Paracrine Apelin signaling regulates endothelial tip cell behavior and invasive migration (322 words)

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During angiogenesis, new blood vessels are formed by the rapid invasion of endothelial tip cells into avascular tissues. This process is tightly regulated by growth factors, which modulate intracellular endothelial signaling cascades. We have previously shown that Apelin signaling is specifically activated in highly migratory endothelial cells and loss of Apelin signaling causes a blunted tip cell morphology and impairs angiogenesis. However, how Apelin signaling regulates tip cell motility is not known. Most studies investigate the dynamic process of sprouting angiogenesis using static tissues, but this approach does not enable the interpretation of dynamic changes of signaling events and cell behaviors. Here, we utilized loss and gain of function analysis, as well as confocal live imaging on zebrafish embryos, to provide a detailed characterization of endothelial tip cell invasion and how this process is controlled by Apelin signaling. Rescue experiments revealed that, unlike previously thought, Apelin does not control tip cell migration via autocrine signaling by endothelial cells, but rather Apelin expressed by a subpopulation of neurons acts as a guidance cue for tip cells. By performing time-lapse imaging, we show that tip cells change their migration behavior in response to Apelin. At the onset of vessel sprouting, both wild-type and *apelin* mutant tip cells migrate with low velocity and exhibit small stochastic filopodia. Wild-type tip cells then become more persistent, change from forming small stochastic filopodia into protruding a long filopodium towards the Apelin expressing cells and elongate by stabilizing and widening the dominant filopodium in an Arp2/3 dependent manner. In contrast, tip cells of *apelin* mutant embryos fail to transition towards a dominant long filopodium and do not elongate. In addition, we noticed that tip cells of *apelin* mutant embryos exhibit a hypercontractile phenotype, where myosin localizes to growing protrusions which are then retracted, leading to a small and round morphology. Our work will provide a deeper understanding of how Apelin signaling regulates angiogenesis by guiding tip cell invasive migration.