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IN THE SPOTLIGHT

Mouse Models Address Key Concerns Regarding Autophagy Inhibition in Cancer Therapy

Ravi Amaravadi[1] and Jayanta Debnath[2]

Summary: With multiple clinical trials under way targeting autophagy against cancer, Yang and colleagues and Karsli-Uzunbas and colleagues address important concerns regarding autophagy inhibition in patients with cancer, using genetically engineered mouse models that more accurately represent the tumor biology found in human patients with pancreatic and lung cancers. *Cancer Discov; 4(8); 873–5. ©2014 AACR.*

See related article by Yang et al., p. 905 (2).

See related article by Karsli-Uzunbas et al., p. 914 (3).

The consideration of autophagy as a therapeutic target in cancer has been mired in the complexities of the pathway and the seemingly dual roles it plays in tumorigenesis. On the one hand, autophagy has been described as a tumor-suppressor mechanism, best exemplified by the fact that *Beclin1* heterozygote mice develop spontaneous tumors (1). However, increasing evidence suggests that, especially at later stages in tumorigenesis, autophagy supports tumor growth. In addition, cytoprotective autophagy is induced by many cancer therapies as a stress response (1). On the basis of these findings, multiple clinical trials targeting autophagy using the antimalarial lysosomal inhibitor hydroxychloroquine are under way, and pharmaceutical companies are developing novel potent and specific autophagy inhibitors. More recently, concerns have arisen regarding whether p53 status affects the anticancer efficacy of autophagy inhibition in certain tumors, as well as whether systemic autophagy inhibition will have deleterious effects on the normal tissues in patients with cancer. Two articles in this issue of *Cancer Discovery*, from Yang and colleagues (2) and Karsli-Uzunbas and colleagues (3), both provide reassurance about targeting autophagy in cancer and motivate the further testing of autophagy inhibitors in the clinic.

In the past few years, a number of investigators have moved beyond cell lines and xenograft mouse models to address the role of autophagy during cancer development and progression, using tumor-specific ablation of autophagy genes, such as *Atg7*, in immunocompetent genetically engineered mouse models (GEMM). In BRAF-driven lung cancer, *Atg7* deficiency initially promoted tumor growth. However, in lung cancers driven by either mutant *Kras* or *Braf*, *Atg7* deletion ultimately stalled tumor growth and promoted oncocytic differentiation with and without p53 (4, 5). These results partially reconciled the dual roles of autophagy during the process of tumorigenesis, but overall supported a role for therapeutic strategies to inhibit autophagy in certain advanced cancers.

However, an important red flag was recently raised by Rosenfeldt and colleagues (6), using a GEMM of pancreas cancer. In this model of pancreas-specific *Kras*-mutant, *Trp53*$^{-/-}$ tumors, genetic ablation of *Atg7* or *Atg5* within tumors or pharmacologic inhibition of autophagy with hydroxychloroquine accelerated the formation of pancreatic ductal adenocarcinomas (PDAC) in mice (Fig. 1A, left; ref. 6). Although these findings illustrated the importance of molecular context in the outcome of autophagy inhibition *in vivo*, they also had the unfortunate consequence of motivating clinical recommendations that patients with *TP53*-mutant pancreas cancer and possibly other *TP53*-mutant cancers avoid clinical trials using hydroxychloroquine (7). Another important concern raised in this study was that pancreas-specific deletion of *Atg7* produced pancreatic atrophy and independently contributed to the death of mice due to exocrine pancreas deficiency. This result was consistent with growing evidence demonstrating the importance of autophagy in maintaining organismal metabolism and tissue function (1, 3). Importantly, to date, most *in vivo* models of autophagy deficiency in cancer have singularly targeted the tumor cell compartment; hence, they have been unable to evaluate the collateral damage to normal tissues that systemic autophagy inhibition may produce in patients.

A major aspect of the *Kras*-mutant, *Trp53*$^{-/-}$ PDAC model in the work of Rosenfeldt and colleagues (6) is that it uses embryonic pancreas-specific homozygous deletion of *Trp53* in the context of *Kras* mutation, resulting in advanced carcinoma during early development (Fig. 1A, left). In contrast, p53 is most commonly found as missense point mutations in *KRAS*-mutant pancreatic cancers. The heterozygous expression of mutant p53 in the setting of oncogenic KRAS is proposed to facilitate the formation of precancerous lesions called pancreatic intraepithelial neoplasias (PanIN); subsequent LOH of the wild-type *TP53* allele drives the progression from PanIN to PDAC (2). Thus, the very rapid and aggressive disease observed in this mouse PDAC model using early developmental deletion of *Trp53* does not fully

