Molecular genetics of familial cerebral cavernous malformations

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Cerebral CMs, or cavernomas, are benign vascular hamartomas that consist of endothelium-lined vascular channels without intervening normal brain parenchyma. The prevalence of CMs in the general population has been estimated at 0.1 to 0.5% based on large autopsy studies. Clinically, cerebral CMs usually present with seizures or hemorrhage. The natural history of symptomatic CMs involves repeated hemorrhages, with a gradual increase in the size and number of the vascular channels. On neuroimaging, cavernomas may be undetectable by computed tomography scanning in up to 30 to 50% of cases. When visualized, they appear as rounded, hyperdense lesions with or without calcium. The classic appearance on magnetic resonance imaging is a “popcorn ball” consisting of a reticulated, heterogeneous core surrounded by a hypointense hemosiderin rim on T₁-weighted images. The T₂-weighted gradient echo images demonstrate a prominent susceptibility effect (blooming) and are the most sensitive diagnostic sequence. Fluid-attenuated inversion-recovery and T₂-weighted magnetic resonance images may show surrounding edema in lesions with acute hemorrhage.

Although CMs are angiographically occult, associated abnormalities such as venous angiomas may be visualized. Cerebral CMs can occur sporadically or as an autosomal-dominant inherited condition. Approximately 50% of Hispanic patients with cerebral CMs have the familial form, compared with 10 to 20% of Caucasian patients.

The exact mechanism of familial CM pathogenesis is still unknown. To date, familial CMs have been attributed to mutations at three different loci: CCM1 on 7q21.2, CCM2 on 7p15-p13, or CCM3 on 3q25.2-q27. The authors summarize the current understanding of the molecular events underlying familial CMs.

Discussion

The CCM1 Locus (KRIT1 Gene)

The CCM1 locus is mutated in nearly all Hispanic-American patients as well as in approximately 40% of all Caucasian patients with familial cavernomas. Although familial and sporadic CMs are phenotypically identical, no mutation in the CCM1 locus has been found in sporadic cases in patients harboring single CMs. The CCM1 locus has been identified as the KRIT-1 gene located on 7q21.2. KRIT-1 is a conserved Ras-family GTPase with unknown cellular function.
The protein KRIT-1, the product of the KRIT-1 gene, has been found in vascular endothelium, astrocytes, and pyramidal cells of the adult brain. The essential function of this protein is demonstrated in KRIT-1 knockout mice, which die at midgestation of vascular pathology. These mice demonstrate marked enlargement of and increased endothelial proliferation in the dorsal aorta, defects that are associated with early downregulation of artery-specific genes.

The KRIT-1 protein has also been shown to associate with the integrin-binding protein ICAP1α. Integrin molecules are transmembrane receptor proteins that play a critical role in endothelial cell–cell and cell–ECM interactions as well as in endothelial cell migration and lumen formation during angiogenesis. The cytoplasmic portion of integrin proteins lacks inherent enzymatic activity and relies on linker proteins for connection to the actin cytoskeleton and for downstream intracellular signaling. The ICAP1α, a broadly distributed 200 amino acid protein, has been identified through yeast hybrid assays to bind avidly to the β1 integrin cytoplasmic domain. The integrin-binding site is located at the amino terminus of the KRIT-1 protein. Mutations of this motif result in complete loss of KRIT-1/ICAP1α interaction. Most mutations in the KRIT-1 gene responsible for familial CM result in early stop codons and truncated protein synthesis, hinting at a defect in ICAP1α-mediated ECM interaction as a possible contributor to CM pathogenesis. Interestingly, results of competition assays support the suggestion that KRIT-1 and β1 integrin compete for the same binding site on ICAP1α, suggesting a possible regulatory mechanism.

In the CCM1 locus, KRIT-1 protein is truncated and lacks the integrin-binding site, which would shift the system toward ICAP1α–integrin interaction. Gunel, et al., have demonstrated through immunohistochemical and coimmunoprecipitation studies that KRIT-1 protein localizes to microtubules. Specifically, KRIT-1 colocalizes with microtubule plus ends during the late phase of mitosis. These authors’ results show that KRIT-1 is a microtubule-associated protein, possibly responsible for microtubule targeting. Furthermore, interaction of KRIT-1 with Krev1 and ICAP1α–integrin suggests that KRIT-1 may help modulate the cytoskeleton, and thus shape endothelial cell morphology and function in response to cell–matrix and cell–cell interactions. Gunel, et al., propose that the loss of this targeting mechanism would lead to abnormal endothelial tube development and the subsequent appearance of cerebral CMs.

