TUMOUR CELL DEATH AND AUTOPHAGY

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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.

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 characterized form of autophagy, and the focus of our work, is macroautophagy, which is often simply referred to as autophagy. The process is characterised by the formation of unique double-membraned vesicles, termed autophagosomes. 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 catabolized 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 destabilize tumour growth and viability by interfering with autophagy, it is imperative that we understand how and at what stages in different tumour types autophagy ceases to be tumour suppressive and switches role to support tumour growth and preservation, enabling appropriate intervention.

Identifying and understanding factors that regulate autophagy

Previous work by our lab, showed that p53 tumour suppressor (Crighton *et al.*, 2006, *Cell*), its related family member p73 (Crighton *et al.*, 2007, *CDD*), the hypoxia inducible transcription factor HIF-1α Wilkinson *et al.*, 2009, *Genes Dev*) and the chromatin modifier BRD4 (Sakamaki *et al.*, 2017, *Mol Cell*) are all regulators of autophagy, indicating that several key cancer-related pathways impact on autophagy. More recently, as well as identifying new autophagy regulators, our attention has also turned to understanding how known autophagy regulators affected the process. An example of this is our work on the leucine-rich repeat kinase 2 (LRRK2), which is frequently mutated in a high percentage of cases of Parkinson's disease and has also been implicated in cancer. In collaboration with the team headed by Prof. Jan Parys at KU Leuven, Belgium, we were interested in the mechanism of autophagy regulation by LRRK2. Our focus was on a cluster of phosphorylation sites where phosphorylation increases upon nutrient deprivation, as can occur in a developing tumour. We found that mutation of these phosphorylation sites impaired autophagy and lysosomal function, implicating the phosphorylation of these sites as a key event in starvation-induced autophagy. Interestingly, inhibition of LRRK2's own kinase activity also resulted in dephosphorylation of the phosphorylation cluster, but did not affect autophagy. As an explanation of these apparently contradictory results, we observed that mutation of the phosphorylation cluster resulted in increased LRRK2 kinase activity that was required to impair autophagy (Kania *et al.*, 2023, *CDDis*). These findings therefore provide insight into an additional control point for autophagy that is relevant to human disease. We continue to work on other known autophagy regulators to see how they function *in vitro* and *in vivo* and how they are affected by the novel autophagy regulators we identify.

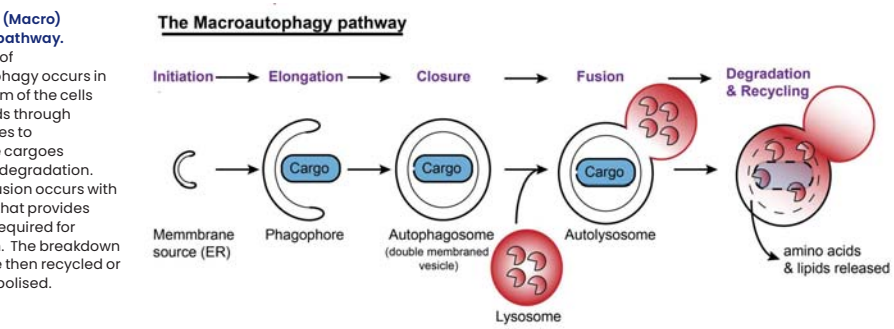

Figure 1: The (Macro) Autophagy pathway.
The process of macroautophagy occurs in the cytoplasm of the cells and proceeds through various stages to encapsulate cargoes destined for degradation. Ultimately, fusion occurs with a lysosome that provides hydrolases required for degradation. The breakdown products are then recycled or further catabolised.

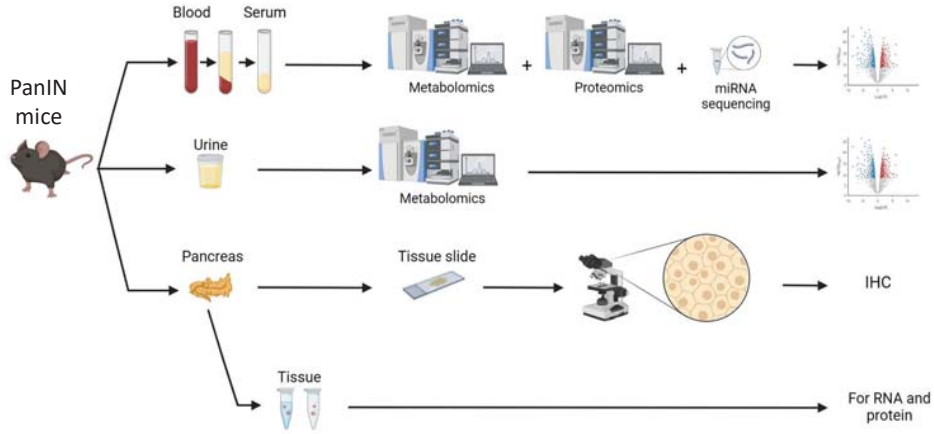

Figure 2: Utilizing autophagy to identify biomarkers of pre-cancerous pancreatic cancer.
Detection of pre-cancerous lesions enables the selection of individuals who are at risk and facilitates tumour formation at an early stage at which it can be treated. Mouse models were used to identify potential biomarkers in a mouse model with excessive Pancreatic interaepithelia neoplasia (PanIN) - the precursors of pancreatic ductal adenocarcinoma (PDAC). Identified factors were triaged and confirmed in an established mouse model of PDAC formation driven by mutant Ras and p53. The subsequent utility of these identified factors to identify human PanINs is currently being examined. IHC, immunohistochemistry.

Identification of potential biomarkers of pre-cancerous pancreatic cancer

It is widely accepted that the early detection of cancer results in more tractable therapeutic strategies, and as a result, better patient prognosis and reduced numbers of cancer-related deaths. Pancreatic ductal adenocarcinoma (PDAC) currently has very poor prognosis with only 7% surviving 5 years after diagnosis. Pancreatic intraepithelial neoplasia (PanIN) are considered to be the precursors of PDAC and the ability to detect PanINs would enable identification of patients at increased risk who can be monitored more frequently for the early stages of PDAC development.

Using a mouse model of autophagy that we previously described as having excess PanIN formation (Rosenfeldt *et al.*, 2013, *Nature*), we have utilized proteomics, metabolic mass spectrometry and microRNAseq to identify potential serum or urine biomarkers of PanIN formation (Figure 2). To triage the hits from these screens, we also analysed the serum and urine from genetically engineered mice that express the tumour-promoting genes mutant Ras and mutant p53 in their pancreata. Having successfully validated a number of hits, we are now examining if these potential biomarkers can be used to identify human PanINs and more importantly PanINs that are likely to progress to PDAC.