atherosclerosis

Atherosclerosis newsletter

Simona Negrini and Arnold von Eckardstein

Volume 373, May 2023 issue

Volume 374, June 2023 issue

In most cases, our newsletter summarizes articles on clinical or epidemiological research published in *Atherosclerosis*. However, our journal also publishes articles on basic and translational research, which deserve and receive attention. As examples, please find below the summaries of five articles published in two recent issues.

Hepatic *Cdkal1* deletion regulates HDL catabolism and promotes reverse cholesterol transport

Associations between variants of *CDKAL1,* encoding Cdk5 regulatory subunit associated protein 1-like 1 (Cdkal1), and cholesterol efflux capacity (CEC) were previously reported. In this study, Bi An et al. aimed to investigate the effects of Cdkal1 deficiency on high-density lipoprotein (HDL) metabolism, atherosclerosis, and related pathways.

Lipid and glucose metabolic profiles, CEC, and *in vivo* reverse cholesterol transport (RCT) were compared in liver-specific *Alb-Cre:Cdkal1*$^{fl/fl}$ and *Cdkal1*$^{fl/fl}$ mice. Aortic atherosclerosis was compared in *Apoe*$^{-/-}$*Alb-Cre:Cdkal1*$^{fl/fl}$*l* and *Apoe*$^{-/-}$ mice fed high-fat diets. HDL subclasses and mediators of HDL metabolism from *Alb-Cre:Cdkal1*$^{fl/fl}$ mice were examined.

The results showed that HDL-cholesterol levels tended to be higher in the *Alb-Cre:Cdkal1*$^{fl/fl}$ *mice*. Glucose and other lipid profiles were similar in the two groups of mice, irrespective of diet. The mean CEC was 27% higher in the *Alb-Cre:Cdkal1*$^{fl/fl}$ mice, as were radioactivity of bile acids and cholesterol from feces. The radioactivity tendency was similar in mice fed a high-fat diet. Atherosclerotic lesion area was smaller in the *Apoe*$^{-/-}$*Alb-Cre:Cdkal1*$^{fl/fl}$ mice than in the *Apoe*$^{-/-}$ mice. Cholesterol concentrations in large HDLs were higher in the *Alb-Cre:Cdkal1*$^{fl/fl}$ *mice* while they were lower in small HDLs. Endothelial lipase and hepatic lipase expression levels were reduced in the Alb-*Cre:Cdkal1*$^{fl/fl}$ mice whereas SR-B1 expression was increased.

The promotion of CEC and RCT in *Alb-Cre:Cdkal1*$^{fl/fl}$ mice confirmed the effect of CDKAL1 seen in the authors' previous human genetic data. These phenotypes were related to regulation of HDL catabolism. This study suggests that CDKAL1 and associated molecules could be targets for improving RCT and vascular pathology.

Serum amyloid A and interleukin -1β facilitate LDL transcytosis across endothelial cells and atherosclerosis via NF-κB/caveolin-1/cavin-1 pathway

A persistent increase in plasma inflammatory factors, including tumor necrosis factor alpha (TNF-α), serum amyloid A (SAA), and interleukin 1β (IL-1β), promotes the formation and progression of atherosclerosis. It was previously demonstrated that TNF-α and SAA stimulated IL-1β production via nuclear factor-κB and Nod-like receptor pyrin domain containing 3 (NF-κB/NLRP3) inflammasome signaling pathway and increased caveolin-1 and cavin-1 expression. Such increase might facilitate the formation of caveolae, which ultimately accelerate LDL transcytosis across endothelial cells. Jia et al. aimed to illustrate the roles of SAA, and IL-1β in low-density lipoproteins (LDL) transcytosis and atherosclerosis.

Effects of SAA and IL-1β on transcytosis of LDL were measured with an *in vitro* LDL transcytosis model. NF-κB/caveolin-1/cavin-1 pathway activation was assessed by Western blots and ELISA. The effect of SAA and IL-1β on the retention of LDL in aortas of C57BL/6J mice was investigated with the IVIS spectrum while the effect of SAA and IL-1β on atherosclerosis in *Apoe*$^{-/-}$ mice was examined by Oil Red O staining.

