Editorial

Untangling the Interplay Between Mitochondrial Fission and NF-κB Signaling in Endothelial Inflammation

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In most if not all cell types, mitochondria are constantly undergoing fission (binary division of a mitochondrion into separate entities) and fusion (the physical merging of 2 originally distinct mitochondria), which is necessary for both the cell and mitochondria to maintain a healthy state.[1] A disturbance in the fission-to-fusion ratio can lead to cellular stress and even cell death. It is now realized that many pathologies, including cardiovascular and neurological diseases, cancers, and diabetes mellitus, are accompanied by an imbalance in fission and fusion that is associated with mitochondrial dysfunction and increased generation of reactive oxygen species. In cardiovascular diseases, mitochondrial dysfunction is frequently found with enhanced mitochondrial fission/fragmentation mediated by the GTPase Drp1 (dynamin-related protein 1). In response to certain stimuli, cytosolic Drp1 translocates to the mitochondrion and oligomerizes at the future fission site to form a ring, the GTP-dependent constriction of which leads to mitochondrial division. However, the mechanistic relationship between mitochondrial fission and endothelial inflammation, as seen for instance with smoking, diabetes mellitus, metabolic syndrome, and hypertension, is poorly understood. In this issue, Forrester et al[2] report the results of a study that addressed this question. One take-home message of their study is the bidirectional nature of the interaction between mitochondrial fission and endothelial inflammation (Figure).

NF-κB (nuclear factor-kappa B) signaling is a major driver of endothelial inflammation and is robustly induced by TNF-α (tumor necrosis factor-α) and lipopolysaccharide.[3] Forrester et al[2] found that these proinflammatory stimuli induced a time-dependent increase in mitochondrial fragmentation in primary rat aortic endothelial cells, with a maximum response at 3 hours. This fragmentation was dependent upon Drp1 as shown by multiple molecular biology and pharmacological means. Blocking Drp1 or one of its mitochondrial outer membrane receptors (ie, Mff [mitochondria fission factor]) also repressed the sustained activation of NF-κB by TNF-α, although the acute translocation of the NF-κB family member p65 to the nucleus was not affected. The sustained NF-κB activity seen with TNF-α and lipopolysaccharide likely induced expression of VCAM-1 (vascular cell adhesion molecule 1) and increased monocyte adhesion, and both of these processes were found to be dependent upon mitochondrial fission in the cultured endothelial cells. Significantly, TNF-α–induced leukocyte vascular adhesion in vivo was suppressed by endothelial cell–targeted genetic reduction of Drp1 or with the Drp1 inhibitor mdivi-1. Thus, their evidence supports the conclusion that mitochondrial fission contributes to sustained NF-κB activation in endothelial cells that is directly tied to inflammation.

What explains the sustained NF-κB activation? Mitochondrial fission in endothelial cells was associated with oxidative stress; however, TNF-α–induced VCAM-1 expression was not blocked with an SOD2 (superoxide dismutase 2) mimic mitoTempo, indicating that reactive oxygen species (at least superoxide) was not responsible. This would seem to be a distinction from what has been observed for certain cancer cells in which NF-κB is activated downstream of enhanced Akt signaling due to reactive oxygen species–mediated inhibition of PTEN (phosphatase and tensin homolog).[4] Autocrine production of an NF-κB–inducing proinflammatory cytokine downstream of NF-κB, such as IL-6 (interleukin-6), is an alternative possibility,[5] but this scenario fails to account for the primacy of mitochondrial fission.

Perhaps the most novel finding of the study by Forrester et al[2] is the observation that canonical NF-κB signaling is required for TNF-α–induced mitochondrial fission. In the canonical pathway, phosphorylation of IκBα (inhibitor of NF-κB) by IKK (IκB kinase) releases the inhibitor from NF-κB, allowing for its degradation and NF-κB activation and nuclear import. Pharmacological inhibitors of IKK-β and of IκBα phosphorylation and degradation, their dominant-negative substitution mutants, and transduction of the Rel homology domain of p65 were all shown by Forrester et al[2] to block TNF-α–induced mitochondrial fission in endothelial cells. Two possible explanations for this observation might be considered.

The first possibility encompasses the nuclear, transcriptional actions of NF-κB and would entail metabolic reprogramming of sorts. Proinflammatory NF-κB signaling was recently shown to promote mitochondrial dysfunction in skeletal muscle that was associated with increased mitochondrial fragmentation, as well as reduced expression of the major regulator of mitochondrial biogenesis, PGC1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha), and that of

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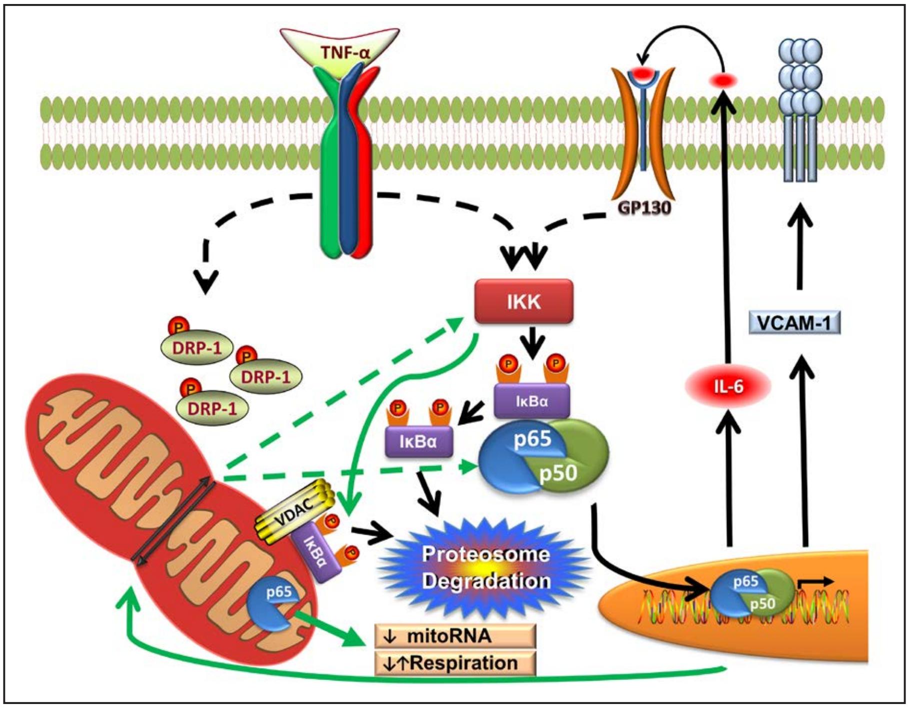

