

Autophagic Punctum

Autophagy facilitates oncogene-induced senescence

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Oncogenic stress triggers a range of intracellular protective responses including DNA damage checkpoints, senescence and apoptosis, depending on the cell type and the severity of the particular stress. Senescent cells are metabolically viable but are stably arrested. Senescence is a collective phenotype, however, that is comprised of various signaling pathways and effector mechanisms. Thus, to understand and manipulate the senescence phenotype, it is critical to find its effector mechanisms and determine the relationships between them. We have recently found that autophagy is activated upon acute induction of senescence and facilitates another effector mechanism: the senescence associated secretory phenotype (SASP).

Senescence was originally described as the phenotype associated with replicative exhaustion of cultured human diploid fibroblasts (HDFs), which we now know to be caused by critically short telomeres. Senescence can also occur prematurely through the activation of oncogenes such as oncogenic *ras*. This is termed oncogene-induced senescence (OIS). In contrast to replicative senescence, OIS is a rapid and dynamic process. The initial response of cells to oncogene activation is hyperproliferation. Senescent cell cycle arrest is then established over the course of several days. Between these two extremes, the initial mitotic burst and the senescence phase, comes the “transition phase” where presumably the most drastic alterations in transcription, cell signaling and cell morphology occur (Fig. 1).

Such alteration in cell signaling includes the triggering of negative feedback regulation in the PtdIns3K/AKT/mTOR pathway, a signaling pathway downstream of *ras*. Consistently during the mitotic phase, when mTOR, a negative regulator of autophagy, is active, autophagy is inhibited. However, activating autophagy

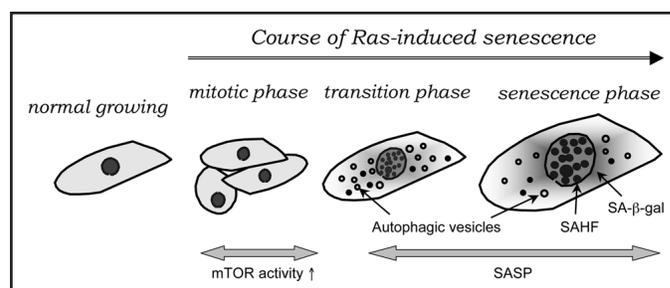


Figure 1. Autophagy in oncogene-induced senescence. Oncogene activation induces rapid cell proliferation (mitotic phase). mTOR activity is initially upregulated in response to the mitotic signal, while it then decreases during the transition phase. This timing correlates with the activation of autophagy and cell cycle exit. The dramatic phenotypic changes that occur during the transition phase are accompanied by the senescence associated secretory phenotype (SASP), senescence associated heterochromatic foci (SAHF), and senescence associated β -galactosidase (SA- β -gal) activity.

during the transition phase was well correlated with the downregulation of mTOR activity. Furthermore, the RNAi-mediated inhibition of autophagy delayed the establishment of senescence in certain HDFs. These data establish functional relevance for autophagy in senescence and potentially suggest a tumor suppressive role for autophagy through senescence.

Autophagy has previously been implicated in cancer, but its precise role is unclear. Autophagy activation can be oncogenic by contributing to tumor cell survival, while the data from some autophagy defective mice also suggest that autophagy shows tumor suppressive activity. Our data might confer a possible explanation for this contradiction. OIS is a very early event during tumorigenesis and is thought to be a critical step in tumor suppression if it is successful. Autophagy might thus contribute to tumor suppression by controlling the senescence phenotype. However, if tumor cells somehow bypass senescence, autophagy would then help such cells to survive metabolic stress, unwittingly facilitating their transformation and contributing to resistance to chemotherapy. Thus it is important to interpret the role of autophagy in the context of the stage of tumorigenesis.

It is also possible that senescence-associated autophagy has a distinct role and is differentially regulated. In fact, triggers of senescence (oncogenic *ras* or DNA damage) and a metabolic

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trigger of autophagy (amino acid starvation) seem to differentially regulate autophagy. mTOR forms two distinct complexes, mTOR complex1 (mTORC1) and mTORC2. While mTORC1 is a negative regulator of autophagosome formation, it has recently been shown that mTORC2 represses some autophagy-related (*ATG*) genes and other autophagy regulators through suppression of a transcription factor, FoxO3. Consistently, our array data show that mRNA of many autophagy associated genes, as well as lysosomal genes, are upregulated during the ras-senescence transition phase, in which the activity of mTORC1 & 2 is suppressed. The expression profile of these genes is distinct between ras-senescence and amino acid starvation. The unique gene expression pattern in senescence might confer a clue to understanding the mechanism by which autophagy is regulated during conditions of oncogenic stress. Of particular interest is the expression pattern of Atg1 homologs.

Atg1 is a serine/threonine protein kinase under the regulation of TOR, and Atg1 is a central node that plays a role in relaying the upstream signals that regulate autophagy. An analysis of the kinome has previously indicated that there are at least five potential human homologs of *Atg1*; *UNC-51-like kinase (ULK) 1-4* and *Fused*. Only *ULK1* and *ULK2*, however, have been clearly implicated in autophagy. In our data, *ULK3* is invariably upregulated during senescence, independent of the trigger. During ras-senescence, however *ULK1* expression displays minimal change while *ULK2* expression is downregulated. In marked contrast, only *ULK1/2* levels are upregulated during amino acid deprivation. Despite apparently limited homology with *ULK1/2* in the kinase domain, *ULK3*, when ectopically expressed, exhibits partial colocalization to the autophagic puncta in HDFs. In addition, overexpression of *ULK3* in HDFs is sufficient to reduce their replicative capacity and to activate autophagy. Although the endogenous level of *ULK3* activity remains to be confirmed, the data raise the interesting possibility that *ULK1/2* and their distant homologs might conceptually share the same function, yet their roles and regulation of expression might vary depending on the cellular context.

How does the bulk protein degradation program contribute to OIS establishment? Under metabolic stress, such as amino acid deprivation, activity of protein translation is typically suppressed to facilitate the catabolic processes that compensate for the lack of an energy source. Ras-senescent cells, on the other hand, contain far more protein than nonsenescent cells and produce a large amount of secretory proteins, suggesting that protein synthesis is also active in senescent cells. These secretory proteins include, among others, IL-6 and IL-8, which are critical components of the senescence-associated secretory phenotype (SASP) that reinforces senescence through autocrine and paracrine signaling. In contrast to typical senescence marker genes, such as *p16^{INK4a}* and *HMGGA2*, the expression pattern of many SASP components is very similar to that of autophagy-associated genes, which show a sharp induction during the transition phase of senescence (Fig. 1). The connection between autophagy and SASP is not only shown by the correlation in mRNA profiles. Our results show that knockdown of *ATG5* or *ATG7* delays synthesis of IL6 and IL8. Importantly, the mRNA levels of these SASP components are not repressed, but are rather

increased by autophagy inhibition. Thus, during senescence, autophagy facilitates the production of IL6/8 at the protein level. It is conceivable that rapid protein turnover during autophagy, coupled with active translation, could handle the sudden demand for these abundant secretory proteins, thus autophagy facilitates senescence establishment, at least in part, through promoting SASP. Autophagy might be a mechanism not only for “quality” control, but also “quantity” control of proteins.