Atherosclerosis newsletter

Simona Negrini and Arnold von Eckardstein

Volume 297, Issue March 2020

Volume 298, Issue April 2020

Atherosclerosis, aortic aneurysm and calcific aortic valve disease are diseases of aging. They share similarities with respect to the involvement of mesenchymal cells. Issues 297 and 298 contain several interesting articles on these pathologies and the role of smooth muscle cells.

Nuclear receptors in abdominal aortic aneurysms

Abdominal aortic aneurysm (AAA) is a common vascular disease where the diameter of the aorta is more than 50% larger than the normal aorta or greater than 3 cm. AAA pose a considerable health burden and are only managed surgically since there is no proven pharmacotherapy that will retard their expansion or reduce the incidence of fatal rupture. This pathology shares several pathophysiological mechanisms with atherosclerosis, such as macrophage infiltration, inflammation, and degradation of extracellular matrix. Therefore, therapeutic targets effective in the treatment of atherosclerosis could also be considered for treatment of AAA. Different members of the nuclear receptor (NR) superfamily have been extensively studied as potential targets in the treatment of cardiovascular disease (CVD) and therefore might also be suited for AAA treatment. This review by Neels et al. summarizes the role of different NRs in CVD, mostly atherosclerosis, and provides compelling evidence to consider several NRs as targets for future treatment of AAA.

Role of *ADAM9* and *miR-126* in the development of abdominal aortic aneurysm

Abdominal aortic aneurysm (AAA) is normally asymptomatic unless aortic rupture occurs, which can be life-threatening. Treatments are available, including specific medicines to help stop AAA from growing, and surgery if AAA grows too fast or has leaked. Although these treatments can control AAA development, the mortality rate of most fatal AAA surgical emergencies is around 90%. Therefore, there is an urgent need to understand the mechanisms of AAA formation at molecular level to develop efficient drug-based therapies. The disintegrin and metalloprotease (ADAMs) proteins are a family of glycosylated type I transmembrane proteins and secreted metalloendopeptidases, Shen et

al. previously showed that disintegrin and metalloprotease 10 (*ADAM10*) play an important role in AAA formation. In this study, they investigate the role of another ADAM protein (ADMA9) in AAA formation.

ADAM9 was overexpressed in an *in vitro* abdominal aortic aneurysm (AAA) model and murine AAA model, suggesting that *ADAM9* may play important roles in AAA formation. Further investigation showed that *ADAM9* induced inflammation leading to increased macrophage infiltration. *ADAM9* was also found to induce cell apoptosis. Bioinformatic analysis showed that the 3' UTR of *ADMA9* was a potential target of *miR-126*, therefore the potential of using *miR-126* to modulate *ADAM9* expression was assessed. The expression level of *miR-126* was decreased and inversely correlated with the expression of *ADAM9* in the *in vitro* model. Further investigation showed that *miR-126* negatively regulated gene expression of *ADAM9* and suppressed the production of inflammatory cytokines. *miR-126* was also found to improve cell survival and significantly reduce AAA formation in murine AAA.

This data reveals a link between *ADAM9* and AAA formation, providing an approach to control AAA development using *miR-126*, possibly through modulation of the expression level of *ADAM9*.

Targeting vascular smooth muscle cell dysfunction with xanthine derivative KMUP-3 inhibits abdominal aortic aneurysm in mice

Inflammation, oxidative stress, matrix degradation, medial calcification and vascular smooth muscle cell (VSMC) loss are prominent features in abdominal aortic aneurysm (AAA). VSMC phenotypic switch to a proinflammatory state and VSMC apoptosis could be targetable mechanisms implicated in the pathogenesis of AAA formation. Lai et al. investigated the hypothesis that a xanthine derivative (KMUP-3) might suppress AAA through inhibition of VSMC phenotypic switch and apoptosis.

β-glycerophosphate was used to induce *in vitro* VSMC calcification while *in vivo* AAA was induced using angiotensin II infusion for 4 weeks in apolipoprotein E-deficient mice.

The results showed that KMUP-3 suppressed VSMC calcification. During VSMC calcification, KMUP-3 inhibited mTOR and β-catenin upregulation, essential for VSMC phenotypic switch, while it enhanced AMP-activated protein kinase (AMPK) activation that protects against VSMC phenotypic switch. Moreover, KMUP-3 attenuated VSMC apoptosis with an increased Bcl-2/Bax ratio and reduced activated caspase-3 expression. During AAA formation, treatment with KMUP-3 inhibited phosphorylated mTOR expression and increased phosphorylated AMPK expression in the medial layer. In addition, KMUP-3 treatment suppressed aortic dilatation together with reduction in proinflammatory cytokines and infiltrating macrophages, attenuation of medial VSMC apoptosis and mitigation of reactive oxygen species generation, matrix-degrading proteinase activities, elastin breakdown and vascular calcification.

