Somatic Mutations in Vascular Malformations

by

Daniel Aaron Snellings

Department of Molecular Genetics and Microbiology
Duke University

Date: ____________________
Approved:

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Douglas Marchuk, Supervisor

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Beth Sullivan

____
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____
Timothy Reddy

____
Craig Lowe

Dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in the Department of Molecular Genetics and Microbiology
in the Graduate School of Duke University
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Abstract

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Abstract

Write your abstract here. You should not include references or mathematical notation.

If you want to dedicate your thesis to anyone do so here

Contents

List of Tables

List of Figures

List of Abbreviations and Symbols

Symbols

Put general notes about symbol usage in text here. Notice this text is double-spaced, as required.

$\mathbb{X}$	A blackboard bold X. Neat.
$\mathcal{X}$	A caligraphic X. Neat.
$\mathfrak{X}$	A fraktur X. Neat.
$\mathbf{X}$	A boldface X.
X	A sans-serif X. Bad notation.
X	A roman X.

Abbreviations

Long lines in the `symbollist` environment are single spaced, like in the other front matter tables.

AR	Aqua Regia, also known as hydrocloric acid plus a splash of nitric acid.
SHORT	Notice the change in alignment caused by the label width between this list and the one above. Also notice that this multiline description is properly spaced.
OMFGTXTMSG4ME	Abbreviations/Symbols in the item are limited to about a quarter of the textwidth, so don't pack too much in there. You'll bust the margins and it looks really bad.

Acknowledgements

Thank anyone you like here. It's good practice to thank every granting agency that's given you money since you've been ABD, any other school you visited during your research, and any professional society that's funded your travel.

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6.1 HHT Pathogenesis

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Although the vascular lesions are the primary sequelae of familial CCM, many groups have noted an increased prevalence of meningioma in individuals with familial CCM—especially those with a mutation in *PDCD10* (Labauge et al., 2009; Riant et al., 2013; Garaci et al., 2015). In addition, we have previously been contacted by an individual whose child had a sporadic CCM that regrew into a meningioma. Unfortunately we were unable to acquire a tissue sample for genetic analysis, however this case along with the strong link between familial CCM and meningioma has fueled my interest in understanding the link between CCM and meningioma.

KLF4

Kruppel-like factor 4 (*KLF4*) is a transcription factor with key roles in the pathogenesis of both CCM and meningioma. In CCM, *KLF4* (along with *KLF2*) is a key player in CCM signaling that is upregulated by loss of the CCM complex or gain of function in *MAP3K3* (Cuttano et al., 2016; Zhou et al., 2016). Indeed, overexpression of *KLF4* in mouse models leads to an aggressive CCM-like phenotype (Ren et al., 2021). In meningioma, mutations in *KLF4* are common and often co-occur with mutations in *TRAF7* (Reuss et al., 2013). The importance of *KLF4* in both CCM and meningioma suggests that dysregulation of *KLF4* may underlie the development of meningioma in individuals with familial CCM.

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GNAQ in uveal melanoma and circumscribed choroidal hemangioma

Link between SWS, UM, and CCH?

6.4.3 The Curious Case of Infantile Hemangioma

Infantile hemangioma (IH) are one of the most common vascular malformations. They occur in children and are typically present at birth as a red spot flush with the surrounding skin. Soon after birth the hemangioma rapidly grows and becomes raised from the skin. They are generally benign and are typically left alone unless they cover the child's mouth, nose, or eyes. What makes IH so interesting compared to other types of vascular malformations is that they almost always completely regress within the first few years of the child's life. While other types of vascular malformations may spontaneously regress (telangiectasia and AVMs), none do so with the consistency of IH. This phenomenon has been of great interest, not for the purpose of developing therapeutics for IH (propranolol is an extremely effective treatment for IH) but for uncovering the mechanism of regression in the hopes that what we learn can be applied to regress other, more nefarious, vascular malformations.

