PERSPECTIVE

Eating to exit: autophagy-enabled senescence revealed

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Autophagy and senescence are two distinct cellular responses to stress that are also tumor suppression mechanisms. In this issue of *Genes & Development*, Young and colleagues (pp. 798–803) discovered that autophagy is induced during and facilitates the process of senescence. Knowing now that these two pathways are functionally intertwined sets the stage for establishing how they function cooperatively in the cancer setting.

An appropriate cellular stress response is critical for maintaining tissue integrity and function and for preventing disease, especially cancer. Cells respond to stress with adaptation, repair, and recovery, or are diverted into irreversible cell cycle exit (senescence) or are eliminated through programmed cell death (apoptosis). These cell fate decisions are critical to deal with the emergence of damaged and potentially dangerous cells that can cause cancer. Defects in the cellular stress response cause the manifestation of cellular damage and/or prevent the senescence or elimination of damaged cells, which causes the accumulation of mutations leading to cancer.

It is becoming clear that the cellular lysosomal degradation pathway of autophagy is also a major component of the cellular stress response. Although initially identified as a cell survival mechanism in times of nutrient starvation, emerging evidence suggests that autophagy can also be an important tumor suppression mechanism (Mathew et al. 2007a; Levine and Kroemer 2008). Autophagy can enable adaptation to stress through the degradation of cellular proteins and organelles to suppress damage, maintain metabolism, and promote cellular viability and fitness. How tumor suppression by autophagy is related to other stress response pathways and mechanisms of tumor suppression is not well understood. Although autophagy can delay apoptosis, a role for autophagy in senescence was not previously known. Young et al. (2009) report here that the stress of oncogene activation also triggers autophagy that is required for efficient establishment of the senescence phenotype. Thus, the two stress pathways of autophagy and senescence are now functionally linked, providing new aspects to their mechanisms of tumor suppression.

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Senescence is a pathway to cell cycle exit, retooling, and tumor suppression

Cellular senescence is a stable form of cell cycle arrest that limits the proliferation of damaged cells. Initially defined by the phenotype of human fibroblasts undergoing replicative exhaustion in culture (Hayflick and Moorhead 1961), senescence can be triggered in many cell types in response to diverse forms of cellular stress, including as a consequence of aberrant hyperproliferative stimuli ("oncogene-induced senescence," OIS) or following DNA damage (Serrano et al. 1997; for review, see d'Adda di Fagagna 2008). As a consequence, cellular senescence imposes a potent barrier to tumorigenesis and contributes to the cytotoxicity of certain anti-cancer agents (Schmitt et al. 2002; Braig et al. 2005; Chen et al. 2005; Collado et al. 2005; Michaloglou et al. 2005). Interestingly, senescent cells have also been observed in certain aged or damaged tissues, and have been suggested to limit cell depletion and the decline of tissue regeneration capacity with age (for review, see Campisi 2007). Senescence may also act as part of a homeostatic mechanism to limit wound healing responses following tissue damage (Krizhanovsky et al. 2008).

The transition from a growing, proliferative state to senescence involves gradual but massive changes in cell physiology and protein expression (for review, see Campisi 2007). Thus, senescent cells often develop a large, flattened morphology and accumulate a senescence-associated β-galactosidase (SA-β-gal) activity that distinguishes them from most quiescent cells. In addition, they often down-regulate genes involved in proliferation and extracellular matrix production, and up-regulate inflammatory cytokines and other molecules known to modulate the tissue microenvironment or immune response. Consistent with the role of cellular senescence as a barrier to

malignant transformation, senescent cells activate the p53 and p16/Rb tumor suppressor pathways and are required to various degrees in different cell types to execute the program (Courtois-Cox et al. 2008). Whereas p53 promotes senescence by transactivating genes that inhibit proliferation, p16INK4a promotes senescence by inhibiting cyclin-dependent kinases 2 and 4, thereby preventing Rb phosphorylation and allowing Rb to promote a global repressive heterochromatin environment that apparently silences certain proliferation-associated genes (Serrano et al. 1997; Narita et al. 2003). Clearly, then, senescence involves substantial cellular remodeling, but how this occurs had not been examined.

Autophagy is a pathway for cellular self-degradation, intracellular recycling, and tumor suppression

The autophagy pathway is a means to capture intracellular proteins, protein aggregates, and organelles in specialized vesicles called autophagosomes, which are then directed to lysosomes for degradation. There are many purposes for autophagy that include maintenance of energy homeostasis through intracellular recycling and damage mitigation through the elimination of unfolded proteins and malfunctioning organelles. Although basal autophagy is an important housekeeping function for normal cells, like senescence, autophagy is induced by stress, including starvation and aging, where it plays a vital role in preserving cellular viability (Levine and Kroemer 2008).

