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TUMOUR CELL DEATH

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[1]Worldwide Cancer Research
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The aim of our group is to understand the factors regulating cell viability in cancer. Since it is known that 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 both known cell death regulators as well as searching for novel proteins and pathways that control cell viability and chemosensitivity. We envisage that the knowledge gained from our studies will be translated and lead to the improvement of existing clinical regimens or new targets for therapeutic intervention.

The role of autophagy during tumour development

There are many pathways in cells that regulate cell viability. One group of processes that promote cell viability by preserving cellular integrity are collectively known as autophagy – literally, 'self eating'. Autophagy, and more specifically the form called macroautophagy (hereafter referred to simply as autophagy), is a mechanism of cellular traffic that delivers cytoplasmic material to lysosomes for degradation (Fig. 1). As such, autophagy is a major mechanism for the removal of damaged proteins and organelles, thereby promoting cellular fidelity. In addition, autophagy can be modulated in response to various forms of stress and can mitigate the effects of this stress to promote cell viability and also cell survival.

It is now well established that autophagy has an important role in both tumour suppression and tumour development. However, the way in which autophagy is controlled in response to various stimuli is currently incompletely resolved, and this area is a major focus of our lab. We hope that the knowledge gained will enable the bespoke modulation of autophagy in a way that potentiates the effectiveness of chemotherapeutic responses in tumours without detrimental effects on normal tissue.

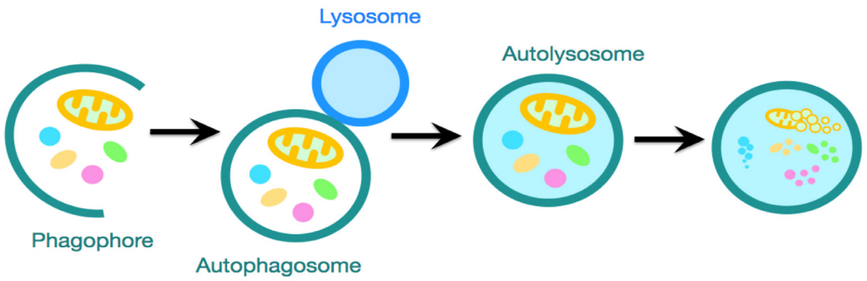

Figure 1
The process of macroautophagy
Within the cytoplasm of cells, membranes nucleate and grow to encapsulate cargoes in double-membraned structures called autophagosomes. Ultimately, autophagosomes fuse with lysosomes to form an autolysosome, within which the cargo is degraded by acidic hydrolases. The breakdown products are then recycled into the cytoplasm where they are either further catabolised or recycled into biosynthetic pathways.

The transcriptional control of autophagy

As an approach to identify new autophagy regulators, we performed an RNA interference (RNAi) screen in *Drosophila* cells. *Drosophila* cells were chosen as a model system due to their relatively low level of functional redundancy and due to the ease with which human counterparts of *Drosophila* proteins can be identified. Through this screen, we identified the product of the *Drosophila* gene *fs(1)h* as a regulator of autophagy.

The human orthologues of fs(1)h are members of the bromodomain and extraterminal (BET) family of proteins. There are four BET proteins in human cells: BRD2, BRD3, BRD4 and BRDT. Our further analysis revealed that BRD4 was also an autophagy regulator, and more specifically we found the protein was a repressor of genes involved in both autophagy and lysosome function.