GLUT1 in IH endothelium

Perhaps one of the most provocative discoveries into the mechanism of IH pathogenesis is the fact that endothelial cells from IHs highly express GLUT1 (North et al., 2000, 2001). GLUT1 is a glucose transporter that has remarkable specificity for the placental endothelium. This finding suggested that the IH may be comprised of cells that dislodged from the maternal placenta, then became hyper-proliferative in a post-fetal environment. If this hypothesis is correct, one would expect to find that the IH is a genetically chimeric growth between fetal and maternal cells. This hypothesis was put to the test using fluorescence *in situ* hybridization to assay the presence of XX cells in IH from a male infant with confirmation by sequencing microsatellites and SNPs that were divergent between mother and child. This analysis found no evidence for maternal-fetal chimerism in IH (Pittman et al., 2006). Despite this counter-evidence, the presence of GLUT1 in IH is strongly indicative of some link with the placenta though unfortunately this link currently remains elusive.

Efforts to find somatic mutations in IH

As it is quickly becoming clear that the vast majority of vascular malformations are the result of somatic mutations—many occurring in known oncogenes—I thought that somatic mutations may also underlie IH. To test this, I sequenced 61 IH lesions on an 'oncopanel' covering many genes that are highly mutated in cancers as well as several genes previously implicated in vascular malformations (*KRIT1,* *CCM2, PDCD10, ACVRL1, ENG, SMAD4,* etc.) Unfortunately after filtering putative variants, no there were no variants with likely functional significance and that occurred in more than a single sample. I am aware of at least 1 other group that has attempted to identify somatic mutations in IH via whole-exome sequencing, however to date there are no known somatic mutations in IH. One important aspect of these studies that must be noted is that they are invariably focused on coding regions of the genome. Non-coding variants are more than capable of causing disease however discovery-focused sequencing studies often ignore non-coding regions both because of the cost of sequencing the entire genome to a depth sufficient to detect somatic mutations, and the challenges associated with functional analysis of non-coding variants. Further studies may find that somatic mutations do cause IH, but they occur in a region of the genome that is missed by the majority of sequencing studies.

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Appendix A

Probability of Multiple Somatic Mutations

Bibliography

Cuttano, R., Rudini, N., Bravi, L., Corada, M., Giampietro, C., Papa, E., Morini, M. F., Maddaluno, L., Baeyens, N., Adams, R. H., Jain, M. K., Owens, G. K., Schwartz, M., Lampugnani, M. G., & Dejana, E. (2016). KLF4 is a key determinant in the development and progression of cerebral cavernous malformations. *EMBO Mol Med*, *8*(1), 6–24.
URL `https://www.ncbi.nlm.nih.gov/pubmed/26612856`

Garaci, F., Marsili, L., Riant, F., Marziali, S., Cecillon, M., Pasquarelli, R., Sangiuolo, F., Floris, R., Novelli, G., Tournier-Lasserve, E., & Brancati, F. (2015). Cerebral cavernous malformations associated to meningioma: High penetrance in a novel family mutated in the *PDCD10* gene. *Neuroradiol J*, *28*(3), 289–93.
URL `https://www.ncbi.nlm.nih.gov/pubmed/26246098`

Labauge, P., Fontaine, B., Neau, J. P., Bergametti, F., Riant, F., Blecon, A., Marchelli, F., Arnoult, M., Lannuzel, A., Clanet, M., Olschwang, S., Denier, C., & Tournier-Lasserve, E. (2009). Multiple dural lesions mimicking meningiomas in patients with CCM3/PDCD10 mutations. *Neurology*, *72*(23), 2044–6.
URL `https://www.ncbi.nlm.nih.gov/pubmed/19506228`

North, P. E., Waner, M., Mizeracki, A., & Mihm, J., M. C. (2000). GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas. *Hum Pathol*, *31*(1), 11–22.
URL `https://www.ncbi.nlm.nih.gov/pubmed/10665907`

