Abstract: **P4913**

Adventitial cytokine production is critical for negative vascular remodelling

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Background: Deciphering the detailed pathophysiological course of vascular remodeling processes is of pivotal importance for the development of novel therapeutic strategies. The impact and the cellular contribution of the vascular adventitial layer on neointima formation are still unknown.

Purpose: 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: We dilated the femoral artery of C57BL/6J mice with a straight spring wire and performed morphometric analysis of the lesion and immunohistochemical staining for the proliferation marker Ki-67. Formation of a profound 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 mm^2 at 0d vs. 0.082±0.013 mm^2 at 7d vs. 0.102±0.029 mm^2 at 14d, n=15, P<0.0001). Further immunohistochemical characterization of the respective proliferating cells revealed them most likely to be fibroblasts. Furthermore, antibody-mediated leukocyte-depletion prevented adventitial cell proliferation.

Complete removal of the adventitial layer diminished 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). Coating of the medial vascular layer of the femoral artery with the aortic adventitia of ubiquitously GFP expressing C57BL/6-Tg (CAG-EGFP)1Osb/J mice restored the negative vascular remodeling process. Importantly, only very view GFP+ cells could be detected in the neointimal layer, indicating that a direct contribution of adventitial cells to the neointimal lesion represents an extremely rare event. Instead, femoral artery dilation of dual color Myh11 creER(T2)−/+ mTmG−/+ double transgenic reporter mice revealed the majority of neointimal SMC to be predifferentiated medial SMCs.

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 adventitial supernatants identified predominantly the up-regulation of interleukin (IL)-6 to trigger SMC proliferation and migration. Conclusively, transplantation of the adventitia of IL-6−/− mice into C57BL/6J wild type mice prevented neointima formation.

Conclusion: Acute vascular injury is immediately followed by adventitial enrichment of cytokine-producing fibroblasts, whose paracrine function is essential for subsequent proliferation and migration of local SMC and

neointima formation.