Research Highlight

Autophagy gets in on the regulatory act

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Autophagy down-regulates the Wnt signal transduction pathway via targeted degradation of a key signaling protein. This may provide an explanation for autophagy’s role in tumor suppression.

The eukaryotic cell has at its disposal two primary methods for getting rid of unwanted proteins: the proteasome and autophagy. The proteasome is a large protein complex comprised of regulatory and proteolytic subunits whose core function is the degradation of damaged or misfolded proteins. It is also utilized in a wide variety of signaling cascades for the regulated degradation of specific target proteins in order to turn off those proteins’ activities. In both cases, the information as to which proteins are to be destroyed by the proteasome is provided by the attachment of a large protein tag, ubiquitin, to the target. In the case of misfolded proteins, cellular chaperones act to recruit ubiquitin ligases to tag these proteins for destruction. With proteins targeted for degradation as the result of a signaling cascade, a wide variety of signals including phosphorylation, the binding of a small molecule, or the binding of another protein partner can specifically promote (or, in other cases, prevent) the recruitment of the ubiquitin ligase and the attachment of the fatal tag.

Macroautophagy, the best-characterized type of autophagy (hereafter referred to as autophagy) is a process where double-membrane vesicles termed autophagosomes are formed to envelop soluble proteins and organelles, which are then delivered to the lysosome and degraded (Mizushima et al., 2008). Autophagy is a conserved response to starvation and can non-selectively degrade cytoplasmic components in order to recycle the resulting macromolecules. However, a basal level of autophagy is present even in non-starved cells, and there are also selective types of autophagy used for the targeting of excess or malfunctioning cellular components to the lysosome. The mechanism of autophagosome formation is such that the sequestering vesicle can accommodate essentially any sized cargo. Accordingly, autophagosomes are used to degrade components that are too big for the proteasome to handle, such as large protein aggregates (e.g., mutant huntingtin) or organelles. Interestingly, the targeting of protein aggregates to the autophagosome also requires their tagging by ubiquitin. Specifically, ubiquitin chains can be recognized by the adapter protein p62, which binds in turn to the autophagy-related (Atg) protein LC3, the homologue of yeast Atg8 (Kirkin et al., 2009). LC3 decorates the forming autophagosomal membrane and acts in part in cargo recognition in various forms of specific autophagy. This means that for misfolded proteins tagged with ubiquitin there are two paths to destruction: via the proteasome and via the autophagosome. However, until now only the proteasome, not the autophagosome, was thought to function in the regulated degradation of fully functional proteins that act as part of a signaling cascade.

The Wnt pathway is a well-studied animal signaling cascade that begins with the binding of a secreted Wnt protein to the extracellular region of a plasma membrane receptor of the Frizzled family. For canonical Wnt signaling, this binding results in the recruitment of the soluble protein Dishevelled (Dvl) to the Frizzled receptor, resulting in the release of active β-catenin, which travels to the nucleus to promote transcription of target genes. The Wnt pathway plays roles in polarity and body plan establishment during embryo development. In addition, this pathway is active in certain tissues in the adult such as the intestinal epithelium, hair follicles and hematopoietic stem cells. In the adult, Wnt largely acts as a pro-proliferative signal in stem cell maintenance, although in some contexts it can also be necessary for terminal differentiation. This role in maintaining cell proliferation is likely the reason why activating mutations in various components of the Wnt signaling cascade are commonly associated with cancer, in particular cancers of the colon (Reya and Clevers, 2005; Clevers, 2006).

Ubiquitination and proteasomal degradation (e.g., of β-catenin) have long been known to play a role in the Wnt pathway, but a paper in the August issue of Nature Cell Biology (Gao et al., 2010) demonstrates for the first time that the pathway is also modulated by autophagy. Induction of autophagy by either starvation or the drug rapamycin significantly reduces the activation of Wnt signaling by Wnt3a in human tissue culture cells. This reduction is eliminated in cells where essential components required for autophagosome formation are absent, showing that this down-regulation is the direct result of autophagy. This effect is correlated with reductions in the levels of Dvl2, a key component of the Wnt signaling pathway, upon autophagic induction, and Dvl2 is targeted to autophagosomes. Dvl2 is also degraded by the proteasome, but use of the proteasome inhibitor MG132 demonstrates that autophagy also plays a significant role. As in the case of protein aggregates, the targeting of Dvl2 to autophagosomes is at least partially mediated by ubiquitination of Dvl2 followed by p62 binding.
pathway by autophagy may represent a crosstalk mechanism, where autophagy stimulated by withdrawal of growth-promoting factors further slows proliferation by down-regulating the Wnt pathway (Figure 1).

The Wnt pathway is active both during embryo development and in maintaining cell proliferation in some tissues in the adult. So far there is no evidence from the analysis of autophagy mutants to suggest that down-regulation of Wnt signaling by autophagy is playing a role in embryo development; however, this possibility may warrant further investigation. A more likely place to see an effect is in adult tissues such as the epithelial lining of the colon, where overactivity of the Wnt pathway is associated with cancer. Indeed, the authors demonstrated that the knockdown of Beclin 1 (Atg6), a protein necessary for autophagy, in a colon cancer cell line increases the number of cancerous colonies formed, and that this increase is dependent on Wnt signaling. Consistent with this, samples from late stage colon tumors show higher levels of Dvl and p62, but lower levels of Beclin 1. Therefore, down-regulation of Wnt signaling is one possible explanation for the well-established, but poorly understood, role of autophagy in tumor suppression (Liang and Jung, 2010).

Figure 1 Starvation, withdrawal of growth factors and other cellular stresses can lead to the induction of autophagy. Gao et al. (2010) have now demonstrated that autophagy leads to the down-regulation of the Wnt signaling pathway via degradation of Dvl2. This mode of crosstalk may be one mechanism for autophagy’s role in tumor suppression.

Surprisingly, however, Dvl2 can also bind directly to LC3 through a WLKI motif in its DEP domain, suggesting that Dvl2 might be a direct autophagy target even without the need for ubiquitination. More work will need to be done to determine the relative contributions of ubiquitination versus direct LC3 binding to the autophagic degradation of Dvl2, perhaps by mutating the ubiquitin conjugation site in the latter. Likewise, the relative roles of the various E3 ligases that have been reported to ubiquitinate Dvl2 (Gao and Chen, 2010) need to be elucidated.

Why might a cell want to down-regulate Wnt signaling upon induction of autophagy? A recent large-scale screen in mammalian cells identified multiple growth signaling pathways as negative regulators of autophagy (Lipinski et al., 2010). Therefore, the regulation of the Wnt pathway by autophagy and autophagosomal targeting of Dvl2 and stabilizes its levels, suggesting that the formation of oligomers is necessary for autophagosomal degradation. This finding is consistent with autophagy’s general role in the degradation of large structures such as protein aggregates. Therefore, signaling cascade components known to form oligomers might be particularly attractive targets to examine for additional instances of autophagic regulation of signal transduction pathways.

Even if autophagic down-regulation of signaling turns out to be a feature only of a few signaling cascades, this will still be an important advance in our understanding of crosstalk between different responses of the cell to its environment. Moreover, given the known connections of both the Wnt pathway and autophagy to cancer, understanding the interplay between the two is of clear medical importance.

References