

IN THE SPOTLIGHT

# Tumor-Promoting and -Suppressive Roles of Autophagy in the Same Mouse Model of *Braf*<sup>V600E</sup>-Driven Lung Cancer

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**Summary:** Although a role of autophagy in cancer development and progression has received increasing appreciation in recent years, there are still significantly uncertain and conflicting results about its tumor-suppressive and -promoting functions, and, more importantly, a lack of understanding of mechanisms underlying these opposing activities. The work presented by Strohecker and colleagues uses an innovative approach to address these challenges by examining the effects of inactivating the key autophagy gene *Atg7* at different stages of oncogenic development in a *Braf*<sup>V600E</sup>-driven mouse lung cancer model. The authors show that autophagy blockage initially accelerated tumor development, but suppressed tumor progression in later stages, converting adenomas to oncocytomas and increasing mouse survival. Importantly, they identify a critical role of glutamine dependency in the suppression of *Braf*<sup>V600E</sup>-induced cancer, thus revealing an important mechanism by which autophagy may promote tumor progression in different cellular contexts. *Cancer Discov*; 3(11); 1225-7. ©2013 AACR.

See related article by Strohecker et al., p. 1272 (6).

Autophagy is a highly conserved cellular process in which cellular components or pathogens are sequestered in autophagosomes and delivered to lysosomes for degradation. Dysregulation of autophagy has been associated with a number of human diseases, including cancer, although the underlying mechanisms are still not well understood (1). The first hint of a potential role of autophagy in cancer came from the observation of frequent monoallelic deletions of the mammalian autophagy gene *Beclin1* in sporadic human breast cancers and ovarian cancers. Indeed, studies using mouse models showed that haploinsufficiency in *Beclin1* promoted spontaneous malignancies, including lung and liver cancers and lymphomas (2). Furthermore, in a series of elegant studies, White and colleagues have shown that blockade of autophagy in various apoptosis-defective tumor cells led to increased neoplastic transformation caused by the accumulation of p62/SQSTM1, damaged mitochondria and reactive oxygen species (ROS), and increased DNA damage, providing significant mechanistic insights into the tumor-suppressive functions of autophagy (1).

Since these early studies, mouse models with conditional knockout of other autophagy genes in different tissues have been generated, but surprisingly, with the exception of benign liver tumors in the mosaic deletion of *Atg5* or liver-specific deletion of *Atg7* (1, 3), these did not result in the development of malignancies as one might expect based on

results from the *Beclin1* haploinsufficiency model. Moreover, although they are associated with susceptibility to various diseases, mutations of other autophagy genes have not been linked directly to human cancers, raising the interesting possibility that only with specific autophagy genes and/or only under particular cellular contexts does autophagy function as a suppressor for tumor initiation. In contrast to the earlier studies, emerging data suggested a paradoxical role of autophagy in promoting the growth and progression of various cancers based on studies both in human cancer cells and new mouse models (1). Elevated autophagy has been observed in various cancers under basal conditions and in tumors treated with chemotherapy or irradiation. More importantly, inhibition of autophagy could sensitize tumor cells to the cytotoxic effects of chemotherapy and ionizing irradiation to benefit cancer treatment. In *Ras*-transformed cells, autophagy has been shown to facilitate tumor cell survival and proliferation through its role in maintaining aerobic glycolysis and the tricarboxylic acid cycle for cellular energy levels (1, 4). Consistent with these data from cancer cell lines and xenograft models in nude mice, a protumorigenic role of autophagy has been shown directly in a recent study using mouse models of breast cancer in animals with intact immune functions (5). Despite these recent advances, the paradoxical role of autophagy in both suppressing and promoting cancer development and progression as well as the molecular mechanisms associated with each remain major challenges in the field.

