Dysregulation of Notch signaling is involved in pathologies of aortic valve such as calcification. As PYK2-mediated phosphorylation of Y657 (human sequence) abrogates the catalytic activity of the endothelial nitric oxide (NO) synthase (eNOS) and decreases NO bioavailability, preventing this post-translational modification may preserve NO output and improve the cellular redox capacity, thereby reducing endothelial dysfunction. Notch signaling and proosteogenic genes are activated in co-culture of human aortic valve endothelial and interstitial cells. The aim of this study was to analyze the impact of the adventitial layer on vascular remodeling processes and to define the underlying cellular mechanisms. The adventitia plays a central role in the development and progression of atherosclerotic disease. To investigate potential paracrine effect of the activated adventitial layer, we explanted adventitial transplants 14 days following injury and transplantation and incubated the respective samples in serum-free media for 24 hours. BrdU incorporation assays and scratch wound assays revealed significantly increased proliferation and migration rates of human coronary artery SMCs in response to the supernatant of adventitial transplants compared to the supernatant of control samples or serum-free media. Further secretome analyses of the same adventitial supernatants identified predominantly interleukin (IL)-6 to trigger SMC proliferation and migration. Accordingly, serum-free media incubated with adventitial grafts of IL-6−/− mice prevented SMC proliferation and migration. Transplantation of the adventitia of IL-6−/− mice into C57BL/6j wild type mice was not sufficient to trigger neointima formation.

Conclusion: Acute vascular injury is followed by an expansion of cytokine-producing adventitial cells, whose paracrine function and especially whose release of IL-6 is essential for the subsequent induction of the proliferation and migration of local SMC and thus for neointima formation.

**Phosphorylation of eNOS on Tyrosine 656 contributes to endothelial dysfunction in vivo**

Primary human aortic valve endothelial and interstitial cells (VEC and VIC) were isolated from normal tricuspid aortic valves. The cells were co-cultured together for 48-96 h in DMEM. Magnetic sorting with anti CD31-conjugated beads was used to separate VEC and VIC after co-culture. Real-time PCR analysis was used to analyze gene expression. Results: Co-culture significantly increased expression of HIF1α and NOTCH2, NOTCH3, NOTCH4, DLL4, JAG1 genes in VEC. Expression of Notch-dependent SLUG and SNAI1 was also upregulated exclusively in VEC. Expression of ACTA2 was upregulated in both VEC and VIC after co-culture. Expression of proosteogenic genes OPN (osteopontin), OPG (osteoprotegrin) and ALP (alkaline-phosphatase) was stimulated in co-cultures of VEC and VIC.

Conclusion: We set up an experimental system for co-culture of human aortic valve endothelial and interstitial cells. We show that interaction of aortic valve endothelial and interstitial cells causes activation of Notch signaling distinctly for either cell type. Endothelial cells activate mesenchymal phenotype in interstitial cells via activation of Notch signaling. Co-culture of VEC and VIC stimulates proosteogenic signaling in both types of the cells. Understanding the mechanisms of Notch action in osteogenic phenotype induction will be important in further studies of aortic valve calcification.

**Adventitial interleukin-6 release is critical for neointima formation**

Introduction: Dysregulation of Notch signaling is involved in pathologies of aortic valve such as calcification. Notch is important for development of aortic valve cusps and also participates in intercellular communications and tissue integrity maintenance during postnatal life. The role of Notch dysregulation in the calcification of aortic valve has been highlighted by recent studies, but the mechanisms of Notch-dependent aortic valve calcification remain largely unknown. The importance of studying Notch in co-culture of mesenchymal and endothelial cells has become recently evident.

Purpose: The aim of the study was to investigate interactions between human aortic valve interstitial cells (VICs) and valve endothelial cells (VECs) to analyze activation of Notch genes and genes related to osteogenesis in co-culture of aortic VIC and VEC.

Methods: Primary human aortic valve endothelial and interstitial cells (VEC and VIC) were isolated from normal tricuspid aortic valves. The cells were co-cultured together for 48-96 h in DMEM. Magnetic sorting with anti CD31-conjugated beads was used to separate VEC and VIC after co-culture. Real-time PCR analysis was used to analyze gene expression.

Results: Co-culture significantly increased expression of HIF1α and NOTCH2, NOTCH3, NOTCH4, DLL4, JAG1 genes in VEC. Expression of Notch-dependent SLUG and SNAI1 was also upregulated exclusively in VEC. Expression of ACTA2 was upregulated in both VEC and VIC after co-culture. Expression of proosteogenic genes OPN (osteopontin), OPG (osteoprotegrin) and ALP (alkaline-phosphatase) was stimulated in co-cultures of VEC and VIC.

Conclusion: We set up an experimental system for co-culture of human aortic valve endothelial and interstitial cells. We show that interaction of aortic valve endothelial and interstitial cells causes activation of Notch signaling distinctly for either cell type. Endothelial cells activate mesenchymal phenotype in interstitial cells via activation of Notch signaling. Co-culture of VEC and VIC stimulates proosteogenic signaling in both types of the cells. Understanding the mechanisms of Notch action in osteogenic phenotype induction will be important in further studies of aortic valve calcification.

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