Autophagy presents Achilles heel in melanoma

Strategies that affect the nutrient-sensing and metabolic pathways of cancer cells are receiving increasing attention. An attractive target in this respect is autophagy — however, autophagy inhibitors have so far shown only modest effects in pre-clinical cancer models. Reporting in *Cancer Cell*, Sabatini and colleagues now show that melanoma cells with an activated RAS–MEK–signalling pathway are particularly sensitive to leucine deprivation and fail to induce autophagy upon leucine withdrawal. Using a mouse xenograft model of melanoma, the authors show that the combination of an autophagy inhibitor and a leucine-free diet can synergistically inhibit tumour growth.

Autophagy is a cytoprotective metabolic pathway that allows cells to survive under nutrient-limiting conditions. It allows the recycling of metabolites such as essential amino acids, and is thought to play a central part in helping cancer cells to survive under nutrient limiting conditions. A key regulator of autophagy is the mammalian target of rapamycin (mTOR) signalling complex 1 (mTORC1), which is sensitive to amino acid levels and represses autophagy under nutrient-rich conditions.

Oncogenic transformation can render tumour cells sensitive to the deficiency of a particular amino acid. In four patient-derived melanoma

cell lines, the authors found that leucine deprivation failed to induce autophagy and triggered caspase-dependent apoptosis instead. Deciphering the complex molecular mechanisms of this process, the selective sensitivity to leucine deprivation was found to depend on the presence of mutated *BRAF*, a component of the RAS–MEK signalling pathway that acts as a critical oncogene in 50–70% of melanoma patients. *In vitro* experiments showed that melanoma cells fail to inactivate mTORC1 upon leucine deprivation, and therefore continue to suppress autophagy. Melanoma cell lines generated from melanocytes by introducing activating mutations to the RAS–MEK pathway were also sensitive to leucine deprivation, whereas untransformed melanocytes were not. Paradoxically, the treatment of these oncogenically activated cells with the MEK inhibitor U0126 or the mTORC1 inhibitor rapamycin, which are normally thought of as anticancer agents, abrogated their sensitivity to leucine deprivation — the treatment reactivated autophagy and led to enhanced survival of these cells.

For *in vivo* experiments, in which leucine-free conditions are difficult to achieve, the authors combined a leucine-free diet with an autophagy inhibitor to sensitize cells to leucine deprivation. Chloroquine, which is better known as a drug for

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the prevention of malaria, inhibits a late stage of the autophagy pathway. In mouse xenograft models of human melanoma, a leucine-free diet or treatment with chloroquine alone had little effect on tumour size, but both in combination synergistically inhibited tumour growth and induced caspase-dependent apoptosis.

This study provides proof of principle that the combination of autophagy inhibitors and strategies to deprive tumours of leucine can be used to inhibit tumour growth. The authors point out that in humans, a leucine-free diet is unlikely to be an ideal approach, but that it may be possible to deliver enzymes that specifically degrade leucine or develop small molecule leucine uptake inhibitors. Alternatively, agents that can deprive tumours of many nutrients, such as angiogenesis inhibitors, could be combined with autophagy inhibitors.

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ORIGINAL RESEARCH PAPER Sheen, J.-H. et al. Defective regulation of autophagy upon leucine deprivation reveals a targetable liability of human melanoma cells *in vitro* and *in vivo*. *Cancer Cell* **19**, 613–628 (2011)