Treatment with KMUP-3 inhibits aneurysm growth possibly through its interference with signaling pathways involved in VSMC phenotypic switch and apoptosis. These findings provide a proof-of-concept validation for VSMC dysfunction as a potential therapeutic target in AAA.

T-cell death-associated gene 8 accelerates atherosclerosis by promoting vascular smooth muscle cell proliferation and migration

Atherosclerosis is a chronic disease characterized by abnormal proliferation and migration of vascular smooth muscle cells (VSMCs) and inflammation. Inflammation and hypoxia may cause an acidic microenvironment, leading to pH values below 6 in plaques. However, the role of low pH values in atherosclerosis is largely unclear. In this study, Chen et al. aimed to investigate the potential effects of T-cell death-associated gene 8 (Tdag8), a proton-sensing receptor, in VSMCs during atherosclerosis.

The expression of Tdag8 in an atherosclerotic model of high-fat-diet-fed *ApoE*$^{-/-}$ mice was assessed, while the role and mechanism of Tdag8 in phenotype transformation, proliferation and migration of VSMCs were investigated in a series of *in vivo* and *in vitro* experiments.

Tdag8 expression at the mRNA and protein level was significantly increased in atherosclerotic *ApoE*$^{-/-}$ mice. Immunofluorescence staining showed that Tdag8 was primarily distributed in proliferating VSMCs. VSMCs phenotypic transformation, proliferation and migration were inhibited by Tdag8 silencing and increased by Tdag8 overexpression. Further mechanistic studies showed that cyclic adenosine monophosphate (cAMP) level is increased in Tdag8-overexpressing VSMCs and *ApoE*$^{-/-}$ mice. However, the protein kinase A (PKA) inhibitor H-89 reversed Tdag8-induced VSMC proliferation and migration, suggesting that Tdag8 promoted proliferation and migration of VSMCs by activating the cAMP/PKA signaling pathway.

The results demonstrate that Tdag8 mediated phenotype transformation, proliferation and migration of VSMCs via the cAMP/PKA signaling pathway, thus contributing to atherosclerosis.

The PGC-1α/NRF1/miR-378a axis protects vascular smooth muscle cells from FFA-induced proliferation, migration and inflammation in atherosclerosis

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is a transcriptional coactivator that works as key regulator of homeostasis affecting multiple cells and organs. Chong et al. investigated the role of PGC-1α in atherosclerosis.

PGC-1α was significantly decreased in the media of human atherosclerotic vessels. To explore whether miRNAs might be regulated by PGC-1α in vascular smooth muscle cells (VSMCs), microarray analysis was performed. Microarray and Pearson's correlation analysis showed that PGC-1α and miR-378a were positively correlated *in vivo* and *in vitro*. As an upstream co-activator, PGC-1α was found

to regulate miR-378a through binding to the transcriptional factor nuclear respiratory factor 1 (NRF1) in VSMCs. Therefore, the decreased expression of PGC-1α might account for suppression of miR-378a in VSMCs in atherosclerosis. Furthermore, insulin-like growth factor 1 (*IGF-1*) and Toll-like receptor 8 (*TLR8*), two genes known to be aberrantly up-regulated in atherogenic vessels, were identified as direct targets of miR-378a. *In vitro* up-regulation of miR-378a markedly inhibited free fatty acid (FFA)-induced VSMC proliferation, migration and inflammation through targeting *IGF1* and *TLR8*.

These findings highlight the protective role of the PGC-1α/NRF1/miR-378a regulatory axis in atherosclerosis progression and suggest miR-378a as potential therapeutic target for atherosclerosis treatment.

l-Arginine prevents inflammatory and pro-calcific differentiation of interstitial aortic valve cells

Calcific aortic valve disease (CAVD) is the leading pathological process of the aortic valve in Western countries. Previous investigations demonstrated that oxidative stress and reduced bioavailability of nitric oxide (NO) could be observed during the initiation and propagation of CAVD. Here, Rattazzi et al. investigated the effects of l-Arginine, the main precursor of NO, on the osteogenic differentiation of aortic interstitial valve cells (VICs).

A clonal population of bovine VICs that expresses osteogenic markers and induces calcification of collagen matrix after stimulation with endotoxin was isolated. VICs were treated *in vitro* with different combinations of lipopolysaccharide (LPS) ± l-Arginine and cell extracts were collected to perform proteomic (iTRAQ) and gene expression (RT-PCR) analysis.

l-Arginine prevented over-expression of alkaline phosphatase (ALP) and reduced matrix calcification in VICs treated with LPS. l-Arginine also reduced over-expression of inflammatory molecules induced by LPS. The proteomic analysis identified 49 proteins with an altered expression profile after stimulation with LPS and significantly modified by l-Arginine. These included proteins involved in the redox homeostasis of the cells, remodeling of the extracellular matrix and cellular signaling.

The results suggest that l-Arginine prevents osteogenic differentiation of VICs and reduces matrix calcification. This effect is achieved through the modulation of proteins involved in the cellular redox system, remodeling of extracellular matrix and inflammatory activation of VICs.