

# Autophagy is required for mitochondrial function, lipid metabolism, growth, and fate of KRAS<sup>G12D</sup>-driven lung tumors

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**Abbreviations:** FAO, fatty acid oxidation; FAs, fatty acids; GEMMs, genetically engineered mouse models; NSCLC, non-small-cell lung cancer; TDCLs, tumor-derived cell lines; TCA, tricarboxylic acid

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**E**vidence suggests that the role of autophagy in tumorigenesis is context dependent. Using genetically engineered mouse models (GEMMs) for human non-small-cell lung cancer (NSCLC), we found that deletion of the essential autophagy gene, *Atg7*, in KRAS<sup>G12D</sup>-driven NSCLC inhibits tumor growth and converts adenomas and adenocarcinomas to benign oncocy-tomas characterized by the accumulation of respiration-defective mitochondria. *Atg7* is required to preserve mitochondrial fatty acid oxidation (FAO) to maintain lipid homeostasis upon additional loss of *Trp53* in NSCLC. Furthermore, cell lines derived from autophagy-deficient tumors depend on glutamine to survive starvation. This suggests that autophagy is essential for the metabolism, growth, and fate of NSCLC.

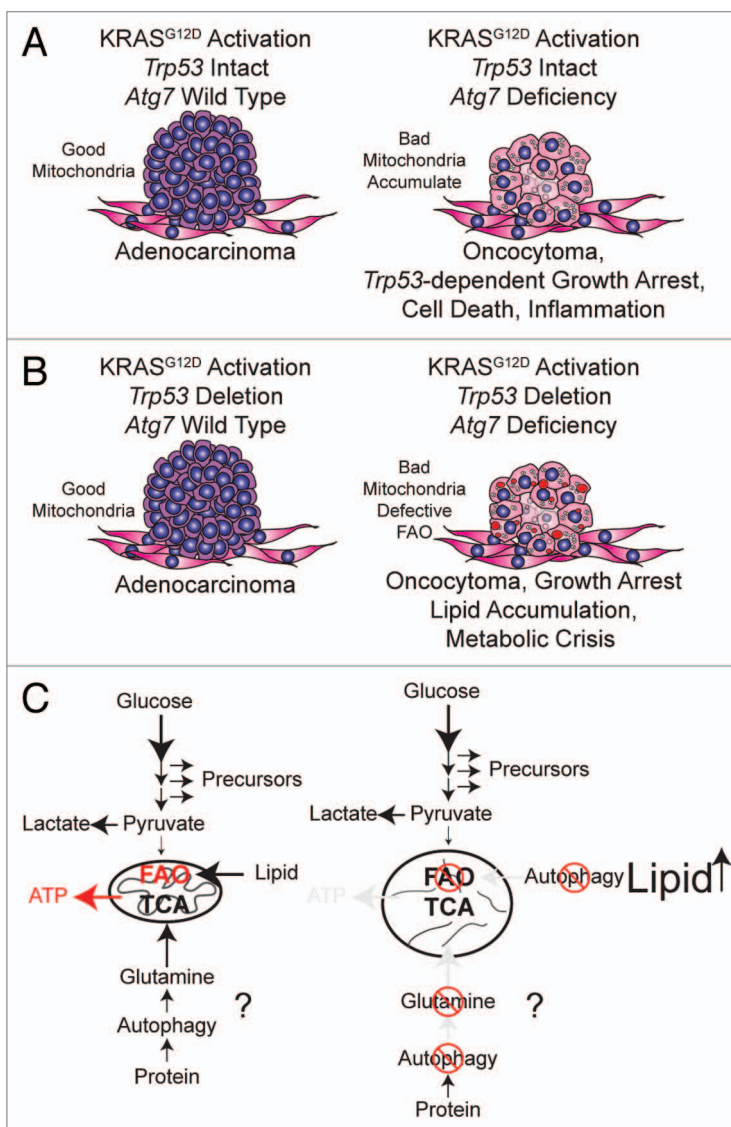
The role of autophagy in cancer is thought to be context dependent. Autophagy can act as tumor suppressor via its quality control function, which removes damaged organelles and protein aggregates to suppress oxidative stress, tissue damage, and genomic instability that can promote tumor initiation. In contrast, in established tumors, autophagy is upregulated in hypoxic regions to support tumor cell survival via recycling cargos and generating substrates such as amino acids, fatty acids (FAs) and nucleotides as well as ATP. In support of the pro-survival role for autophagy in cancer, in our previous work we found that RAS activation upregulates basal autophagy, and human cancer cells with activated RAS have high basal autophagy and depend on

