Microvasculature and vascular malformations

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1. Introduction

Cerebral blood vessels are composed of endothelial cells (ECs), vascular smooth muscle cells (SMCs), and pericytes. To date, most brain arteriovenous malformation (bAVM) research has focused on the endothelium. Histologic evaluation has described significant endothelial heterogeneity.[1] Disruption of the blood-brain barrier (BBB) has been well documented,[2] and microhemorrhages are frequently observed in unruptured bAVMs and may predict future rupture[3]. (Fig.1) In this lecture, the author aims to clarify the role of microvasculature in the pathogenesis of vascular malformations (bAVM and cerebral cavernous malformation [CCM]).

2. Microvasculature

1.1 Endothelial cells

Brain ECs form a monolayer cell lining of the vascular lumen, serving as the vital interface between the blood and brain parenchyma known as the BBB. At the molecular level, ECs express higher levels of pro-angiogenic factors[4]; thus, they frequently assume a pro-angiogenic phenotype in bAVMs. The experimental models reproduce some features observed in human bAVM (dilated vessels, an arteriovenous shunt, a high-flow lesion, and formation of the nidus). Adenovirus-mediated EC-selective ALK1 deletion and overexpression of vascular endothelial growth factor induces lesions resembling human bAVM as well,[5] suggesting the involvement of changes in EC function and angiogenesis in the pathogenesis.

1.2 Smooth Muscle Cells

Vascular SMCs are the predominant cellular constituents of the vessel wall in the arteries and veins. SMCs derived from bAVMs formed tubes in culture, which were longer than those formed by normal brain vascular SMCs. The migration and proliferation of SMCs in bAVMs exceeded those in normal brain vascular SMCs. The reduction in vascular SMCs in bAVMs has been described.[6]

1.3 Pericytes

Pericytes were named by Zimmermann in 1923 and embedded within a vascular basement membrane that is shared with the adjacent endothelium. A growing body of evidence has revealed that pericytes play various important roles in the development and maintenance of the BBB, regulation of the

neurovascular system (vascular stability, vessel formation, and cerebral blood flow), trafficking of inflammatory cells, clearance of toxic waste products from the brain, and acquisition of stem cell-like properties. The close spatial proximity during vascular remodeling between sprouting ECs and neighboring pericytes suggests a '**crosstalk**', and potentially a reciprocal signaling relationship between these two cell types during vascular remodeling.[7]

In the capillaries, the vessel size was further decreased, and the endothelial layer was intermittently surrounded by pericytes. Compared to the peripheral vascular beds, **central nervous system (CNS) capillaries have higher pericyte-to-endothelial cell ratios (1:1 to 1:3),** and approximately 70–80% of the capillary surface area is covered with pericyte cell processes.[8] The crosstalk between pericytes and endothelial cells is indispensable for angiogenesis, vascular stability, and BBB formation. (Fig.2) Understanding the function of the factors involved in pericyte-endothelial cell interactions can help in designing therapies to prevent vascular permeability and destabilization in bAVM.

Pericytes play an important role in promoting vascular stability and maturation throughout the human body.[10] Reduction in pericyte reduction has been described in both human bAVMs and rodent models and is greatest in ruptured human AVMs.[2] In unruptured AVMs, the magnitude of pericyte loss correlates with the severity of BBB disruption and microhemorrhage.[2] Since pericytes have multifunctional properties and contribute to various neurological disorders, pericytes as a therapeutic target can be approached from various aspects: (1) prevention of BBB dysfunction, (2) promotion of angiogenesis and vascular stability, (3) reduction of pericyte constriction under pathological conditions, (4) control of inflammation, and (5) implantation therapy through multipotential stem cell properties.[11]

3. Cerebral Cavernous Malformation (CCM)

CCMs are the second most common type of vascular lesions in the central nervous system and account for approximately 8–15% of all neurovascular malformations. CCMs are low-pressure lesions with no high-pressure arterial supply or distinct venous drainage. The most common clinical manifestations of CCMs include epileptic seizures (50%), symptomatic intracerebral hemorrhage (ICH, 25%), and non-hemorrhagic focal neurologic deficits (NH-FNDs, 25%). A significant number of patients with CCM (20–50%) were asymptomatic. CCM-related symptoms can be attributed to intralesional or extralesional hemorrhage, mass effect, or other mechanisms, including perilesional hemosiderin deposition.

