Braking self-cannibalism in cancer

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Autophagy is a catabolic process involving the degradation of a cell's own components through the lysosomal machinery. The process is tightly regulated and plays a normal part in cell growth, development, and homeostasis. Autophagy helps to maintain a balance between the synthesis, degradation, and subsequent recycling of cellular products. Autophagy is also a major mechanism by which starving cells reallocate nutrients from unnecessary processes to more-essential processes. The best-known autophagic mechanism involves the formation of a membrane around a targeted region of the cell that separates the contents from the rest of the cytoplasm. The resultant vesicle then fuses with a lysosome that subsequently degrades the contents (Fig. 1). Autophagy was described already 40 years ago; however, many questions remain about the actual processes and mechanisms. Autophagy may help to prevent or halt the progression of some diseases such as some types of neurodegenerative disorders and cancer. Autophagy may play a protective role against infection by intracellular pathogens; however, in other situations, autophagy could actually contribute to the development of diseases, such as cancer [1].

Autophagy is particularly active during metabolic stress. In the cancer cell, it fulfills a dual role, having tumor-promoting and tumor-suppressing properties. On the one hand, autophagy prevents necrosis and inflammation, which can lead to genetic instability. On the other hand,

autophagy might be important for tumor progression by providing energy through its recycling mechanism during unfavorable metabolic circumstances. A central checkpoint that inversely regulates autophagy is the mammalian target of rapamycin (mTOR) pathway. Anticancer drugs inhibiting the phosphoinositol-3 kinase (PI3K), protein kinase B (AKT), and mTOR axis influence autophagy [2]. However, whether autophagy contributes to the antitumor effects of these drugs, or to drug resistance, is largely unknown. The antimalarial drug chloroquine inhibits autophagy, leading to increased cytotoxicity when combined with several anticancer drugs.

In many cancers, activating mutations occur in signaling molecules that are activated by receptor tyrosine kinases. One such mutation involves the Kirsten rat sarcoma viral oncogene homolog (KRAS). RAS proteins are GTPases that are tethered to cell membranes by means of an isoprenyl group on the C terminus. KRAS is normally activated, through signals from an activated receptor tyrosine kinase (like epidermal growth factor for instance) and signals via intermediary proteins to RAF, MEK, and ERK, a cascade commonly called the RAF–MEK–ERK signaling pathway (Fig. 2, left panel). The signaling pathway begins when a growth factor (such as epidermal growth factor) binds to the receptor on the cell surface and ends when the DNA in the nucleus expresses a protein and produces some change in the cell, such as cell division. The pathway includes many proteins, including MAPK (originally called ERK), which communicate by adding phosphate groups to a neighbor protein. These signals act as “on” or “off” switches. When one of the proteins in the pathway is mutated (in this case KRAS), the cascade can be stuck in the “on” position. Drugs to inhibit the epidermal growth factor receptor are useless in patients with KRAS mutations, since the activation occurs downstream of the receptor.

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Fig. 1 There are four stages of autophagy. First, following external/internal stimuli, such as nutrient depletion or ischemia, mTOR is inhibited that leads to induction of autophagy. Key genes in yeast are Atg1 and Atg13. Second, autophagosomes are formed. A double-membrane vesicle that may arise from the endoplasmic reticulum sequesters cytosolic proteins and organelles (mitochondria shown here). Formation of this vesicle is coordinated by Atg proteins. Third, docking and fusion with the lysosome occurs. Fourth, the autophagic vesicle breaks down within the lysosome. Note the pivotal role of the mTOR pathway

There are numerous modulators influencing the RAF–MEK–ERK pathway. In addition to kinases capable of regulating RAS (not shown), RAF is modulated by AKT, which has an inhibitory effect on RAF. AKT in turn is influenced by PI3K. PI3K activation, via RAS, activates

AKT, which in turn activates mTOR (Fig. 2, right panel). AKT is a survival signal. In many cancers, this pathway is overactive, thereby reducing apoptosis and allowing proliferation. Thus, some experimental cancer drugs are aimed to inhibit the mTOR signaling sequence at various points.

