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Myeloid Cell PKM2 Deletion Enhances Efferocytosis and Reduces Atherosclerosis

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Background: The glycolytic enzyme pyruvate kinase muscle 2 (PKM2) is upregulated in monocytes/macrophages of patients with atherosclerotic coronary artery disease. However, the role of cell type-specific PKM2 in the setting of atherosclerosis remains to be defined. We determined whether myeloid cell-specific PKM2 regulates efferocytosis and atherosclerosis.

Methods: We generated novel myeloid cell-specific PKM2$^{-/-}$ mice on LdlR-deficient background (PKM2$^{mye\text{-}KO}$Ldlr$^{-/-}$). Controls were littermate PKM2^{WT}Ldlr$^{-/-}$ mice. To rule out sex-based differences, male and female mice were placed on a high-fat Western diet for 14 weeks, starting at 8 weeks.

Results: PKM2 was upregulated in macrophages of Ldlr$^{-/-}$ mice fed the Western diet compared with a control chow diet. Myeloid cell-specific deletion of PKM2 led to a significant reduction in lesions in the whole aorta and aortic sinus despite high cholesterol and triglyceride levels. Furthermore, we found decreased macrophage content in the lesions of myeloid cell-specific PKM2$^{-/-}$ mice associated with decreased MCP-1 levels in plasma, reduced transmigration of macrophages in response to MCP-1, and an impaired glycolytic rate. Macrophages isolated from myeloid-specific PKM2$^{-/-}$ mice fed the Western diet exhibited reduced expression of proinflammatory genes, including MCP-1, interleukin-1β, and interleukin-12. Myeloid cell-specific PKM2$^{-/-}$ mice exhibited reduced apoptosis concomitant with enhanced macrophage efferocytosis and upregulation of LRP1 in macrophages in vitro and atherosclerotic lesions in vivo. Silencing LRP1 in PKM2-deficient macrophages restored inflammatory gene expression and reduced efferocytosis. As a therapeutic intervention, inhibiting PKM2 nuclear translocation using a small molecule reduced glycolytic rate, enhanced efferocytosis, and reduced atherosclerosis in Ldlr$^{-/-}$ mice.

Conclusions: Genetic deletion or limiting PKM2 nuclear translocation in myeloid cells reduces atherosclerosis by suppressing inflammation and enhancing efferocytosis.

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Novel Atheroprotective Role of Chk1-induced Senp2 S344 Phosphorylation under Laminar Flow

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Background: Atherosclerosis tends to form at arterial regions exposed to disturbed (DF) but not laminar (LF) flow. Previously, we reported the significant role of SUMOylation in DF-induced endothelial cell activation and subsequent atherosclerosis formation via upregulating p53 and ERK5 SUMOylation. LF can inhibit the basal levels of p53 and ERK5 SUMOylation, but how LF inhibits SUMOylation remains unknown. A chemical genetics approach with high-resolution mass spectrometry revealed that the cell-cycle checkpoint kinases CHK1 can phosphorylate SENP2 S344 site, but the functional role remains largely unknown. We aimed to study the functional role of CHK1 in SENP2 function and subsequent atherosclerosis.

Methods: First, we generated phospho-specific SENP2 S344 antibody, and found that LF, but not DF, increases SENP2 S344 phosphorylation. We found the increase of CHK1 S280 phosphorylation (related to nuclear translocation) after LF, suggesting LF-induced nuclear translocation of CHK1. The CHK1-specific inhibitor of GDC0575 and the depletion of CHK1 inhibited LF-induced SENP2 S344 phosphorylation and increased both p53 and ERK5 SUMOylation in ECs. These data suggested the crucial role of CHK1 in LF-induced SENP2 S344 phosphorylation and upregulating de-SUMOylation activity. Next, we generated CRISPR/Cas9-induced *Senp2* S344A knock-in (KI) and found that the mutation of SENP2 S344A accelerated p53 and ERK5 SUMOylation. LF-induced reduction of p21, cleaved caspase 3, and ICAM-1 were all reversed by SENP2 S344A mutation. Last, we found that the significant acceleration of atherosclerosis formation in both ascending aorta/arch (DF area) and descending aorta (LF area) in KI mice compared with wild-type (WT) mice after receiving AAV-PCSK9 and high fat diet. We also performed bone marrow transplantation (BMT) after 13 Gy whole body radiation, and larger atherosclerosis but only in the aortic arch was observed in BMT mice from WT donor to KI recipient, but not in BMT mice from WT donor to WT recipient, supporting the role of endothelial SENP2 S344 phosphorylation on atherosclerosis. The radiation reduced CHK1 expression in ECs, which may explain the different regulatory pattern under BMT or non-BMT.

Conclusions: Taken together, these results suggest the critical role of CHK1-mediated SENP2 S344 phosphorylation on LF-induced anti-atherosclerosis effects.

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Natural Vascular Scaffolding Suppresses Experimental Abdominal Aortic Aneurysms

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Background: Proteolytic destruction of aortic extracellular matrix is central to abdominal aortic aneurysm (AAA) pathogenesis. Matrix metalloproteinase inhibition with doxycycline failed to limit AAA progression. However, photochemical modification of collagen and elastin fibers may provide an alternative approach to extracellular matrix stabilization. We investigated the effectiveness of this treatment in limiting experimental AAA progression.

Methods: AAAs were created in 8- to 10-week-old male C57BL/6J mice via intra-aortic elastase infusion. Natural vascular scaffolding (2 mg/mL, Alucent Biomedical, Salt Lake City, UT) or vehicle solution was applied to the abluminal aortic wall immediately following elastase infusion and aortotomy closure and exposed to laser light activation. AAA progression was assessed via serial ultrasound aortic diameter measurements and histopathologic analysis at humane killing.

Results: Ultrasound examination confirmed progressive aortic enlargement and AAA formation in all vehicle-treated mice within 14 days following elastase infusion. Natural vascular scaffolding treatment substantially attenuated AAA development and progression with reduced medial elastin degradation and smooth muscle cell depletion, as well as mural neovessel development. No difference was seen in aortic CD4 or CD8 T accumulation between the two treatment groups.