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AUTHOR'S VIEWS

Sometimes even apoptosis fails: implications for cancer

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ABSTRACT

Apoptosis is vital for the correct morphogenesis of multi-cellular organisms. However, like most physiological programs, the cell's ability to commit suicide is hijacked by cancer in its own proliferative and invasive interest. We recently showed that inefficient execution of apoptosis (or failed apoptosis) is used by cancer to boost invasiveness.

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Author's views

Successful cancer treatment largely relies on efficient induction of cancer cell apoptosis. In most cells, this well described form of regulated cell death requires two interconnected processes: widespread mitochondrial permeabilization and efficient caspase protease activation that will demolish host cells within minutes. Recently, the infallible execution of apoptosis has come under debate: in certain settings, cells can sustain limited mitochondrial permeabilization and non-lethal activation of otherwise deadly caspases.[1-3] Maintaining mitochondrial permeabilization and caspase activity under a lethal threshold accounts for the well-described vital functions of caspases in various physiological processes, ranging from immune cell function, stemness, neuronal axon branching to muscle cell differentiation.[4] Although well defined in physiological settings, the relationship between non-lethal engagement of apoptosis and cancer is poorly understood. In a recently published article, we argue that failure to completely execute apoptosis (so-called failed apoptosis) has a profound impact on melanoma cancer aggressiveness (Figure 1).[1,5]

To better characterize the consequences of failed apoptosis on cancer cells, we first set up a cellular model to precisely visualize, quantify and ultimately isolate cancer cells which maintained effector caspase activation at non-lethal levels. This is based on the stable expression of a switch-on fluorescence reporter for effector caspases alongside a tunable doxycycline-inducible system for expressing the pro-apoptotic protein tBID (truncated BH3-Interacting Domain Death Agonist protein).[6] By incrementally reducing the concentration of doxycycline, we obtained and isolated by cell sorting, a discrete cell population characterized by caspase activity, in the absence of hallmarks of apoptosis (annexin V positivity and nuclear membrane permeabilization). An alternative approach to inducing failed apoptosis was obtained by dialing-down chemotherapy doses. By using bulk RNA sequencing analysis of melanoma cells experiencing failed apoptosis, we defined a transcriptomic signature specifically enriched in cell motility-related genes. Consistently, failed apoptotic melanoma cells displayed a sharp increase in their capacity to migrate and invade. This was also validated in three *in vivo* animal models of metastasis: zebrafish, chicken embryos and mice. Most of the genes composing the failed apoptosis signature are transcriptionally regulated by the AP-1 (Activating Protein-1) transcription factor that is orchestrated by upstream activation of JNKs (c-Jun N-terminal kinases), corroborating recent publications.[7,8] This signature may be clinically relevant since was also detected in most melanoma cells in a metastatic tumor that was analyzed by single cell RNA sequencing. In addition, the failed apoptosis transcript signature discriminated between primary and metastatic melanoma. Aside from cancer cells, this effect of failed apoptosis may be applicable to other cell types such as stem cells, neurons or muscle fibers, in which a more detailed investigation into the non-lethal roles of caspases is needed. The ability to isolate failed apoptotic cells by cell sorting could facilitate their analysis by single cell RNA sequencing, and may enable us to address an unanswered question: at a given time, what drives most of the cells to undergo efficient apoptosis while some cells experience failed apoptosis. Moreover, it remains to be established whether changes in gene expression in cells evading other forms of programmed cell death such as necroptosis are similar to those observed in failed apoptotic cancer cells. In a recent insightful review on the future of cell death research, Douglas Green presented five unanswered riddles.[9] The first one is how deadly is death and more precisely, how deadly is mitochondrial outer-membrane permeabilization (or MOMP)? Up until recently, MOMP was considered to be the "point-of-no-return" for intrinsic apoptosis. Considering our recent results, should we set a new "point-of-no-return" for apoptosis? Can a dying cell escape death, recover and live? For apoptosis, MOMP is no longer a death sentence when triggered partially. In certain settings, some mitochondria avoid permeabilization and then repopulate a cell.[2] Furthermore, if the apoptotic stimuli do not reach lethal levels, only some mitochondria undergo permeabilization (minority MOMP), activate low levels of effector caspases that can then be

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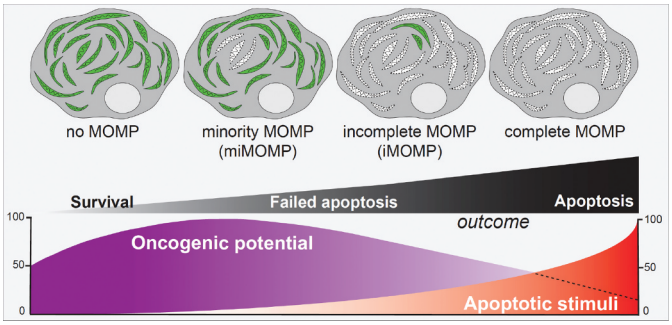

Figure 1. Repositioning the point-of-no-return in apoptosis. MOMP (mitochondrial outer-membrane permeabilization) is not simply an ON/OFF event. Depending on the intensity of the apoptotic stimuli, there are several possible scenarios: first, there is no permeabilization; second, some mitochondria undergo permeabilization (minority MOMP or miMOMP); third, some of them escape permeabilization (incomplete MOMP or iMOMP) and, fourth, all mitochondria permeabilize triggering rapid apoptosis. Lack of MOMP and subsequent cell survival contribute to uncontrolled cancer cells proliferation while complete apoptosis slows down tumorigenesis. Failed apoptosis, a phenomenon originating from minority MOMP, enhances cancer cell aggressiveness.

mutagenic and pro-oncogenic (Figure 1). This scenario was also demonstrated for non-lethal treatment with extrinsic apoptosis inducers.[3] The rigidity of the "point-of-no-return" for other types of cell death was also recently questioned. In necroptosis and pyroptosis, components of the ESCRT (endosomal sorting complexes required for transport) machinery can repair the plasma membrane of certain necroptotic and pyroptotic cells and ensure their survival.[9]

We believe that the tipping point between life and death is no longer set in stone, implying that cell death is a more plastic phenomenon. Even though we keep redefining and repositioning this "point-of-no-return", we still lack mechanistic knowledge into how it is precisely regulated and why it differs from one cell to another.

Another important aspect in need of further investigation is whether all types of cell death are permissive to scenarios of failed execution and what would be the phenotypic consequences. In the case of failed apoptosis, a very important question remains unanswered: what maintains otherwise deadly effector caspases at non-apoptotic levels? Hints as to the mechanisms underlying this phenomenon were provided in a recent study in *Drosophila*, in which, the orthologue of caspase-9 is maintained in close proximity to the basal side of the plasma membrane, where it sustains the vital function of apoptosis-induced proliferation.[10]

Overall, even though we do not exactly know what settings are permissive for failed apoptosis, the most significant message emerging from our study is that apoptosis can be both water and fire for oncogenesis.

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