Autophagy is induced in metabolically stressed, hypoxic regions of tumors, where it supports tumor cell viability (Mathew et al. 2007a). The tumor suppression function of autophagy may result from preventing the accumulation of damaged proteins and organelles, which may be a source of oxidative stress and lead to genome damage (Karantza-Wadsworth et al. 2007; Mathew et al. 2007b). Furthermore, the recycling of intracellular components sustains cell metabolism during deprivation that may have an indirect role in cellular protection (Jin and White 2008). This protein and organelle quality control function of autophagy can be thought of ultimately as a means of cellular quality control. As such, autophagy may preserve cellular health and fitness in stress through catabolism, thereby limiting tumorigenesis. The damage mitigation and metabolic homeostasis provided by autophagy highlights the importance of the lysosomal compartment in this process and is a conceptually novel tumor suppression mechanism.

Autophagy induction and role in OIS

Young et al. (2009) examined human diploid fibroblasts induced into senescence by activation of the H-*ras* oncogene and found stimulation of autophagosome formation. In this OIS, cells typically undergo a burst of proliferation ("the mitotic phase") that in this experimental paradigm spans day 1, followed by the transition phase on days 2–4, and senescence on days 5–6. The activation of autophagy peaks in the transition phase of senescence and correlates with down-regulation of mammalian target of rapamycin (mTOR) and cell cycle exit. Surprisingly, Young et al. (2009) found a subset of autophagy-related genes (*ATG*) are up-regulated in senescence, including *ULK3*, *LC3B*, *BNIP3*, and *BNIP3L*. *ULK3* is one of the three mammalian homologs of yeast *ATG1* that encodes a protein kinase responsible for activating autophagy, *LC3B* encodes an autophagosome component, and *BNIP3* and *BNIP3L* encode proteins important for directing mitochondria to autophagosomes for degradation. *ATG* genes were initially suppressed in the mitotic phase then up-regulated in the transition phase, correlating with inhibition of mTOR and induction of the transcription factor FoxO3a that induces *ATG* gene expression. Importantly, the coincidence of increased autophagy with cellular senescence is not limited to cell culture settings involving oncogene overexpression. Hence, chemically induced skin papillomas, which harbor activated ras mutations and are loaded with senescent cells (Collado et al. 2005), also show high autophagic activity (Young et al. 2009).

Although these are interesting associations, the significance of autophagy induction during OIS was revealed with autophagy inhibition, which delayed senescence and the accumulation of senescence-associated secreted proteins (Young et al. 2009). Thus autophagy is an important component of the senescence program required for efficient establishment and quality of the senescence phenotype. It is provocative to speculate that impairment of autophagy facilitates escape from senescence, and that this contributes to the increased tumorigenesis that results from autophagy defects (Mathew et al. 2007a; Levine and Kroemer 2008). Defining which aspects of autophagy are required for senescence and if or how this impacts tumor suppression through senescence are the next important issues.

Is senescence-associated autophagy a specialized form of autophagy?

Although autophagy is induced in response to a wide variety of stresses, it is not clear that the process of autophagy is the same in all cases. In starvation, for example, the recycling role of autophagy may be paramount, whereas in response to hypoxia, the elimination of mitochondria through autophagy to suppress oxidative stress may be more critical. During senescence, induction of *ULK3* was sufficient to stimulate autophagy and premature senescence (Young et al. 2009). Thus, like ULK1 and ULK2, ULK3 is an autophagy regulator. The induction of ULK3 in OIS is associated with a gene expression signature of autophagy-related genes that is distinct from that induced by starvation. This suggests that ULK3 and autophagy induction in OIS may be a specialized form of autophagy that acts to facilitate cellular remodeling. Going forward, it will be interesting to test if different forms of autophagy exist and how these may accomplish their distinct objectives. Furthermore, it will be interesting to examine if expression of specific *ULK*s is an essential determinant, and what aspects of autophagy are required

and perhaps specific for establishing the senescence phenotype.

Role of autophagy in senescence

The current study establishes the requirement for autophagy in the efficient execution of the senescence program. Two main remaining questions are how autophagy facilitates senescence, and whether there is a consequence to oncogenesis when senescence is bypassed by defects in autophagy. While the answers to these questions are not yet known, some potential outcomes can be extrapolated from the current state of knowledge.

