Inflammatory actions of chemokines and extracellular versicles in pathological tissue remodeling

Summary

The present thesis describes the initiation of atherogenesis with the focus on calcification of blood vessels. The role of platelet and VSMC-derived extracellular vesicles (EVs), and chemokines in this process was especially emphasized. Moreover, presented work aimed to elucidate the role of platelet-derived chemokines in the vasculature with the focus on vascular smooth muscle cells (VSMCs) and endothelial cells (ECs). Finally, the role of steatotic hepatocyte-derived EVs in modulating stellate cells was investigated.

Chapter 1 gives the reader the general overview on the importance of extracellular vesicles, platelets and smooth muscle cells in the process of calcification of blood vessels that occurs in various pathological states, including atherosclerosis.

Chapter 2 addresses the importance of extracellular vesicles derived from platelets and smooth muscle cells in the initiation and propagation of vascular calcification. The involvement of perivascular mesenchymal stem cell (MSC)-like cells in this process is also addressed.

Chapter 3 explains the importance of calcification in the initiation of atherogenesis. Moreover, the mechanisms of calcification on a cellular level and the concept of reversing calcium/phosphate crystals from the vasculature is discussed.

Chapter 4 emphasizes the role of platelet-derived chemokines (CXCl4/CXCL4L1) in vascular remodeling and calcification *in vitro*. In this experimental work various cell-based assays were used to elucidate the functional differences between CXCL4 and CXCL4L1 in their ability to modulate human VSMCs. The proliferation, gene expression of selected contractile markers, transcription factors and cytokines were investigated. Also, the role of CXCL4/CXCL4L1 in initiation of calcification of VSMCs as well as their receptors playing a role in endocytosis were examined. Overall, CXCL4 and CXCL4L1 interacted differently with VSMCs and may thus play distinct roles during vascular remodeling.

Chapter 5 describes the fate of CCL5 and CXCL4 after deposition on endothelial cells. Chemokine uptake was analyzed by microscopy and by ELISA. Intracellular calcium signaling and monocyte arrest was evaluated under laminar flow upon chemokine treatment. The mechanism of endocytosis of CCL5 and CXCL4 was investigated as well as the accumulation of chemokines in various cell organelle fractions. Overall, even though CCL5 and CXCL4 treatment does not affect monocyte adhesion to endothelium, they are rapidly and actively internalized by ECs where they are directed to the nucleus.

Chapter 6 investigates the interplay between hepatocytes and hepatic stellate cells under normal and steatotic conditions and the role of EVs in this process. Migration of the stellate cell line TWNT4 towards control or steatotic EV as well as sera of NASH patients was investigated. TWNT4 phenotype alterations after incubation with EV was determined by qPCR, western blotting and immunofluorescence staining. Overall, EVs from steatotic HepG2 cells can influence the behavior and phenotype of TWNT4 cells as well as the expression of remodeling markers and guide directed migration.

Finally, the results are critically discussed and translated to a clinically relevant context in **Chapter 7.**