North, P. E., Waner, M., Mizeracki, A., Mrak, R. E., Nicholas, R., Kincannon, J., Suen, J. Y., & Mihm, J., M. C. (2001). A unique microvascular phenotype shared by juvenile hemangiomas and human placenta. *Arch Dermatol*, *137*(5), 559–70.
URL `https://www.ncbi.nlm.nih.gov/pubmed/11346333`

Pittman, K. M., Losken, H. W., Kleinman, M. E., Marcus, J. R., Blei, F., Gurtner, G. C., & Marchuk, D. A. (2006). No evidence for maternal-fetal microchimerism in infantile hemangioma: a molecular genetic investigation. *J Invest Dermatol*, *126*(11), 2533–8.
URL `https://www.ncbi.nlm.nih.gov/pubmed/16902414`

Ren, A. A., Snellings, D. A., Su, Y. S., Hong, C. C., Castro, M., Tang, A. T., Detter, M. R., Hobson, N., Girard, R., Romanos, S., Lightle, R., Moore, T., Shenkar, R., Benavides, C., Beaman, M. M., Mueller-Fielitz, H., Chen, M., Mericko, P., Yang, J., Sung, D. C., Lawton, M. T., Ruppert, M., Schwaninger, M., Korbelin, J., Potente, M., Awad, I. A., Marchuk, D. A., & Kahn, M. L. (2021). *PIK3CA* and CCM mutations fuel cavernomas through a cancer-like mechanism. *Nature*.
URL `https://www.ncbi.nlm.nih.gov/pubmed/33910229`

Reuss, D. E., Piro, R. M., Jones, D. T., Simon, M., Ketter, R., Kool, M., Becker, A., Sahm, F., Pusch, S., Meyer, J., Hagenlocher, C., Schweizer, L., Capper, D., Kickingereder, P., Mucha, J., Koelsche, C., Jager, N., Santarius, T., Tarpey, P. S., Stephens, P. J., Andrew Futreal, P., Wellenreuther, R., Kraus, J., Lenartz, D., Herold-Mende, C., Hartmann, C., Mawrin, C., Giese, N., Eils, R., Collins, V. P., Konig, R., Wiestler, O. D., Pfister, S. M., & von Deimling, A. (2013). Secretory meningiomas are defined by combined *KLF4* K409Q and *TRAF7* mutations. *Acta Neuropathol*, *125*(3), 351–8.
URL `https://www.ncbi.nlm.nih.gov/pubmed/23404370`

Riant, F., Bergametti, F., Fournier, H. D., Chapon, F., Michalak-Provost, S., Cecillon, M., Lejeune, P., Hosseini, H., Choe, C., Orth, M., Bernreuther, C., Boulday, G., Denier, C., Labauge, P., & Tournier-Lasserve, E. (2013). CCM3 Mutations Are Associated with Early-Onset Cerebral Hemorrhage and Multiple Meningiomas. *Mol Syndromol*, *4*(4), 165–72.
URL `https://www.ncbi.nlm.nih.gov/pubmed/23801932`

Zhou, Z., Tang, A. T., Wong, W. Y., Bamezai, S., Goddard, L. M., Shenkar, R., Zhou, S., Yang, J., Wright, A. C., Foley, M., Arthur, J. S., Whitehead, K. J., Awad, I. A., Li, D. Y., Zheng, X., & Kahn, M. L. (2016). Cerebral cavernous malformations arise from endothelial gain of MEKK3-KLF2/4 signalling. *Nature*, *532*(7597), 122–6.
URL `https://www.ncbi.nlm.nih.gov/pubmed/27027284`

Biography

Your biography is limited to one page and must contain

1. Full name
2. Date and place of birth
3. Every degree you've earned, including this one, and where you earned it from.

Mostly, that information is to narrow down which John Smith wrote that dissertation on the mating habits of sea cucumbers. Sexy!

You may also include

1. Any awards you've won related to your discipline since your undergraduate degree.
2. Any fellowships you've held
3. Anything you've published (papers, books, book chapters). Don't be afraid to cite it here, so that the full bibliographic record of your article appears in the bibliography!
4. Where your next job will be, if you know