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Autophagy in the Tumor or in the Host: Which Plays a Greater Supportive Role?

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Summary

Autophagy has been identified as a potential therapeutic target in pancreatic ductal adenocarcinoma, one of the most lethal cancers, with few therapeutic options. Yang and colleagues successfully created a genetically engineered mouse model focused on the autophagy gene *Atg4b* that allows the study of therapeutic autophagy inhibition in fully formed tumors. Using this tool, they demonstrated that selective autophagy inhibition in either the tumor cells, normal host cells, or both suppresses tumor growth.

Macroautophagy (autophagy hereafter) is a complex catabolic process initially described in yeast that performs homeo-static functions in the cell, protein, and organelle turnover. In genetically engineered mouse models (GEMM) of tumorigen-esis, loss of autophagy genes such as *Atg7*, *Atg5*, or *Fip200* has demonstrated that in most cases, autophagy suppresses early tumor formation, but promotes the growth of established tumors. Although these models have shed light on the role of autophagy during tumorigenesis, the effects of targeting autophagy once tumors are already present—more akin to what happens in patients—have been studied mostly in xenograft models. In this issue, Yang and colleagues (1) report a mouse model of pancreatic cancer in which inducible inhibition of ATG4B function can be accomplished in fully formed tumors. This model of cancer therapy provides new insights into the role of autophagy in the tumor and host cells and further supports autophagy inhibition as a therapeutic strategy in cancer.

As a survival mechanism, autophagy plays a crucial role in the tumor microenvironment, preventing cancer cell death in the face of hypoxia, nutrient starvation, and antineo-plastic drugs. One key concern raised in these models is that autophagy inhibition will enhance the development of benign tumors. Even putting this concern aside, another major question is which node of the autophagy pathway to target in cancer. Autophagy is a complex pathway with many enzymes coordinately regulating the sequestration of cellular cargo in autophagic vesicles, fusion with the lysosome, degradation of the cargo, and recycling of the cargo constituents. Druggable targets include but are not limited to (i) ULK1, part of the ULK1

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complex, which initiates autophagic vesicle membrane formation; (ii) VPS34, part of the VPS34–Beclin complex, which prepares membranes for the conjugation machinery; (iii) ATG7 and ATG4B, both part of the LC3 conjugation machinery, which conjugate the protein LC3 to lipids on the autophagic vesicle; and (iv) the lysosome, which is the rate-limiting degradative step for autophagic flux (Fig. 1A; ref. 2).

A major emphasis has been placed on targeting the lyso-some to inhibit autophagy through the use of chloroquine derivatives. These lysosomal inhibitors have been used for the treatment of malaria and rheumatoid arthritis for more than 50 years and are available for laboratory studies. Based on promising *in vivo* results in dozens of models, clinical trials have been launched combining anticancer agents with hydroxychloroquine (HCQ). One major drawback to this approach is that the molecular target of HCQ is not yet known. Although more potent derivatives have been made of HCQ, the lack of a molecular target makes developing more potent and specific agents difficult. Inroads into this problem have been made through the recent identification of palmitoyl-protein thioesterase (PPT1) as the molecular target of dimeric quinacrine autophagy inhibitors that target the lysosome in a similar fashion as HCQ (3).

Although efforts to target the lysosome in cancer continue, efforts to target core autophagy enzymes have also been tackled by a number of groups (4). Groundbreaking work by White's lab (5) found that systemic knockdown of *Atg7* in mice bearing *Kras*-driven lung cancer resulted in substantial tumor shrinkage. However, after months, these animals with systemic and complete elimination of *Atg7* died of increased susceptibility to *Streptococcus* infections, glucose homeostasis imbalance, and neurodegeneration. ATG7 is an E1-like ligase and could be a druggable target; however, to date no tool compound has been reported that can effectively inhibit ATG7. In contrast, some groups are working on tool compounds to inhibit ATG4B (6, 7), a cysteine protease that cleaves and recycles LC3 (Fig. 1A).

In this issue, Yang and colleagues report the first pancreatic cancer GEMM in which a dominant-negative form of ATG4B (ATG4B^{DN}) is inducibly expressed either within the tumor or systemically (1). In this work, a construct in which ATG4B^{DN} is expressed under a Cre-regulated tetracycline responsive element was generated, to develop a system in which autophagy inhibition could be controlled both spatially and temporally. ATG4B^{DN} sequesters free LC3B, preventing its lipidation, and consequently impairs autophagosome maturation. After demonstrating that expression of *Atg4b*^{DN} in pancreatic cells results in effective autophagy inhibition, they crossed this allele with a pancreatic cancer GEMM (*LSL-Kras*^{G12D}, *Trp53*^{lox/+}, *p48Cre*⁺), so ATG4B^{DN} was coexpressed with mutant *Kras* and *Trp53* in all pancreatic cells by administering doxycycline through the diet.

In mice containing one copy of *Atg4b*^{DN}, tumor growth was impaired initially, but after day 5, tumor growth resumed due to the selective pressure that resulted in loss of the *Atg4b*^{DN} allele in growing tumor cells. In mice harboring two copies of the mutant allele, no reactivation of autophagy and complete suppression of tumor growth was observed after doxycycline induction. However, as expected, sustained blockade of autophagy induced benign tumor growth in *Kras*-mutant/*Trp53*-mutant cells in the pancreas evidenced as metaplasia histologically. In this study, the authors included an important control by

examining the effects of expression of ATG4B^{DN} in mice which did not harbor a *Kras* mutation in every pancreatic cell, and found no evidence of metaplasia. Thus, the investigators were faced with an artifact of the model system that did not reflect the human condition, where normal pancreatic cells do not harbor *KRAS* mutation and therefore would not be driven to metaplasia with an ATG4B-targeted autophagy inhibitor (Fig. 1B).

