Astrocyte Endothelial cell interactions in VHL disease

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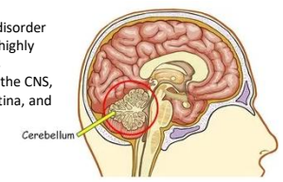
Background

Von Hippel-Lindau (VHL) disease

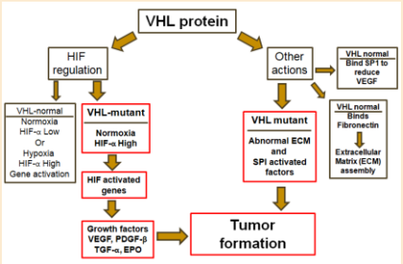
VHL syndrome is an inherited disorder characterized by formation of highly vascular tumors. These tumors (hemangioblastomas) occur in the CNS, particularly the cerebellum, retina, and spinal cord.



https://brainmadesimple.com/cerebellum/

Main hemangioblastoma cell types

1) Abnormal leaky capillaries composed of endothelial cells
2) Pericytes associated with capillaries
3) Stromal cells
4) Mast cells (immune cells)
5) Astrocytes



Hypothesis: Normal communications between cerebellar astrocytes and endothelial cells (EC) are disrupted in VHL disease

Experiment Plan

Obtain Transgenic Mice

Breed mice with an inducible VHL deficiency

Co-culture astrocytes and endothelial cells

Determine extent to which astrocytes induce blood vessel malformations

Cross 1

$VHL^{fl/fl}$ × $Cre^{+/-}$ $VHL^{wt/wt}$ =

- 50% $VHL^{fl/wt}$
- 50% $Cre^{+/-}VHL^{fl/wt}$

Cross 2

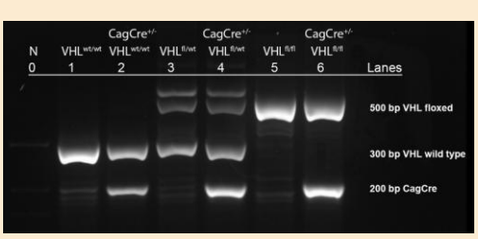
$VHL^{fl/fl}$ × $Cre^{+/-}$ $VHL^{fl/wt}$ =

- 25% $VHL^{fl/wt}$
- 25% $Cre^{+/-}VHL^{fl/wt}$
- 25% $Cre^{+/-}VHL^{fl/fl}$
- 25% $Cre^{+/-}VHL^{fl/fl}$

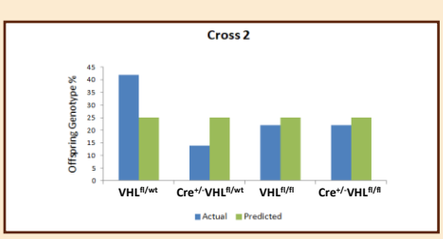
Cross 3

$VHL^{fl/fl}$ × $Cre^{+/-}$ $VHL^{fl/fl}$ =

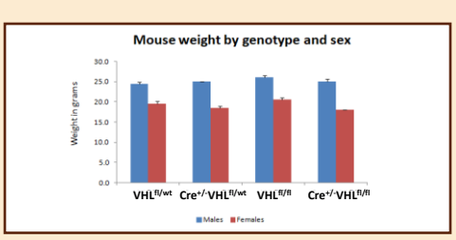
- 50% $Cre^{+/-}VHL^{fl/fl}$
- 50% $Cre^{+/-}VHL^{fl/fl}$



Representative genotyping gel showing PCR bands from 6 different mouse genotypes used in this study, lanes 1-6. N (lane 0) is negative control.



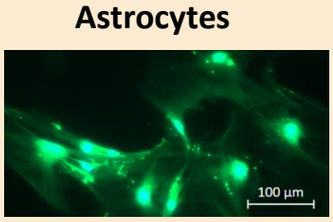
Genetic distribution actual versus predicted for 50 mice produced from Cross 2. On average equal numbers of males and females were produced.



Mice on average weighed 25.1 (males) and 19.1 grams (females) at 10 weeks of age. Weights were similar between genotypes (N=1 to 7 per type, 28 total mice). Occasional $Cre^{+/-}VHL^{fl/fl}$ mice appeared smaller than their littermates.

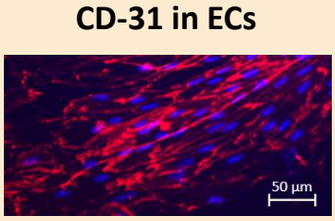
Cerebellar Astrocyte and Endothelial Cell Culture

Astrocytes

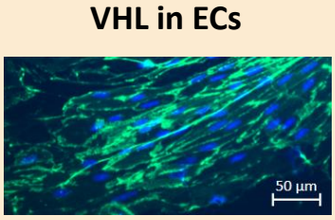


GFAP positive astrocytes (green)
Nuclei stained with DAPI (blue)

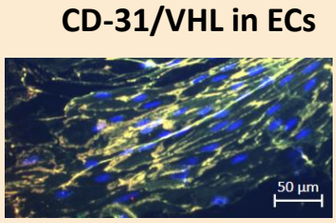
CD-31 in ECs



VHL in ECs



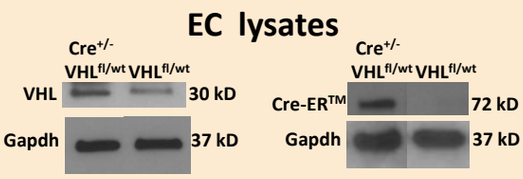
CD-31/VHL in ECs



CD31 positive endothelial cells (red) also showed positive staining for VHL protein (green). Cultures fixed with ice cold methanol followed by Invitrogen rabbit polyclonal anti-VHL (1:200) and rat polyclonal anti CD-31 (1:500). Invitrogen secondary antibodies (1:1000). Nuclei stained with DAPI (blue) and imaged with Zeiss fluorescent light microscope at 20x.

Cerebellar lysates

EC lysates



Nonadjacent bands from same blots show expression of Cre-ER in $Cre^{+/-}VHL^{fl/wt}$ but not $VHL^{fl/wt}$ cerebellar and EC lysates. VHL protein was detected in both genotypes. Gapdh was used as the internal control.

Ongoing work

The culture of astrocytes and ECs from $Cre^{+/-}VHL^{fl/fl}$ mice is ongoing with the goal of inducing culture specific VHL deficiencies to determine the extent to which VHL deficiencies in astrocytes induce blood vessel malformations.

Acknowledgements

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