

Laboratory for Vascular Morphogenesis

RIKEN Center for Biosystems Dynamics Research



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The establishment and maintenance of a network of blood vessels is crucial throughout the lifetime of a vertebrate. During development, blood vessels supply oxygen and nutrients to meet the demands of growing tissues such that inadequate blood vessel formation can lead to embryonic lethality. In the adult, the circulatory network of blood vessels caters for the metabolic needs of tissues and organs; serve as conduits through which immune cells travel to sites of infection; and, importantly, new blood vessel formation is necessary for tissue repair. The formation of new blood vessels frequently occurs through sprouting angiogenesis, where new vessels are generated from pre-existing ones. Sprouting angiogenesis is a multicellular process that is tightly regulated in time and space beginning with the formation of new vascular sprouts composed of endothelial cells. Together, endothelial tip and stalk cells migrate in a collective manner to invade hypoxic tissues. The polarised migration of endothelial cell collectives requires the coordinated behaviour of individual endothelial cells that is mediated through cell-cell junctions, which act as sites of mechanocoupling to transmit force from one cell to another. Endothelial cells also undergo extensive changes in cell shape that is adapted to function. Live imaging has revealed that local and transient cell shape changes underlie migration, cell rearrangements, anastomosis and lumen formation, which are cellular behaviours that are critical for building a multicellular tubular vascular network.

My lab is interested in understanding the morphogenetic processes of blood vessel formation and maintenance. Using the zebrafish as a model system, we employ genetics, molecular biology, optical and pharmacological approaches with high resolution time-lapse imaging to investigate how endothelial cell behaviours are regulated and coordinated to build vessels of specific size and architecture.

Endothelial cell shape regulation

Endothelial cells undergo extensive cell shape changes required to drive specific cellular processes. In our lab, we seek to understand how the actin cytoskeleton regulates endothelial cell shape plasticity. We have previously shown that during sprouting angiogenesis, the generation of actin bundles in filopodia facilitates efficient cell migration and anastomosis (Phng et al., 2013). During lumen formation, transient polymerization of actin at the apical membranes controls lumen expansion (Gebala et al., 2016) while actin cables at endothelial cell-cell junctions stabilize newly-formed

tubules to produce a functional vascular network (Phng et al., 2015). Our work therefore demonstrates that actin cytoskeleton of different dynamics and localization drive distinct steps of vessel morphogenesis. Future studies in the lab include understanding how the actomyosin cytoskeleton is remodelled and organized to generate specialised subcellular structures that drive cell shape changes during angiogenesis, and, upon the establishment of a patent vascular network, how endothelial cells maintain their shape and the vessel retain its structure.

Endothelial cell mechanoreponse to haemodynamic forces

Once blood vessels become lumenized, endothelial cells are exposed to haemodynamic forces such as fluid shear stress and blood pressure. Previous work demonstrates that blood pressure locally deforms the apical membrane of endothelial cells to generate inverse blebs during lumen formation (Gebala et al., 2016). In turn, endothelial cells counteract the deforming forces by triggering an actomyosin-dependent repair mechanism to retract the blebs. More recently, we discovered that endothelial cells adapt to increasing haemodynamic forces by generating a cortex composed of a balanced network of linear and branched actin bundles that resist fluid forces (Kondrychyn et al., 2020). When the balance of linear and branched actin bundles is skewed towards linear by the over expression of the actin bundling protein, Marcks11, the endothelial cell cortex becomes weaker and more deformable, leading to ectopic membrane blebbing and cell enlargement when subjected to haemodynamic forces. This work therefore highlights the importance of cortical actin organization in modulating endothelial cell mechanoreponse to blood flow to regulate cell and vessel shape. Future work in the lab is aimed at quantifying the types and magnitude of haemodynamic forces (wall shear stress, luminal pressure) that endothelial cells are exposed to during vessel morphogenesis using computational fluid dynamics modelling, and how endothelial cells sense and respond to changes in haemodynamic forces.

Regulation of vessel remodelling by blood flow

After the formation of blood vessels, the primitive vascular network is further remodelled in a blood-flow dependent manner to generate a hierarchical network of larger arteries and veins and smaller caliber capillaries of optimal branching pattern. This is achieved through alteration in vessel diameter, which requires changes in endothelial cell shape and size, and vessel pruning, when endothelial cells migrate from a vessel segment with low blood flow to a segment with higher flow. However, the

mechanism by which haemodynamic forces regulate endothelial cell behaviours to modulate blood vessel diameter and network pattern is still unresolved. In this project, we seek to unravel how haemodynamic forces remodel endothelial cell actomyosin organization and junctions to regulate endothelial cell behaviors (such as size and shape) and control blood vessel diameter.



Figure 1.: Blood vascular network in a developing zebrafish embryo



Figure 2. Actin cytoskeleton in a zebrafish intersegmental vessel.

Seeing is believing.

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