Primary cilia on endothelial cells : component of the shear stress sensor localized to athero-prone flow areas

Heiden, K. van der

Summary

In a number of studies on the influence of hemodynamics on the cardiovascular system it has been shown that abnormal changes in blood flow and ensuing shear stress are related to the emergence of congenital cardiovascular anomalies. In this thesis, the intracellular shear stress sensing machinery is investigated with special emphasis on the role and presence of primary cilia on endothelial cells.

Chapter 1 provides background information on shear stress, its role in cardiovascular development and pathology, and on cilia structure and function. Endothelial mechanotransducing components and the potential pathway from shear stress sensing to gene expression are presented.

Chapter 2 shows the distribution pattern of endothelial primary cilia during normal chicken cardiovascular development (HH24, 28, 30). A shear stress-related occurrence of protrusions on endothelial cells is demonstrated. One of these cell protrusions is identified as a primary cilium. The distribution pattern of the primary cilia is inversely correlated to the expression pattern of the high shear stress marker *Krüppel-like factor-2* (*KLF2*). As expression of *KLF2* is positively regulated by high steady and pulsatile shear stress and negatively regulated by low and oscillatory shear stress it can be concluded that ciliation is restricted to areas of low and oscillatory shear stress.

In **Chapter 3** the direct effect of altering intracardiac flow by venous clip on shear stress in the outflow tract (OFT) of the heart and on the distribution pattern of endothelial primary cilia in the chicken embryonic heart is shown. Venous clip appears to increase shear stress levels in the OFT, as determined by micro particle image velocimetry (μPIV). The distribution pattern of endothelial primary cilia in a normal and clipped HH17 chicken embryonic heart is provided. The apparent increase in shear stress level does not affect endothelial ciliation.

Chapter 4 is a review that focuses on the effects of shear stress on endothelial cells of the cardiovascular system and the role of shear stress in looping of the embryonic chicken heart. During early development the heart remodels from a straight tube into a c-shaped tube. The sharp curvature, small vessel dimensions and embryonic blood characteristics direct the highest shear stress towards the inner curvature of the looped heart, which is very important in cardiac morphogenesis as experimentally-induced alterations in shear stress mainly affect inner curvature remodeling.

As endothelial ciliation in the chicken embryonic cardiovascular system is restricted to regions of low and oscillatory shear stress, the correlation between the prevalence of primary cilia and different flow profiles is investigated in **Chapter 5**. In addition, the structure of endothelial primary cilia, as determined by electron microscopy, is shown. Cultured endothelial cells were subjected to steady, pulsatile, or oscillatory flow in a bioreactor, and subsequently analyzed for ciliation. Endothelial ciliation is dependent on the pattern of shear stress rather than the level. Furthermore, bidirectional (oscillatory), but not unidirectional (steady or pulsatile), shear stress induces the prevalence of primary cilia on endothelial cells. Finally, endothelial cilia are indeed primary cilia as they have a 9+0 configuration of microtubules in their axoneme.

In **Chapter 6** the role of primary cilia in mechanosensing is investigated. Naturally ciliated and non-ciliated, and chemically de-ciliated endothelial cells were exposed to shear stress and subsequently analyzed for *KLF2* expression. Furthermore, as cilia are connected to the cytoskeleton the role of cytoskeletal components in shear stress sensing was analyzed, by subjecting cultured endothelial cells to shear stress after their cytoskeletal components were chemically targeted. It is demonstrated that the endothelial shear stress response depends on an intact microtubular cytoskeleton and that shear-induced expression of *KLF2* is significantly higher in ciliated cells than in non- or de-ciliated cells.

Chapter 7 is an editorial accompanying an article by Nauli *et al*. in *Circulation*. They describe the presence of polycystin-1 in endothelial primary cilia and show that the shear-induced Ca^{2+} transient is dependent on polycystin-1 containing primary cilia. In combination with the data presented in this thesis a double mechanistic role for endothelial primary cilia in shear stress sensing is evident. (I) An immediate response, as determined by a Ca^{2+} transient, is generated by polycystins in the ciliary membrane. (II) A prolonged response on gene expression is mediated by conformational changes of the cytoskeleton, which are amplified by the primary cilium.

The shear-related distribution of ciliated endothelial cells in the chicken embryonic cardiovascular system is reminiscent of the shear-related distribution of atherosclerotic lesions in the adult arterial system, as lesions develop exclusively at sites of low and oscillatory shear stress. Therefore, we determined whether a link exists between the prevalence of endothelial primary cilia and atherogenesis in **Chapter 8**. The distribution of endothelial primary cilia in the aortic arch and common carotid artery of wild-type and apolipoprotein-E-deficient (*Apoe*$^{-/-}$) adult mice is presented. In addition, the effect of experimentally-altered shear stress on primary cilia distribution was analyzed by placement of a restrictive cast around the common carotid artery. It is demonstrated that ciliated endothelial cells are located at atherosclerotic predilection sites in wild-type mice and up- and downstream of atherosclerotic lesions in *Apoe*$^{-/-}$ mice, which have significantly more primary cilia in the aortic arch than wild-type mice. Furthermore, experimentally-induced flow disturbance leads to induction of primary cilia.

As ciliated cells had been shown to propagate the flow-induced Ca^{2+} signal to neighboring cells by intercellular communication through gap junctions, the effect of a reduction of gap junction structural proteins (connexins) on endothelial ciliation was investigated in **Chapter 9**. The effect of a reduction of connexin (Cx) levels on ciliation of a mouse endothelial cell line (clone bEnd.3) and the effect of Cx37 deficiency on endothelial ciliation in the aortic arch of *Apoe*$^{-/-}$ mice was analyzed. The prevalence of endothelial primary cilia is identical in the Cx-deprived group compared to the control group.

Chapter 10 provides a general discussion of the data presented in Chapter 2-9. The occurrence and characteristics of primary cilia on endothelial cells are described and confirmed and potential functions of endothelial primary cilia and their involvement in cardiovascular development, health, and disease are discussed.