In this issue of *Cancer Discovery*, Strohecker and colleagues (6) describe an innovative approach to addressing these challenges by examining the effects of inactivating the key autophagy gene *Atg7* at different stages of the oncogenic process in a mouse model of *Braf*<sup>V600E</sup>-driven lung cancer. They showed that deletion of *Atg7* accelerated tumor development initially, but suppressed tumor progression in later stages,

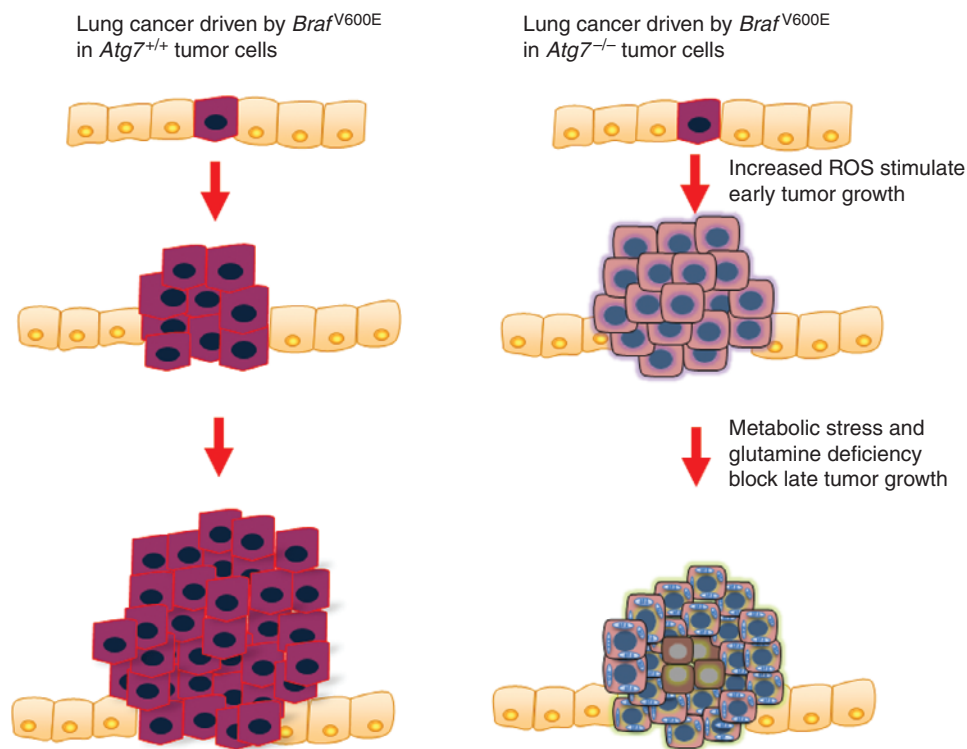
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**Figure 1.** Differential effect of *Atg7* deletion on the early and late stages of *Braf*<sup>V600E</sup>-driven lung cancer. Autophagy blockage by *Atg7* deletion in *Braf*<sup>V600E</sup>-driven lung tumor cells increased tumor growth at the early stage due to failure to remove dysfunctional mitochondria and consequent ROS accumulation. At the late stage, however, impairment of mitochondrial function caused by metabolic stress, and particularly glutamine limitation, becomes a dominant effect in the *Atg7*-null tumors, leading to their drastically decreased progression.

converting adenomas to oncocytoomas and increasing mouse survival (Fig. 1). Thus, these studies reconcile the paradoxical roles of autophagy in cancer development and progression in the same *in vivo* model, providing novel insights into our understanding of the context-dependent role of autophagy in cancer.

More importantly, Strohecker and colleagues (6) delved deeper into deciphering the mechanisms by which autophagy deficiency affected cancer development and progression differentially using the unique mouse models as well as tumor cells derived from these mice. On the basis of previous data from cancer cell lines (1), one potential mechanism of the accelerated tumorigenesis resulting from *Atg7* knockout is a deficiency of *Atg7*-null tumor cells in removing dysfunctional mitochondria and consequent accumulation of excess ROS, which can promote tumorigenesis through increased DNA damage and gene mutations. However, the authors also explored the alternative possibility of a role for NRF2 in mediating the tumorigenic effect of *Atg7* deletion in these cells. This is based on a very interesting recent report that activating a ROS-detoxification program by increasing NRF2 expression contributed to tumorigenesis in tumor models driven by classical oncogenes, such as *Kras*<sup>G12D</sup>, *Braf*<sup>V619E</sup>, and *Myc* (7). In addition to activation by oxidative stress to regulate the transcription of antioxidant-defense genes, NRF2 has been shown recently to be regulated by p62 through its interaction with KEAP1, an inhibitor of NRF2. p62 interacts with KEAP1 through a KEAP1 interaction

region, which leads to the release (from KEAP1 binding in the cytoplasm) and activation (in the nucleus) of NRF2 (8). Indeed, the authors observed increased Nrf2 accumulation in the nucleus, which is correlated with p62 accumulation in *Atg7*-deficient tumor cells, raising the possibility that activated NRF2 might contribute to the increased tumorigenicity of these cells. Nevertheless, deletion of *Nrf2* did not rescue the phenotype of *Atg7*-deficient tumors, suggesting that the upregulation of NRF2 and its targets for antioxidant defense mechanism are unlikely to be major players in the increased tumorigenesis observed in the *Atg7*-deficient tumors.