autophagy for survival. The mechanism behind this “autophagy addiction” is that cancer cells require autophagy to maintain the pool of functioning mitochondria and cellular metabolism that are necessary for tumor cell growth and survival. Autophagy is particularly critical to the survival of cancer cells during metabolic stress, which is a common feature of the tumor microenvironment.

To further understand the role of autophagy in RAS-driven cancers in a physiological setting, we examined the consequence of autophagy ablation in spontaneously arising RAS-driven cancers in immune-competent mice, the *LSL-KRAS<sup>G12D</sup>* GEMM for NSCLC. Mice were also engineered to additionally delete the *Trp53* tumor suppressor gene in tumor cells, which alters metabolism and accelerates progression to adenocarcinoma, potentially influencing autophagy.

## Autophagy is Required to Maintain KRAS<sup>G12D</sup>-Driven NSCLC Tumor Growth and Fate

To test if autophagy blockade suppressed tumorigenesis in spontaneously arising RAS-driven cancers, we crossed the *LSL-KRAS<sup>G12D</sup>* GEMMs for human NSCLC to *Atg7<sup>lox/flox</sup>* mice possessing conditional deficiency in *Atg7* in the presence or absence of *Trp53*. Initiation of tumorigenesis by *KRAS<sup>G12D</sup>* activation, without or with *Trp53* deletion, without or with *Atg7* deletion, was achieved by intranasal delivery of adenovirus-Cre to the mice. *Atg7* wild-type, *KRAS<sup>G12D</sup>*-driven NSCLC tumor progression begins with hyperplasia that gradually progresses to ade-



**Figure 1.** Autophagy maintains functioning mitochondria to support *KRAS<sup>G12D</sup>*-driven NSCLC tumor metabolism, growth, and fate. **(A)** When *Trp53* is intact in *KRAS<sup>G12D</sup>*-driven NSCLC, *Atg7* deficiency causes accumulation of dysfunctional mitochondria and converts adenomas and adenocarcinomas to oncocyomas, which results in tumor growth arrest and tumor atrophy. **(B)** With the additional loss of *Trp53*, *Atg7* deficiency still converts adenocarcinomas to oncocyomas, but also causes defective mitochondrial FAO, leading to tumor cell lipid accumulation and metabolic impairment. **(C)** Tumor cell metabolism regulated by autophagy is oncogene- and tumor suppressor gene-dependent: with the additional loss of *Trp53*, autophagy preserves mitochondrial FAO and may provide metabolic substrates such as glutamine from protein degradation. RAS-driven cancer cells increase glucose metabolism to support their high energetic and biosynthetic demands. Increased glycolysis enhances conversion of pyruvate to lactate, resulting in insufficient pyruvate to support mitochondrial tricarboxylic acid (TCA) cycle metabolism upon nutrient deprivation. In this case, activated autophagy will degrade proteins and organelles to supply glutamine to maintain mitochondrial metabolism and FAO. When autophagy is blocked, however, depletion of the metabolic substrate glutamine, important for TCA cycle anaplerosis and defective FAO, will lead to tumor cell metabolic catastrophe.