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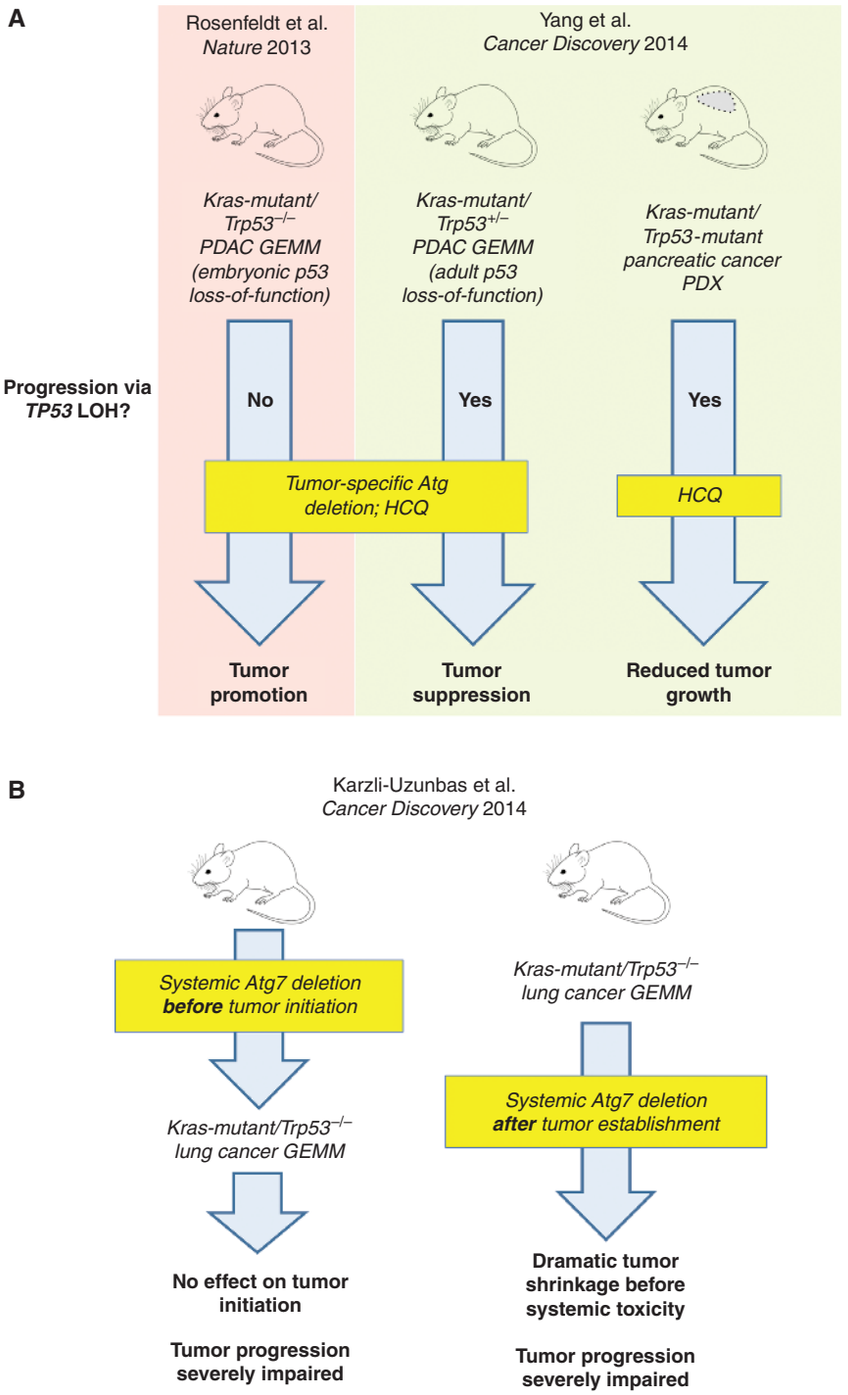

Figure 1. Effects of autophagy inhibition in mouse models of pancreatic and lung cancer. **A,** in GEMMs and patient-derived xenografts (PDX) of *KRAS*-mutant PDAC, both genetic autophagy ablation and hydroxychloroquine (HCQ) treatment elicited contrasting effects on tumorigenesis. The phenotypes observed upon autophagy inhibition in each model are likely the result of the developmental stage at which p53 tumor suppression function is lost (embryonic or during tumor progression), and whether PDAC progression involves *TP53* LOH. **B,** in a *Kras*-mutant, *Trp53*$^{-/-}$ lung cancer GEMM, the effects of systemic autophagy inhibition were analyzed following inducible genetic *Atg7* deletion in the entire adult mouse. Systemically inhibiting autophagy before tumor onset did not affect early initiation; rather, it severely impaired the progression to advanced cancers (left). In mice with established lung tumors, acute autophagy deletion led to significant tumor regression, which occurred before the onset of any systemic toxicity or normal tissue degeneration secondary to the complete loss of autophagy in the whole animal (right).

recapitulate the typical stepwise progression of pancreas cancer found in humans.

