scription are still unknown. However, upregulation of Pax gene expression is clearly involved in rhabdomyosarcomagenesis. Pax3 is known to regulate c-met gene transcription, overexpression of which is occasionally seen in human RMS. Overexpression of HGF/SF in transgenic mice also caused RMS to develop in 4% of mice (Merlino and Helman, 1999). More recently, RMS is frequently observed in mice carrying overexpression of HGF/SF gene in an *Ink4a/Arf* null background (Sharp et al., 2002). These reports strongly suggest that rhabdomyosaromagenesis implicates Fos, Pax, c-met, and HGF/SF signaling pathways. Although the origin of RMS cells is assumed to be from myogenic cells such as satellite cells, recent work demonstrates existence of novel stem cells in muscle, which give rise to myogenic cells during muscle regeneration (Asakura et al., 2002; Polesskaya et al., 2003). Therefore, the novel stem cells in muscle such as side population (SP) cells may be a developmental origin for RMS cells, in which Fos/Pax/c-met pathway is involved. In the near future, finding the molecular mechanisms of Fos/Pax/c-met pathway involved in rhabdomyosarcomagenesis and the developmental origin of RMS would not only facilitate our understanding of the mechanisms of rhabdomyosarcomagenesis but also develop novel therapeutic applications for human RMS patients.

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**Selected reading**

Acquaviva, C., Bossis, G., Ferrara, P., Brockly, F., Jariel-Encontre, I., and Piechaczyk, M. (2002). Ann. N Y Acad. Sci. *973*, 426–434.

Asakura, A., Seale, P., Girgis-Gabardo, A., and Rudnicki, M.A. (2002). J. Cell Biol. *159*, 123–134.

Buckingham, M. (2001). Curr. Opin. Genet. Dev. *11*, 440–448.

Fleischmann, A., Jochum, W., Eferl, R., Witowsky, J., and Wagner, E.F. (2003). Cancer Cell *4*, this issue.

Khan, J., Bittner, M.L., Saal, L.H., Teichmann, U., Azorsa, D.O., Gooden, G.C., Pavan, W.J., Trent, J.M., and Meltzer, P.S. (1999). Proc. Natl. Acad. Sci. USA *96*, 13264–13269.

Kirkwood, T.B. (2002). Bioessays *24*, 577–579.

Merlino, G., and Helman, L.J. (1999). Oncogene *18*, 5340–5348.

Polesskaya, A., Seale, P., and Rudnicki, M.A. (2003). Cell *113*, 841–852.

Seale, P., Sabourin, L.A., Girgis-Gabardo, A., Mansouri, A., Gruss, P., and Rudnicki, M.A. (2000). Cell *102*, 777–786.

Sharp, R., Recio, J.A., Jhappan, C., Otsuka, T., Liu, S., Yu, Y., Liu, W., Anver, M., Navid, F., Helman, L.J., et al. (2002). Nat. Med. *8*, 1276–1280.

White, J.D., Rachel, C., Vermeulen, R., Davies, M., and Grounds, M.D. (2002). Int. J. Dev. Biol. *46*, 577–582.

# Defective autophagy leads to cancer

**Cellular proteins are degraded within two distinct compartments: the proteasome and the lysosome. Alterations in proteasomal degradation can contribute to carcinogenesis. In contrast, alterations in autophagic protein degradation through the lysosome have not been linked to cancer. Now two reports demonstrate that the autophagic gene, Beclin 1, is a haploinsufficient tumor suppressor gene. These new data suggest that autophagic degradation provides an important mechanism to prevent cellular transformation.**

The accumulation of individual proteins within eukaryotic cells reflects a tightly controlled balance between protein synthesis and degradation. Two major pathways for the degradation of proteins have been described: cytosolic degradation by the proteasome and autophagic degradation of proteins and organelles within the lysosome (Klionsky and Emr, 2000). Considerable evidence suggests that the proteasome is responsible for the regulated degradation of short-lived proteins involved in cell cycle control as well as proteins that participate in stress responses such as DNA damage-induced cell cycle arrest or adaptation to hypoxia. A number of examples linking alterations in proteasomal protein degradation to the pathogenesis of cancer exist (Pagano and Benmaamar, 2003). In contrast, alterations in autophagic degradation of cellular proteins has not been linked to the causation of cancer. Rather, autophagy has been demonstrated to be important in developmental/differentiative remodeling of cells. Autophagy is also required for the cellular adaptation to nutrient deprivation and the elimination of damaged organelles (Figure 1; Klionsky and Emr, 2000).

