Drainage versus defense

Summary

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Cardiovascular disease (CVD) remains the most common cause of death in Europe and worldwide accounting for 31% of all deaths. Importantly, atherosclerosis is by far the most important cause of CVD, accounting for 80% atherosclerosis of CVD worldwide. Atherosclerosis is a lipid-driven chronic inflammatory disease, leading to the formation of plaques at vital segments of the arterial tree. Atherosclerotic plaques are deposits of cholesterol, lipids and cellular debris in the artery wall, resulting in narrowing of the larger arteries. Initial atherosclerotic lesions are characterized by subendothelial accumulation of macrophages and macrophage-derived foam cells. In progressive stages of disease development, medial smooth muscle cells will proliferate, migrate into the intima and produce collagen, forming a fibrous cap that covers the plaque (i.e. stable lesion). The vulnerable plaque is characterized by an extensive lipid core in the central atheroma, a high level of inflammation and a thin and inflamed fibrous cap covering the large necrotic core (thin-cap fibroatheromas; TFCA). In the final disease stage this vulnerable plaque will rupture leading to thrombus formation which can occlude the lumen causing distal ischemia and acute cardiovascular events (e.g. myocardial infarction (MI), stroke).

Endothelial cells play an important role in maintaining cardiovascular homeostasis. In this thesis we have focused on the molecular regulators thereof. The aim of this thesis is two-fold: 1) to study endothelial cells (EC) dysfunction mediated by impaired NO in heart failure, and 2) to define new molecular regulators of lymphatic EC (LEC) in atherosclerosis.

The first part of the thesis involves a study of endothelial dysfunction in the heart and vessels, which is often characterized by impaired NO bioavailability, leading to vasoconstriction, coagulation and inflammation. When chronic, this can lead to several pathological conditions, including MI, hypertension and atherosclerosis. In this dissertation it is hypothesized that modulation of endothelial nitric oxide synthase (eNOS) can protect against pressure overload-induced left ventricular hypertrophy.

In the first part of this thesis (**chapter 2-5**) are focused on the molecular mechanism of eNOS and its essential cofactor tetrahydrobiopterin (BH4), as well as potential therapeutic interventions to modulate eNOS in the pathogenesis of myocardial and endothelial dysfunction. As discussed in **chapter 2**, BH4 is a critical regulator of cardiovascular homeostasis, and substantial evidence implicates BH4 as a key regulator of eNOS in the setting of cardiovascular disease. Strategies to maintain BH4 bioavailability may be achieved by 1) enhancing the regeneration of BH4 from the inactive form BH2, 2) chemically stabilizing BH4, and 3) reducing oxidative degradation. Indeed, eNOS-generated NO has a crucial role in the regulation of endothelial function. **Chaper3** provides an extensive overview of the important role

of eNOS-uncoupling in the pathogenesis of endothelial dysfunction. Evidence suggests that modulation of eNOS by stabilizing eNOS function, and suppressing eNOS-derived ROS is a promising therapeutic target for endothelial dysfunction. Subsequently, in **chapter 4**, two, novel pharmacologic small molecule compounds that transcriptionally enhance eNOS gene expression, AVE9488 and AVE3085, are discussed. These compounds are designed to increase eNOS transcription and enhance NO signaling and bioavailability. Interestingly, AVE have been shown to also increase BH4 bioavailability reversing eNOS uncoupling, and to augment eNOS activity. Although their precise mode of action remains to be clarified, AVE are likely to interact with eNOS uncoupling. Subsequently, in **chapter 5,** we test if AVE3085 can prevent pressure overload-induced left ventricular (LV) hypertrophy. We have demonstrated that AVE 3085 attenuates the pathological LV changes caused by pressure overload. Surprisingly, these effects are seen in the absence of transcriptional upregulation of eNOS. Importantly, we provide a novel beneficial function of AVE3085 treatment on eNOS stability, demonstrating by an increase in eNOS dimer to monomer ratio and by a decrease in myocardial ROS generation. This effect is likely the result of protection of BH4 against oxidation by ROS. Possible mechanisms for AVE3085 mediate modulation/dampening of transverse aortic constriction -induced inflammatory responses and fibrosis may result from (I) indirect effects secondary to inhibition of macrophage infiltration and inflammatory signaling mediated by NO and/ or BH4, or (2) pleiotropic effects of AVE3085 on anti-inflammatory and anti-fibrotic effect.

The second part of this thesis is centered on the role of LEC in atherosclerosis. Angiogenesis, the sprouting of new blood vessels from the pre-existing ones, is essential for physiological development. Pathological angiogenesis contributes to pathogenesis of cardiovascular diseases. Although plaque angiogenesis has been linked to proinflammatory and proatherosclerotic effects, lymphatic vessels have only recently been implicated in the pathogenesis of MI and atherosclerosis, where proper lymphatic drainage seemed to protect against disease. The lymphatic system is increasingly recognized as a critical process in atherosclerosis to transport cholesterol and immune cells to the lymph nodes and eventually to the circulation. However, the precise driving factors and consequences of lymphangiogenesis in the context of human atherosclerosis still remain to be elucidated. Plaque-resident factors specifically involved in pathologic lymphangiogenesis during atherosclerosis development and progression may well represent novel approaches for therapeutic modulation of plaque lymphangiogenesis. In this part, it is hypothesized that gene network analysis can provide powerful predictions of candidate genes and those genes represent potential novel targets for regulating lymphangiogenesis-associated atherosclerosis in human (approaches will be described below).

In **chapter 6** we attempt to identify novel players in pathologic lymphangiogenesis in atherosclerosis. We employ a bioinformatics approach, weighted gene coexpression network analysis (WGCNA), to identify networks and candidate genes relevant to plaque lymphangiogenesis in humans. In this study, we correlate phenotypic traits i.e. LVD, identified by D2-40^{+} in human atherosclerotic tissue with transcription profiles, derived from the same tissue. We are able to identify modules with high correlation to LVD, unveiling a novel role for the phosphatase TNIP2, as a VEGF- independent key regulator of plaque lymphangiogenesis in humans *ex vivo*, in LEC *in vitro* and in zebrafish *in vivo*. In addition, we also provide transcription factor binding sites, miRNAs, and potential pathways which may be involved in TNIP2-mediated plaque lymphangiogenesis. TNIP2-responsive genes are enriched in lymphatic signature genes and suggest the involvement of type I/II IFN signaling, and lipid/cholesterol homeostatic regulation in TNIP2 functional effects.

Finally, in **chapter 7** we have discussed the most relevant findings of this thesis and provided the future perspectives. Altogether, this thesis affords novel insights into the molecular regulation of endothelial cells in CVD, with particular focus on eNOS and TNIP2. In conclusion, enhancement of functional eNOS can ameliorate endothelial and heart function in heart failure. In vessels, TNIP2 induction could represent an interesting target to stimulate plaque lymphangiogenesis, thereby promoting inflammation resolution in plaque. Considering the therapeutic potential of my findings we may conclude that (I) AVE3085 helps maintaining eNOS functionality and thus may improve eNOS/NO signaling dependent cardiovascular function, and (II) TNIP2 induction will increase lymphangiogenic responses in plaque, which could improve lymphatic drainage of constituents, cytokines and inflammatory cells from plaque ,and thus may dampen atherosclerosis progression. Therefore, the next challenge is to translate these findings to clinical applications for cardiovascular diseases prevention and/or treatment.