To address this salient issue, Yang and colleagues (2) used a pancreas-specific *Kras*-mutant *Trp53*$^{+/-}$ mouse model that exhibits LOH of the wild-type *Trp53* allele during PDAC progression, thereby reproducing the stepwise human development of pancreas cancer more faithfully than the *Kras*-mutant, *Trp53*$^{-/-}$ model. When *Atg5* was genetically ablated in the *KRAS*-mutant, *Trp53*$^{+/-}$ model, the number of PanIN lesions was increased, but the progression of PanIN to PDAC was significantly prevented, and mice with autophagy-deficient tumors survived longer. Importantly, the investigators confirmed that *Trp53* LOH was responsible for the conversion of PanIN to PDAC in the context of *Atg5* deficiency (Fig. 1A, right). These beneficial effects of autophagy inhibition were also observed upon treating *KRAS*-mutant PDAC tumor cells, possessing either deleted or mutant p53, with chloroquine. In addition, this article interrogated a large collection of genetically characterized patient-derived xenografts (PDX) treated with hydroxychloroquine *in vivo* (Fig. 1A, right). Impressive

tumor growth reduction was observed uniformly in 100% of *KRAS*-mutant, *TP53*-mutant pancreatic PDX lines. Altogether, this focus on a key aspect of p53 genetics in mouse models convincingly ameliorates concerns about developing autophagy inhibitors for *TP53*-mutant pancreas cancers and possibly other *TP53*-mutant tumors. It also teaches us a major lesson that extrapolation of mouse model data to help make clinical decisions for therapeutic strategies needs to be done with careful consideration, and that the details of the model really do matter. Although it now clearly seems premature to turn patients with *TP53*-mutant tumors away from clinical trials of autophagy inhibitors, the overall results from these two seminal studies in pancreatic cancer highlight the importance of obtaining *TP53* genotypes in patients enrolled in autophagy inhibitor trials (2, 6).

The development of a mouse model that better recapitulates the human response to systemic therapy allowed another group of investigators to tackle the concern that autophagy inhibition may be too toxic to pursue in the clinic due to the protective roles of autophagy in normal cells. Karsli-Uzunbas and colleagues (3) developed an inducible model of systemic *Atg7* ablation that allowed the study of systemic autophagy inhibition in adult mice, as well as the effects of acute autophagy ablation on the initiation, progression, and maintenance of KRAS-driven lung cancers. The systemic loss of ATG7 in adult mice surprisingly showed little toxicity for 5 weeks, although over 2 to 3 months, these autophagy-null mice did develop liver and muscle injury, depletion of white adipose tissue, and lethal neurodegeneration. Moreover, these mice did not tolerate fasting, and rigorous metabolic analysis uncovered a critical role for ATG7 in this context for the maintenance of glucose homeostasis in starved animals. Next, the investigators systemically deleted *Atg7* and then initiated lung cancer by activating *Kras* and deleting *Trp53* (Fig. 1B). Although early tumor initiation was independent of ATG7 status, the lung tumors that did arise in the setting of systemic ATG7 deficiency failed to progress to aggressive cancers and displayed the histologic features of benign oncocytomas.

Finally, when *Kras*-mutant, *Trp53*$^{-/-}$ lung cancers were allowed to grow in adult mice, upon which *Atg7* was systemically ablated in the context of established tumors, there was substantial antitumor activity, with tumor growth arrest, increased apoptosis, and loss of RAS-driven oncogenic signaling (Fig. 1B). Tumor clusters that were examined by histology consisted largely of dead cells and cells with oncocytic features. Importantly, the antitumor effects of systemic autophagy inhibition occurred rapidly and before the onset of any normal tissue degeneration attributable to systemic *Atg7* deletion. Notably, this last experiment most closely recapitulates the clinical scenario of treating patients with advanced *KRAS*-mutant lung cancer with pharmacologic autophagy inhibitors, including antimalarials such as hydroxychloroquine. Although the multiorgan toxicity associated with long-term *Atg7* deletion continues to illustrate that certain toxicities should be considered when applying autophagy inhibitors to humans, it is very important to recognize that pharmacologic autophagy inhibitors, both present and future, are unlikely to achieve the complete and irreversible autophagy-null phenotype obtained in this model. In addition, due to disruption of protein–protein interactions, the complete loss of the ATG7 protein may have unique implications in comparison with either pharmacologic inhibition of ATG7 enzymatic activity or lysosomal inhibition using hydroxychloroquine or other antimalarial agents.

The important findings of these two mouse models arrive at an opportune time, as the first series of hydroxychloroquine clinical trials in patients with cancer has been recently published; three examples include references 8–10. In general, the nonhematologic toxicity profile across these clinical trials was mild and manageable, despite achieving very high doses of hydroxychloroquine in certain instances. Remarkably, there was no evidence of extensive metabolic problems, liver injury, or neurologic impairment. Taken together with the GEMM data, it now seems that ample evidence supports moving forward with autophagy inhibition in patients with cancer, with either antimalarials or the next generation of more specific upstream autophagy inhibitors.

Disclosure of Potential Conflicts of Interest

J. Debnath has received honoraria from the speakers' bureaus of Amgen and Novartis. No potential conflicts of interest were disclosed by the other author.

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