TUMOUR CELL DEATH AND AUTOPHAGY

Our group is focused on understanding the factors regulating cell viability in cancer. Since inhibition of cell death mechanisms is a common event in tumour development, this poses problems for many forms of chemotherapy that utilise cell death pathways, leading to drug resistance.

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We are investigating known cell viability and integrity regulators in several processes including apoptosis and autophagy, as well as searching for novel proteins and pathways that control cell homeostasis, tumour growth and chemosensitivity. We envisage knowledge gained from our studies will be translated and lead to improvement of existing clinical regimens or new targets for therapeutic intervention.

Autophagy in cancer

Autophagy (literally, 'self-eating') is a major catabolic process in the cell whereby cellular cargoes are delivered to and degraded in lysosomes allowing the cell to remove misfolded/damaged proteins and organelles that would otherwise be toxic for the cell. As such, autophagy is highly homeostatic and a significant factor in the preservation of cellular integrity.

The most characterised form of autophagy, and the focus of our work, is macroautophagy, simply referred to as autophagy. The process is characterised by the formation of unique double-membraned vesicle, termed the autophagosome. The formation of autophagosomes is orchestrated via a series of evolutionarily-conserved **A**u**T**opha**G**y-related (ATG) proteins and as they grow they encapsulate cellular cargoes that are destined for degradation in the lysosome. Upon cargo digestion, the constituent parts of macromolecules are delivered back into the cytoplasm and can then either be recycled in biosynthetic pathways or further catabolised for the production of energy (Figure 1).

Due to its role in the preservation of cellular health and viability, autophagy protects against various forms of disease. In the context of cancer, the role of autophagy becomes complex. The consensus is that autophagy is tumour suppressive in normal cells and in the early stages of cancer. However, in established tumours, autophagy in tumour cells and associated stroma sustains the viability of tumour cells, hence in this context it promotes tumour maintenance. As a result, if we aim to destabilise tumour growth and viability by interfering with autophagy, it is imperative to understand how and at what stages in different tumour types autophagy ceased to be tumour suppressive and switches role to support tumour growth and preservation, so we can decide on the appropriate intervention.

The complex role of autophagy in cancer development

Previous work by our lab, showed that p53 tumour suppressor status could determine how autophagy affected the development of pancreatic ductal adenocarcinoma (PDAC) (*Nature*, 2013). These previous studies involved activation of mutant Ras, and deletion of p53 and essential autophagy genes *in utero*, and while this is common in mouse models of cancer, it did not best recapitulate the progression of PDAC in human. In addition, in human PDAC, p53 is rarely deleted, but if often retained, but mutated. As a result, we decided to test the involvement of autophagy in a system that was more in line with normal PDAC development and utilised a tamoxifen inducible Cre recombinase to cause more focal activation of mutant Ras, mutant p53 and impairment of autophagy in adult mice. This revealed, similar to what we had observed in mice that had gene recombination *in utero* that deletion of the essential gene *Atg7*, resulted in a higher percentage of mice development PDAC, as well as pre-cancerous lesions (*PNAS* 2022). To our surprise, however, we also found that hemizygous deletion of *Atg7* also resulted in enhanced tumour development, which was not expected, as loss of one allele of *Atg7* should not, and we found did not, ablate autophagy.

We were intrigued by these observations as this indicated that ATG7 had roles in tumour development beyond its role in autophagy. Because $Atg7^{-/-}$ mice were autophagy competent, they did not undergo the pancreatic destruction observed upon loss of autophagy in $Atg7^{-/-}$ mice. This enabled us to study the further progression of mutant Ras- and mutant p53-driven PDAC tumours in $Atg7^{-/-}$ animals; and we were again surprised to observe that loss of one allele of *Atg7* in PDAC driven by mutant Ras and mutant p53 reduced the number of mice with PDAC metastasis when compared to *Atg7* wild-type animals. This therefore indicated that it could potentially be possible to partially inhibit Atg7 function to inhibit metastasis, while circumventing the detrimental effects of inhibition of autophagy in the rest of the body.

Identification of novel autophagy regulators

It is undisputed that autophagy has a role in the prevention of tumour development, but also in the maintenance of established tumours. As a result, we have a constant quest to identify autophagy regulators that have either a selective or a metered impact on autophagy, which potentially could be targeted therapeutically. Our entry into the autophagy field began with our discovery of the Damage-Regulated Autophagy Modulator (DRAM) as a target gene of p53 (*Cell* 2006). DRAM, now renamed DRAM-1, was subsequently found to be a member of a family, which has 5 members in human. We previously characterised DRAM-2 and DRAM-3 and more recently, we turned our interest to DRAM-4 and DRAM-5. We found that different to DRAM-1, DRAM-4 and DRAM-5 are not induced by p53 but were instead induced by nutrient deprivation. Nonetheless, we found that over-expression of either protein, like DRAM-1, resulted in induction of autophagy. Seemingly paradoxically, however, CRISPR-mediated deletion of DRAM-4 also resulted in induction of autophagy. We found, however, that deletion of DRAM-4 caused compensatory up-regulation of DRAM-5, which induced autophagy. The consequence of these effects was revealed when we examined cell survival upon nutrient deprivation. Deletion of DRAM-4 promoted cell survival upon deprivation of amino acids, glucose or serum. This effect was, however, completely reversed by concomitant deletion of DRAM-5, highlighting new inter-connected players in the regulation of nutrient-deprived conditions as occurs in the development of most solid tumours.

