Astrocyte Endothelial Cell Interactions in VHL Disease

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Background: Von Hippel-Lindau (VHL) disease is caused by a mutation in the VHL gene resulting in formation of highly vascular tumors called hemangioblastomas. These CNS tumors form primarily in eye, cerebellum, and spinal cord. Although the role of VHL protein in regulating cellular responses to hypoxia and extracellular matrix formation have been identified, the cellular mechanisms by which mutant VHL protein lead to tumor formation remain to be elucidated. We hypothesize that normal communications between cerebellar brain astrocytes and endothelial cells of the microvasculature are disrupted in VHL disease. To study these astrocyte-endothelial cell interactions, we have developed an *in vitro* model system using tamoxifen inducible Cre recombination to cause a VHL deficiency in primary cultures of cerebellar endothelial cells and astrocytes.

Methods: VHL exon-1 floxed and tamoxifen inducible CagCre transgenic mice were purchased from Jackson laboratory and crossed to produce a strain of inducible VHL deficient mice. Cerebellar endothelial and astrocyte cultures were isolated from these mice for the induction of VHL deficiency by administration of 4-hyroxytamoxifen (OHT).

Results: First, homozygous $VHL^{fl/fl}$ floxed females were crossed with heterozygous $CagCre^{+/-}$ males to produce $VHL^{fl/wt}$-$CagCre^{+/-}$ offspring. Second, homozygous $VHL^{fl/fl}$ floxed females were crossed back to $VHL^{fl/wt}$-$CagCre^{+/-}$ males to produce VHL homozygous floxed ($VHL^{fl/fl}$-$CagCre^{+/-}$) and VHL heterozygous floxed ($VHL^{fl/wt}$-$CagCre^{+/-}$) inducible VHL deficient mice. Crosses utilizing females with the $CagCre^{+/-}$ genotype and males with the $VHL^{fl/fl}$ genotype were generally unsuccessful. Cultures of cerebellar endothelial cells and astrocytes were isolated and characterized from mice with a variety of genotypes including $VHL^{fl/wt}$-$CagCre^{+/-}$. Investigations continue to determine how induction of VHL deficiency in these cultures alters expression of HIF-1α, HIF-2α, fibronectin, and various growth factors such as VEGF and PDGFβ.

Conclusions: Characterization of primary VHL deficient cerebellar endothelial cells and astrocytes lays the foundation for future investigations of astrocyte endothelial cell interactions in VHL disease.