Public Abstract
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The formation of blood vessels is a crucial step during early development so that newly formed tissues are able to undergo nutrient and gas exchange to support life. Fundamental to this process is the ability of endothelial cells (ECs) to change their shape and assemble into cell-lined capillary tube structures in a three-dimensional (3D) environment. Important steps during EC tube formation involve the proper spatial orientation of these cells in 3D environments, termed polarity, so that separate plasma membrane surfaces can be created to interface either with blood flow or the extracellular matrix environment that provides structural and biochemical support to cells. These separate membrane surfaces have important functional differences through the localization of key signaling molecules to either surface to maintain proper blood vessel function. While several key molecules have been identified to regulate blood vessel development, the basic molecular and cell-signaling mechanisms controlling this process are not well understood.

The Ras superfamily of small GTPase molecules act as molecular switches to control an array of cell-signaling processes regulating things such as growth, motility, membrane trafficking events and organization of cell cytoskeletal proteins that control cell shape. Activity of these GTPases is regulated by guanine nucleotide exchange factors (GEFs) that help to turn GTPases “on” and GTPase activating proteins (GAPs) that turn GTPases “off” by regulating GTPase association with the nucleotide GTP, and nucleoside GDP. When in an activated state, the GTPases then associate with various downstream effector molecules to regulate different cell-signaling pathways.

Utilizing a 3D collagen gel cell culture model to induce EC tube formation and capillary assembly in concert with gene expression knockdown techniques, we demonstrate key roles for the GTPases Rac2, k-Ras and Rap1B in addition to the previously identified requirement for Cdc42 and Rac1 activity in controlling EC tube formation. We also identify several key effectors acting downstream of these GTPases being IQGAP1, MRCK?, ?-Pix and GIT1. Furthermore, we show that the GAPs Arhgap31 and Rasa1 are key negative regulators of this process by inactivating Cdc42/Rac and k-Ras respectively. Additionally, the GTPases Rab3A, Rab3B, Rab8A, Rab11A, Rab27A, RalA and RalB along with the protein Caveolin-1 were shown to regulate key membrane trafficking and fusion events involved in EC tube formation. By using different techniques to identify proteins with fluorescent markers, we show that several key proteins controlling EC tube formation also regulate EC polarity during this process.

Together, the activity of these molecules and other crucial co-regulators function to coordinate cell-signaling pathways to control EC polarization, membrane trafficking, and tube formation involved in the development of a functional capillary network. It is of great importance to understand how these separate events are controlled so that it will lead to more directed diagnosis and treatment of disease associated with capillary malfunction as well as advancements in aspects of blood vessel and tissue bioengineering.