6) (12). The TMD is responsible for the ER targeting (33, 35) and for the oligomerization of the InsP<sub>3</sub>R into tetramers, which occurs co-translationally (20). Lastly, TM5 and 6 forms the pore through which Ca<sup>2+</sup> is conducted (37). The loop between TM5 and 6 contains a selectivity filter (GVGD; similar to the super family of cation selective channels) that provides some degree of cation selectivity to the InsP<sub>3</sub>R (14). However, it is poorly Ca<sup>2+</sup> selective and allows conduction of monovalent cations (Ca<sup>2+</sup>:K<sup>+</sup> = 6:1). In fact, it is believed that “functional” Ca<sup>2+</sup> selectivity of InsP<sub>3</sub>R is primarily determined by virtue of SERCA being a highly selective Ca<sup>2+</sup> pump and Ca<sup>2+</sup> being by the far the most abundant cation in the ER. To “gate” and open the channel, evidence suggest that the SD interacts with the cytosolic loop between TM4-5, and that InsP<sub>3</sub> binding results in a conformational change that moves TM5 away from TM6 and opens the channel (38). The CT tail (last 160 AA) and the large (1700 AA) but less conserved modulatory domain contains putative binding sites for the numerous modulators of InsP<sub>3</sub>R activity (56). These modulators, which include Ca<sup>2+</sup>, ATP and PKA, all contribute in distinct ways to the differential Ca<sup>2+</sup> release profiles encoded by the 3 isoforms.

### **3. Tools for study of Beclin 1**

#### **a. cDNA clones**

Two human Beclin 1 clones are available from OriGene (Cat. #[SC117750](#) and #[SC324265](#)). Also OriGene offer a GFP-tagged clone

(Cat #[RG201629](#)), a Myc-DDK-tagged clone (Cat.#[RC201629](#)). None of these have been checked by us.



**Figure 2.** AR42J rat acinar cell (A); 293T Human Embryonic Kidney (B); and HeLa human cervical cancer cell line were transfected with the CFP-Beclin 1 expression plasmid. Confocal microscope image, inverted LSM Olympus FV1000, Buenos Aires, Argentina.

#### **b. Antibodies**

Rabbit antibody raised against Beclin 1 have been used to identify Beclin 1 by western blotting, IP, immunofluorescence (Sigma Aldrich Cat. B6186). We used this antibody for western blot, IP and immunofluorescence at a dilution of 1:100-1:500. We also used anti-Beclin 1 (Santa Cruz Biotechnology, Inc. Cat. sc-11427) with the SNAP i.d. system for western blot at a dilution of 1/500.

#### **c. Viral Vectors**

The human BECN1 cDNA was cloned into the pReceiver-Lv105 lentivirus vector (GeneCopoeia Cat. LP-M0768-Lv105-0200-S) (39).

#### **d. Mouse Models**

Beclin 1 is a novel Bcl-2-homology (BH)-3 domain only protein. The embryonic phenotype of Beclin 1 null mice is even more severe than that of other autophagy gene-deficient mice, which die in early embryonic development (E7.5 or earlier) with defects in proamniotic canal closure (37). Heterozygous *beclin 1*<sup>+/-</sup> mice have reduced autophagy activity and increased incidence of spontaneous tumors.

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