Cerebral cavernous malformation 3 and cerebrovascular disease

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ABSTRACT
Cerebral cavernous malformations (CCMs) are vascular lesions characterized by enlarged and irregular structure of small blood vessels in the brain, which can result in increased risk of stroke, focal neurological defects and seizures. CCMs can occur as a sporadic or familial autosomal dominant form. Three different genes, CCM1/KRIT1, CCM2/MGC4607 and CCM3/PDCD10, have now been identified as the main targets which are involved in the CCMs’ progression. These three CCM proteins have similar or unique function in maintaining the normal structure of small blood vessels. However, CCM3 mutation results in a more severe form of the disease which may suggest we should pay more attention on the area of CCM3. The current research focused on the angiogenic function and mechanisms of CCM3 including endothelial cell junction, proliferation, migration and permeability, and these findings may offer some potential targets for CCMs’ therapy.

KEYWORDS: CCM3, PDCD10, cell junction, angiogenesis, GCKIII, EndMT.

1. Introduction
Cerebral cavernous malformations are collections of capillaries in the brain which are abnormal in structure. These capillaries have abnormally thin vessel walls, and they lack support tissues like elastic fibers which make them stretchy, resulting in increased risk of stroke, focal neurological defects and seizures. CCMs have been reported to affect about 0.5 percent of the population worldwide. Most CCMs occur in the central nervous system except for some that are located in the retina or skin [1]. There is no medicine available to treat CCMs yet, and the only treatment for CCMs is surgical resection for now.

CCMs may be familial or sporadic. Familial cases are caused by mutations in one of three CCM genes: CCM1/KRIT1, CCM2/malcavernin, or CCM3/PDCD10. These CCM patients usually develop multiple lesions in the brain. Whereas sporadic CCMs occur in people with no family history of the disorder. These individuals often present with a single lesion. Three different genes are involved in familial CCMs: KRIT1 (Krev interaction trapped 1, also known as CCM1), CCM2 (MGC4607) and PDCD10 (programmed cell death 10, also known as CCM3) [2-4]. Loss-of-function mutations in any one of the three genes can result in the formation of CCMs, which suggests there is an essential pathway involving all three CCM proteins [5]. However, mutations in CCM3 are often associated with higher risk of early-onset cerebral hemorrhage [6] and more severe form of the disease [7], suggesting a separate or unique role of CCM3 compared with CCM1 and CCM2.

Bergametti et al. [4] first identified PDCD10 as the third CCM gene, which is located on 3q25.2-27. This locus was identified within a 22-cM
interval flanked by D3S1763 and D3S1262 [8]. CCM3 has no parologue in the human genome; it seems to be highly conserved in vertebrates and invertebrates according to database searching [9]. The highly conserved gene encodes a 212-amino acid protein which is ubiquitously expressed. It contains an N-terminal dimerization domain and a C-terminal focal adhesion targeting-homology (FAT-H) domain [10, 11]. CCM3 involves in different pathway by the interaction of the two domains and their target proteins. CCM3/PDCD10 was originally proved to be a protein inhibiting the natural cell death of 293 cells [12]. After the relationship between PDCD10 and CCMs was elucidated, more and more functions of this protein related to CCMs have been illuminated. This review will mainly focus on the role of CCM3 in affecting cell junction, maintaining normal structure and function of vascular endothelial cells, and regulating angiogenesis, which all can be involved in the progression of CCMs.

2. CCM3 and the cerebrovascular disease

2.1. Two-hit mechanism in CCM3 mutation

CCMs have been proved to develop through a ‘two-hit’ mechanism; patients with CCMs get one mutated allele of one of the CCM genes from their parents, and somatic mutation at the second allele make the complete loss-of-function of one of the CCM genes [13, 14]. More than 150 different mutations of CCM1/CCM2/CCM3 have been found to date [15-17]. Those mutations almost all lead to a premature termination code through different mechanisms including nonsense, splice-site and frameshift mutations [18].

