atherosclerosis

Atherosclerosis newsletter

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Calcification is a hallmark of advanced atherosclerotic plaques. The two issues contain several articles reporting research data on the pathogenesis and diagnostic exploitation of calcification.

Soluble RAGE attenuates Ang II-induced arterial calcification via inhibiting AT1R-HMGB1-RAGE axis

Vascular calcification (VC) is the deposition of calcium phosphate crystals in the aortic wall and cardiovascular tissue, mainly caused by aging, atherosclerosis, diabetes, and chronic kidney disease. VC is a strong independent predictor of increased cardiovascular morbidity and mortality. Arterial calcification (AC), which is an important process in the pathogenesis of atherosclerosis, is accelerated by angiotensin II (Ang II), a critical effector of the renin-angiotensin system (RAS). Receptor for advanced glycation end-product (RAGE) is an important pattern recognition receptor downstream of Ang II. Although recent studies have suggested an association between RAGE-mediated signaling and RAS in AC, the detailed mechanism, particularly in relation to Ang II, remains unclear. Jeon et al. investigated the role of RAGE-mediated signaling pathways and the therapeutic efficacy of soluble RAGE (sRAGE) in Ang II-induced AC, using both a human aortic smooth muscle cell (HAoSMC) model, and an *in vivo* apolipoprotein E knockout (*ApoE* KO) mouse model.

Ang II significantly increased the calcification of HAoSMCs, and the associated activation of RAGE was mediated by subsequent high-mobility-group-protein B1 (HMGB1) release through Angiotensin II type 1 receptor activation. Both HMGB1 neutralizing antibody and sRAGE inhibited Ang II-induced calcium deposition. Furthermore, sRAGE attenuated HMGB1 secretion and the activation of RAGE-mediated signaling. The *in vivo* study indicated that Ang II significantly induced calcium deposition in the aorta, and this was significantly attenuated by sRAGE.

These findings suggest that RAGE may potentially be a therapeutic target for VC treatment.

Irisin alleviates vascular calcification by inhibiting VSMC osteoblastic transformation and mitochondria dysfunction via AMPK/Drp1 signaling pathway in chronic kidney disease

Cardiovascular disease (CVD) is the major cause of mortality and morbidity in patients with chronic kidney disease (CKD). Vascular calcification (VC) is a highly prevalent complication and an independent predictor of cardiovascular mortality in CKD. VC is an intricate active process, significantly

controlled by vascular smooth muscle cells (VSMCs). Accumulating evidence indicates that mitochondrial dysfunction plays a pivotal role in VC and VSMCs osteoblastic transformation. Irisin is a newly identified myokine that regulates energy metabolism. It could stimulate osteoblasts and enhance bone formation, which suggests that Irisin may be a potent mediator to modulate bone turnover. Wang et al. previously reported that decreased levels of Irisin were independently associated with VC in hemodialysis patients. The present study aimed to investigate the role of Irisin in VC, especially in VSMCs osteoblastic transformation and mitochondrial function.

VSMCs calcification was induced *in vitro* by β-glycerophosphate, while *in vivo* VC was triggered by adenine and high phosphorus diet. Alizarin red and Von Kossa staining was performed, and calcium and alkaline phosphatase (ALP) activity assessed to test VC. Western blot and immunohistochemical staining were used to analyze the expression of proteins associated with VSMCs osteoblastic transformation and 5' AMP-activated protein kinase (AMPK) signaling. Mitochondrial membrane potential (MMP) and structures were observed with immunofluorescence staining.

Irisin alleviated VSMCs calcification induced by β-glycerophosphate. Mechanistically, Irisin activated AMPK and downregulated the expression of dynamin-related protein 1 (DRP1), further alleviating mitochondria fission and VSMCs osteoblastic transformation. *In vivo*, Irisin decreased serum creatinine, urea and phosphorous levels in CKD mice. Importantly, Irisin treatment postponed CKD-associated VC with upregulation of smooth muscle alpha-actin (αSMA) and p-AMPK expression, and downregulation of Runt-related transcription factor 2 (RUNX2) and Drp1 expression.

These results show that Irisin inhibits CKD-associated VC. Irisin suppresses VSMCs osteoblastic transformation and mitochondria dysfunction via AMPK/Drp1 signaling.

PCSK9 promotes arterial medial calcification

A complex interplay among chronic kidney disease (CKD), lipid metabolism and aortic calcification has been recognized. Lupo et al. investigated the influence of kidney function on proprotein convertase subtilisin/kexin type 9 (PCSK9) levels and its potential direct action on smooth muscle cells (SMCs) calcification.