Upon histological examination, CCMs present as clusters of ectatic, endothelium-lined sinusoidal channels without interposing neural or glial tissue embedded in a connective tissue matrix. There is no evidence of normally developed vessel layers or structures, for example, intervening tight junctions, mural muscular fibers, or elastic fibers. Since blood flow within these channels is stagnant, subsequent undulating episodes of thrombosis and recanalization result in the characteristic magnetic resonance imaging (MRI) appearance of intralesional blood at various stages of thrombosis and organization.[12]

3.1. Pathogenesis

Somatic mutation

CCMs occur in both sporadic and familial forms. Heterozygous loss-of-function mutations in at least three different CCM genes, that is, CCM 1 (KRIT 1), CCM 2 (MGC 4607), and CCM 3 (PDCD 10), have been identified in both sporadic and familial forms. Somatic mutations in CCM genes were identified in the endothelial cells of CCM lesion tissue, i.e., **endothelial cells are the primary site of CCM lesion pathogenesis**. All three genes are likely involved in the same molecular pathway providing an interplay between the neural and glial elements (neurons and astrocytes) and the endothelium of the CNS.[13] Biallelic somatic mutations of the same genes in cavernoma endothelial cells likely contribute to lesion genesis in both familial and sporadic forms of the disease.[14] Carriers of the mutated genes have cavernomas on MRI in up to 69% of cases. Thus, the presence of mutations in these genes is necessary; however, it not sufficient for the development of the CCM. CCM lesion genesis is thought to follow a "two-hit" mechanism, requiring a biallelic germline and somatic mutations in one of the known CCM genes.

Useful insights into innovative approaches for CCM disease prevention and treatment are emerging from a growing understanding of the biological functions of the three known CCM proteins. Biochemical, molecular, and cellular studies have shown that these proteins are involved in the endothelial cell-cell junction and blood-brain barrier stability maintenance through the regulation of major cellular structures and mechanisms, including endothelial cell-cell and cell-matrix adhesion, actin cytoskeleton dynamics, autophagy, and endothelial-to-mesenchymal transition, suggesting that they act as pleiotropic regulators of cellular homeostasis and open novel therapeutic perspectives.

3.2. Symptoms

The distinctions and overlaps between ICH, NH-FND, and focal neurological deficit not otherwise specified (NOS-FND) are shown in Fig. 3. Surgery is indicated in cases of space-occupying extralesional CCM hemorrhage.

The **CCM1 gene** (alternative name KRIT 1) is located at chromosome locus 7q and stabilizes the inter-endothelial junctions associated with actin stress fibers. It is expressed in the arterial and microvascular endothelium of the CNS, and more than 90 mutations in CCM1 have been reported. The natural history of cavernomas originating from CCM1 seems to be the least severe when compared to CCM2 and CCM3 gene mutations. The **CCM2 gene** (or malcavernin) located at 7p probably determines cellular responses to osmotic stress. In a study by Plummer et al., CCM2 expression in the brain was found to be primarily neuronal, but not endothelial.[15] This finding suggests that cavernomas may arise from abnormalities in surrounding neuronal and glial cells rather than in the vascular endothelium.[16]

The **CCM3 gene** is located at chromosome locus 3q (called programmed cell death 10 or PDCD10) and is found in up to 15% of familial cavernomas. It determines cell proliferation and transformation (cancer cell lines) and modulates extracellular signal-regulated kinases. CCM3 mutation carries a greater chance of spontaneous mutation, an increased cavernoma burden, and a younger mean age

of presentation, which is often associated with clinical hemorrhage.
The impact of CCM3 on the natural history of the disease may be devastating as was shown by Shenkar et al. who found an exceptionally high aggressiveness of the disease in this cohort of patients.[17] According to their study, lesion burden on susceptibility-weighted imaging MRI was extraordinarily high, with 33% of CCM3 mutation carriers harboring more than 100 lesions and 78% harboring more than 20 lesions. The mean number of lesions on T2-MRI was 31, whereas the mean lesion count in individuals with CCM1 and CCM2 mutations was only 5.1. Moreover, the annual rate of de novo lesions in the CCM3 cohort was eight times as high as in CCM1 and CCM2 group (2.36 vs. 0.3, respectively).[17]

