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Inhibition of autophagy attenuates pancreatic cancer growth independent of *TP53/TRP53* status

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Basal levels of autophagy are elevated in most pancreatic ductal adenocarcinomas (PDAC). Suppressing autophagy pharmacologically using chloroquine (CQ) or genetically with RNAi to essential autophagy genes inhibits human pancreatic cancer growth in vitro and in vivo, which presents possible treatment opportunities for PDAC patients using the CQ-derivative hydroxychloroquine (HCQ). Indeed, such clinical trials are ongoing. However, autophagy is a complex cellular mechanism to maintain cell homeostasis under stress. Based on its biological role, a dual role of autophagy in tumorigenesis has been proposed: at tumor initiation, autophagy helps maintain genomic stability and prevent tumor initiation; while in advanced disease, autophagy degrades and recycles cellular components to meet the metabolic needs for rapid growth. This model was proven to be the case in mouse lung tumor models. However, in contrast to prior work in various PDAC model systems, loss of autophagy in PDAC mouse models with embryonic homozygous *Trp53* deletion does not inhibit tumor growth and paradoxically increases progression. This raised concerns whether there may be a genotype-dependent reliance of PDAC on autophagy. In a recent study, our group used a *Trp53* heterozygous mouse PDAC model and human PDX xenografts to address the question. Our results demonstrate that autophagy inhibition was effective against PDAC tumors irrespective of *TP53/TRP53* status.

Pancreatic ductal adenocarcinoma is a highly lethal tumor, and novel therapeutic approaches are needed to improve patient outcomes. Over 90% of PDAC possess activating mutations in the *KRAS* oncogene and studies in mouse models have shown that it is critical for tumor initiation and can result in the generation of pancreatic intraepithelial neoplasias (PanIN), a benign precursor of PDAC. Additional mutations in tumor suppressors are required for efficient progression from PanIN to PDAC. One such tumor suppressor gene is *TP53* which is mutated or lost in the majority of human PDAC. Such alterations typically occur through loss of heterozygosity (LOH) during tumorigenesis.

Elevation of basal autophagy level is a key characteristic of PDAC cells and is important to sustain growth in human tumor cell lines, xenografts, and mouse models, the majority of which have *TP53/Trp53* alterations. Recently, a study showed that autophagy loss can accelerate tumor progression in a PDAC mouse model where both copies of *Trp53* are simultaneously deleted during embryogenesis. We hypothesized that the differences between these and results from the previous work of our group and others were due to the nature of the model used. In particular, in contrast to human PDAC, tumors in this model do not undergo LOH of the *Trp53* allele. To fully understand how autophagy affects tumorigenesis, we inhibited autophagy by breeding a conditional *Atg5* null allele to a widely used PDAC mouse model with heterozygous *Trp53* deletion and *Kras* mutation conditionally expressed in pancreas using a *Pdx1-Cre* allele. The second copy of *Trp53* in this model is lost as an obligate step for tumor progression.

Keywords: PDAC, *Trp53*, *TP53*, *Atg5*, autophagy, chloroquine, pancreatic cancer