Publications listed on page 110

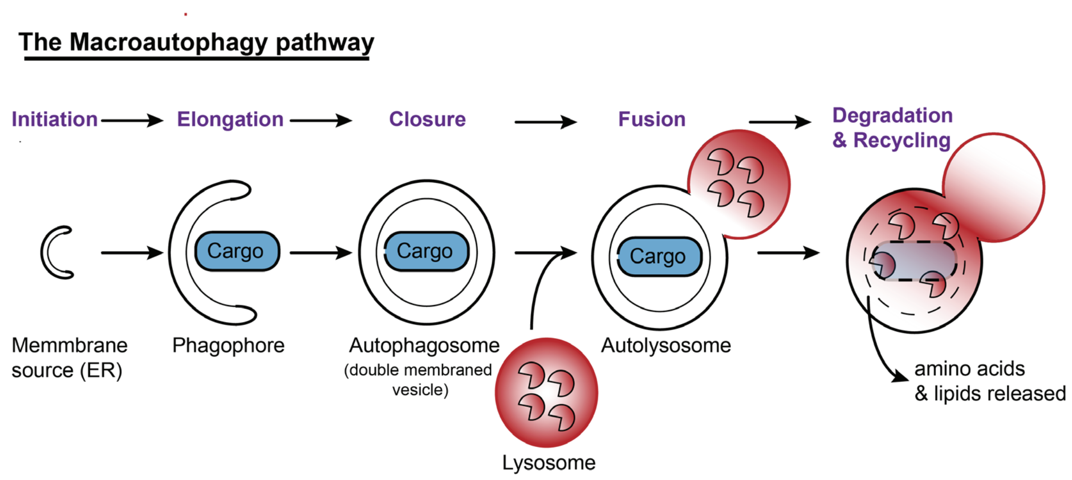

Figure 1
The autophagy pathway
The autophagy pathway is a lysosomal degradation pathway that the cell utilises to maintain cellular homeostasis and acts as an adaption mechanism for dealing with stress. Proteotoxic stress (accumulation of aggregates), reactive oxygen species (ROS), infection and nutrient depravation (starvation) all act to initiate (1) the formation of the double membrane structure, termed the autophagosome. After initiation, the phagophore undergoes an expansion (2) to engulf cellular cargo (including pathogens, damaged organelles and protein aggregates) prior to sealing (3) and closure to form the autophagosome. This then fuses (4) with the lysosome, to form the autolysosome where the contents and inner membrane are degraded (5) and recycled (6) back to the cell as basic 'building blocks' such as amino acids, lipids and other nutrients.

Figure 2

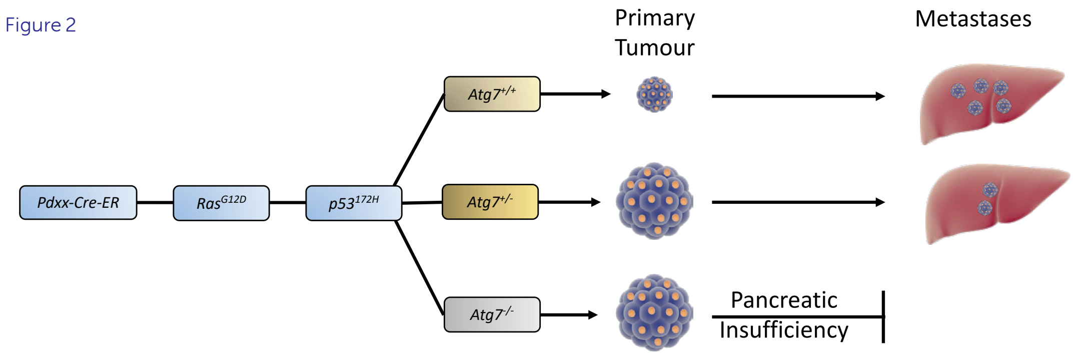

Figure 2
Gene dosage of Atg7 regulates tumour development and metastasis
Studies of the essential autophagy gene Atg7 in the development of PDAC revealed autophagy-independent effects caused by its hemizygous deletion. This mono-allelic loss did not inhibit autophagy, but enhanced PDAC tumour development and conversely resulted in fewer mice with metastatic disease.

Figure 3

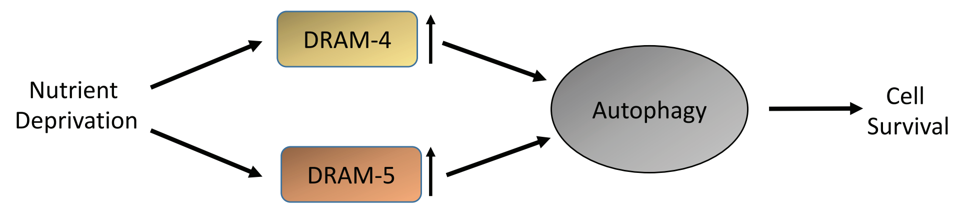

Figure 3
DRAM-4 and DRAM-5 regulate cell survival in response to glucose deprivation
DRAM-4 and DRAM-5 are novel autophagy regulators that are related to DRAM-1, and which are induced by nutrient deprivation. Over-expression of DRAM-4 or DRAM-5 induces autophagy. In addition, deletion of DRAM-4 also induces autophagy to promote cell survival under nutrient deprivation, but this occurs through compensatory up-regulation of DRAM-5.