***Regulation of autophagy***

During autophagic degradation, cytosolic proteins and/or organelles are first sequestered within double membrane vesicles, which are then fused to the lysosome (Klionsky and Emr, 2000). The vesicular contents are broken down by pH-sensitive lysosomal hydrolases, and the degradation products are recycled for use in macromolecular synthesis and/or bioenergetics. Relatively little is known about how protein complexes and organelles are specifically targeted for degradation through autophagy. However, much of the molecular machinery required for autophagic vacuole formation and fusion with the lysosome has been identified through genetic screens

to identify proteins necessary for cell survival when external nutrient supplies are low. To date, more than 20 genes havebeen identified which are required to induce autophagy in response to starvation conditions (Klionsky and Emr, 2000).

Recently, it has been demonstrated that protein turnover by autophagy is differentially regulated by type I and type III PI3 kinases (Ogier-Denis and Codogno, 2003). Type I PI3 kinases and their downstream signal transduction components, Akt and TOR, have been implicated in suppressing autophagy, while the tumor suppressor, PTEN, acts as a negative regulator of the activity of type I PI3 kinases and acts as an activator of autophagy. In contrast, type III PI3 kinases have been shown to be required for both autophagic vesicle formation and vesicular transport to the lysosome.

### *Beclin 1, a haploinsufficient tumor suppressor gene that regulates autophagy*

One type III PI3 kinase-interacting protein that participates in the induction of autophagy in response to starvation is Apg6. Recently, the mammalian ortholog of Apg6, Beclin 1, was found to be monoallelically deleted in a high percentage of ovarian, breast, and prostate cancers, and established tumor cell lines from these tissues express low to undetectable levels of Beclin 1 protein (Liang et al., 1999). Furthermore, transfection of Beclin 1 into a transformed breast carcinoma cell line decreased its tumorigenic potential in nude mice. To investigate whether Beclin 1 acts as a tumor suppressor and whether loss of Beclin 1 would contribute to an increased incidence of cancer, two groups have now generated *beclin 1*-deficient mice (Qu et al., 2003; Yue et al., 2003). These mice demonstrate that Beclin 1 loss is associated with a reduction in autophagic vacuole formation, that Beclin 1-mediated regulation of autophagy is required for normal mammalian development, and that animals with reduced levels of Beclin 1 display a pronounced increase in epithelial and hematopoietic malignancies.

*Apg6* is not an essential gene in yeast. Similarly, Yue et al. found that *beclin 1* is not required for ES cell growth under standard culture conditions. Furthermore, it does not appear to be absolutely required for autophagic vacuole formation, although loss of *beclin 1* suppresses the autophagic response to nutrient limitation, suggesting that *beclin 1* plays a regulatory rather than required role in autophagy. Despite the fact that Beclin 1 is not required for cellular survival, *beclin 1*-deficient embryos did not develop past embryonic day 8.5 because of their inability to undergo remodeling and proper differentiation of ventral endoderm (Yue et al., 2003). These results confirm that autophagy plays a role in developmental/differentiative tissue remodeling. However, the most surprising result of both studies is that *beclin 1*-haploinsufficient animals, as they age, displayed a pronounced increase in the incidence of lymphoma and carcinoma of the lung and liver. In addition, mammary tissues in these animals displayed hyperproliferative, preneoplastic changes in reponse to *beclin 1* deletion (Qu et al., 2003).

On the surface, the ability of Beclin 1 deficiency to increase tumorigenesis is counterintuitive. Autophagy is an adaptive response to nutrient deprivation, allowing cells to persist for prolonged periods under suboptimal nutrient conditions. Cancer cells are frequently subjected to nutrient limitation, as growth of the tumor frequently outstrips the ability of the vasculature to supply oxygen and essential nutrients to maintain macromolecular synthesis and bioenergetics. Therefore, it might have been expected that an intact autophagic pathway would be essential for tumor cells to adapt and survive in vivo. Although it remains possible that Beclin 1's role as a tumor suppressor does not reflect its role in autophagy, there are several potential mechanisms by which inhibition of autophagic degradation might contribute to cellular transformation.