Furthermore, despite the apparent higher disease severity in familial CCM cases, up to 70% of mutation carriers remain asymptomatic or minimally symptomatic throughout life. Moreover, large variability in disease severity is observed even among family members of similar ages carrying the same disease-associated genetic defect, including wide inter-individual differences in lesion number, size, and susceptibility to ICH, suggesting that additional factors other than the disease-causing mutation can contribute to the pathogenesis of CCM.[18]

3.3. Therapeutic targets (Table)

Currently, there are no direct therapeutic approaches for CCM. A recurring theme dominating the CCM scientific literature is the causal link between the loss of function of CCM proteins and hyperactivation of the small GTPase RhoA and its effector Rho kinase (ROCK). RhoA activation increases cellular contractility and destabilizes endothelial adherens junctions, thereby reducing endothelial barrier function and increasing vascular permeability.[19] Potential therapy for CCM disease based on inhibitors of the RhoA-ROCK signaling, includes statins (simvastatin, fluvastatin, and atorvastatin)[20] and fasudil [20],[21].

Statins have long been posited as potential therapeutic drugs to stabilize CCM. Vulnerability can then lead to extravasation of iron and blood components, eliciting a neoangiogenic process and CCM formation.[22] Currently, the therapeutic potential of statins is being evaluated in the AT CASH EPOC trial, evaluating the potential for atorvastatin to reduce lesional iron deposition, indicative of CCM hemorrhage.[23] A related issue is the safety of aspirin and anticoagulants in these patients, which is particularly pertinent given that CCMs have a higher prevalence in older patients. Schneble et al. have shown that antiplatelet medications may be safe and associated with a modest decrease in the incidence of symptomatic hemorrhages.[24] Patients with a CCM receiving therapy with both aspirin and statins were less likely to present at diagnosis with acute hemorrhage.[25]

4. AVM

Please refer to the NNAC2020 proceeding.
http://www.nicheneuroangio.com/pdf/2020nnac/2020%2012%20ota.pdf

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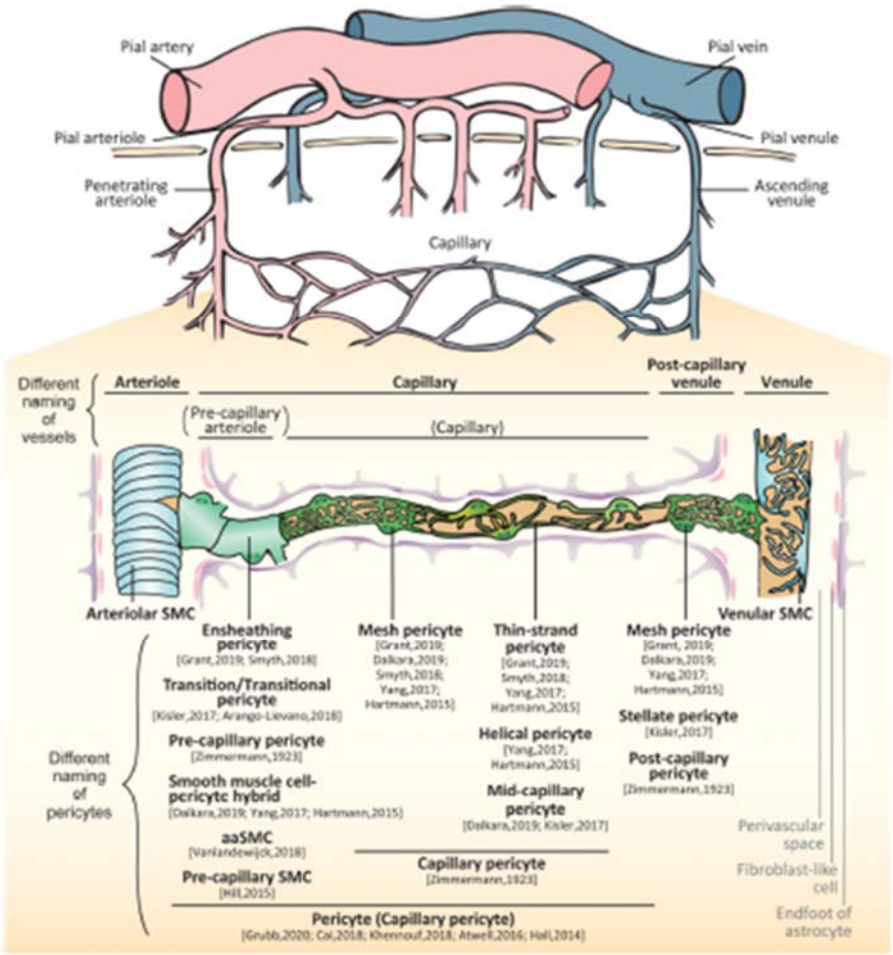

