Regulating the mitochondrial decision to die

Background

Mitochondria are tiny organelles present in every cell in our body. They are essential for energy production, generated in the form of a molecule called ATP. However, mitochondria are also often essential to kill a cell, through a form of cell suicide called apoptosis. During apoptosis, mitochondria become leaky, releasing proteins that actively kill cells. Apoptosis is key to keeping humans healthy. In cancer, apoptosis can be inhibited allowing cells to become cancerous and also allowing them to become resistant to conventional chemo- and/or radiotherapy. As such, intense interest surrounds mechanisms regulating mitochondrial permeabilisation in order to target this process in health and disease. This Marie Curie project was aimed at further understanding if differences in the speed and extent of mitochondrial permeabilisation can impact on cell death.

Results and Future Work

During our research we developed a new way to detect mitochondrial permeabilisation making use of fluorescent fusion proteins (Figure 1). In this method, GFP re-localises onto RFP labelled mitochondria when they are permeabilised, this leads to mitochondria turning yellow – we can detect this by microscopy.

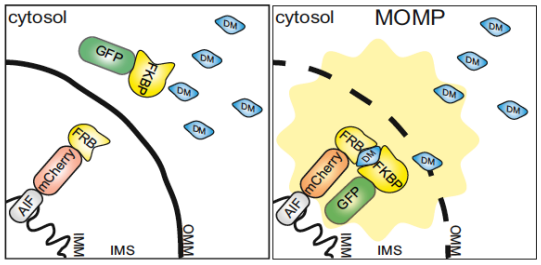

Figure 1 (see text for details).

Using this approach we have found that sub-lethal stresses can engage mitochondrial permeabilisation in a minority of mitochondria, a process we call minority MOMP. Importantly minority MOMP does not lead to cell death but instead triggers DNA-damage. These data argue that apoptosis signalling is not always anti-cancer *per se* and instead may promote cancer in some circumstances – we are investigating this further (Figure 2) [1].

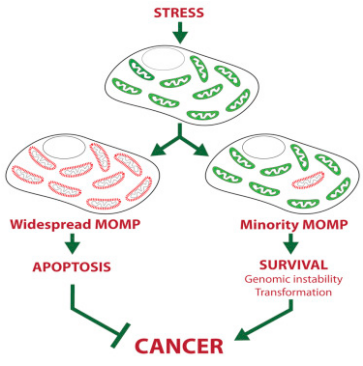

Figure 2 (see text for details)

Intense interest surrounds that targeting of proteins, called Bcl-2 proteins, that prevent mitochondrial permeabilisation. Indeed, recently developed drugs called BH3-mimetics target Bcl-2 proteins and in doing so, sensitise to apoptosis. The BH3-mimetic compound called Venetoclax has recently been clinically approved for the treatment of specific type of leukaemia. Unfortunately, the Bcl-2 protein family is relatively diverse such that no-one inhibitor can inhibit them all – this represents a source of resistance. The ability to screen for specific new inhibitors, as well as investigate resistance mechanisms is therefore key. Driven by this, and the desire to develop a clean-system to trigger mitochondrial apoptosis we developed a method we

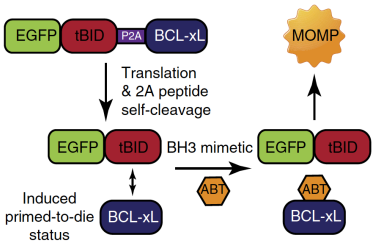

Figure 3 (see text for details)

call "mito-priming" [2]. In this we co-express a pair of proteins, one a pro-apoptotic killer BH3-only protein the other an anti-apoptotic Bcl-2 protein. Cells expressing this combination are viable but highly sensitive to the addition of BH3-mimetic compounds. Following treatment, cells rapidly succumb to cell death. The specific combination of killer and protector can be altered. This method serves both as a powerful research tool as well as a means to rapidly screen for new and specific Bcl-2 targeting inhibitors.

References

1. Ichim, G. et al. Limited mitochondrial permeabilization causes DNA damage and genomic instability in the absence of cell death. *Mol Cell* **57**, 860-72 (2015).
2. Lopez, J. et al. Mito-priming as a method to engineer Bcl-2 addiction. *Nat Commun* **7**, 10538 (2016).

Contact Details

Stephen Tait

Cancer Research UK Beatson Institute
Institute of Cancer Sciences
University of Glasgow
Garscube Estate, Switchback Road
Glasgow, G61 1BD
United Kingdom

tel – +44 (0)141 330 8703
e-mail – stephen.tait@glasgow.ac.uk