nomas and then to adenocarcinomas after acquisition of *Trp53* mutations. However, *Atg7* deficiency converts adenomas and adenocarcinomas to low-grade, papillary, oncocyctic neoplasms or oncocyomas composed of tumor cells with a swollen, grainy, eosinophilic cytoplasm (oncocytes). Importantly, *Atg7*-deficient tumors accumulate respiration-defective mitochondria, as demonstrated by loss of enzymatic activity for mitochondrial cytochrome c oxidase. The conversion of adenomas and carcinomas to oncocyomas upon *Atg7* deletion in NSCLC results in a drastic reduction in tumor cell proliferation that dramatically decreases tumor burden. Thus, autophagy is required for *KRAS*-driven tumor mitochondrial metabolism, growth, and fate (Fig. 1A and B). These findings suggest that

mutations in essential autophagy genes may be the genetic basis for the development of human oncocyomas, and that converting adenomas and carcinomas to more benign oncocyomas by inhibiting autophagy or mitophagy might be a potential therapy for the treatment of NSCLC.

### Autophagy is Required for Lipid Homeostasis with Loss of *Trp53* in NSCLC

*Trp53* deficiency accelerates NSCLC and regulates cancer metabolism by increasing glucose uptake, directing glucose to lipid storage, and suppressing FAO. We found that in the *KRAS<sup>G12D</sup>*-driven GEMMs for NSCLC, lipid accumulation was observed only in *Atg7*-deficient tumors with *Trp53* deletion, and not in

*Atg7*-deficient or wild-type tumors with intact *Trp53*. This suggests that *Atg7* is required for lipid homeostasis in NSCLC specifically in the absence of *Trp53* (Fig. 1B).

To elucidate the mechanism by which autophagy maintains lipid homeostasis in *Trp53*-deficient NSCLC, we generated tumor-derived cell lines (TDCLs) from the GEMM lung tumors and found that *atg7<sup>-/-</sup>* TDCLs were extremely sensitive to starvation-induced cell death and were defective for tumor growth as we expected. As we reported previously for autophagy-deficient *HRAS* or *KRAS*-transformed baby mouse kidney cells, the *atg7<sup>-/-</sup>* TDCLs were also defective for oxygen consumption in starvation. Similar to *Atg7*-deficient tumors lacking *Trp53*, *atg7<sup>-/-</sup>* TDCLs accumulate neutral lipids and FAs. Importantly, *atg7<sup>-/-</sup>* TDCLs fail to respire when palmitate is provided during starvation, indicative of defective mitochondrial FAO. This suggests that *Atg7* deficiency causes NSCLC cells to become defective for mitochondrial FAO

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identifying the major reason for impaired mitochondria metabolism that leads to lipid and FA accumulation (Fig. 1C). Additionally, *atg7<sup>-/-</sup>* TDCLs are more sensitive to further inhibition of FAO during starvation than *Atg7<sup>+/+</sup>* TDCLs, indicating combination therapy involving autophagy and FAO inhibition is a strategy for *KRAS*-driven lung cancers.

Upon metabolic stress, autophagy is activated and degrades intracellular components to provide fuel to support cell metabolism, and amino acids are one important fuel. We found that glutamine, but not glucose, supplementation successfully rescues survival of

*atg7<sup>-/-</sup>* TDCLs during Hank's Balanced Salt Solution induced-starvation, suggesting that in addition to preserving FAO, autophagy may also supply metabolic substrates such as glutamine from protein degradation to maintain metabolism (Fig. 1C).

Although the detailed mechanism behind the role of autophagy in tumorigenesis still needs to be further investigated, we provide the first evidence that autophagy is required to maintain the pool of functional mitochondria for *KRAS*-driven tumor growth in a physiological setting. We are also the first to report that autophagy is required to

maintain NSCLC fate, and lipid and glutamine metabolism, and that functions of autophagy are oncogene- and tumor suppressor gene-specific (Fig. 1).

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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