Chapter 8

EPILOGUE
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OUTCOME OF THIS THESIS

In this thesis, we studied the mechanisms that safeguard endothelial homeostasis with a focus on the signaling induced by laminar shear stress. We uncovered an intrinsic multi-layered mechanism that encompasses classical signal transduction and converges with regulatory mechanisms depending on epigenetics and posttranscriptional repression. Dysregulation of these mechanisms culminate in endothelial to mesenchymal transition (EndMT) and might contribute to the development of intimal hyperplasia and cardiac fibrosis. The in-depth knowledge on the factors that induce, facilitate or aggravate EndMT may contributes to the development of novel therapeutic approaches to treat intimal hyperplasia and cardiac fibrosis in the future.

MOLECULAR INSIGHTS ON ADVERSE ENDOTHELIAL PLASTICITY – POSSIBLE TARGET?

Despite the fact that endothelial cells throughout the body have the same origin – the hemangioblast (1) - there are distinct functional and structural differences between endothelial cells originating from their function in specific organs or the microenvironment the endothelial cells are in (2). This cellular heterogeneity is a reflection of the physiological endothelial plasticity and enables the optimal functioning of the residing organ(3). For instance, the endothelium in the blood-brain barrier forms a non-fenestrated endothelial layer which is impermeable to toxic substances thereby safeguarding the proper functioning of the brain(4), whereas a highly fenestrated endothelial layer is present in the liver sinusoid that enables the fast uptake of metabolites, plasma proteins and even drug molecules by the hepatocytes and hepatic satellite cells(5).

In the medium and large sized arteries, non-fenestrated endothelial cells reside on the basement membrane and align to the direction of blood flow. The biomechanical signal originating from the laminar shear stress sensed by the endothelium, is a key driving force of signaling pathways by which the endothelium safeguards the vascular integrity (e.g. endothelial cell-cell junctions and anti-inflammatory signaling) and maintains the blood flow (e.g. vasodilatory and anti-thrombogenic pathways). It is well established that at sites of vascular curvatures and bifurcation, endothelial cells are exposed to low and oscillatory shear stress(LOSS)(6, 7) and might undergo endothelial-mesenchymal transition(8), an adverse form of plasticity. Endothelial cells exposed to LOSS induce the expression of proinflammatory genes while reducing antioxidant gene expression (9-11). This distinct change in gene expression profile can partly be explained by the activities of the LOSS-induced transcription factors SMAD2/3 (12), SNAI1 (8), TWIST (13) and others. We uncovered that EZH2-dependent (Chapter 4 and 5) H3K27me3 bears important role in modulating the expression of protein coding genes as well as the microRNAs in response to the loss of laminar shear stress, thereby modulate endothelial quiescence (14).

EndMT contributes to the development of several pathologies including intimal hyperplasia, atherosclerosis, cardiac fibrosis and other fibroproliferative diseases. The intrinsic EndMT inhibitors therefore bear a therapeutic potential, which needs to be
further characterized in atherosclerosis and cardiac fibrosis models. The future looks bright because we and other researchers recognized intrinsic protectives signaling molecules such as pMAPK7(15), BMP7(16) and FGFRI (17) are capable of inhibiting EndMT. In this thesis, we uncovered that a combination of microRNA miR-101, miR-200a and miR-141 can preclude EndMT in endothelial cell models. Moreover, we uncovered that the antimir-374b inhibits the induction of EndMT in endothelial cell. These data suggest that microRNA mimetics or antimiRs might have anti-atherosclerosis potential through the maintenance of endothelial homeostasis. MicroRNAs “fine-tune” key signaling cascades and their therapeutic potential is exemplified in preclinical atherosclerosis models (18). For instance, miR-92a is a flow responsive microRNA that targets the mRNA of transcription factors KLF2 and KLF4, which are essential to endothelial homeostasis. The specific inhibition of miR-92a ameliorates endothelial activation and reduces plaque size in LDLR/- mice (19). In humans, synthetic antisense oligonucleotides similar in chemistry to miR mimics or antimiRs have been therapeutically used. Mipomersen, a second-generation anti-sense oligonucleotide molecule targeting the messenger RNA encoding for ApoB, effectively reduces the LDL-C level among patients suffering from familiar hypercholesterolemia and coronary heart disease patients non-responding to the maximum tolerated dose of other LDL-C lowering medications (20, 21), providing support for future RNA-based treatment possibilities.

