Vascular Remodeling in Cardiovascular Disease

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231 Absence of PI3Kg leads to increased reendothelialization in mice through modulation of IP-10 secretion.
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The main way of treating symptomatic atherosclerosis is angioplasty with stent placement. This intervention injures the vascular wall, causing endothelial loss, inflammation and intimal hyperplasia (IH), which frequently cause restenosis, a de novo obstruction of the lumen. To prevent this complication, Drug Eluting Stents have been coated with antiproliferative agents. Although they have proven their efficiency, their use is associated with increased early neointima formation and, although rare long term thrombosis leading to fatal side effect. This side effect is in correlation with an impaired endothelial healing due to the lack of specificity of the antiproliferative drugs. So it is of major importance to find new therapeutic targets to prevent restenosis without interfering with a correct reendothelialization. We have previously shown that the invalidation of the kinase activity of phosphoinositide 3-kinase gamma (PI3Kg) efficiently prevented intimal hyperplasia in mice after arterial injury. Interestingly, immunohistochemical staining strongly suggested that endothelial coverage was increased in mice lacking PI3Kg activity (PI3KgKD, kinase dead) compared to WT controls. PI3Kg is especially known for its inflammatory and immune roles. Yet, no causal link between endothelial healing and immune-inflammatory processes has been previously reported. We aimed to study the mechanisms by which PI3Kg is involved in endothelial healing. For this purpose, mice were subjected to an endovascular mechanical injury of the carotid artery. Intravenous injection of Evans Blue, allowing the staining of the denuded area, showed a 2 fold increase in reendothelialization rates in PI3Kg KD mice compared with WT, demonstrating a deleterious role of PI3Kg activity upon endothelial healing. Bone marrow transfer experiment showed that this role was attributable to PI3Kg activity in the medullar compartment. A screen at genetic and protein levels showed a PI3Kg dependent increase in the expression and secretion of IP-10, (IFNγ-induced protein 10) in injured carotid arteries, a chemokine previously identified as a possible regulator of endothelial cell proliferation. Moreover, injection of IP-10 neutralizing antibodies accelerates reendothelialization as the same level than observed in absence of PI3Kg activity. Our results demonstrate that PI3Kg invalidation improves endothelial healing through an indirect mechanism involving IP-10 secretion. When added to our previous results, the inhibition of PI3Kg represents a way of preventing complication of arterial angioplasty such as neointima hyperplasia and late stent thrombosis.

Results: We could demonstrate that DPP4 inhibition tremendously reduced HD-induced neo-atherosclerosis in the ApoE-/- mice. Gliptin-mediated protective effects were reverted by addition of the CXCR4 blocker AMD3100, which clearly proved the SDF-1α/CXCR4-signaling as the therapeutic relevant gliptin-mediated pathway. We could further show that CXCR4 is highly expressed on the surface of cholesterol-exporting M2 macrophages and that the number of M2 macrophages in the aortic wall of Stat1-deficient mice was significantly higher than in placebo-treated animals on HD. While the number of M2 macrophages inversely correlated to total plaque area, AMD3100 inhibited the mural enrichment of these cells. Additional in vitro analyses showed that gliptin-mediated enrichment of mural M2 macrophages occurred due to induction of monocyte differentiation rather than induction of cell recruitment. Regarding endothelial recovery, we were able to show that an accelerated reendothelialization of denuded arterial blood vessels via inhibition of DPP4 is mediated by the enhanced recruitment of circulating progenitor cells. Interestingly, reendothelialization occurred only from the borders of the injured area, which supports the notion that local proliferating endothelial cells may have regenerated the endothelial coverage.

Conclusion: Different gliptins show a protective effect on arterial blood vessels. Depending on the mechanism of injury (acute endothelial vs. chronic atherosclerotic damage) different cellular mechanisms appear to be responsible for the DPP4-dependent vascular protection. Thus, pharmacological inhibition of DPP4 may depict a future option for the prevention of ischemic cardiomyopathy in diabetics and non-diabetic patients.

232 DPP4 inhibition mediates vascular protection in acute and chronic vascular injury.
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Background/Purpose: Available therapies for vascular diseases at present focus on the treatment of vascular stiffness and reduction of cardiovascular risk factors only. In this project, we want to establish the pharmacological inhibition of DPP4 as a new therapeutic concept focusing on inhibition of atherosclerosis development and improvement of endothelial healing.