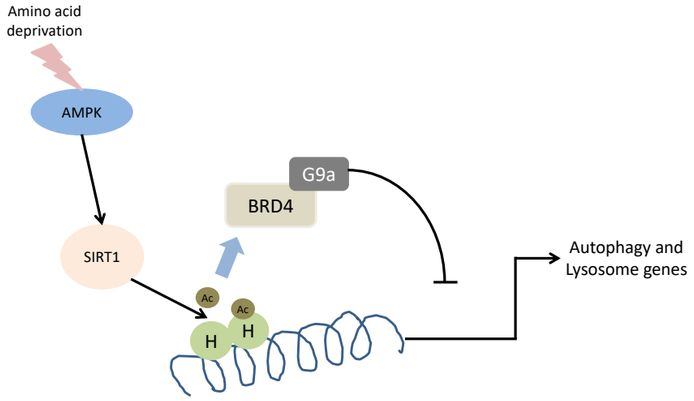

Figure 2
BRD4 represses expression of genes involved in autophagy and lysosome function. Amino acid deprivation signals histone deacetylation via AMPK and SIRT1. This causes displacement of the BRD4-G9a complex from chromatin, resulting in de-repression of genes involved in autophagy and lysosome function. H, histones; Ac, acetylation.

BRD4 is a chromatin reader protein which binds to acetylated histones. The protein then recruits proteins which can modulate gene expression, such methyltransferases. We were therefore interested to understand how this mechanism of gene regulation might be affected under a physiological autophagic response. Amino acid deprivation elicits an evolutionarily conserved autophagic response, and we found that under these conditions, BRD4 dissociates from chromatin through the deacetylation of histones. This response involves the histone deacetylase SIRT1 and the energy-sensing AMP-activated protein kinase (AMPK) (Fig. 2).

In most situations, BRD4 has been reported to be involved in the activation of gene transcription, but in the case of autophagy and lysosome genes, we found BRD4 to be a repressor. This indicated that BRD4 must bind a repressor of gene transcription to mediate this effect. Through analysis of the literature, we learnt that the methyltransferase G9a can act as both an activator and repressor of gene transcription, and our subsequent studies revealed that G9a was indeed a repressor of autophagy and lysosome genes via BRD4.

The main purpose of our study was to identify autophagy regulators that may modulate autophagy in a specific manner. To this end, we examined if BRD4 was a repressor of autophagy in response to additional autophagic stimuli. This revealed that in addition to amino acid deprivation, inhibition of BRD4 augments autophagy induced by glucose starvation, hypoxia, oncogenic Ras and the protein aggregates associated with Huntington's disease. In contrast, inhibition of BRD4 has no effect on the autophagic clearance of bacteria or mitochondria.

BET domain proteins are considered promoters of tumour development in a variety of cancers. In particular, a chromosome translocation involving *BRD4* and a gene called *NUT* produces a fusion protein called BRD4-NUT, which is considered the driver of a specific type of cancer called NUT midline carcinoma. As a result, several BET inhibitors have been developed for the treatment of this and other cancers. We were therefore interested to know whether BRD4-NUT and also BET inhibitors have an effect on autophagy. In line with what we observed upon knockdown of BRD4, knockdown of BRD4-NUT caused a marked induction of autophagy. Similarly, treatment of cells with various BET inhibitors also caused induction of autophagy. Since in many cases autophagy is cytoprotective, this result opens up the exciting possibility of combing BET inhibitors with inhibitors of autophagy to give an enhanced therapeutic response.

Application of CRISPR reveals new roles for autophagy

The CRISPR (Clustered Regulatory Interspaced Short Palindromic Repeats) system has revolutionised the ability to investigate gene function in cells and animals. The system can be used to disrupt or mutate endogenous genes very efficiently. We applied this system to delete essential autophagy genes, enabling us to understand the role of autophagy in cancer cells under various settings. Against common belief that the majority of human cancer cells are dependent on autophagy, we found that acute CRISPR-mediated disruption of either *Atg5* or *Atg7* (two genes essential for macroautophagy) was consistent with cell viability in a panel of human tumour cell lines. These findings have important implications for targeting autophagy in human cancer, although the survival of these cells under conditions of tumour-associated stress is yet to be determined.

Using these autophagy-deficient cell systems, we have also been able to assess the role of autophagy in therapeutic situations. In this regard, we found that the drug Verapamil – a cardiac drug which is being considered for cancer treatment – was a potent inducer of autophagy. This drug also causes apoptosis, and treatment of autophagy-deficient cells with Verapamil resulted in an enhanced cytotoxic effect, indicating that combinations of Verapamil with autophagy inhibitors is worthy of further investigation.

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