2.2. The role of CCM3 in cell junction

The normal type of cell junction of endothelial cells is important to maintain the normal structure and function of blood vessels, which were often disrupted in CCM lesions. Cell-cell junction especially tight junction is essential to prevent blood-borne compounds from leaking to brain parenchyma, which further leads to inflammatory responses, endothelial injury and lesion progression [19-21]. Tight junction is built on the interactions between transmembrane tight junction proteins (claudin-5, occludin, JAM-A), scaffolding proteins (ZO-1, -2, -3, Af-6, VASP, Par3, Par6) and the actin cytoskeleton [22-23]. Stamatovic et al. [24] found that CCM3 regulates the integrity of brain endothelial barrier and the organization of tight junction complex. Loss of CCM3 activates ERK1/2; this induces Ser phosphorylation (pS405) of cortactin (a cortical actin ring protein), which leads to cortactin degradation. Increased cortactin degradation is associated with loss interaction of the cortical actin ring and ZO-1: actin, which is essential for providing physical support and anchoring of tight junction proteins. The reduced anchoring of ZO-1 to the actin cytoskeleton impact organization of tight junction proteins, and eventually disrupt the tight junction complex.

It has been demonstrated that CCM2 knockdown in brain endothelial cells resulted in the activation of RhoA by dysregulating Smurf1, a ubiquitin-protein ligase (E3) that controls RhoA degradation [25-26]. Overabundant RhoA can inhibit vessel-like tube formation and increase endothelial monolayer permeability. Borikova et al. [27] proved that CCM3 knockdown in endothelial cells also show RhoA overexpression and activation. Overexpressed RhoA activates ROCK, which phosphorylates several substrates like myosin light chain and LIM kinase to regulate cytoskeleton dynamics. Activated ROCK also regulates vascular permeability which is essential for the normal function of blood vessels [29]. By phosphorylating the myosin light chain, ROCK can increase the formation of actin stress fiber (microtubules) [30]; p-MLC enables myosin to bind to the actin filaments, and therefore increase the cellular contraction rate. The increased contractility in cells impact β-catenin and VE-cadherin lining the vascular walls [31], then the cell-cell adhesion of the endothelial cells is disrupted, which result in enlargement of blood vessels and unstable vessel structure, and thus leading to vascular leakage and initiation of CCM progression [25]. Faurobert et al. [32] proved that extracellular matrix (ECM) aberrant remolding is associated with CCM progression. CCM1-CCM2 complex directly binds to ICAP-1 [33], a negative regulator of β1 integrin, and loss of CCM1 or CCM2 will destabilize ICAP-1, and hence β1 integrin will be activated after the destabilization of ICAP-1, and then activated β1 integrin activates RhoA and ROCK pathway. β1 integrin also affect normal
DLL4 may determine endothelial angiogenesis by regulating the balance of VEGFR1 and VEGFR2. Impaired DLL4-Notch signaling also activated Erk1/2, thus affecting cell junction, and cell permeability. You et al. [41] also found that silencing of CCM3 in endothelial cells significantly activates EphB4 kinase activity by up-regulating EphB4 mRNA and protein expression, accompanied by activation of Erk1/2, and hence CCM3 may regulate angiogenesis by endothelial signaling pathway of CCM3-DLL4/Notch-EphB4-Erk1/2.

CCM3 can be involved in different signaling pathways as an anchor protein which can bind to different target proteins. Hence the role of CCM3 in endothelial cells may be controversial when different pathways are coexistent. The best-characterized interaction between CCM3 and its target proteins lies in the dimerization-domain-mediated interaction with the GCKIII group of kinases, MST4/MASK, STK24/MST3 and STK25/YSK1/SOK1 [42]. Chan et al. [43] proved that CCM3 interaction with GCKIII is critical in lumen formation in Drosophila, and this result may suggest a similar function of CCM3 in human cells. But it’s known that the GCKIII family is represented by a single protein in Drosophila, whereas a complex in mammalian, and it seems that the three kinases are functionally redundant in mammalian cells because loss of any single of the kinases doesn’t affect lumen formation; only when loss of STK25 along with loss of either STK24 or MST4 will reproduce the lumen formation defects, and hence these data may suggest a more reliable and robust system in maintaining the normal lumen formation in mammalians due to the complex vasculature. Each of the GCKIII family kinases have been implicated in regulating different cellular functions [44-46]. Zhang et al. [47] proved that CCM3 associates with STK24 and regulates exocytosis in neutrophils. STK24 binds to UNC13B and STK24, and inhibits UNC13B-mediated intracellular molecules exocytosis.