In a cohort of 594 subjects, a negative association between glomerular filtration rate and plasma PCSK9 was found. Atherosclerotic cardiovascular disease, as co-morbidity, further increased PCSK9 plasma levels. Diet-induced uremic condition in rats led to aortic calcification and increased total cholesterol and Pcsk9 levels in plasma, livers, and kidneys. Both human and rat SMCs overexpressing human PCSK9 ($SMCs^{PCSK9}$), cultured in a pro-calcific environment showed a significantly higher extracellular calcium (Ca^{2+}) deposition compared to control SMCs. The addition of recombinant human PCSK9 did not increase the extracellular calcification of SMCs, suggesting the involvement of intracellular PCSK9. Accordingly, the further challenge with evolocumab did not affect calcium

deposition in hSMCs^{PCSK9}. Under pro-calcific conditions, SMCs^{PCSK9} released a higher number of extracellular vesicles (EVs) positive for three tetraspanin molecules, such as CD63, CD9, and CD81. EVs derived from SMCs^{PCSK9} tended to be more enriched in calcium and alkaline phosphatase (ALP), compared to EVs from control SMCs. In addition, PCSK9 was detected in SMCs^{PCSK9}-derived EVs. SMCs^{PCSK9} showed an increase in pro-calcific markers, namely bone morphogenetic protein 2 and ALP, and a decrease in anti-calcific mediator matrix GLA protein and osteopontin.

This study shows a direct role of PCSK9 on vascular calcification induced by higher inorganic phosphate levels associated with renal impairment. The effect appears to be mediated by a switch towards a pro-calcific phenotype of SMCs associated with the release of EVs containing Ca^{2+} and ALP.

Machine learning for atherosclerotic tissue component classification in combined near-infrared spectroscopy intravascular ultrasound imaging: Validation against histology

The assessment of coronary plaque composition is essential in treatment planning and predicting cardiovascular events. Calcific atherosclerotic plaques have been associated with a higher risk of procedural complications, stent under-expansion and suboptimal percutaneous coronary intervention (PCI) results. Intravascular ultrasound (IVUS) has limited efficacy in assessing tissue types, while near-infrared spectroscopy (NIRS) provides complementary information to IVUS but lacks depth information. Bajaj et al. aimed to train and assess the efficacy of a machine learning classifier for plaque component classification that relies on IVUS echogenicity and NIRS-signal, using histology as reference standard.

Matched NIRS-IVUS and histology images from 15 cadaveric human coronary arteries were analyzed (10 vessels were used for training and 5 for testing). Fibrous/pathological intimal thickening (F-PIT), early necrotic core (ENC), late necrotic core (LNC), and calcific tissue regions-of-interest were detected on histology and superimposed onto IVUS frames. The pixel intensities of these tissue types from the training set were used to train a J48 classifier for plaque characterization (ECHO-classification). To aid differentiation of F-PIT from necrotic cores, the NIRS-signal was used to classify non-calcific pixels outside yellow-spot regions as F-PIT (ECHO-NIRS classification). The performance of ECHO and ECHO-NIRS classifications were validated against histology.

262 matched frames were included in the analysis (162 constituted the training set and 100 the test set). The pixel intensities of F-PIT and ENC were similar and thus these two tissues could not be differentiated by echogenicity. With ENC and LNC as a single class, ECHO-classification showed good agreement with histology for detecting calcific and F-PIT tissues but had poor efficacy for necrotic cores. Similar results were found when F-PIT and ENC were treated as a single class. ECHO-NIRS classification improved necrotic core and LNC detection, resulting in an increase of the overall accuracy of both models, from 81.4% to 91.8%, and from 87.9% to 94.7%, respectively. Comparable

performance of the two models was seen in the test set where the overall accuracy of ECHO-NIRS classification was 95.0% and 95.5%, respectively.

The combination of echogenicity with NIRS-signal appears capable of overcoming limitations of echogenicity, enabling more accurate characterization of plaque components. This approach may be advantageous for vulnerable plaque detection and for monitoring plaque evolution.

Carotid plaque is strongly associated with coronary artery calcium and predicts incident coronary heart disease in a population-based cohort

Coronary artery calcium (CAC) is a sign of advanced atherosclerosis and a risk factor for incident coronary heart disease (CHD). Carotid artery plaque is also a known predictor of CHD. Results on the association between CAC and carotid plaque have been based on relatively few population based studies or studies on selected patient groups with prevalent CHD or other important co-morbidities. Gudmundsson et al. investigated associations between CAC and carotid plaque in asymptomatic individuals also in relation to predicted CHD-risk and incident events. A secondary aim was to compare predictive value between CAC, carotid plaque, and total carotid plaque area (TPA) as predictors for future CHD events.

The REFINE (Risk Evaluation For Infarct Estimates)-Reykjavik study, conducted by the Icelandic Heart Association, is a prospective and population-based study. A total of 948 individuals without clinical CHD were included. CAC scores were categorized into 0,1–100,101-300 and > 300, and carotid plaque into none, minimal and significant. Three models were applied adjusted for age, sex, and each of the Framingham risk score (FRS), local CHD risk score and established CHD risk factors.

Combined carotid plaque- and CAC-presence was highly prevalent, 69.5% for males and 41.7% for females. TPA outperformed base models in CHD prediction, resulting in statistically significant area under the receiver operator characteristic curve (AUC) increase ranging from 0.02 to 0.05. Most CHD-events in females occurred in individuals classified as low-risk with respect to traditional risk factors but with a gradient in observed risk across carotid plaque categories.

Carotid plaque was strongly associated with the presence and extent of CAC in asymptomatic individuals in a population-based cohort. Carotid plaque predicts incident CHD events over risk scores and may be useful for refined risk prediction in females.