To glean further therapeutic insight despite the limitations of this model, the investigators reasoned that drug therapy can almost never achieve complete constant target suppression in clinical practice. So they tested the effects of intermittent doxycycline administration. This approach completely impaired tumor growth in a sustained manner and prolonged survival in mice, mitigating the toxicity observed with chronic continuous doxycycline dosing in mice expressing *KRAS* in all pancreatic cells.

Recently, Kimmelman's group demonstrated the ability of autophagy in stroma cells to support growth by supplying alanine to neighboring pancreatic cancer cells (8). To determine if the growth-suppressive effects of ATG4B^{DN} were primarily occurring within the cancer cell or were driven by supporting cells within the tumor microenvironment, the investigators grew tumor xenografts from cell lines derived from *Atg4b* wild-type (WT) and *Atg4b*^{DN} tumors and found a significant but much less dramatic growth impairment in nude mice than in the GEMM. In the GEMM, no significant differences in T cells were observed, but an increased number of infiltrating macrophages was observed in tumors in which ATG4B^{DN} was expressed in tumor cells. Tumor growth impairment associated with expression of *Atg4b*^{DN} was abrogated when macrophages were depleted. Along these lines in the xenograft model in which expression of *Atg4b*^{DN} was less effective at impairing tumor growth, no differences in the macrophage infiltrate were observed between tumors expressing *Atg4b* WT and *Atg4b*^{DN}.

Next, the effects of whole-body inhibition of ATG4B^{DN} were undertaken by crossing the conditional inducible *Atg4b*^{DN} locus with mice harboring a ubiquitously expressed tamoxifen-inducible Cre recombinase. The specifics of this model are important because there was no expression of Cre in brain or muscle, and the expression of Cre in other tissues was mosaic, significantly limiting the potential toxicity of whole-body ATG4B inhibition. Orthotopic implantation of *Kras*-driven pancreatic cancer cells that either did or did not express *Atg4b*^{DN}, in hosts which systemically did or did not express *Atg4b*^{DN}, demonstrated that stromal cell ATG4B serves to enhance tumor establishment at early time points but, at later time points, plays less of a role than ATG4B^{DN} tumor cell does to slow tumor growth (Fig. 1C).

Taken together, this work is a major step forward in the field for a number of reasons. This is the first GEMM in which autophagy can be inhibited at the level of ATG4B and adds to a growing list of models to study autophagy in cancer. The work is the first to demonstrate that intermittent autophagy inhibition slows tumor growth and promotes regression, without lethal toxicity. Further, this work demonstrates that ATG4B blockade does not accelerate metaplasia in pancreatic cells lacking oncogene expression, addressing a major concern about the therapeutic liability of autophagy inhibition. This work also compares the relative contribution of host and tumor cell autophagy to tumor growth and confirms that

surrounding cells play a major role in tumor implantation and a minor role in supporting established tumor growth. Finally, the recruitment of antitumor macrophages into tumors in which tumor cells are deficient in autophagy is fascinating and requires further work.

These findings raise many new questions: How does tumor cell autophagy blockade result in increased antitumor macrophage influx? Why is this blunted in nude mice lacking T cells? Would complete loss of *Atg4b* produce similar results as expression of this dominant-negative mutant? Will small-molecule inhibitors of ATG4B reproduce these findings *in vivo*? Or is the dominant-negative construct and its ability to sequester LC3 a unique biological tool that is not clinically translatable? Is intermittent autophagy inhibition the best strategy to pursue moving forward? Is the mosaic model representative of what would happen therapeutically? Are these findings related to ATG4B modulation specific to *KRAS*-driven tumors, or is ATG4B a promising target in other oncogenic contexts? Regardless, the generation of this new model of therapeutic autophagy inhibition and the fundamental insights into tumor cell biology it has shed light on bring us a step closer to new drugs to target autophagy, a major resistance mechanism to cancer therapy.

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Figure 1.
A, Targeting *Atg4b* in the tumor and in the host. ATG4B cleaves LC3 to form LC3-I. Then, ATG7 along with a cascade of other proteins conjugates LC3-I with phosphatidylethanolamine (PE) to form LC3-II, which is required for cargo recruitment and autophagosome maturation. The autophago-some fuses the lysosome to enable cargo degradation and finally the cargo constituents are recycled. Dominant-negative ATG4B (ATG4B^{DN}) protein sequesters free LC3, preventing autophagosome maturation. **B**, Expression of *Atg4b*^{DN} in pancreatic cells, which harbor mutations in *Kras* and *Trp53*, produces tumor growth suppression but is also associated with acinar–ductal metaplasia that disrupts normal exocrine pancreas function. In pancreatic cells that harbor wild-type (WT) *Kras* and *Trp53* and expression of *Atg4b*^{DN}, no metaplasia or functional pancreas impairment is observed. **C**, Schematic representation of results of an orthotopic pancreatic cancer model in which *Atg4b*^{DN} can be expressed in tumor cells, the entire body or both. Tumor growth at early and late time points. ✓, intact autophagy; ✗, deficient autophagy.