2.3. The role of CCM3 in angiogenesis

Angiogenesis is a process that involves endothelial cells proliferation, migration and morphology remodeling. He et al. [38] found that CCM3 knockdown in HUVECs significantly reduced endothelial cell proliferation and induced cell apoptosis, and also inhibited VEGF-induced endothelial cell cord formation. CCM3 specifically associates with VEGFR2 and was required for stabilization of VEGFR2, thereby maintaining the VEGF signaling pathway which is essential for angiogenesis. But there were different views about the role of CCM3 in angiogenesis. You et al. [39] proved that knockdown of CCM3 in HUVECs significantly stimulates angiogenesis behaviors including proliferation, migration and sprouting. They found that silencing of CCM3 in HUVECs significantly downregulates DLL4 expression and impaired DLL4-Notch signaling therefore activating endothelial angiogenesis. Deletion of DLL4 reduced the expression of VEGFR1 but increased VEGFR2, thereby stimulating cell proliferation, migration and sprouting [40]. These data suggest the level of DLL4 may determine endothelial angiogenesis by regulating the balance of VEGFR1 and VEGFR2.
2.4. Potential mechanisms by which CCM3 regulates CCM progression

Endothelial-to-mesenchymal transition (EndMT) has been demonstrated with cardiac fibrosis and cancer progression, and it is characterized by the acquisition of mesenchymal and stem-cell-like features of endothelium [53, 54]. The progression of EndMT leads to disrupted cell junction organization, loss of cell polarity, increased cell proliferation and migration [55]. Maddaluno et al. [56] found EndMT exists in CCM1 ECKO mice with disorganized VE-Cadherin and significantly up-regulated N-Cadherin, and this progress is mediated by the upregulation of endogenous BMP6, which in turn activates the TGF-β (transforming growth factor-β) and BMP (bone morphogenetic protein) signaling pathway. Inhibiting TGF-β or BMP pathway prevents EndMT and reduces CCM lesion in mice. They also found that endothelial cells from CCM3 ECKO mice present a similar phenotype to those from CCM1 ECKO mice, which means EndMT is a common feature of loss-of-function mutation-induced CCMs. Studies have proved that loss of CCM proteins induced inhibition of Notch, and upregulated KLF4 can activate BMP pathway, thus resulting in EndMT [57, 58]. Autophagy is also related to CCM formation and EndMT [59]. CCM1-deleted endothelial cells present suppression in autophagy with increased levels of p62 (p62 is a receptor for ubiquitinated cargoes and delivers them to autophagosome, p62 itself is degraded by autophagy) and total LC3 (autophagy protein microtubule-associated protein 1 light chain 3), the mechanism may lie in CCM1 deletion induced up-regulation of mTOR-ULK1 pathway. They also found that down-regulation of autophagy gene ATG7 in HUVECs (human umbilical vein endothelial cells) suppressed autophagy and was associated with EndMT progression, and down-regulation of autophagy-related protein p62 in CCM1-deleted endothelial cells suppressed the expression of mesenchymal markers such as PAI1, Cd44 and Id1, and these suggest autophagy is associated with EndMT. Bravi et al. [60] found that Wnt-independent stimulation of β-catenin transcription activity in CCM3-deficient endothelial cells and β-catenin transcriptional activity promotes TGF-β/BMP signaling and consequent EndMT.
CCM2 interacts with TrkA, a receptor tyrosine kinase involved in prosurvival signaling in the nervous system, through the PTB domain [61]. CCM3-GCKIII kinase indirectly links with TrkA by interaction with CCM2, and hence CCM3 may regulate the cell death signaling pathway in neural cells. Louvi et al. [62] found that CCM3 deletion in neural cells results in a vascular phenotype that resemble human CCMs, which suggests CCM3 may affect CCM progression through cell death pathway. ROS (Reactive oxygen species) have been proved to affect tight junctions in endothelial cells by impairing the cytoskeleton and blood-brain barrier, which are related to CCMs. Fidalgo et al. [36] found that CCM3-GCKIII kinase (MST4) mediates ERM(ezrin/radixin/moesin) phosphorylation and cell survival after ROS stimulating, and it has been proved that disturbed Notch signaling is related to ROS accumulation [63], all of these suggesting ROS may be a factor implicated in loss of CCM3-dependent CCMs progression.