Fig.1 Brain vessels and mural cells. (Uemura MT, *Front. Aging Neurosci*. 12.80.2020)

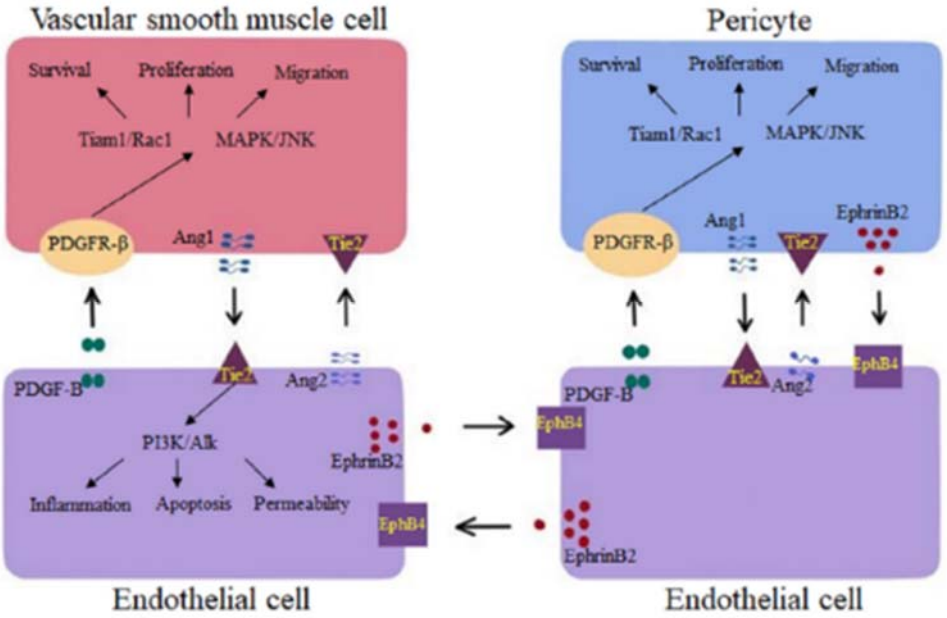

Fig.2 Summary of key signaling pathways involved in the regulation of mural cell recruitment

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during angiogenesis and vasculogenesis[9]

