Macrophage mitochondrial-derived reactive oxygen species (mtROS) enhances early atherosclerogenesis

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Excessive accumulation of mitochondrial-derived reactive oxygen species (mtROS) occurs in different types of cells in the atherosclerotic lesions of both humans and animal models. However, evidences on the causative roles of myeloid cell specific mtROS in atherosclerosis are limited. Mechanistically, lesional factors that stimulate mtROS remain to be determined, and the potential pathogenic intracellular signals require further investigation. We have recently provided new evidences that arteriosclerotic lesions, monocyte infiltration-associated chronic inflammation are enhanced by mtROS accumulation in lesional macrophages. Additionally, our study has revealed an important signaling pathway linking mtROS to the activation of an essential transcription factor NF-κB (RelA) and its downstream chemokine MCP-1. This pathway drives monocyte infiltration and amplifies the chronic inflammation during the early atherosclerosis.


Mitochondrial reactive oxygen species (mtROS) are natural by-products of the electron transportation chain (ETC), a key step in the generation of ATP through oxidative phosphorylation (OXPHOS) [1]. Due to its physical proximity of to ETC-derived ROS, mitochondrial DNA (mtDNA) is highly vulnerable to oxidative damage. We and others have provided several lines of evidences which indicate the occurrence of excessive mtROS-induced oxidative damage in atherosclerotic lesions of both animal models and humans [2]. For example, a 5-kb mtDNA deletion, referred to as “the common mtDNA deletion,” has been identified in mouse and human atherosclerotic lesions and was found to be positively associated with the atherosclerosis progression [3]. Furthermore, human and rabbit atherosclerotic lesions react with an antibody that recognizes an oxidized form of cardiolipin, a phospholipid exclusively expressed in mitochondria, suggesting excessive mtROS [4]. We used 8-hydroxy-deoxyguanosine (8-OhdG or 8-OhoG) as a marker for ROS accumulation [5]. 8-OhdG is one of the major products formed upon oxidative damage of DNA in various pathological conditions [6, 7]. 8-OHdG accumulation has been observed in circulating leukocytes and in various cell types in atherosclerotic lesions of human and animal models [8-10]. In line with these reports, we found that the level of nuclear 8-OHdG (an indicator of nuclear DNA oxidative damage) in lesion macrophages increased with lesion progression. More importantly, we first reported that the level of non-nuclear 8-OHdG (an indicator of mitochondrial DNA oxidative damage) in lesional macrophages also positively correlated with atherosclerosis lesion progression [3]. Therefore, these data validate the use of WD-fed Ldlr−/− mice [5]. Therefore, these data validate the use of WD-fed Ldlr−/− model to further study the progressive increase in lesional mtROS, a known feature of human atherosclerotic lesion [3].

To further address whether myeloid cells mtROS were sufficient to promote atherosclerosis, we quelled mtROS in macrophages using a mitochondria-targeted transgenic
catalase (mCAT) model. In this model, the human catalase is targeted to the mitochondria matrix to facilitate the reduction of $\text{H}_2\text{O}_2$, prevent the accumulation of the highly reactive products such as hydroxyl radical ($\cdot\text{HO}$) and peroxinitrile (OONO$^-$), and consequently protects against oxidative damage to mitochondrial DNA, mitochondrial-localized proteins or lipids \cite{11}. In combination with a macrophage-specific LysMCre model or bone marrow transplantation, we showed that mCAT macrophages in lesions were protected from mtDNA oxidation (reduced non-nuclear 8-OhdG). After 8wk WD, these mice had smaller atherosclerosis in the $\text{Ldlr}^{-/-}$ background, less monocyte infiltration, decreased pro-inflammatory chemokine/cytokines expression and decreased activation of the pro-inflammatory transcription factor RelA NF-κB in the atherosclerotic lesions. Interestingly, quenching mtROS in macrophages does not change the levels of other chemokines that have been indicated to promote monocyte infiltrations, such as CCL5, CXCL1 or CX3CL1 \cite{12-14}. Thus, our initial in-vivo findings primarily establish a very specific, but essential signaling pathway linking mtROS to monocyte infiltration, namely mtROS-NF-κB-MCP-1 pathway.

Further mechanistic studies with cultured macrophages suggested that mtROS can be stimulated by numerous factors in atherosclerotic lesions, including Toll-like receptor activators (oxLDL and LPS), saturated fatty acid (Lp (a)) and oxidized phospholipids (oxPAPC). Quenching mtROS in cultured macrophages suppressed oxLDL-induced pro-inflammatory chemokine MCP-1 transcription through preventing the activation of the NF-κB (RelA) pathway. In contrast, we found that the suppression of cytosolic ROS, mainly generated by NADPH oxidase (NOX), did not affect MCP-1 expression \cite{15}. Thus, the source and/or intracellular location of oxidative stress can have distinct effects on activation of RelA mediated pro-inflammatory chemokine MCP-1 transcription.
To summarize, our study is the first myeloid cell-specific causation study to address the pathological significance of endogenous mtROS in early atherosclerogenesis in vivo. We demonstrate that (1) mtROS in lesional macrophages are simulated by a number of factors in atherosclerotic lesions (2) mtROS promote the additional monocyte entry, amplifies the inflammatory milieu of lesions, and enhance the early atherosclerosis through mtROS-NF-κB-MCP-1 pathway (Figure 1). These data reveal a potentially new therapeutic target to prevent the early progression of atherosclerosis. As macrophage mtROS are positively associated with the lesion progression, further studies will be conducted to investigate the role of macrophage mtROS in the advanced stage of atherosclerosis, with a focus on the necrotic core formation, atherothrombosis and other features of vulnerable plaques. It is also important to note that in addition to atherosclerosis, the mtROS-NF-κB-MCP-1 signaling in macrophages is likely playing a similar role in other chronic inflammatory diseases.

References