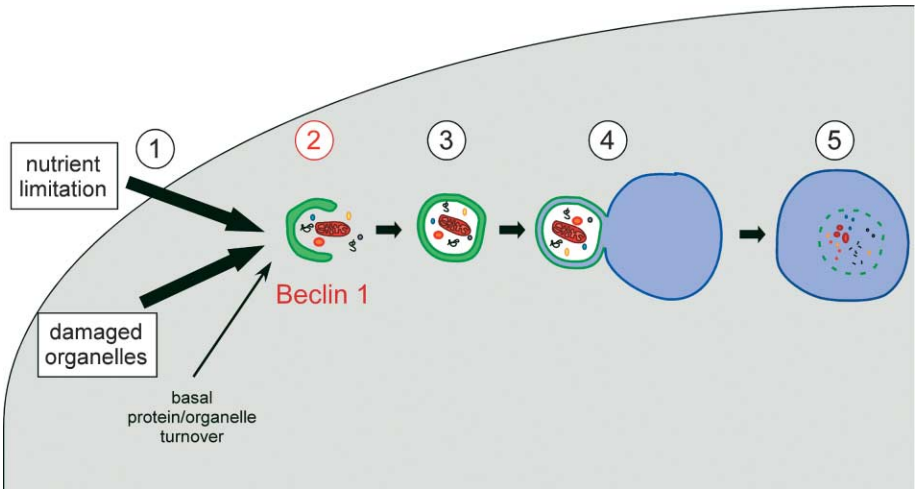


**Figure 1.** Beclin 1 is required for a proximal step in autophagy

Autophagy consists of five basic steps: (1) in addition to constitutive, housekeeping degradative functions, autophagy is induced by nutrient limitation or to remove damaged or excess organelles; (2) a double membrane bound autophagosome forms enclosing organelles, proteins, and cytosol; (3) the autophagosome is trafficked to the lysosome; (4) the autophagosome fuses with the lysosome; and (5) the contents of the autophagosome are degraded. Beclin 1 appears to be involved in the formation of the autophagosome and thus blocks autophagy at an early step.

### *Autophagy as an alternative form of programmed cell death*

Recently, an alternative form of programmed cell death that occurs independently of caspase activation has been described in neurons (Klionsky and Emr, 2000). This cell death has been termed type II programmed cell death and is morphologically associated with massive induction of autophagic vacuole formation. Based on this morphologic finding, it has been suggested that unrestrained autophagic proteolysis represents an alternative pathway for programmed cell death and thus limits cell-autonomous survival. The fact that neither Qu et al. or Yue et al. found any protection from programmed cell death in cells deficient in *beclin 1* argues against this explanation for increased tumor formation in Beclin 1-deficient mice. A more definitive test of this hypothesis is likely to be forthcoming. Although Yue et al. produced *beclin 1*-deficient ES and demonstrated that they exhibit a pronounced reduction in autophagic vesicle formation in response

to nutrient deprivation, the group did not evaluate whether this reduction in autophagy was associated with enhanced cellular survival. It will be important to determine whether the inhibition of autophagy associated with Beclin 1 deficiency increases cellular survival as would be predicted if autophagy is the mediator of type II programmed cell death, or whether inhibition of autophagy decreases cell survival in response to conditions of cellular stress or nutrient deprivation as would be predicted from comparable studies in yeast.

***Autophagy as a mechanism to decrease genotoxic stress***

Autophagy plays a critical role in removing damaged or surplus organelles in order to maintain cellular homeostasis. For example, by removing damaged mitochondria, autophagy may limit the exposure of cellular DNA to genotoxic stresses such as free radicals. The removal of damaged mitochondria through autophagic degradation would thus decrease the basal mutation rate and suppress oncogenesis. This model is consistent with the speculation that the levels of endogenous cellular oxidants produced during normal physiology or in response to extrinsic damage may be the major contributors to the basal mutation rate observed in cells (Ames et al., 1995). Thus, removal of sources of oxidant stress such as damaged mitochondria or endoplasmic reticulum by autophagy might limit genotoxic damage.

