

Apoptosis and Tumour Metabolism

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In order to engage in fast replicative division, a cancer cell must duplicate its genome, synthesise proteins and lipids, and assemble these components to form daughter cells. These activities require increased uptake of nutrients to be used as biosynthetic precursors and an energy source. However, rapid tumour growth surpasses the required blood supply and exposes cancer cells to extreme conditions of metabolic deficit and stress. Therefore, cancer cells have developed traits that cater for their unique metabolic conditions. Targeting these survival mechanisms would help curb cancer growth. To this end, my laboratory studies the relationship between cancer, cell metabolism and cell death.

Apoptotic events on the mitochondria

Apoptosis is a highly regulated pathway by which cells participate in their own killing. It is essential for the development and preservation of multicellular organisms, eliminating cells that are superfluous or defective. Most apoptotic cascades require an early mitochondria-dependent step involving the release of apoptogenic factors (such as cytochrome c) from the mitochondria into the cytosol, where they activate the cellular programme that commits a cell to die. The permeabilisation of the mitochondrial outer membrane to these

proteins is regulated by the Bcl-2 family of proteins that comprises both pro-apoptotic (e.g. Bak and Bid) and anti-apoptotic (e.g. Bcl-2) proteins.

Glucose metabolism has been demonstrated to play a crucial role in apoptosis – linking apoptosis to evolutionarily conserved, nutrient-dependent signalling cascades (King and Gottlieb, *Curr. Opin. Cell Biol.* 2009; 21: 885). It has recently been shown that in the apoptotic network, lipids too are important. Cardiolipin, a mitochondria-specific phospholipid has emerged

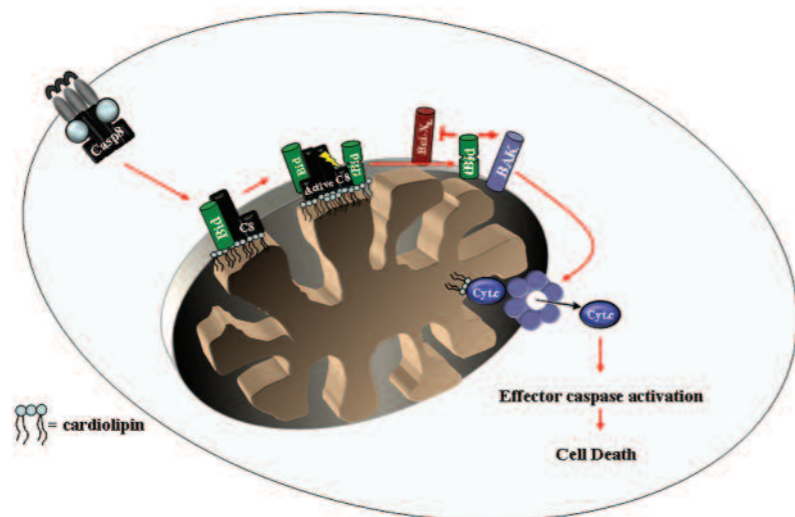


Figure 1
Caspase-8 forms a native complex with Bid on the mitochondria. The first step entails the initial activation of apoptosis by an external signal such as Fas or TRAIL and the formation of the death inducing signalling complex (DISC) on the plasma membrane. The next step is the stable insertion of active caspase-8 into the mitochondrial membrane. In the third step, active caspase-8 cleaves BID within the native complex on the mitochondrial membrane. tBID dissociate from the caspase-8/BID complex and shifts to separate complexes on the mitochondrial membrane where it interacts with BAK and/or BCL-XL to activate mitochondria outer membrane permeabilisation. Finally, apoptogenic factors, such as cytochrome c and Smac/DIABLO, are released from the mitochondria to activate effector caspases and cell death.

