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Innate Immune Recognition of mtDNA—An Undercover Signal?

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In addition to their roles in cellular metabolism and apoptosis, mitochondria function as signaling platforms in the innate immune response. In *Nature*, West et al. (2015) demonstrate that mitochondrial stress triggers a type I interferon response and confers viral resistance via release of mtDNA and activation of the cGAS–STING pathway.

Although known as cellular powerhouses, mitochondria also regulate apoptotic cell death pathways. Recently, mitochondria were shown to help elicit cellular inflammation, particularly by inducing antiviral signaling pathways. The cytosolic sensors RIG-I and MDA5 signal through mitochondrial antiviral-signaling protein, an adaptor on the outer mitochondrial membrane (Yoneyama et al., 2015). Damaged mitochondria have been implicated in the induction of the NLRP3 inflammasome through the production of reactive-oxygen species and/or the release of mitochondrial DNA (mtDNA) (Zhou et al., 2011). In a recent issue of *Nature*, West and colleagues (West et al., 2015) demonstrated that disruption of mtDNA stability, such as that caused by heterozygosity of the histone-like mitochondrial transcription factor TFAM, results in the release of mtDNA into the cytosol via an unknown mechanism. Once in the cytoplasm, mtDNA elicits interferon (INF) production. Similar to foreign bacterial and viral DNA in mammalian cells, mtDNA is recognized by the cytosolic DNA sensor cGAS. Activation of cGAS promotes signaling through the adaptor STING and the transcription factor IRF3, resulting in the induction of type I IFNs and IFN-stimulated genes (Figure 1).

The physiologic relevance of this pathway was highlighted in the context of viral infection. In *TFAM*$^{+/-}$ mouse embryonic fibroblasts, the enhanced basal levels of IFN-stimulated genes prevented infection by either DNA or RNA viruses. Although this scenario could occur under certain pathophysiological conditions in which damaged mitochondria pre-exist in the host, the authors also demonstrated that members of the herpes virus family caused mitochondrial damage in a UL12 viral protein-dependent manner. Expression of UL12 alone was sufficient to induce mitochondrial stress and TFAM depletion, which enhanced the sensing of herpes virus infection and increased antiviral gene production. Again, it is unclear how such stress results in the release of mtDNA, though the effects are evident. UL12 mutant herpes simplex virus 1 was less immunostimulatory and replicated more efficiently in vitro and in vivo. These findings suggest that a pathogen-induced mitochondrial response resulting in the release of mtDNA is an evolutionarily beneficial mechanism in the host that amplifies antiviral signaling in response to pathogen invasion. However, the aberrant accumulation of damaged mitochondria and the leakage of mtDNA into the cytosol may also cause autoinflammatory or autoimmune syndromes.

Two recent articles have highlighted the role of mtDNA sensing by the cGAS-STING pathway. White et al. (White et al., 2014) and Rongvaux et al. (Rongvaux et al., 2014) concurrently published that the activation of caspases involved in the intrinsic pathway of apoptosis (caspase-3, caspase-7, and caspase-9) prevents activation of the IFN response in cells undergoing apoptosis mediated by Bax and Bak. These studies proposed a model in which the formation of Bax/Bak pores mediates the release of mtDNA, thereby triggering cGAS-STING signaling, IRF3 activation, and type I IFN production. In most cells, this IFN response is mitigated by the concurrent release of cytochrome *c*, formation of the apoptosome, and activation of the downstream effectors caspase-3 and caspase-7, resulting in apoptotic cell death. Similar to that of West et al., the article by Rongvaux et al. demonstrated a profound protection from both DNA and RNA virus infection after deletion or inhibition of caspase activation during Bax/Bak activation. Intriguingly, the presence of mtDNA in the cytoplasm of caspase-deficient mice did not alter the maximal INF response to virus infection; instead, it resulted in a pre-existing state of antiviral readiness due to increased basal stimulation of cGAS-STING-mediated activation of the IFN pathway. The cGAS DNA-sensing pathway also triggers autophagy (Liang et al., 2014), which can eliminate cytosolic pathogens. Therefore, it is tempting to speculate that the pathway elucidated by West and colleagues, Rongvaux and colleagues, and White and colleagues represents an evolutionary adaptation of a host-pathogen response arising from the incorporation of a bacterial endosymbiont (i.e., mitochondria precursor) in an archaeal host (Gray, 2012).