Fig. 2 Receptor tyrosine kinase activation initiates MAPK pathway signaling, which leads to cell cycle progression and proliferation. MAPK pathway activation can also augment PI3K signaling via intermediates. Receptor tyrosine kinases bind with ligand and signal intermediate molecules. PI3K becomes activated, which results in phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP₂) to phosphatidylinositol 3,4,5-trisphosphate (PIP₃). At the cell membrane, PDK1 and AKT become phosphorylated. PDK1 can also

phosphorylate critical residues on AKT. The tumor suppressor complex, TSC1/TSC2, normally inhibits mTOR activation. Activated AKT prevents this inhibition, leading to activation of the mTOR/Raptor complex known as mTOR complex 1 (mTORC1). This complex can be inhibited by rapamycin and its analogs. Ultimately, mTORC1 leads to the activation of downstream proteins involved in the initiation of protein synthesis, resulting in cellular growth (figure adapted from [3])

Activating mutations in the PI3K catalytic alpha subunit have been described [3]. These mutations activate the mTOR pathway and contribute to carcinogenesis. Needed are tools to inhibit components of the pathways resulting in interactions between the RAF–MEK–ERK pathway and mTOR (Fig. 2, right panel). Since mTOR is a regulator of autophagy, we would expect effects on that process by such strategies.

In this issue of J Mol Med, Mirzoeva et al. [4] studied pancreatic ductal adenocarcinoma cells (PDA) to test the notion that PI3K/AKT/mTOR inhibition at two sites provides an advantage in inhibiting PDA cells and how the consequences of this inhibition influence autophagy. In PDA cells, the hallmark molecular alteration is an activating mutation in KRAS and a subsequent activation of the RAF–MEK–ERK pathway. The authors focused on the mTOR pathway. They found that a dual-targeted PI3K and mTOR inhibition was more effective in inducing PDA cells to undergo apoptosis, than targeting either of these components alone. However, the dual inhibition also resulted in increased autophagy in their cells, more than by targeting either component alone. As mentioned, mTOR is a suppressor of autophagy. Thus, inhibiting mTOR, similar to starvation, would be expected to stimulate autophagy. Administering an autophagy inhibitor resulted in increased antitumor activity in vitro and in a mouse in vivo model. The authors suggest that including autophagy inhibition, which can be achieved by administering chloroquin, might result in an augmented anticancer therapy. Recently, Yang et al. showed that pancreatic cancers require autophagy for tumor growth [5]. Most importantly, they also reported that inhibition of autophagy by genetic means or chloroquine treatment led to robust tumor regression and prolonged survival in pancreatic cancer xenografts and in genetic mouse models.

Interestingly, Di Nicolantonio et al. explored the interactions between the RAF–MEK–ERK signaling pathway and the PI3K–AKT–mTOR pathway in a similar cell-based study [6]. They studied a cancer cell line harboring a mutated PI3K catalytic a subunit (PIK3CA) that was mentioned above. Their cells were particularly sensitive to mTOR inhibition, whereas wild-type cells were not. In their cells, the simultaneous presence of a KRAS mutation resulted in resistance to mTOR inhibition. The experiments provided evidence that mutant KRAS led to mTOR-independent protein synthesis, possibly through activation of the ribo-

somal S6 kinase. KRAS mutations, in and of themselves, can stimulate autophagy [7]. The PDA cells employed by Mirzoeva et al. harbor a mutant KRAS. Thus, the fact that simultaneous inhibition of PI3K and mTOR by XL765 was necessary for a successful effect is not surprising. The novelty of the current report is the demonstration that inhibition of autophagy could limit tumor growth when the PI3K/mTOR pathway is adequately inhibited. Important will be subsequent studies of survival in animal tumor models. Whether or not chloroquin will have clinical utility as an autophagy inhibitor remains to be seen. The drug has well-known toxicities. One that is lesser appreciated is the development of restrictive cardiomyopathy [8]. However, against a devastating lethal disease, the drug could make a welcome comeback.

Respectfully,
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