Deadly encounter: Endosomes meet Mitochondria to initiate Apoptosis

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Mitochondrial outer membrane permeabilization (MOMP) is a crucial event enabling apoptotic cell death. In this issue of Developmental Cell, Wang et al. (2020) reveal a previously unknown interaction contributing to full MOMP execution, which depends on endosomes accumulating on apoptotic mitochondria. This causes mitochondrial lipid alterations that may contribute to functional pore assembly.

Apoptosis is an evolutionarily conserved cell death pathway resulting in cell self-destruction and clearance without the release of cellular constituents into the surrounding area. By ensuring the death of unnecessary and damaged cells, apoptosis plays an indispensable role in maintaining organismal homeostasis and ensuring proper tissue development. Deregulation of apoptosis drives various pathologies, either via insufficient cell removal promoting cancer and autoimmunity or excessive cell death contributing to neurodegeneration and immunodeficiency (Singh, Letai et al. 2019).

Mitochondrial outer membrane permeabilization (MOMP) is a crucial event in apoptosis. Mitochondrial outer membrane integrity is regulated via two members of the BCL-2 (B cell lymphoma 2) protein family: BAX (BCL-2-associated X protein) and BAK (BCL-2 antagonist or killer) (Singh, Letai et al. 2019). In healthy cells, BAX shuttles between the mitochondrial outer membrane and cytoplasm. After apoptotic stimuli, BH3 (BCL-2 homology 3)-only proteins bind BAX, leading to its activation and accumulation on the mitochondrial surface. Membrane-associated BAX/BAK dimers undergo allosteric changes, leading to oligomerization and the formation of proteo-lipid macropores that cause mitochondrial outer membrane permeabilization. This results in the release of the apoptogenic proteins cytochrome c and Smac from the intermembrane space into the cytosol. The coordinated action of cytosolic cytochrome c and Smac culminates in the activation of the caspase cascade that degrades cellular components leading to cell death.

More sophisticated events are believed to occur at the mitochondrial membrane that determine whether apoptosis is induced or not. While multiple studies have shown that mitochondrial BAX activation and pore assembly depend on inhibition of pro-survival Bcl2 family proteins (O'Neill, Huang et al. 2016) and on membrane remodelling at the site of the

apoptotic pore formation (Giacomello, Pyakurel et al. 2020), less is known about how crosstalk between apoptotic mitochondria and other organelles affects this process.

Previous studies have demonstrated that mitochondrial contact with the endoplasmic reticulum influences MOMP via lipid and calcium exchange and via mitochondrial fragmentation and cristae remodelling (Giacomello, Pyakurel et al. 2020). Close contacts between apoptotic mitochondria and endosomes have been visualised in several scenarios (Calore, Genisset et al. 2010, Hamacher-Brady, Choe et al. 2014) and the use of high-resolution imaging showed that endosomal proteins are restricted to domains close to BAX clusters on the outer mitochondrial membrane when apoptosis is induced, suggesting that a biochemical transformation of the mitochondria surface occurs at the sites of contact.

Here, Wang et al. (2020) uncover a previously unknown fundamental role for endosome-mitochondrial interactions in the MOMP execution. The authors found that pharmacological inducers of intrinsic apoptosis increased transient interactions between endosomes and mitochondria. Using high-resolution imaging techniques, they were able to show that Rab5-positive endosomes accumulated in close proximity to BAX clusters at the outer mitochondrial membrane. Live cell microscopy recording of cells exposed to apoptotic stimuli revealed a striking temporal correlation between BAX clustering and Rab5 targeting to the mitochondria surface. Both events precede cytochrome c and Smac release and culminate in the accumulation of endosomes within the entire mitochondrial compartment (Figure 1).

Cells with defective endosomal dynamics, depleted of either the endosomal regulator Rab5 or of the Rab5 activator Rabex-5, displayed significantly reduced levels of cell death in response to apoptotic treatments. Interfering with Rab5 activation or with Rab5 endosome recruitment to apoptotic mitochondria (by depleting the endosomal mobility regulator USP15) impaired mitochondrial pore formation and the release of cytochrome c and Smac. Wang et al. show that although these perturbations impacted differently on the BAX recruitment to mitochondria, they all affected the ability of BAX to undergo allosteric changes required for functional pore assembly.