Cellular remodeling

Senescence is a remarkable cellular reprogramming event that involves both epigenetic changes in gene expression and cellular remodeling. Some of the changes in gene expression during OIS are responsible for autophagy induction. What is unusual is that the lysosomal degradation of cellular components by progressive autophagy typically results in cell shrinkage due to self-consumption (Lum et al. 2005; Degenhardt et al. 2006). In contrast, senescence results in the generation of large cells with dramatically altered morphology. If senescence-associated autophagy is a distinct process from other forms of autophagy, it may be that specific lysosomal degradation events are required for the physical remodeling that generates the senescence phenotype.

Although autophagy can be a bulk degradation process, evidence is emerging that there is also targeted degradation of cellular components though autophagy. In the case of specific protein degradation by autophagy, polyubiquitination tags proteins for recognition by the adaptor protein p62, which directs them to autophagosomes for degradation (Pankiv et al. 2007). Cytoskeletal proteins of the keratin family, for example, are degraded by autophagy along with p62 and accumulate aberrantly when autophagy is defective (Komatsu et al. 2005, 2006, 2007; Hara et al. 2006). It is possible that some of the phenotypic changes during senescence require targeted degradation of specific cellular components, perhaps of the cytoskeleton, to achieve efficient remodeling associated with senescence.

Energy homeostasis and intracellular recycling

Catabolism through autophagy sustains metabolism and viability, which is important in starvation and potentially in situations where energy demand is high (Kuma et al. 2004; Tsukamoto et al. 2008). Oncogene activation may increase bioenergentic stress, and the process of senescence itself may increase energy demand. Senescent cells are metabolically and biosynthetically active and secrete inflammatory mediators essential for entry and maintenance of senescence (Acosta et al. 2008; Kuilman et al. 2008; Wajapeyee et al. 2008) and immune surveillance (Xue et al. 2007; Krizhanovsky et al. 2008). Through intracellular recycling, autophagy provides building blocks for macromolecular synthesis that may support cellular metabolism for protein synthesis and secretion. Assessment of the metabolic state of senescent cells and autophagy dependence and identification of the specific origin of the secretory impairment when autophagy is suppressed should illuminate this issue.

Damage mitigation

It is clear that autophagy is required for the degradation of polyubiquitinated proteins and organelles, that this requirement is increased with stress, and that failure of this process can lead to cell damage and death. This damage mitigation function is likely responsible for preventing neurodegeneration, liver damage, and cancer (Hara et al. 2006; Komatsu et al. 2006, 2007; Karantza-Wadsworth et al. 2007; Mathew et al. 2007b). Preservation of the health of senescent cells through autophagy damage mitigation may be similarly important to prevent cancer and liver fibrosis. It is not yet known if inhibition of autophagy during senescence produces the buildup of polyubiquitinated proteins, p62, damaged mitochondria, and activation of the DNA damage response, but this would be a first step toward addressing this question.

Does autophagy suppress tumorigenesis by maintaining cellular quality control and remodeling?

Senescence is an early barrier to oncogenesis, and impairment of the efficiency or quality of senescence by autophagy defect may increase cancer incidence. The potential role for autophagy in preventing senescence bypass can begin to be assessed by comparing cancer incidence following oncogene activation and induction of OIS in mice with an autophagy wild-type and defective (*beclin1*$^{+/-}$) genetic background. Nonetheless, the successful management of stress is critical for tumor suppression and autophagy is a fundamental part of stress management. Stressed cells activate autophagy, which prevents damage and maintains metabolism though lysosomal turnover of cellular components (Fig. 1). Autophagy can facilitate senescence or limit damage and delay apoptosis to allow recovery of normal cell function as tumor suppression mechanisms. In overwhelming stress, apoptosis may independently function in tumor suppression (Fig. 1). Autophagy can thereby be thought of as regulating cellular quality control, both of senescent and normal cells that may ultimately limit tumorigenesis. While there are still many unanswered questions, this provides a unifying theme linking the stress response pathways of senescence, autophagy, and apoptosis with tumor suppression.

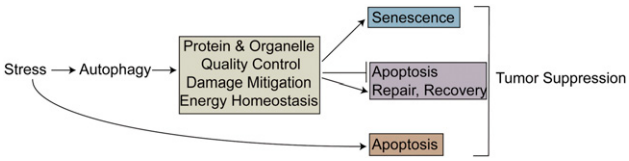

Figure 1. Role of autophagy, apoptosis, and senescence in tumor suppression. See the text for detail.

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