Unique and Redundant Roles of Notch1 and Notch4 in Retinal Angiogenesis

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Angiogenic processes are involved in embryological development, but also in inflammation, wound healing, and the metastasis of cancers. Notch signaling plays a critical role in the mediation of angiogenesis. Of the four Notch genes, the mechanisms of the Notch1 pathway are most well-understood, whereas the regulatory impact of Notch4 on the vasculature is poorly studied and contradictory. Using the retinal angiogenic model, our lab has determined that loss of Notch4 has substantially different effects than loss of Notch1 in developmental angiogenesis. Loss of Notch4 has been shown to be anti-angiogenic, characterized by reduced radial outgrowth of vessels and decreased vessel density, in contrast to the loss of Notch1, which results in hypersprouting and increased density. Our preliminary data also suggests that Notch4 regulates macrophage numbers and position. However, the structural similarity and common transcriptional targets of Notch1 and Notch4 suggests they may have overlapping functions that are not apparent when either is present. We hypothesize that Notch1 and Notch4, while distinct in their roles as angiogenic regulators**,** have redundant proangiogenic impacts. We therefore expect to see a more severe reduction in vascular development if both signaling pathways are hindered. To investigate this further, we generated mice with endothelial-specific knockouts of Notch1 (Notch1ECKO), Notch4 (Notch4ECKO), or both (double-mutant Notch1Notch4ECKO). At postnatal days P1-3, we inject mice with tamoxifen (100ug) to stimulate the splicing of our target gene's exons. Harvested P5 retina are stained for Isolectin B_4 to identify vasculature, which will be analyzed for vascular growth measures including density, outgrowth, and tip cell counts. In preliminary experiments, I have partially optimized antibodies for macrophage detection in the retina. To determine the mechanism of interaction between Notch1 and Notch4, we will be exploring the vascular and macrophage changes unique to Notch4ECKO, Notch1ECKO, and Notch1Notch4ECKO that will clarify each gene's individual function.