***Beclin 1 as a regulator of selective turnover of proteins involved in cell growth and proliferation***

As mentioned above, Beclin 1 is not required for autophagic degradation but instead appears to play a more specialized role, possibly regulating the magnitude of the autophagic response or restricting degradation to proteins from specific cellular compartments or to specific cellular conditions. For example, Beclin 1 could be involved in the turnover of proteins required for the positive regulation of cell growth and proliferation. Evidence that Beclin 1 can play a specific role in protein degradation is demonstrated by the fact that the overexpression of the Beclin 1 binding protein, CAL (also known as PIST), decreases the cell surface expression of the CFTR chloride channel, while levels of P glycoprotein were unaffected (Cheng et al., 2002). Thus, a Beclin 1-containing protein complex may regulate the trafficking and turnover of other plasma membrane proteins that are involved in signal transduction and/or nutrient acquisition. Consistent with this possibility, another gene involved in the regulation of autophagy, the small GTPase Rab7, has recently been implicated in the degradation of nutrient transporters in response to growth factor withdrawal (Edinger et al., 2003). Under these conditions, Rab7 deficiency leads to prolonged cell-autonomous survival in the absence of growth factors. Loss of Rab7 also promoted in vitro cellular transformation by E1A. Together, the data suggests that when the expression of growth-promoting proteins is uncoupled from the signal transduction events that initiate or promote their activities, the enhanced/prolonged expression of such proteins may contribute to cellular transformation. Consistent with this possibility, enhancement of cellular translation through upregulation of the limiting translation factor, eIF4E, was demonstrated to promote transformation (Sonenberg and Gingras, 1998). The ability of eIF4E overexpression to promote transformation is consistent with the above model, illustrating the "flip side" of the coin. eIF4E is the limiting factor for the translation of growth-promoting mRNAs. Enhanced expression of eIF4E appears to override the ability of cells to turn off a growth-promoting cellular program, and thus eIF4E acts as an oncogene to maintain cell growth and proliferation.

***Conclusions***

Like many important initial observations, the increased tumor incidence associated with the loss of the ability to properly regulate autophagy appears to raise more questions than it answers. It seems likely that a more precise understanding of the cellular role of Beclin 1 may provide important insights into how regulation of protein turnover through autophagy contributes to cellular homeostasis. Further elucidating the molecular basis for the regulation of autophagy by Beclin 1 is also likely to shed light on the mechanism by which Beclin 1 suppresses tumor formation.

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**Selected reading**

Ames, B.N., Gold, L.S., and Willett, W.C. (1995). Proc. Natl. Acad. Sci. USA *92*, 5258–5265.

Cheng, J., Moyer, B.D., Milewski, M., Loffing, J., Ikeda, M., Mickle, J.E., Cutting, G.R., Li, M., Stanton, B.A., and Guggino, W.B. (2002). J. Biol. Chem. *277*, 3520–3529.

Edinger, A.L., Cinalli, R.M., and Thompson, C.B. (2003). Dev. Cell *5*, 571–582.

Klionsky, D.J., and Emr, S.D. (2000). Science *290*, 1717–1721.

Liang, X.H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., and Levine, B. (1999). Nature *402*, 672–676.

Ogier-Denis, E., and Codogno, P. (2003). Biochim. Biophys. Acta *1603*, 113–128.

Pagano, M., and Benmaamar, R. (2003). Cancer Cell *4*, 251–256.

Qu, X., Yu, J., Bhagat, G., Furuya, N., Hibshoosh, H., Troxel, A., Rosen, J., Eskelinen, E.-L., Mizushima, N., Ohsumi, Y., et al. (2003). J. Clin. Invest., in press. Published online November 24, 2003. 10.1172/jci200320039.

Sonenberg, N., and Gingras, A.-C. (1998). Curr. Opin. Cell Biol. *10*, 268–275.

Yue, Z., Shengkan, J., Yang, C., Levine, A.J., and Heintz, N. (2003). Proc. Natl. Acad. Sci. USA, in press. Published online December 1, 2003. 10.1073/pnas.2436255100.