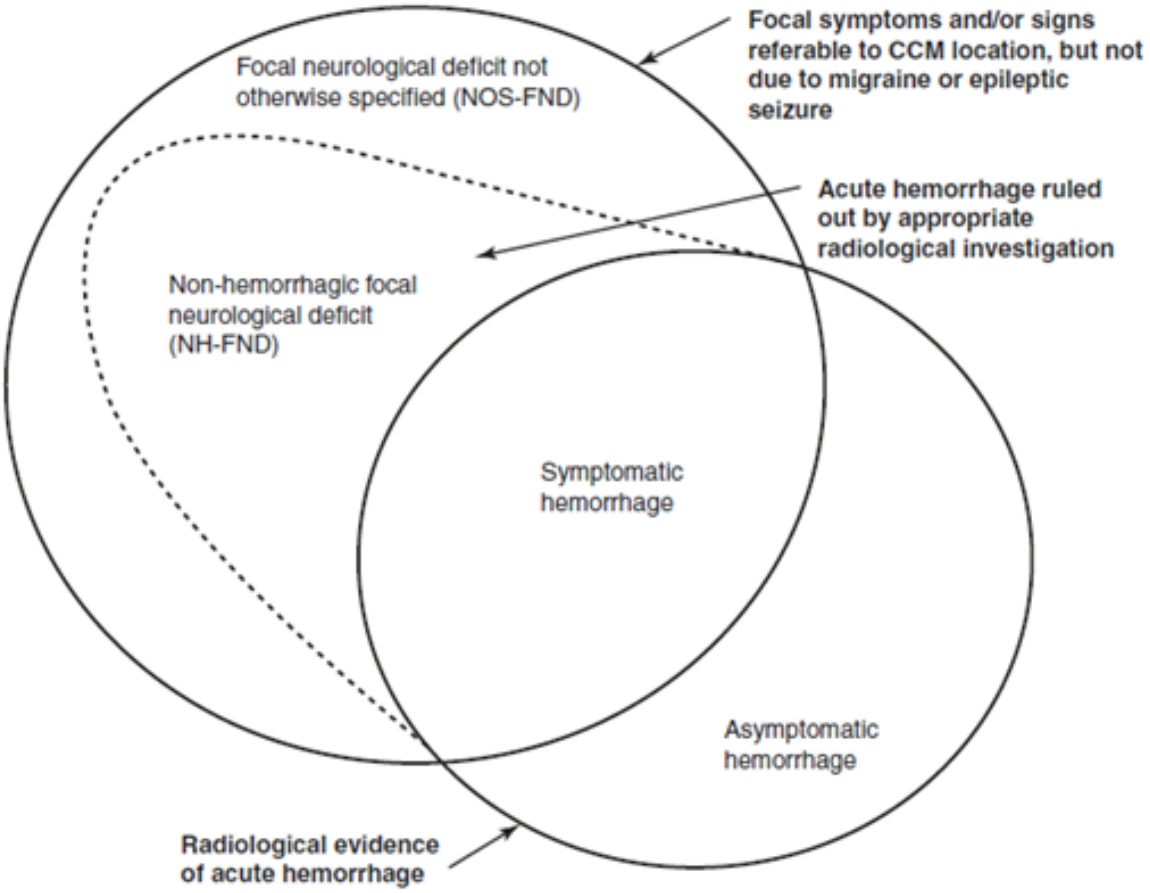

Fig.3 Radiological and clinical reporting standards on cerebral cavernous malformation (CCM) presentation. (Al-Shahi R, Stroke 39:3222-3230, 2008)

Table 1
Candidate therapies in CCM based on molecular pathways altered by loss of function of CCM genes

Molecular pathway	Mutated gene	Pharmacological approach
Adherens junctions	*CCM1*, *CCM2*, *CCM3*	
Autophagy	*CCM1*, *CCM2*, *CCM3*	mTOR inhibitors (Rapamycin, Torin 1)
Endothelial-to-mesenchymal transition (EndMT)	*CCM1*, *CCM3*	Inhibitors of TGF-β pathway (sulindac sulfide and its analogs)
Angiogenesis and VEGF	*CCM1*, *CCM3*	Angiogenesis inhibitors (semaxanib, ponatinib, bevacizumab, propranolol)
ANGPT2	*CCM3*	ANGPT2-neutralizing antibodies
TSP-1	*CCM1*	TSP-1 replacement (3TSR)
TM and EPCR	*CCM1*, *CCM3*	TM and EPCR inhibitors
Inflammation	*CCM3*	Anti-BR3 antibody (B-cell depletion); Avenanthramide; Toll-like receptor 4 (TLR4) antagonists; alteration of microbiome
β1 integrin adhesion	*CCM1*, *CCM2*	
JNK/c-Jun	*CCM1*	Antioxidant compounds (N-acetylcysteine)
FoxO1	*CCM1*, *CCM2*	Antioxidant compounds (N-acetylcysteine, Avenanthramide)
NADPH oxidase (Nox4)	*CCM1*	Nox inhibitors; Avenanthramide
KLF2/KLF4	*CCM1*, *CCM2*, *CCM3*	Ponatinib (inhibitor of MEKK3-KLF signaling with anti-angiogenic effects)
MEKK3	*CCM1*, *CCM2*, *CCM3*	Ponatinib (inhibitor of MEKK3-KLF signaling with anti-angiogenic effects)
Notch	*CCM1*, *CCM3*	Sorafenib (multikinase inhibitor)
RhoA/ROCK	*CCM1*, *CCM2*, *CCM3*	Inhibitors of Rho signaling and multitarget compounds (Statins, Fasudil, Tempol, Vitamin D3)
Reactive Oxygen Species (ROS)	*CCM1*, *CCM2*, *CCM3*	Antioxidant compounds and autophagy inducers (N-acetylcysteine, Avenanthramide, Tempol, Vitamin D3, Torin 1, Pt NPs)
STRIPAK	*CCM3*	