Previously, the same group described outer mitochondrial membrane marker discontinuities where BAX clusters and Rabex5 labelling appear following apoptotic treatments (Hamacher-Brady, Choe et al. 2014). Wang et al. (2020) found an enrichment of cholesterol-loaded endosomes associated with these discontinuities and an increased sensitivity of outer mitochondrial membrane to the cholesterol-selective detergent saponin in cells treated with apoptotic stimuli. This sensitivity is strongly reduced in cells depleted of Rab5 and Rabex5. The authors propose that upon apoptosis induction, changes in lipid composition at mitochondrial membrane microdomains contribute to *in situ* BAX activation and that targeting endosomes to apoptotic mitochondria results in lipid membrane alterations at apoptotic foci via cholesterol transfer at membrane contact sites. Even if previous studies have correlated mitochondrial accumulation of cholesterol and BAX-mediated MOMP (Christenson, Merlin et al. 2008), whether the transfer of cholesterol is the main function of contacts between endosomes and mitochondria at the onset of MOMP remains to be shown.

Multiple questions emerge from the findings presented by Wang et al. (2020), such as the nature of the endosomes that are targeted to the apoptotic mitochondria and what marks the site of endosome-apoptotic mitochondria contacts.

Decoration of mitochondria by Rab5-positive endosomes is not necessarily a death signal, as inhibition of endosome-mitochondria contact by knockdown of the Rab5-GEF ALS2/Alsin enhances susceptibility to oxidative stress-mediated apoptosis (Hsu, Spannl et al. 2018).

Interestingly, Wang et al (2020) found that Rabex-5 depletion potently blocked not only cytochrome c release, but, unlike Rab5 silencing, it also prevented mitochondrial BAX accumulation, suggesting that other Rabs other than Rab5 are also involved in MOMP. Strong candidates are Rab21 and Rab17, which were previously shown to be targeted by Rabex-5 (Delprato, Merithew et al. 2004) and are particularly relevant for trafficking in developing neurons.

The nature of the endosomes redirected towards the mitochondria and how these are selected is unclear. The Wang et al (2020) study shows that the targeting of Rab5-positive endosomes to apoptotic mitochondria depends on the deubiquitinating enzyme USP15, whose role is the release of endoplasmic reticulum-associated endocytic vesicles (Jongsma, Berlin et al. 2016). Thus, a possible scenario is that the endosomes relevant for MOMP are the endosomes anchored to the ER. On the other hand, Rabex-5 targeting to apoptotic mitochondria is regulated by binding of ubiquitinated mitochondria (Hamacher-Brady, Choe et al. 2014). One interesting possibility raised by this study is that ubiquitination pathway and endoplasmic reticulum might have a role in the selection process.

Figure 1: **Mitochondria-endosome contacts promote mitochondrial outer membrane permeabilization.** Upon apoptotic stimuli, endosomes are enriched on the mitochondrial surface and endosomal constituents appear to facilitate the assembly of BAX-pores and the release of pro-apoptotic proteins to initiate the caspase cascade in the cytosol. Perturbations of Rab5-positive endosomal dynamics, either by silencing the endosomal GTPases Rab5A and Rab5C or the mobility regulator USP15 or the Rab5 activator Rabex-5, significantly reduced BAX-pore forming activity and cytochrome c release. Intriguingly, Rabex-5 silencing interfered with MOMP execution more potently by blocking mitochondrial BAX recruitment. Also, Rab5A and Rab5C and USP15 knockdown do not obviously affect BAX recruitment to mitochondria. This makes conceivable that Rabex-5, unlike Rab5 and USP15, regulates MOMP acting on factors that are upstream BAX recruitment.

