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# MITOCHONDRIA AND CANCER CELL DEATH


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The best way to treat cancer is to kill it. Indeed, most cancer therapies work by killing tumour cells, be it directly or indirectly. Nevertheless, issues of toxicity and resistance limit the effectiveness of anti-cancer therapies. To address these, our research centres on understanding how mitochondria regulate cancer cell death and inflammation, with the ultimate goal of improving cancer treatment.

## Mitochondria, cell death and cancer

Apoptosis requires caspase protease activity, leading to widespread substrate cleavage and rapid cell death. During apoptosis, mitochondrial outer membrane permeabilisation (MOMP) occurs, a crucial event that is required for caspase activation. Following MOMP, mitochondrial intermembrane space proteins, such as cytochrome *c*, are released into the cytoplasm where they cause caspase activation and apoptosis. Given its key role in controlling cell survival, mitochondrial outer membrane integrity is highly regulated, largely through interactions between pro- and anti-apoptotic Bcl-2 proteins. Cancer cells often inhibit apoptosis by preventing MOMP, often through upregulation of anti-apoptotic Bcl-2 proteins. Importantly, this can be exploited therapeutically – newly developed anti-cancer therapeutics called BH3-mimetics target these apoptotic blocks.

## How a cancer cell dies matters

Irrespective of caspase activity, widespread MOMP commits a cell to die and is therefore a point-of-no-return. As long as a cancer cell dies, should we care how it dies? Our recent findings argue a resounding yes. We have found that

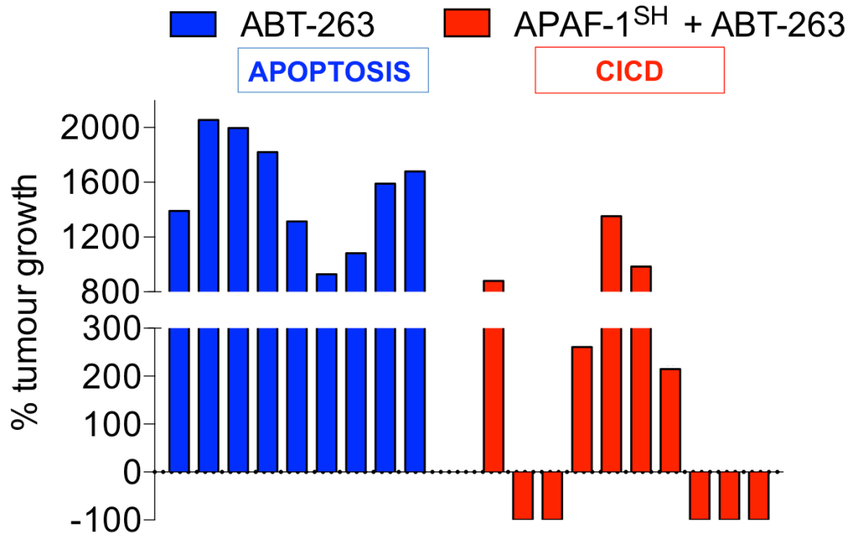


Figure 1
Caspase-independent cell death is more effective than apoptosis at inhibiting tumour growth
Control, apoptosis-proficient CT26 colorectal cancer cells were rendered CICD proficient through shRNA knockdown of APAF-1. Cells were implanted into syngeneic, immunocompetent BALB/c recipient mice. Following tumourigenesis, mice were treated with the BH3-mimetic ABT-263 to engage apoptosis (blue) or CICD (red). Tumour volume was measured over time; individual tumour growth (%) is shown. Inducing CICD inhibited tumour growth in all cases, where complete tumour regression was observed in 50% of mice.