There is, however, a fundamental flaw in the idea that Bax and Bak release mtDNA. While activated, Bax and Bak clearly permeabilize the outer mitochondrial membrane, but the inner mitochondrial membrane, which lies between the mtDNA and the cytosolic sensors, is not disrupted (Tait and Green, 2010). Thus, this mechanism is unlikely.

Another potential mechanism is the so-called mitochondrial-permeability transition (MPT), which is caused by high

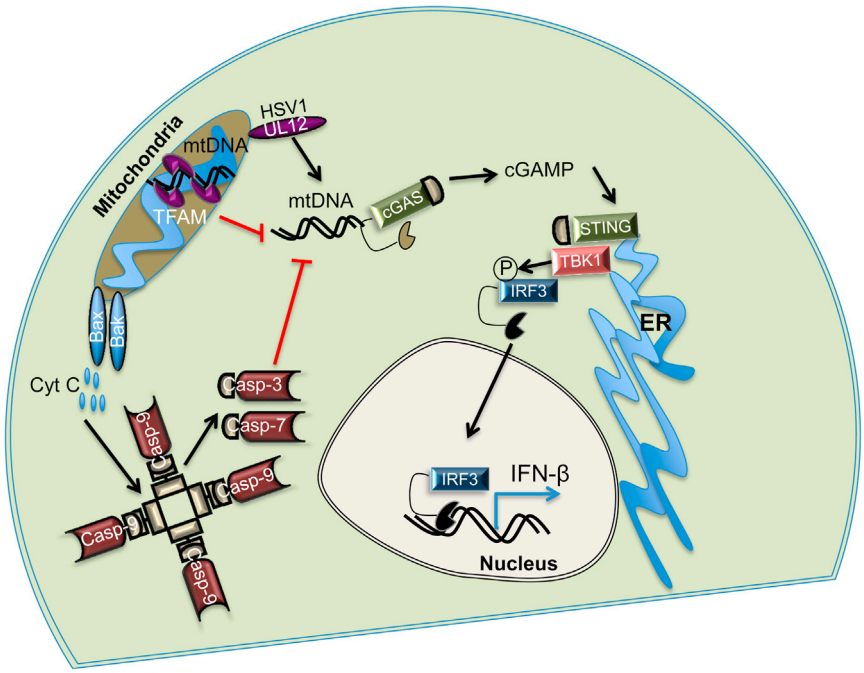

Figure 1. Innate Immune Recognition of mtDNA

Under normal conditions, West et al. (2015) demonstrated that the presence of the mitochondrial transcription factor TFAM prevents mitochondrial DNA (mtDNA) damage and release of mtDNA into the cytoplasm. However, in the absence of TFAM or in the presence of viral proteins like herpes simplex virus 1 (HSV-1) and UL12 M185 (UL12), mitochondrial damage occurs and mtDNA released into the cytosol is detected by the cGAS-STING pathway, resulting in IRF3-dependent expression of type I interferons and other interferon-stimulated genes. Similarly, two recent publications (White et al., 2014; Rongvaux et al., 2014) demonstrated that the initiation of apoptosis appears to result in mtDNA leakage into the cytosol with subsequent activation of the same cGAS-STING pathway if caspase-3 and caspase-7, or caspase-9 were inhibited or deleted. However, the exact mechanisms of mtDNA release remain to be determined.

concentrations of calcium and other signals at the inner mitochondrial membrane. During apoptosis, permeabilization of the outer mitochondrial membrane allows cytosolic proteases (e.g., caspases and calpains) to access the inner mitochondrial membrane, and this can trigger an MPT (Sun et al., 2007). However, MPT opens a small inner-membrane channel (approximately 1.5 kDa in diameter), which is not predicted to release mtDNA.

Another possible pathway for intact mtDNA to be released is mitochondria-derived vesicles, which sort specific protein and lipid cargo from mitochondria into small vesicular carriers (Sugiura et al., 2014). However, there is no evidence of mtDNA incorporation into those vesicles, nor is it clear how mtDNA would escape from those vesicles and activate cGAS. Perhaps additional insight remains to be gained by investigating mechanisms of DNA transfer in bacteria. Regardless of the mechanism by which mtDNA is released from mitochondria and the nature of the mtDNA, cytosolic mtDNA can obviously activate the innate immune response. Although mechanisms are in place to keep this response in check, disruption of those processes may contribute to aberrant immune response to pathogens and the etiology of autoimmune disorders. The therapeutic potential of activating or inhibiting this pathway remains to be seen.

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