Generation and distribution of endothelium-derived contractile forces and their impact on vascular permeability

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Summary

All blood vessels of the human body are covered with a specialized cell layer, the endothelium, which forms a physical barrier between the vessel lumen and the surrounding tissues. This continuous monolayer is semi-permeable and actively controls the transport of oxygen, nutrients and waste products to meet the needs of the underlying tissues. Under normal physiological conditions endothelial cells are tightly connected to their basal substrate and neighboring cells and prevent blood plasma leakage. However, as described in **Chapter 1**, in pathological conditions these cells can become activated resulting in the formation of intercellular gaps, by which additional transendothelial permeability is mediated. This leakage is a direct threat for the homeostasis of the vasculature and forms a hallmark of many life-threatening inflammatory diseases. Despite the intense research and medical need, no specialized therapies are available to prevent or reduce hyperpermeability. One important similarity between all heterogeneous inflammatory syndromes is that it involves the generation of acto-myosin-based contractile forces by the endothelium itself. Hereby, monolayer tension is increased which can cause the loss of endothelial integrity by the disruption of cell-cell interactions. A better understanding of these contractile forces, in relation to the cell-cell and cell-matrix interactions and the underlying molecular pathways, will provide new insights into the mechanisms that drive vascular leakage.

To study endothelial derived forces a biophysical device is needed which enables measurements within a confluent cell monolayer under resting and activated conditions. Different potential techniques to quantify forces are reviewed in **Chapter 2,** as well as the important regulators of endothelial contractility, the Rho-GTPases. After comparing the different options, we concluded that the most suitable techniques to answer our questions is the traction force microscopy method, which is based on the displacement of fiducial markers at the top of a deformable substrate. By implementing this technique we obtained the ability to study the spatiotemporal distribution and stimulus-induced reorganization of traction forces. Subsequently, we showed that integral traction forces are heterogeneously distributed underneath the endothelial monolayers, with nuclear areas showing lower and cell-cell junctional regions higher forces than the whole-monolayer average. Moreover, a good correlation between force vector orientation and the organization of the F-actin cytoskeleton was found. In the final part of **Chapter 3** we revealed that unstable areas, showing high force fluctuations within the monolayer, are prone to form inter-endothelial gaps after the stimulation with a pro-inflammatory mediator.

The usefulness of measuring traction forces was demonstrated in **Chapter 4** in which we studied the isoforms specific contribution of Rho kinase (ROCK), a well-known and prominent inducer of actomyosin-generated forces. We were able to show that thrombin-mediated hyperpermeability is primarily ROCK2 dependent and involves a reduced passive junctional tension in the endothelium. However, to acquire a normal response to thrombin, also ROCK1 is required, contributing to prolongation of the hyperpermeability response by the formation of F-actin stress fibers.

Subsequently, **Chapter 5** relates Abl-related gene (Arg) activity in endothelial cells to an altered

cell-matrix interaction, in which the internalization of α5β1 integrins facilitates cell retraction and agonist-induced endothelial barrier disruption. Moreover, this mechanism is shown to be relevant in inflammatory disorders *in vivo* and associates with endothelial barrier dysfunction. By the previously shown pharmacological inhibitor potential, Arg is a valuable new drug target with important clinical implications in the field of vascular leak.

In **Chapters 6 and 7** we addressed two physical parameters that can influence endothelial barrier function: blood flow and impaired oxygenation. In **Chapter 6,** we show that the biomechanical force applied by the flow of blood on endothelial cells (shear stress)- and Krüppel-Like Factor 2 (KLF2)-induced formation of actin shear fibers is specifically Diaphanous Related Formin 2 (DRF2) dependent. However, counter intuitively, the appearance of these actin structures was not accompanied by higher contractile forces exerted on the matrix and did not contribute to the thrombin-induced endothelial contraction. DFR2-dependent actin reorganization and stabilization of focal adhesions enables the endothelium to resist shear stresses and maintain integrity of the endothelium.

In **Chapter 7,** we show that the barrier protective effect under hypoxic conditions is mediated via increased VE-cadherin expression in the adherens junctions, resulting in improved cell-cell contacts. This effect is regulated by the stabilization of Hypoxia-inducible factor (HIF)-2α, but not HIF-1α, in the absence of oxygen. Moreover, we observed decreased cell motility, - micromotion and lamellipodia formation, without significantly affecting the generation of traction forces after incubation in hypoxia. The hypoxia-mimetic DMOG improved the endothelial integrity in a similar, HIF-dependent fashion, but triggers additional effects on the cell-matrix interactions and traction force fluctuations, which probably relate to a rearrangement of F-actin cytoskeleton.

Finally, the implications of the studies presented in this thesis are discussed in **Chapter 8**. Here, we also elaborate on our initial hypotheses, new models and the identification of promising new candidates for future drug development against vascular leakage.