SAA and IL-1β stimulated LDL transcytosis across endothelial cells (ECs), which was accompanied by an increase in LDL uptake by ECs. SAA and IL-1β enhanced the activity of NF-κB, consequently facilitating the upregulation of proteins involved in caveolae formation, including caveolin-1 and cavin-1, along with the assembly of NLRP3 inflammasome. Furthermore, SAA- and IL-1β-induced effects were blocked by NF-κB subunit p65 siRNA. SAA- and IL-1β-induced LDL transcytosis was effectively blocked by *caveolin-1* or *cavin-1* siRNAs. Interestingly, SAA and IL-1β facilitated LDL entering into the aorta of C57BL/6J mice. In *Apoe*$^{-/-}$ mice, SAA and IL-1β increased the areas of lipid-rich atherosclerotic lesions in both the ascending and root of aorta. Furthermore, a significant increase in the NLRP3 inflammasome, accompanied by accumulation of cavin-1 and caveolin-1, was observed in the aortic endothelium of *Apoe* $^{-/-}$ mice.

In conclusion, SAA and IL-1β accelerated LDL transcytosis via the NF-κB/caveolin-1/cavin-1 axis.

Exercise-induced endothelial Mecp2 lactylation suppresses atherosclerosis via the Ereg/MAPK signalling pathway

Post-translational modifications (PTM) provide a precise mechanism to regulate protein function, including regulating protein stability, subcellular localisation, and enzymatic activity, and controlling protein–protein and protein–DNA interactions. Lactylation, a recently identified PTM, plays a central role in the regulation of multiple physiological and pathological processes. Exercise is known to provide protection against cardiovascular disease. However, whether exercise-generated lactate changes lactylation and is involved in the exercise-induced attenuation of atherosclerotic

cardiovascular disease (ASCVD) remains unclear. Wang et al. investigated the effects and mechanisms of exercise-induced lactylation on ASCVD.

Using the high-fat diet-induced apolipoprotein-deficient mouse model of ASCVD, the authors found that exercise training promoted Mecp2 lysine lactylation (Mecp2k271la); it decreased the expression of vascular cell adhesion molecule 1 (Vcam-1), intercellular adhesion molecule 1 (Icam-1), monocyte chemoattractant protein 1 (Mcp-1), interleukin (IL)-1β, IL-6, and increased the level of endothelial nitric oxide synthase (Enos) in the aortic tissue of mice. To explore the underlying mechanisms, mouse aortic endothelial cells (MAECs) were subjected to RNA-sequencing and CHIP-qPCR, which confirmed that Mecp2k271la repressed the expression of epiregulin (Ereg) by binding to its chromatin, demonstrating Ereg as a key downstream molecule for Mecp2k271la. Furthermore, Ereg altered the mitogen-activated protein kinase (MAPK) signalling pathway through regulating the phosphorylation level of epidermal growth factor receptor, thereby affecting the expression of Vcam-1, Icam-1, Mcp-1, IL-1β, IL-6, and Enos in endothelial cells (ECs), which in turn promoted regression of atherosclerosis. In addition, increasing the levels of Mecp2k271la by exogenous lactate administration *in vivo* inhibited the expression of Ereg and MAPK activity in ECs, resulting in repressed atherosclerotic progression.

This study provides a mechanistic link between exercise and lactylation modification, offering new insight into the anti-atherosclerotic effects of exercise-induced PTM.

Sprouty1 has a protective role in atherogenesis and modifies the migratory and inflammatory phenotype of vascular smooth muscle cells

Sprouty1 (Spry1) is a member of the family of feedback inhibitors of receptor tyrosine kinases that are important in vascular development and diseases. Spry1 is required to maintain the vascular smooth muscle cells (VSMC) contractile phenotype *in vitro*, and plays an important role in regulation of injury-induced artery restenosis. In this study, Yang et al. investigated the role of Spry1 in hypercholesterolemia-induced VSMC inflammatory phenotypic transition, and in the pathology of atherosclerosis.

Spry1 null mouse, and *Myh11-Cre^{ERT2}, ROSA26-STOP$^{fl/fl}$-tdTomato-Spry1$^{fl/fl}$* mice were used to allow for lineage tracing and conditional *Spry1* deletion in VSMC. Atherosclerosis was induced by injection of a mutant form of mPCSK9D377Y-AAV followed by Western diet. Human aortic VSMC (hVSMC) with shRNA targeting of Spry1 were also analyzed.

