Endothelial cells drive organ fibrosis and dysfunction by inducing the transcription factor Sox9

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Introduction. Fibrotic remodeling is a critical maladaptive feature in several diseases. Previously, we suggested that endothelial cells (ECs) contribute to extracellular matrix (ECM) deposition during cardiac fibrotic remodeling by transient induction of mesenchymal genes. However, the functional importance of this event for the development of organ fibrosis and disease remains elusive.
Aim of the study was to investigate the contribution of ECs to organ fibrosis and function by analyzing the role of the fibrogenic transcription factor Sox9 in ECs.
Methods & Results. Adult mice were challenged either with transverse aortic constriction (TAC) or a combination of high fat diet (HFD) and L-NAME in order to induce systolic or diastolic heart failure, resp. Early during each entity, we found a significant induction of *Sox9* mRNA in isolated cardiac ECs. Similarly, Sox9 was induced not only in murine ECs during fibrotic liver (through CDAA diet) and lung disease (through Bleomycin application) but also in human cardiac ECs from fibrotic explants as shown by single-cell RNA sequencing and histologic staining.
Endothelial cell-specific, *Cdh5*-promoted overexpression of Sox9 in transgenic mice resulted in fibrotic and hypertrophic remodeling of heart, lung, liver and spleen. Bulk and single-cell RNA sequencing of isolated ECs showed that ECs were the origin of ECM deposition. On functional level, we found impaired blood oxygen saturation, increased pulmonary artery resistance, cardiac diastolic dysfunction and eventually systolic functional impairment.
In contrast, mice with an inducible, EC-restricted knock-out of *Sox9* were protected from fibrotic heart, lung and liver remodeling following TAC, HFD/L-NAME, Bleomycin injection, or CDAA diet, resp., as indicated by reduced matrix displacement, decreased maladaptive remodeling and maintained organ function.
RNA-sequencing of isolated ECs from those organs upon Sox9 overexpression or deletion in disease showed that Sox9 directly induced the expression of mesenchymal genes in ECs, but also triggered the expression of paracrine growth factors. Likewise, mono-cultured ECs showed mesenchymal gene activation upon adenoviral Sox9 overexpression, but in addition activated mesenchymal gene transcription and migration in co-cultured fibroblasts.
Conclusions. Endothelial Sox9 is a master regulator of mesenchymal activation of ECs, hence driving organ fibrosis and failure, by both distinct ECM deposition and fibroblast activation. Absence of Sox9 in ECs, in turn, ameliorates fibrotic remodeling and maintains organ function.