Likewise, small molecule drugs that alter the activity of specific epigenetic enzymes might have an anti-atherosclerosis potential and are starting to enter the clinics. Although data on cardiovascular endpoints are scarce in the initial clinical studies, these drugs have proven safe in oncology trials, which warrant their transition for treatment of cardiovascular disease. This transition allows to test the hypothesis that treating epigenetic dysregulations in endothelial cells (e.g. SIRT1 deactivation or EZH2 innervation in atherosclerosis), precludes the development of atherosclerosis and potentially reduces the disease burden. The review only addressed to the early stage of atherosclerosis and the effect may differ late stages of atherosclerosis, especially in vulnerable plaques.

Also, we found that inhibition of intracellular Gal-3 attenuated EndMT. This finding can be linked with study which showed Gal-3 knockdown in mice lead to less cardiac fibrosis and improved cardiac function (22). Since reduction of GAL-3 reduces nuclear accumulation of β-catenin/TCF4 in colorectal cells (23) and induces gene expression of SNAI1, SNAI2 and TWIST1 in endothelial cells, the beneficial effect may explained via occurring through modulation of EndMT transcription factors.

TARGETING ADVERSE ENDOTHELIAL PLASTICITY IN ATHEROSCLEROSIS AND CARDIAC FIBROSIS

There is ample experimental and clinical evidence that high laminar shear stress benefits endothelial function and homeostasis and suppresses adverse endothelial plasticity and dysfunction. The easiest, reliable and risk-free way to physically induce shear stress magnitude is doing optimal regular physical exercise, which results in favorable effects in heart and vessel (24, 25). Thereby supporting physical activity among general population is important for endothelial health.
Although, vascular bifurcations and curvatures remain at risk due to their geometry – and these areas are atheroprone areas. In order to tackling this issue, we stated our prospect of ameliorating atherosclerosis via targeting pro-atherogenic endothelium using pre-exemplifying epigenetic enzymes namely SIRT1 activation and EZH2 inhibition in Chapter 2. Treatment against adverse endothelial plasticity is also inquired after successful coronary artery bypass graft (CABG) or percutaneous coronary intervention (PCI) to prevent from vein graft failure or restenosis, since the underlying pathology of these complications are due to the EndMT-derived intimal hyperplasia (30). Targeted delivery to endothelium such as with immunoliposomes (26) is obligatory to deliver intrinsic EndMT inhibiting molecules in vivo. Because we need to take into account that targets of miRNAs and functions of epigenetic enzymes vary in different cell types, systemic administration of these molecules may result in adverse off-target effects. The promising targeting delivery method is immune-liposome based technology which can selectively deliver siRNAs to the activated endothelial cells using surface markers such as E-selectin (27, 28).

Regarding cardiac fibrosis, targeting endMT during cardiac fibrosis may be a novel therapeutic strategy, because 30% of the myofibroblasts are derived via EndMT (16). Successful inhibition of EndMT in heart may ameliorate fibrosis in certain extend. The net result may be not only happening through the inhibition of EndMT-derived myofibroblast differentiation but also reducing hypoxia via maintaining endothelial phenotype. As hypoxia is well established to promote myofibroblast differentiation.

CONCLUDING REMARKS

Adverse endothelial plasticity is fundamental basis of multiple adult pathologies including atherosclerosis, intimal hyperplasia and cardiac fibrosis. We uncovered a number of signaling mechanisms, including signaling intermediates, epigenetics and post-transcriptional silencing that might preclude the development or progression of atherosclerosis and cardiac fibrosis. These mechanisms need to be investigated in future research by interventional studies in diseased animal models. Advances in the field of targeted drug delivery are essential to deliver therapeutic molecules in the cells they need treatment and enable to avoid undesirable side effects.
REFERENCE


