SPECIFIC AIMS

Cerebral cavernous malformations (CCM) are vascular lesions of the central nervous system that increase the risk of stroke, epilepsy, recurrent headaches, and paralysis. Familial cases of CCM develop multiple lesions following an autosomal dominant inheritance pattern. Mutations in three genes have been shown to cause the familial form of the disease – *CCM1* (*KRIT1*), *CCM2 (malcavernin)* and *CCM3 (PDCD10)*, the first two of which were discovered by our lab. We have recently found somatic mutations in late-stage human lesions, suggesting that CCM follows a two-hit mechanism where each of the two allelic copies of a CCM gene must be inactivated for lesion genesis to occur. A major drawback of studying CCM pathogenesis in humans is that the lesion samples available are late-stage, multicavernous and hemorrhagic, containing many alterations from normal physiology that may or may not reflect the primary cause of CCM lesion genesis. What little is known about early-stage lesions is based on autopsy reports where information about how these early lesions may progress to the late-stage entity is purely speculative.

Our lab was the first to create knockout mouse models for two CCM genes. Homozygous knockout of either *Ccm1* or *Ccm2* results in embryonic lethality, but mice heterozygous for these genes show no obvious phenotype. Following the two-hit mutation hypothesis, the heterozygous mice were crossed into "sensitized" backgrounds (*Trp53*-/- or *Msh2*-/-) to promote somatic mutation throughout the genome. The resultant mice exhibit both early- and late-stage CCM lesions that demonstrate many of the hallmarks of the human disease, thereby becoming the first authentic animal model of CCM. These mouse models provide a unique opportunity, not only to study late-stage CCM lesions, but also to investigate the molecular and genetic mechanisms underlying CCM lesion genesis and progression.

Specific Aim 1: To study the effects of ROCK inhibition on mouse CCM lesion progression.

RhoA is a regulator of the actin cytoskeleton that is involved in endothelial cell lumen formation and vascular permeability. A downstream effector of RhoA, Rho Kinase (ROCK) shows increased activity in CCM lesion endothelial cells, but it is unclear which pathological features of the mature lesion are direct results of ROCK activation. I hypothesize that ROCK activity is central to CCM lesion progression. I propose to inhibit ROCK in our mouse models using the ROCK inhibitor fasudil and examine the effects on CCM lesion genesis, growth and maturation to evaluate the centrality of Rho and ROCK in CCM pathogenesis. To accomplish this, I will examine CCM lesions from mice treated with fasudil for alterations known to occur in late-stage CCM lesions, such as iron deposition and endothelial cell proliferation. From this work, I will determine if ROCK inhibition can prevent CCM lesion genesis, halt lesion growth at an early stage or prevent particular histopathological phenotypes from developing.

Specific Aim 2**:** To investigate the two-hit mutation mechanism in early-stage mouse CCM lesions.

All studies of human CCM lesions have used only late-stage, surgically resected lesion tissue. In these samples it is difficult to determine which features are causes of CCM and which are resultant effects. Using our new mouse models, I propose to investigate the two-hit mutation hypothesis in mouse CCM lesions in order to determine if the genetic, somatic mutation is a primary cause of or a secondary event in CCM lesion growth. I hypothesize that, by immunohistochemistry and deep resequencing, I will find evidence of a second-hit mutation in late-stage, multicavernous lesions as well as in early-stage, isolated caverns.

Differentiating causes of CCM from derived effects is essential for development of effective treatments. The proposed work will further understanding of CCM lesion pathogenesis by deducing which elements are specific to the disease pathway and which are downstream effects. This knowledge will, in turn, directly inform potential therapies for CCM.