2.5. Progression in CCM therapy

The only treatment for CCM disease is surgical resection so far, although there is high risk for cerebral operation. To date, no medical therapy has been approved. Based on the researches of molecular mechanism which regulates CCM progression, some drugs which affect intracellular signaling pathway can be effective in animal trials. Administration of fasudil, a Rho-kinase inhibitor, resulted in attenuated CCM lesion in mice with CCM1 mutations [30]. Statin inhibits HMG-CoA reductase, which reduces RhoA-dependent small GTPase activation, and presents a symptomatic improvement in mouse model [25], but administration of statin was associated with increased risk of intracerebral hemorrhage [64], and hence more researches should be done before the application of statin in CCM therapy. Inhibition of TGF (transforming growth factor) signaling also presents exciting results, while administration of LY-364947, an inhibitor of TGF-β type 1 receptor, significantly inhibited EndMT transition in CCM1 mouse model [57]. Sulindac, an anti-inflammatory drug, can attenuate CCM development by suppressing β-catenin activity [61]. ANGPT2-neutralizing antibody significantly reduces CCM lesion formation in CCM3ECKO mice [48]. TLR4 (Toll-like receptor 4) antagonists and alteration of microbiome can affect CCM formation in mice [65]. Many advances have been made, but the cure for CCM lesion is still unknown. Further study is still needed to uncover novel mechanisms regulating CCMs and possible drugs preventing the progression of CCMs.

3. Conclusion

As an anchor protein, CCM3 binds to different types of proteins, which enable it takes part in different intracellular signaling which affect cell junction, angiogenesis, apoptosis and stress action (Figure 1). When we focus on different pathways, some controversial result may exist, but we cannot ignore that CCM lesion is a comprehensive result of all different pathways in cells, not only endothelial cells. Despite the mounting knowledge about the role of CCM3 in the progression of CCMs, there are still many unclearness that needs to be clarified. We still don’t know why a ubiquitously expressed CCM3 protein involves in the progression of CCMs exclusively in CNS (central nervous system), although some explanations lie in the relationship between neural cells and endothelial cells, but details are still unknown. We don’t know the effect of CCM3 on translational and post-translational modification of those tight junction proteins, and how CCM3 regulates β1 integrin signaling. We don’t know the detailed relationship between CCM3-UNC13B-ANGPT2-Tie2 axis and cell junction which affect CCM lesion development, and how Golgi polarization which is regulated by CCM proteins contributes to vascular defects and CCMs. Recent study uncovered a relationship between innate immune/microbiome and CCM lesion in mice with CCM1/2 deficiency, and CCM3 may be linked to the innate immune pathway as loss of any one of the three CCM genes leads to a similar phenotype. We also should notice that there will be a possibility of other CCM-related genes, as mutations in CCM1/CCM2/CCM3 don’t cover all the familiar cases. There is no doubt that further studies are necessary to better understand the mechanism of the progression of CCMs and find a non-invasive therapy for CCM disease.
Figure 1. The possible role of CCM3 in different pathways regulating CCM formation. CCM3 maintains cell junction by inhibiting ERK1/2 phosphorylation, CCM3 inhibits stress fiber migration and endothelial permeability by inhibiting RhoA signaling, CCM3 regulates angiogenesis with or without binding to GCKIII kinases, and CCM3 also involves in EndMT transition, autophagy and ROS stimulation.

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CONFLICT OF INTEREST STATEMENT
The authors declare no competing financial interests.

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