REFERENCES

Calore, F., C. Genisset, A. Casellato, M. Rossato, G. Codolo, M. D. Esposti, L. Scorrano and M. de Bernard (2010). "Endosome-mitochondria juxtaposition during apoptosis induced by H. pylori VacA." Cell Death Differ **17**(11): 1707-1716.

Christenson, E., S. Merlin, M. Saito and P. Schlesinger (2008). "Cholesterol effects on BAX pore activation." J Mol Biol **381**(5): 1168-1183.

Delprato, A., E. Merithew and D. G. Lambright (2004). "Structure, exchange determinants, and family-wide rab specificity of the tandem helical bundle and Vps9 domains of Rabex-5." Cell **118**(5): 607-617.

Giacomello, M., A. Pyakurel, C. Glytsou and L. Scorrano (2020). "The cell biology of mitochondrial membrane dynamics." Nat Rev Mol Cell Biol **21**(4): 204-224.

Hamacher-Brady, A., S. C. Choe, J. Krijnse-Locker and N. R. Brady (2014). "Intramitochondrial recruitment of endolysosomes mediates Smac degradation and constitutes a novel intrinsic apoptosis antagonizing function of XIAP E3 ligase." Cell Death Differ **21**(12): 1862-1876.

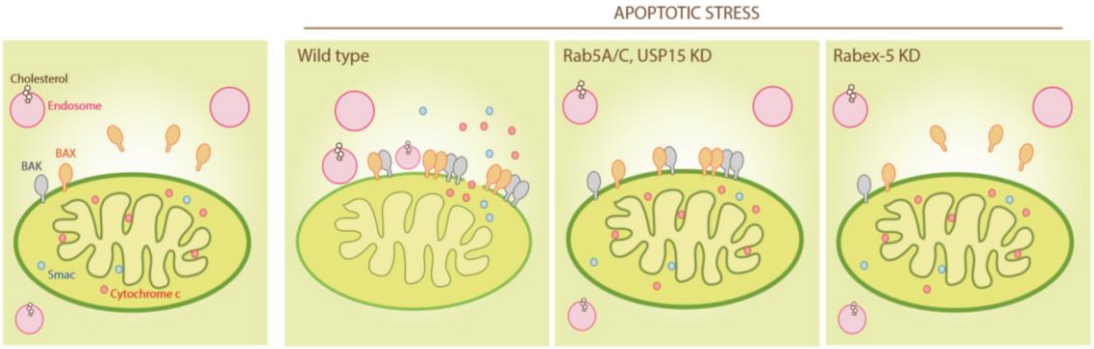
Hsu, F., S. Spannl, C. Ferguson, A. A. Hyman, R. G. Parton and M. Zerial (2018). "Rab5 and Alsin regulate stress-activated cytoprotective signaling on mitochondria." Elife **7**.

Jongsma, M. L., I. Berlin, R. H. Wijdeven, L. Janssen, G. M. Janssen, M. A. Garstka, H. Janssen, M. Mensink, P. A. van Veelen, R. M. Spaapen and J. Neefjes (2016). "An ER-Associated Pathway Defines Endosomal Architecture for Controlled Cargo Transport." Cell **166**(1): 152-166.

O'Neill, K. L., K. Huang, J. Zhang, Y. Chen and X. Luo (2016). "Inactivation of prosurvival Bcl-2 proteins activates Bax/Bak through the outer mitochondrial membrane." Genes Dev **30**(8): 973-988.

Singh, R., A. Letai and K. Sarosiek (2019). "Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins." Nat Rev Mol Cell Biol **20**(3): 175-193.

Wang, T.S., I. Coppens, A. Saorin, N.R. Brady, and A. Hamacher-Brady (2020). "Endolysosomal Targeting of Mitochondria is Integral to BAX-mediated Mitochondrial Permeabilization During Apoptosis Signaling." Dev Cell XXXX


APOPTOTIC STRESS
Cholesterol
Endosome
BAX
BAK
Smac
Cytochrome c
Wild type
Rab5A/C, USP15 KD
Rabex-5 KD