The CCM2 Locus (MGC4607 or malcaverin Gene)

The CCM2 locus has been identified as the MGC4607 or malcaverin gene located on 7p15-p13. It encodes a protein containing a putative phosphotyrosine-binding domain. Although protein sequencing fails to show any known conserved regions that might define the function of the CCM2 gene, a clue may come from the CCM2 mouse homolog, the OSM. The OSM is involved in the cellular response to osmotic insults, and is required for MEKK3-mediated activation of p38 in response to cellular stress. It is known that p38, a member of the MAPKs, is one of several intracellular kinases that transduce signals essential for vascular remodeling and maturation. The p38 kinase negatively regulates endothelial cell survival, proliferation, and differentiation in fibroblast growth factor–stimulated angiogenesis. This kinase is also an important mediator of vascular endothelial growth factor activation of the COX-2 expression in endothelial cells during angiogenesis. The p38 MAPK also mediates tumor necrosis factor–induced apoptosis in endothelial cells through two known mechanisms: 1) phosphorylation and downregulation of the antiapoptotic protein Bcl-xL; and 2) phosphorylation of the proapoptotic protein Bad through suppression of the MEK1/2–ERK1/2 survival pathway (Fig. 1).

Using in situ hybridization, Seker, et al., found significant colocalization of CCM2 with CCM1 messenger RNA in embryonal and postnatal mice. This was confirmed at the protein expression level, with both proteins present in arterial endothelium as well as in pyramidal neurons and the foot processes of astrocytes. Coimmunoprecipitation and fluorescence resonance energy transfer studies showed that the CCM1 and CCM2 gene products interact with each other. In much the same way that CCM1 and β1 integrin interact with ICAP1α, this interaction is dependent on the phosphotyrosine-binding domain of CCM2 and is inhibited by a familial CCM2 missense mutation, suggesting that loss of this interaction may be another step in the pathogenesis of familial cavernomas. The parallel expression and mutual binding of CCM1 and CCM2 suggests that they may function through the same regulatory pathways in cavernoma formation (Fig. 2). Moreover, CCM1, CCM2, and MEKK3 bind in a ternary complex, suggesting that the pathways are not simply parallel but probably converge.

In the cell, the OSM localizes to Rac-containing membrane ruffles (Rac is a Rho-GTPase that is known to regulate endothelial motility and morphology during angiogenesis). The ICAP1α, which binds KRIT-1, is also a known inhibitor of Rac signaling. Modulation of Rac-mediated angiogenesis may represent another potential point of convergence in action for CCM1 and CCM2 (Fig. 2). The CCM3 Locus (PCD10 Gene)

Approximately 40% of kindred with familial CMs show linkage to the CCM3 locus. The CCM3 locus has been identified as the PCD10 gene located on 3q25.2-27. The PCD10 gene codes for a 212 amino acid protein lacking any known domains. This protein has been linked to apoptosis, which is an essential process in arterial morphogenesis. Interestingly, apoptosis in smooth-muscle cells has been shown to be mediated by a β1 integrin signaling cascade, providing a possible link with CCM1 and CCM2 in the formation of cerebral CMs.

Conclusions

The exact mechanism of familial CM pathogenesis is
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Fig. 1. Flow chart summarizing the role of the p38 MAPK pathway in angiogenesis and cavernoma formation. Gene names are in parentheses. VEGF = vascular endothelial growth factor.

Fig. 2. Flow chart summarizing the signaling pathways in familial CMs.
still unknown. Mutation at three distinct loci (CCM1, CCM2, and CCM3) has been shown in cases of familial cerebral CM. Because familial cavernomas caused by different gene mutations are pathologically and phenotypically indistinguishable, it has been postulated that the three CCM genes act through the same molecular pathway.12,13,15 There is growing evidence that CCM1 may play a role in regulating β1 integrin-mediated angiogenesis through KRIT-1, which is involved with a bidirectional signaling pathway between the ECM and the cytoskeleton that uses an integrin-mediated cascade.23 Future studies detailing this signaling cascade will shed new light on cerebral CM pathogenesis.

The p38 MAPK is a ubiquitously expressed intracellular kinase that regulates endothelial cell survival, proliferation, and differentiation.20 Regulation of the p38 MAPK cascade may be one of the final common pathways for modulation of angiogenesis by the CCM genes. The CCM1 and CCM2 gene products influence the p38 MAPK pathway by activating its upstream kinase (MEKK3). Immunohistochemical and binding studies could be used to investigate whether the CCM3 protein also participates in the interaction required for MEKK3 and p38 activation. Any interaction of the CCM gene products with MEKK6, the other upstream regulator of p38, needs to be clarified. Cross-talk of p38 with other members of the MAPK pathway, such as MEK/ERK, needs further elucidation as well.

Development of dominant negative CCM genes, if possible, would be valuable for future studies. Endothelial cell lines could then be transfected with the dominant negative CCM genes, and the activated phospho-p38 MAPK levels, as well as apoptotic or differentiation markers, could be assayed with Western blot analysis. The convergence of CCM2 and CCM3 on the apoptotic mechanisms is yet another area that requires further study.

It has been shown that CCM1 knockout mice die in utero. Future development of CCM2 or CCM3 knockout mice, especially if they were to survive, would also allow better characterization of the intracellular signaling pathways, including p38 MAPK. Finally, modulation of Rac-mediated angiogenesis may represent another potential point of convergence in action for the CCM genes that warrants further study (Fig. 2).25

References

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