Figure. Mitochondrial fission helps sustain inflammatory signaling in the endothelium. In the canonical pathway, activation of the kinase IKK (IκB kinase) leads eventually to the proteasomal degradation of the inhibitor IκBα (inhibitor of NF-κB [nuclear factor-kappa B]), thereby permitting translocation of the p65/p50 heterodimer to the nucleus and gene expression. Canonical NF-κB signaling downstream of TNF-α (tumor necrosis factor-α) or lipopolysaccharide receptor activation may lead to proinflammatory cytokine expression (eg, IL [interleukin]-6 or TNF-α) that contributes to autocrine induction of NF-κB activity. Enhanced NF-κB–mediated gene transcription enhances the expression of VCAM-1 (vascular cell adhesion molecule 1), leading to increased leukocyte adhesion. TNF-α receptors activate mitochondrial fission as well through Drp1 (dynamin-related protein 1) recruitment (the exact posttranslational modification was not assessed), which further enhances canonical NF-κB signaling. Notably, canonical NF-κB signaling is implicated in mitochondrial fission. This may occur at the level of the mitochondrion, although the exact mechanism is not defined, and involve increased instability of the outer mitochondrial membrane due to loss of voltage-dependent anion channel 1–IκBα interactions or a direct effect of p65 NF-κB on mitochondrial gene expression or respiration. Sustained NF-κB–mediated gene transcription may also couple to mitochondrial fission through increased expression of fission proteins, such as Drp1 or FIS-1 (fission 1 protein). Green arrows indicate postulated signaling events that sustain mitochondrial fission and endothelial inflammation. See text for additional details.

key mitochondrial components.[6] Moreover, at least in mice, elevated levels of IL-6—a gene target of NF-κB—are associated with enhanced Drp1 and mitochondrial FIS-1 (fission 1 protein) expression.[7] Forrester et al[2] combined shotgun and gene ontology analysis to demonstrate that mitochondrial fission contributed to proinflammatory reprogramming in endothelial cells. Compared with cells in which fission was blocked by a dominant-negative Drp1 mutant, control cells treated for 6 hours with TNF-α exhibited upregulated proteins involved with aging, apoptotic mitochondria, and mitochondria. Forrester et al[2] concluded that a proinflammatory response of endothelial cells to TNF-α, which included inflammageing and metabolic reprogramming, was "dictated by the status of mitochondrial fission and Drp1 activity." But the converse must be true as well, viz, that TNF-α or canonical NF-κB signaling has an impact on mitochondrial fission. A more detailed time course and focus on expression levels of proteins involved in the fission process are needed.

For the second—perhaps more intriguing—possibility, a direct role for the components of canonical NF-κB signaling at the level of the mitochondrion was considered. In support of this, both IκBα and p65 NF-κB family member were detected in endothelial mitochondria by Forrester et al.[2] Notably, TNF-α induced mitochondrial IκBα degradation. In cancer cells, IκBα was found to associate with voltage-dependent anion channel 1 at the outer mitochondrial membrane and thereby inhibit apoptosis independently of NF-κB cytosolic retention.[8] Mitochondrial p65 was also reported to inhibit mitochondrial RNA expression and to either enhance or reduce respiration.[9] It is also possible that posttranslational modification of Drp1 important for mediating mitochondrial fission is facilitated by signal cross talk between mitochondrial p65 and Drp1. However, the role for IκBα and p65 in mitochondrial fission awaits future investigation. A judicious and rigorous approach is warranted given issues that have arisen regarding the reality of mitochondrial STAT3 (signal transducer and activator of transcription 3) in cardiomyocytes.[10]

In conclusion, Forrester et al[2] are to be congratulated for a thorough and innovative study that addresses the relationship between mitochondrial fission and canonical NF-κB signaling in endothelial inflammation. They moreover show that targeting the latter by inhibiting IKK-β with the nonsteroidal anti-inflammatory drug sodium salicylate was effective in vivo in attenuating TNF-α–induced leukocyte adhesion in the mouse mesenteric microcirculation, along with attenuating endothelial mitochondrial fragmentation and VCAM-1 expression in vitro. Their study highlights as well the possibility of pharmacologically targeting mitochondrial fission/fusion in endothelial inflammation. Still to be explored is the possible direct role of NF-κB signaling at the level of the mitochondrion in affecting mitochondrial dynamics. Investigating that question might lead to innovative approaches to treat endothelial inflammation. Mitochondrial fission is reported to occur in other cardiovascular cell types as well (eg, cardiac myocytes and fibroblasts) under conditions that are associated with inflammation, but its role as a driver of the inflammatory process in those cases requires investigation.

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Disclosures

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