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Novel insights in signaling pathways in endothelial dysfunction

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Notch signaling and proosteogenic genes are activated in co-culture of human aortic valve endothelial and interstitial cells

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Introduction: Dysregulation of Notch signaling is involved in pathologies of aortic valve such as bicuspid aortic valve and aortic valve 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 (VEC): 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 VIC and VEC after co-culture. Real-time PCR analysis was used to analyze gene expression.
Results: Co-culture significantly increased expression of HEY1 and NOTCH2, NOTCH3, NOTCH4, DLL4, JAG1 genes in VEC. Expression of Notch-dependent SLUG and SNAIL1 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 (osteoprotegerin) 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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Adventitial interleukin-6 release is critical for neointima formation

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Background: 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.
Methods: and Results: The femoral artery of C57BL/6J mice was dilated with a straight spring wire and morphometric analysis of the lesion and immunohistochemical staining for the proliferation marker Ki-67 was performed 7, 14, and 21 days following injury. Formation of a neointimal lesion at 21 days was preceded by high adventitial proliferation rates and massive adventitial thickening at 7 and 14 days (adventitial area: 0.036±0.015 mm2 at 0d vs. 0.082 ± 0.013 mm2 at 7d vs. 0.102 ± 0.029 mm2 at 14d, $n = 15$, $P < 0.0001$).
Complete removal of the adventitial layer prevented neointima formation, attributing pivotal importance to the adventitial layer (luminal stenosis: 71.73 ± 3.77 % vs. 7.44 ± 1.71 %, $n = 5$, $P < 0.0001$). Re-transplantation of the aortic adventitia of ubiquitously GFP expressing C57BL/6-Tg(CAG-EGFP)1Osb/J mice around the medial vascular layer of the femoral artery where the native adventitia has been removed completely restored neointima formation. Importantly, only very view GFP+ cells were present in the neointimal layer, indicating that a direct contribution of adventitial cells to the neointimal lesion represents an extremely rare event.
To investigate a 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.

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

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Background: Increased oxidative stress is a hallmark of most cardiovascular diseases including hypertension and atherosclerosis and is associated with impaired endothelial function. Proline-rich-tyrosine-kinase 2 (PYK2) is considered to be a redox-sensitive kinase that is activated in response to angiotensin II (AngII) and elevated oxidative stress.
Purpose: As PYK2-mediated phosphorylation of Y657 (human sequence) abrogates the catalytic activity of the endothelial nitric oxide (NO) synthase (eNOS) and decreases NO bioavailability, we asked whether the phosphorylation of eNOS on Y657 contributes to endothelial dysfunction and cardiovascular disease in vivo.
Methods: and Results: Non-phosphorylatable eNOS knock-in (Y657F) mice displayed lower blood pressure and enhanced ACh-induced vascular relaxation compared to wild-type (WT) mice. Angiotensin II increased Y656 phosphorylation and induced hypertension in WT mice but the increase in pressure was less pronounced in the Y656F mice. On the ApoE-/- background, partial left carotid artery (LCA) ligation combined with the Paigen diet impaired ACh-induced vascular relaxation and increased the expression of ICAM-1 and VCAM-1 and infiltration of neutrophils. These effects were improved in Y656F–ApoE-/- mice. Atherosclerotic plaque development in the LCA 21 days after ligation or in the aorta and aortic arch of mice fed a high-fat diet for four or six months was also reduced in the Y656F–ApoE-/- group. At the molecular level, agonist-induced NO production was greater in cells expressing the Y657F compared to the WT enzyme and was associated with increased binding of Hsp90 and calmodulin, whereas NO synthesis was entirely abrogated in cells expressing the Y657D eNOS phosphomimetic mutant, due to deficient binding of flavin mononucleotide. In addition, depletion of tetrahydrobiopterin resulted in the uncoupling of WT but not Y657F eNOS enzymes. In growth factor-stimulated endothelial cells, Hsp90 mediated the increased association between the Y657F eNOS and pyruvate kinase (PKM2), a rate limiting enzyme at the end of the glycolytic pathway. The S-nitrosation of PKM2 was higher in cells expressing the Y657F compared to the WT eNOS and resulted in the reduction of PKM2 activity.
Conclusions: Phosphorylation of eNOS on Tyr656 in vivo is associated with endothelial dysfunction and development of cardiovascular disease. Preventing this post-translational modification may preserve NO output and improve the cellular redox capacity, thereby reducing endothelial dysfunction.