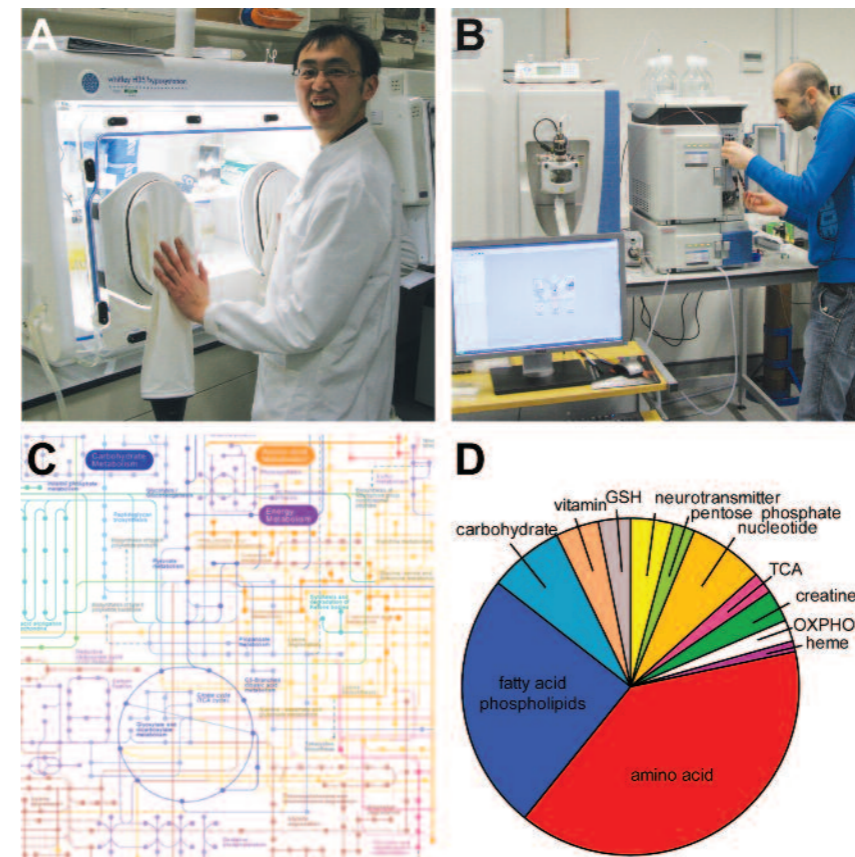


Figure 2
Studying metabolomic profiles of cells under hypoxia. Cells cultured under controlled low oxygen conditions (A) were extracted for metabolite profiling by LC-MS (B) where the steady-state levels of 300 different metabolites were identified. Each metabolite was assigned to known metabolic pathways based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. (C) and statistical methods, specifically designed to identify pathway activation, were applied to classify the metabolic signature of hypoxic cells (D).

as a player in many of the mitochondrial-dependent steps of apoptosis (Schug and Gottlieb, *Biochim. Biophys. Acta* 2009; 1788: 2022). We have recently shown that cardiolipin is required for the mitochondria-dependent extrinsic apoptotic pathway. In particular, cardiolipin is fundamental to the formation of an apoptotic signalling platform on the mitochondrial outer membrane supporting the recruitment, processing and activation of caspase-8, a necessary step for an efficient apoptotic response that is initiated by an external death signal (Gonzalvez et al., *J. Cell Biol.* 2008; 183: 681). However, the purpose of activating caspase-8 on the mitochondrial membrane remains unknown. We have now identified a native complex containing caspase-8 and Bid on the mitochondrial membrane (Schug et al., *Cell Death Differ.*, in press). Furthermore, we showed that apoptotic cells stabilise active caspase-8 on the mitochondria in order to specifically target mitochondria-associated Bid and that Bid cleavage on the mitochondria is essential for cytochrome c release (Fig. 1). Activation of caspase-8 within this native complex induced the cleavage of Bid to its active, truncated form tBid that then moved to a separate mitochondria-associated complex containing Bak. Our findings indicate that during the extrinsic apoptotic response, caspase-8 can specifically target Bid where it is needed, on the surface of mitochondria.

Metabolism and cancer

It is now well established that cancer cells undergo many metabolic changes (collectively

known as the 'metabolic transformation' process) that support their growth and survival (Tennant et al., *Carcinogenesis* 2009; 30: 1269). Studying the metabolic profiles of cancers is of extreme importance in identifying new targets for therapy (Tennant et al., *Nature Rev. Cancer* 2010; 10: 267). Our work involves a team of biologists, chemists and computer scientists who develop new and sophisticated tools for investigating metabolic changes in cancer. This requires the employment of new technologies in analytical chemistry and the development of new tools to examine and decode the data. Quantifying hundreds of different metabolites (metabolomics) is a technological challenge, while understanding the changes in the metabolome within the context of a large metabolic network, which consists of thousands of enzymatic reactions, is a computational one. Nevertheless, both chemical and computational methods are needed for accurate prediction of new targets in the metabolic network of cancer. We are taking these systems biology approaches to study metabolic alterations in cancer cells with inborn metabolic defects or in cells exposed to environmental stress.

Low oxygen availability (hypoxia) is one of the features of poorly vascularised areas of solid cancers. Yet, cancer cells can survive in these hypoxic regions despite the very low oxygen tension. The adaptation to hypoxia requires both biochemical and genetic responses that result in a metabolic rearrangement to counter-balance the decrease in energy supply from mitochondrial respiration. However, the exact nature of the metabolic adaptation of cancer cells to hypoxia is not fully known. We assessed the effects of hypoxia on cellular metabolism in colorectal cancer cell lines. Prolonged hypoxia led to an increase in glucose metabolism and a decrease in respiratory rate accompanied by mitochondrial dysfunction. In order to better define the metabolic signature of the hypoxic cells, an unbiased metabolomic analysis was performed using liquid chromatography–mass spectrometry (LC-MS) (Fig. 2). We found that the metabolomics fingerprint of hypoxic cells was dominated by catabolic products of lipids and proteins, suggesting that hypoxic cells might rely on autophagy, the process of 'self-eating', as an energy source. Indeed, we demonstrated that the inhibition of autophagy led to an increase in cell death under hypoxia. These results illustrate the importance of synchronised and regulated catabolism as a mechanism of adaptation to bioenergetic stress, and suggest that inhibiting autophagy may be used to target hypoxic regions of tumours, the most notoriously resistant areas to chemo- and radiotherapy.

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