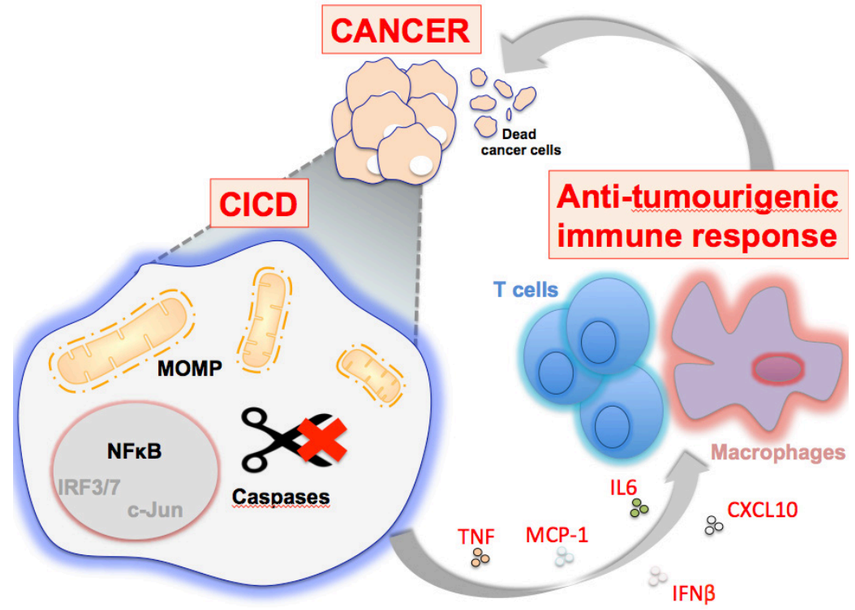


Figure 2
Caspase-independent cell death: an anti-cancer double whammy
Following widespread mitochondrial permeabilisation, cancer cells die regardless of caspase activity. Inhibiting caspases, leading to caspase-independent cell death (CICD), has multiple beneficial effects. First, it inhibits caspase-associated toxicity. Second, cancer cells undergoing CICD are immunogenic – this requires NF-κB-dependent cytokine upregulation. By dying in this manner, CICD triggers host anti-tumour immunity that can kill remaining tumour cells.

under caspase-inhibited conditions following MOMP, cells still die through caspase-independent cell death (CICD) but produce a variety of pro-inflammatory cytokines; these can stimulate an immune response towards the dying cell. As such, unlike apoptosis, CICD can be considered an immunogenic form of cell death. Importantly, we have found that CICD can elicit anti-tumour immunity. Using an *in vivo* model that mimics partial therapeutic responses, we have found that CICD is much more effective than apoptosis at reducing tumour growth – often CICD led to complete regression (Fig. 1). These beneficial effects are entirely dependent on intact immunity, consistent with CICD being an immunogenic cell death.

## Mitochondria drive immunogenic cell death

Investigating how CICD could be immunogenic, we focused on the role of mitochondria. Interestingly, we find that, under caspase-inhibited conditions, mitochondrial permeabilisation leads to activation of the NF-κB transcription factor pathway. This, in turn, is required for inflammatory signaling during CICD. Mechanistically, mitochondria activate NF-κB by releasing proteins that downregulate cIAP1/2, resulting in NIK and NF-κB activation. Similar to others, we have found that permeabilised mitochondria, by releasing mtDNA, can also activate cGAS/STING, triggering an interferon response. As such, while mitochondrial apoptosis is largely viewed as a non-inflammatory type of cell death, the central event that initiates it – MOMP – is in itself pro-inflammatory. Key questions currently being investigated include 1) are there additional inflammatory signals initiated by mitochondria? and 2) how do caspases suppress these effects? Finally, in addition to targeting these effects to improve cancer treatment, we are investigating roles for these inflammatory effects in different areas of health and disease.

## Caspase-independent cell death – an anti-cancer double whammy

We have previously found that sub-lethal caspase activity can promote DNA damage and genomic instability. Moreover, other studies have shown that caspase-dependent effects may contribute to tumour growth as well as the toxicity of chemotherapy. Coupled to our recent findings discussed above, this suggests that the benefits of inhibiting caspase function in cancer therapy would be multi-fold. Not only will it block unwanted toxicity but, by engaging anti-tumour immunity, caspase inhibition enhances therapy-induced killing (Fig. 2). Based on this, we are investigating the potential benefit of targeting CICD in a range of cancer types.


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