Global loss of *Spry1* increased inflammatory markers ICAM1 and Cox2 in VSMC. Conditional deletion of *Spry1* in VSMC had no effect on early lesion development, despite increased Sca1^{high} cells. After 26 weeks of Western diet, mice with VSMC deletion of *Spry1* had increased plaque burden, with reduced collagen content and smooth muscle alpha actin (SMA) in the fibrous cap. Lineage tracing via

tdTomato marking Cre-recombined cells indicated that VSMC with loss of Spry1 had decreased migration into the lesion, noted by decreased proportions of tdTomato+ and tdTomato+/SMA + cells. Loss-of-function of Spry1 in hVSMC increased mesenchymal and activation markers, including Kruppel-Like factor 4 (KLF4), platelet-derived growth factor receptor beta (PDGFRb), intercellular adhesion molecule 1 (ICAM1), and cyclooxygenase-2 (Cox2). Loss of Spry1 enhanced the effects of platelet-derived growth factor-BB (PDGF-BB) and tumor necrosis factor alpha (TNF-α) on hVSMC.

Loss of Spry1 in VSMC aggravated plaque formation at later stages, and increased markers of instability. These results indicate that Spry1 suppresses the mesenchymal and inflammatory phenotype of VSMC, and its expression in VSMC is protective against chronic atherosclerotic disease.

Sirt4 deficiency promotes the development of atherosclerosis by activating the NF-κB/IκB/CXCL2/3 pathway

As a member of mitochondrial sirtuins, Sirt4 plays a vital role in cellular metabolism and intracellular signal transduction and has been shown to play an essential role in cardiovascular disease; however, its effect on atherosclerosis is unclear. Chang et al. aimed to explore the effect of Sirt4 on atherosclerosis and its underlying mechanism.

In vivo, *Apoe*$^{-/-}$ and *Apoe*$^{-/-}$*/Sirt4*$^{-/-}$ mice were fed a high-fat diet to induce atherosclerosis. *In vitro,* peritoneal macrophages from two mouse types were extracted and treated with oxidized low-density lipoprotein to establish a cell model. THP-1 cells were used to observe the effect of Sirt4 on the adhesion ability of monocytes. The growth and composition of aortic plaques in two mouse types were analyzed by hematoxylin and eosin staining, Oil Red O staining, Dil oxidized low-density lipoprotein, immunohistochemistry, real-time quantitative polymerase chain reaction and enzyme-linked immunosorbent assay. Transcriptome analysis and Western blotting were performed to explore the specific mechanisms.

Sirt4 deficiency aggravated atherosclerosis in mice. *In vivo,* aortic plaque size, lipid content, and expression of related inflammatory factors in *Apoe*$^{-/-}$*/Sirt4*$^{-/-}$ mice were higher than those in the control group, whereas the content of collagen I and smooth muscle actin-α was significantly lower. Sirt4-deficient macrophages exhibited stronger lipid phagocytosis *in vitro,* and the adhesion ability of monocytes increased when Sirt4 expression decreased. Transcriptome analysis showed that the expression of C-X-C motif chemokine ligand 2 and 3 (CXCL2 and CXCL3) in Sirt4-deficient peritoneal macrophages increased significantly, which may play a role by activating the nuclear factor- κB (NF-κB) pathway. In further analysis, the results *in vitro* and *in vivo* showed that the expression of VCAM-1 and pro-inflammatory factors, such as interleukin 6 (IL-6), tumor necrosis factor α (TNF-α) and IL-1β, increased, whereas the expression of anti-inflammatory factor IL-37 decreased in Sirt4-deficient

peritoneal macrophages and tissues. After blocking the effect with NK-κB inhibitor BAY11-7082, the inflammatory reaction in Sirt4 deficient macrophages was also significantly decreased.

This study demonstrates that Sirt4 deficiency promotes the development of atherosclerosis by activating the NF-κB/IκB/CXCL2/3 pathway, suggesting that Sirt4 may exhibit a protective effect in atherosclerosis, which provides a new strategy for clinical prevention and treatment of atherosclerosis.