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AUTOPHAGY

Limiting factors

NRAS, KRAS and HRAS are often deregulated in cancer; however, increased RAS activity does not guarantee tumour formation, as other pathways, such as cell death or senescence, limit the effects of oncogenic RAS. Three recent papers have indicated that autophagy is induced by oncogenic RAS and both limits and enables cellular survival.

Human ovarian surface epithelial (HOSE) cells expressing an inducible *HRAS*^{V12} gene undergo cell cycle arrest and show reduced colony formation *in vitro*, so Seamus Martin and colleagues investigated the nature of this withdrawal from the cell cycle. They found that after approximately 1 week of HRAS^{V12} expression most of the cells underwent non-apoptotic cell death and that prior to this they appeared highly vacuolated — a characteristic of autophagy. Indeed, HRAS^{V12}-expressing cells also had increased levels of beclin 1, and knockdown of beclin 1 and other autophagy-associated genes inhibited HRAS^{V12}-induced cell death. Cells expressing HRAS^{V12} also had increased expression of the BH3-only protein NOXA, which was induced as a result of increased ERK activity downstream of HRAS^{V12}. Further experiments showed that induction of NOXA displaces beclin 1 from its interaction with the BCL-2 family member MCL1 and is associated with the degradation of this pro-survival protein. Thus, in the absence of other cooperating mutations, oncogenic activation of HRAS can induce autophagic cell death. But what happens when cooperating mutations are present, such as in established tumours *in vivo*?

Eileen White and colleagues looked at the role of autophagy in models of aggressive cancers that express either HRAS^{V12} or KRAS^{V12}. Expression of either form of RAS in immortalized baby mouse kidney (iBMK) cells increased rates of basal autophagy, even in the presence of nutrients. Moreover, the tumorigenicity of these cells in nude mice was substantially impaired by reduced expression of the essential autophagy genes *Atg5* and *Atg7*. Increased levels of autophagy were also evident in a number of human cancer cell lines with mutations in RAS family members, and suppression of autophagy by RNA interference induced cell death in some of these cell lines. The reasons for reduced viability in the absence of autophagy seemed to stem from a shortage of metabolites exclusively produced by the tricarboxylic acid cycle in mitochondria, impairment of mitochondrial respiration and accumulation of damaged mitochondria. In short, RAS expression under starvation conditions or during tumour growth amplifies energy depletion, and autophagy is required to balance this.

The clinical importance of these findings was underlined by Alec Kimmelman and colleagues, who examined autophagy in pancreatic ductal adenocarcinoma (PDAC), which often harbour mutated KRAS. Having established that PDAC cell lines have high basal levels of autophagy, these authors examined 25 biopsy samples of pancreatic intraepithelial neoplasia (PanIN; a pre-cancerous lesion) and 80 PDAC biopsy samples. Normal pancreatic epithelium and low-grade PanIN1 and PanIN2 did not have increased levels of autophagy, but the PanIN3 samples and PDAC samples did, as did lymph node metastases. Inhibition of autophagy using chloroquine or RNA interference against autophagy genes substantially reduced the proliferation of PDAC cell lines. These authors also found evidence for reduced mitochondrial respiration when autophagy was inhibited, and they proposed that autophagy is required to maintain ATP production. Mice with PDAC xenografts or orthotopic pancreatic tumours responded to treatment with chloroquine, and this drug significantly increased survival in genetically engineered mice with KRAS-driven PDAC.

All three papers indicate that oncogenic activation of RAS increases autophagy. In cells *in vitro* with no other oncogenic mutations, this can result in cell death. However, in tumour cells, RAS-driven autophagy becomes essential to ensure energy balance, making inhibition of autophagy in established tumours a therapeutic target.

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ORIGINAL RESEARCH PAPERS Guo, J. Y. *et al.* Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes Dev.* 11 Feb 2011 (doi:10.1101/gad.2016311) | Yang, S. *et al.* Pancreatic cancers require autophagy for tumour growth. *Genes Dev.* 15 Mar 2011 (doi:10.1101/gad.2016111) | Elgendy, M., Sheridan, C., Brumatti, G. and Martin, S. J. Oncogenic Rax-induced expression of Noxa and Beclin 1 promotes autophagic cell death and limits clonogenic survival. *Mol. Cell* 23 Feb 2011 (doi:10.1016/j.molcel.2011.02.009)