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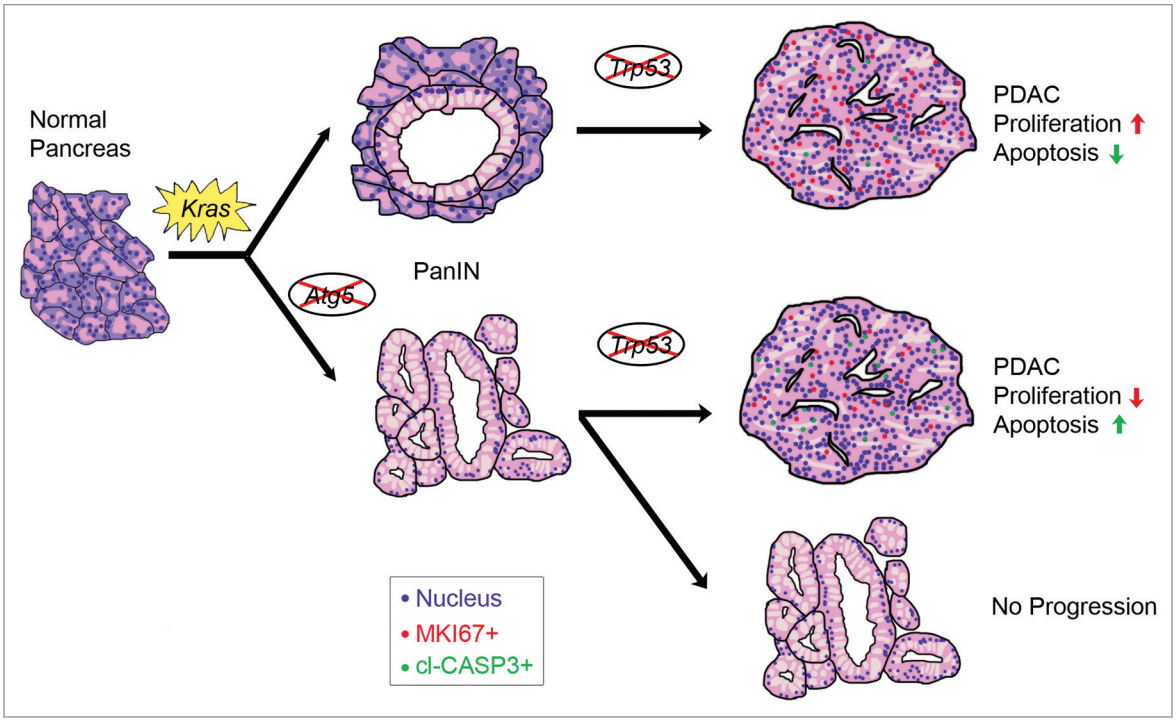

Figure 1. Abrogation of autophagy affects PDAC tumorigenesis. Activation of *Kras* initiates transformation of normal pancreas to benign PanIN lesions. *Atg5* deletion further promotes PanIN formation (more than 50 per mouse) compared with a wild-type mouse (2-10 PanINs per mouse). At later stages, *Trp53* is lost via LOH resulting in PanIN transformation to invasive PDAC. PanINs in *Atg5*-deleted mice were impaired in their ability to progress to PDAC. Those that did develop PDAC had less proliferation and more apoptosis.

Despite a small portion of mice dying prematurely from exocrine pancreas disruption, the autophagy-deficient cohort with *Kras* mutation and *Trp53* heterozygous deletion exhibited longer survival times. Examination of pancreata at early and late timepoints showed that *Atg5* null mice were more susceptible to early tumor initiation with a significantly increased number of PanINs, but were resistant to tumor progression, as invasive PDAC incidence was significantly diminished. Those tumors that did form in autophagy-incompetent pancreata showed increased DNA damage and apoptosis, with lower proliferation (**Fig. 1**).

To specifically examine if *Trp53* status affected response of tumor cells to acute autophagy inhibition, we performed studies using CQ treatment to block autophagosome degradation as well as RNAi to critical autophagy genes in PDAC cell lines derived from *Trp53* heterozygous mice, *Trp53* homozygous mice and $Trp53^{R172H/+}$ mutated mice. These results were consistent with our previous findings that autophagy inhibition reduced both colony formation as well as oxidative phosphorylation in all PDAC cell lines independent of the original *Trp53* genetic status.

Finally, we performed efficacy studies using a panel of PDAC patient-derived xenografts (PDXs) using the CQ-derivative hydroxychloroquine (HCQ) as this is currently being tested in multiple clinical trials. Responses were seen in nearly every PDX line (11 of which had mutated *TP53*). Interestingly, the only PDX line that did not respond to HCQ treatment was *KRAS* wild type and had minimal basal levels of autophagy. Assessment of the xenografts showed that HCQ effectively inhibited autophagy as demonstrated by increased LC3 puncta. Similar to the *Atg5*-deleted PDAC tumors, HCQ treated PDXs had elevated apoptosis and reduced proliferation.

Together our data shows that *Trp53* status does not influence the response of PDAC to autophagy inhibition. While the concurrent homozygous deletion of *Trp53* and *Atg5* or *Atg7* during embryogenesis may accelerate PDAC progression, this is a different situation than what is seen in human PDAC where *TP53* is lost by LOH. In this scenario, autophagy appears to have a predominantly pro-tumorigenic role. Clinical trials in PDAC patients are currently assessing the efficacy of HCQ in combination with other therapies. Whether these will be successful will depend on a variety of factors, including the ability of HCQ to effectively inhibit autophagy in human patients. However, we think that our data do not support the exclusion of patients with *TP53* mutations from these trials.

Disclosure of Potential Conflicts of Interest

ACK is a consultant for Forma Therapeutics.

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