The observation of an inhibitory effect on tumor growth and progression at late stages upon *Atg7* deletion is consistent with recent findings in a breast cancer model (5) and similar to a recent article by the same group showing that *Atg7* deletion also suppressed progression of oncogenic *Kras*-induced lung cancer cells, converting them to oncocytoomas (9). However, it is very interesting to note that the current studies identified a critical role of glutamine dependency, rather than defective fatty acid oxidation as shown in the previous studies in *Kras*-induced tumors, in the suppression of *Braf*<sup>V600E</sup>-induced cancer. The authors found that autophagy-deficient *Braf*<sup>V600E</sup> tumor cells displayed a significant decrease in survival upon starvation *in vitro* and in nude mice, and, strikingly, the addition of glutamine, but not glucose, was sufficient to rescue starvation-induced cell death. In contrast, addition of the ROS scavenger NAC (*N*-acetyl-L-cysteine) did not

rescue the survival deficiency of *Atg7*-null tumor cells, suggesting that increased ROS and oxidative stress (responsible for the increased tumorigenesis of autophagy inhibition in early stages) is not involved in the decreased tumor growth and progression at late stages. Along with the previous findings, the current study supports the notion that autophagy may promote tumor progression through different mechanisms in various cellular contexts (e.g., tumors driven by different oncogenic events and/or in different tumors) and may influence cancer development and progression through differential mechanisms. Nevertheless, future studies will be necessary to clarify the molecular and cellular basis for such differences.

Although the data are compelling that defective autophagy is responsible for the observed phenotypes in the *Atg7*-null mouse model, it should be noted that ATG7 has been shown to regulate p53 to control cell cycle and death during metabolic stress in an autophagy-independent manner (10). Future studies will be necessary to evaluate whether such an autophagy-independent mechanism may also contribute to the observed phenotype during early and/or late stages in this study. Indeed, given that almost all autophagy genes are involved in cellular processes other than autophagy, a major challenge in autophagy research now is to determine whether phenotypes observed in this and various other gene knockout mouse models are due to disruption of autophagy *per se* or possibly due to the loss of other, autophagy-independent functions of these genes (11). Future development of mouse models that specifically disrupt autophagy function by knockin of mutant alleles of autophagy genes may resolve these critical questions. Likewise, generation of inducible knockout mouse models enabling autophagy to be selectively blocked at different stages of tumorigenesis will be very helpful in dissecting the role of autophagy functions in cancer *in vivo*.

Other remaining puzzles to be resolved include the mechanisms underlying the transition from stimulation of tumorigenesis in early stages to inhibition of tumor growth and progression in late stages in *Atg7*-null tumor cells. Although the increased ROS production could induce more DNA damage for the initial increase in tumorigenesis, the authors suggested that this is unlikely to be a major player given that the increased tumor growth is transient, but that other oncogenic signaling pathways activated by ROS may be responsible. An alternative possibility is that the changes (either increased mutations or other activated signaling pathways) may persist, but the glutamine deficiency caused by mitochondrial malfunction could become a more dominant defect in later stages to inhibit tumor growth in *Atg7*-null tumors. Either of these possibilities remains to be explored.

Despite the need to resolve these and other mechanistic issues, the current study further highlights the critical role of autophagy in maintaining mitochondrial function to

sustain tumor cell growth *in vivo*. We anticipate that effective and novel treatments targeting autophagy and the metabolic pathways it controls in “autophagy-addicted” cancers will be developed on the basis of this and other related studies. It will be also interesting to determine whether the autophagy-dependent glutamine-addiction mechanism observed here is specific to oncogenic BRAF-driven lung cancers or can be extended or generalized to other cancers with *BRAF* mutation and/or tumors driven by other oncogenic mutations for potential new therapies based on this exciting study.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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