Methods: ApoE-/- mice were treated with a DPP4 inhibitor (- AMD3100 (in ablzet osmotic pumps) or placebo starting before initiation of high-cholesterol diet (HD)-induced atherosclerosis. Aortic plaque development was quantified after 12 weeks of treatment using the Oil-Red O staining. Mechanistic analyses comprised FACS analyses for macrophage subtypes isolated from the diseased aortic wall and detailed in-vitro monocyte/macrophage differentiation assays.

To evaluate endothelial healing under treatment with different DPP4 inhibitors the common carotid artery of wildtype mice was denuded at a length of 4 mm using an electric injury model. Evans blue staining was used to quantify endothelial recovery after 3 and 6 days of treatment. Further mechanistic analyses were performed using FACS, ultrasound and enzymatic assays.

Results: We could demonstrate that DPP4 inhibition tremendously reduced HD-induced neo-atherosclerosis in the ApoE-/- mice. Gliptin-mediated protective effects were reverted by addition of the CXCR4 blocker AMD3100, which clearly proved the SDF-1α/CXCR4-signaling as the therapeutic relevant gliptin-mediated pathway. We could further show that CXCR4 is highly expressed on the surface of cholesterol-exporting M2 macrophages and that the number of M2 macrophages in the aortic wall of Stat1-deficient mice was significantly higher than in placebo-treated animals on HD. While the number of M2 macrophages inversely correlated to total plaque area, AMD3100 inhibited the mural enrichment of these cells. Additional in vitro analyses showed that gliptin-mediated enrichment of mural M2 macrophages occurred due to induction of monocyte differentiation rather than induction of cell recruitment. Regarding endothelial recovery, we were able to show that an accelerated reendothelialization of denuded arterial blood vessels via inhibition of DPP4 is mediated by the enhanced recruitment of circulating progenitor cells. Interestingly, reendothelialization occurred only from the borders of the injured area, which supports the notion that local proliferating endothelial cells may have regenerated the endothelial coverage.

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233 Effects of transforming growth factor beta signalling on smooth muscle cell phenotype in the angiotensin II-induced abdominal aortic aneurysm model.
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Introduction: Abdominal aortic aneurysm (AAA) is a leading cause of sudden death in aged people. Vascular smooth muscle cells (VSMC) experience phenotypic adaptations to a variety of pathophysiological conditions of alterations underlie progressive aortic dilation. TGFβ modulates the transition between VSMC contractile and synthetic phenotypes. Increased TGFβ signalling results in thoracic aortic aneurysm. In contrast, its bas in the development of AAA report this growth factor as a protective cytokine in the development of AAA.

Purpose: To study the role of TGF-beta in phenotypic alteration of VSMC in angiotensin II (Ang II) AAA model.

Methods: CS7BL6 mice received Angl (1.8 μg/hour, 2 wk) infused with osmotic pumps. A neutralizing Anti-TGFβ antibody was injected intraperitoneally (24 mg/kg/wk). AA diameter was measured by echography. Aortic tissue was processed for gene quantification (qPCR) and transmission electron microscopy (TEM). VSMC isolated from human AA were treated with recombinant TGFβ1 and AngII for 24h and processed for gene quantification (qPCR) and immunofluorescence. The morphological phenotype and contractile, synthetic and proliferation markers were determined.

Results: AngII infusion to mice induced suprarenal aortic dilation (4±6%). Treatment with TGFβ1–Ab plus AngII potentiated the dilation (8±13%) and the mortality due to AAA rupture. mRNA levels of collagen and fibronectin, as well as a-SMA were increased by TGFβ1–Ab plus AngII when compared with AngII alone. TEM of the aortic wall confirmed higher synthetic and contractile VSMC phenotypes, ECM accumulation and elastic disarray in the latter group. In cultured human VSMC, treatment with either AngII or TGFβ1 induced a contractile phenotype (α-SMA: 2±0.2). Conversely, the combined treatment with AngII plus TGFβ1 reduced the expression of contractile markers (calponin1: 0.3±0.9; α-SMA: 0.6±0.2; connective tissue growth factor: 0.5±0.12), and increased the proliferation marker, cyclin D1.

Conclusion: Neutralization of TGFβ1 potentiates AAA dilation, ECM deposition and contractile phenotype in AngII-infused mice. On the contrary, association of TGFβ1 to AngII in cultured VSMC downregulates genes encoding contractile markers and collagen. We suggest that TGFβ protects against AAA by decreasing intracellular and extracellular matrix contributors to aortic wall stiffness.

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