A Dual Role for the HFE2 Protein in Retinal Angiogenesis

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Introduction: Angiogenesis, the formation of new vessel sprouts from pre-existing vasculature, plays a crucial role in vascularization and specialized structure generation for the developing retina. Manipulating angiogenic mechanisms holds promise for treating retinal diseases including diabetic retinopathy, age related macular degeneration, and retinal vein occlusions. Our study focuses on the role of Hemojuvelin (HFE2), a protein produced and secreted by the liver and muscle tissue, revealing its dual role in angiogenic activity. We found that liver-secreted HFE2 acts as a pro-angiogenic factor, while muscle-derived HFE2 suppresses angiogenesis in the developing retina. Differential modifications of liver- and muscle-derived HFE2 likely contribute to these distinct effects. Our aim is to establish that these modifications may lead to preferential binding to HFE2's receptors, Neogenin (NEO1), and Bone Morphogenic Protein (BMP), providing insights into the underlying mechanisms modulating angiogenesis.

Methods: Using mouse models with liver and muscle HFE2 knockouts (HFE2fl/fl-ΔAlb-Cre and HFE2fl/fl-ΔActa-Cre), we studied the in vivo function of these proteins in the developing retina (P6-P11) with whole mount staining protocols for angiogenic markers (ESM1, ERG, PH3, CD31). The tube formation assay was used to assess if these effects can be replicated in vitro. Reporter assays, Western blots, ELISA binding assays, and RT-qPCR were done to understand the underlying mechanism behind this observed phenomenon.

Results: Assessment of the retinal vascular layers at critical stages of development showed a decrease in angiogenic activity in HFE2fl/flΔAlb-Cre mice while HFE2fl/flΔActa-Cre exhibited the opposite effect. These findings were replicated in our in vitro tube formation assay, where human brain endothelial cells treated with muscle or liver derived HFE2 protein displayed a decrease or increase in vascular formation and growth, respectively. Through western blot analysis, we discovered that muscle derived HFE2 lacks the ability to undergo cleavage at its autocatalytic site, crucial for the binding to receptor Neogenin. Furthermore, ELISA binding assays revealed that liver derived HFE2 preferentially binds to Neogenin. Reporter assays and RT-qPCR analysis revealed that treatment with muscle-derived HFE2, but not liver-derived HFE2, increases Notch activity through a BMP-mediated pathway.

Conclusion: This research uncovers a novel role for HFE2 on regulating retinal vascularization. We will investigate HFE2 expression levels throughout mouse development using BioOrthogonal Non-Canonical Amino Acid Tagging. This research deepens the understanding of angiogenic processes, providing insights into underlying mechanisms and potential therapeutic options for retinal diseases. Moreover, this research reveals how distinct organ systems influence retinal angiogenesis, laying a broader foundation for clinical intervention.