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REVIEW

PTEN/PI3K/Akt/VEGF signaling and the cross talk to KRIT1, CCM2, and PDCD10 proteins in cerebral cavernous malformations

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Abstract Cerebral cavernous malformations (CCM) are common vascular malformation of the brain and are associated with abnormal angiogenesis. Although the exact etiology and the underlying molecular mechanism are still under investigation, recent advances in the identification of the mutations in three genes and their interactions with different signaling pathways have shed light on our understanding of CCM pathogenesis. The phosphatidylinositol 3-kinase (PI3K)/Akt pathway is known to play a major role in angiogenesis. Studies have shown that the phosphatase and tensin homologue deleted on chromosome ten (PTEN), a tumor suppressor, is an antagonist regulator of the PI3K/Akt pathway and mediates angiogenesis by activating vascular endothelial growth factor (VEGF) expression. Here, we provide an update literature review on the current knowledge of the PTEN/PI3K/Akt/VEGF signaling in angiogenesis, more importantly in CCM pathogenesis. In addition to reviewing the current literatures, this article will also focus on the structural domain of the three CCM proteins and their interacting partners. Understanding the biology of these proteins with respect to their signaling counterpart will help to guide future research towards new therapeutic targets applicable for CCM treatment.

Keywords Cerebral cavernous malformations · Angiogenesis · PTEN · VEGF

Introduction

Cerebral cavernous malformations (CCM) are vascular lesions of the brain affecting 0.4–0.8 % of the general population [1, 3, 52]. These lesions account for 5–13 % of all the vascular malformation in the central nervous system (CNS) [9, 53] and manifest a great spectrum of clinical symptoms such as headaches, motor and sensory deficits, epilepsy, and hemorrhagic strokes [4, 62, 87]. Histologically, CCM have dilated thin-walled capillaries with clusters of enlarged endothelial channels (caverns) lacking intermediate brain parenchyma [12, 16] and tight junctions [32, 64]. CCM can be classified as either sporadic or familial with 80 % accounting to the former and 20 % to the latter [18, 58]. Unlike the sporadic lesions which are mostly accompanied with a single lesion, familial cases of CCM are characterized by the presence of multiple lesions with autosomal dominant pattern of inheritance [58]. Such familial types of lesions are associated with loss of function mutations in the following three genes: *KRIT1* (Krev interaction trapped 1) [13], *CCM2*/*OSM*/*MALCAVERNIN* [45], and *PDCD10* (programmed cell death 10)/*TFAR15* [2]. Similar to other tumor suppressor genes, CCM pathogenesis also follows the Knudsonian two-hit mechanism, in which loss of one allele due to a germline mutation in an affected cell (first hit) is accompanied with somatic mutation in the other (second hit) [58]. Research in the past few years have shown extensively how different signaling pathways are regulated by the product of these mutated genes. However, understanding how and where these signaling pathways cross talk may shed light on their function and help therapeutically to patients suffering from CCM. Phosphatase and tensin homologue deleted on chromosome ten (PTEN) has a crucial role to play in the regulation of angiogenesis in tumor tissues, but its relevance to CCM pathogenesis remains unexplored. In this review, we will discuss the recent insights and provide an update literature reviews into the role of PTEN/PI3K/Akt/VEGF pathway in CCM pathogenesis. In the first section, current understanding of the brief molecular architecture of the CCM proteins is reviewed. Next, recent updates into the PTEN protein

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structure and its interplay with the PI3K/Akt/VEGF signaling during angiogenesis and CCM pathogenesis are discussed. Finally, in the concluding part of this article, we will focus on how such pathways could help to better understand the biology of CCM that may favor future therapeutic treatments.

Structure of CCM proteins

This section will discuss briefly the structural domain and the molecular interaction of three CCM proteins: KRIT1, CCM2, and PDCD10.

KRIT1

Among the three CCM genes known, *KRIT1* remain the first gene to be identified and its linkage mapping to chromosome 7q has been broadly studied [1, 23, 34, 61]. *KRIT1* gene encodes KRIT1, a 736-amino acid protein consisting of a C-terminal FERM domain (F for 4.1 protein, ezrin, radixin, moesin), an N-terminal region consisting of three NPxY motif (NPAY195, NPLF234, NPYF253) and 3 ankyrin repeating domains [24, 60, 81] (Fig. 1). In the NPxY motif, NPAY195 interacts with ICAP1 (integrin cytoplasmic-associated protein) and regulates vascular development, cell migration, and angiogenesis [48]. The FERM domain of KRIT1 comprises of three subdomains, F1, F2, and F3, in which the F3 subdomain possess a phosphotyrosine-binding fold (PTB) [17]. A large spectrum of protein binds to these domains in order to regulate cell-cell interactions and vascular permeability [22]. The F1 subdomain utilizes an ubiquitin-like shape, the F2 subdomain folds similar to acyl-CoA homology and the F3 subdomain simulate a PTB/pleckstrin homology [57]. Recent studies have shown that KRIT1 FERM domain is able to form complexes with the N-terminus of the protein, the heart of glass (HEG1) C-terminus, and the small GTPase Rap1 [17, 20, 21, 37]. The GTPase Rap1 binds to both the F1 and F2 subdomains of KRIT1 FERM domain, while HEG1 interacts within the F1 and F3 interface. The binding of KRIT1 protein and the Rap1 is important for endothelial cell (EC) stabilization [22]. KRIT1 is primarily localized in the cytoplasm and encodes for nuclear localization sequence (NLS) and a nuclear export sequence (NES) [1]. This sequence helps the intracellular protein to shuttle between the cytoplasm and the nucleus [79]. Liu et al. showed that direct binding of Rap1 to KRIT1 FERM domain enables it to localize to the cell-cell junctions and suppress Rho kinase signaling [47]. Moreover, KRIT1 interacts with CCM2 through two of its Npxy motifs, NPLF234 and NPYF253 [82], and regulate cellular functions such as endothelial cell-cell junction stabilization and vascular permeability.

CCM2

CCM2 is important for the regulation of cell-cell interactions and endothelial cell lumen formation. It is a 444 amino acid protein and is known to interact with KRIT1 through its putative PTB domain (Fig. 1). CCM2 is thought to mediate its binding to PDCD10 through its putative C-terminus located slightly before the PTB domain, although the detailed mechanism of interaction is not well understood [18]. The KRIT1-CCM2-PDCD10 complex formation, therefore, is achievable only in the presence of CCM2 which acts as the hub bringing together KRIT1 and PDCD10. Zhu et al. also demonstrated that silencing CCM2 gene in endothelial cells activate the Akt signaling proteins [89], indicating that CCM2 regulates PI3K/Akt signaling. Notwithstanding that the C-terminal region of the CCM2 domain is approximately 200-amino acid region of unknown function, a recent study have shown that CCM2 C-terminus is able to induce cell death through TrkA receptor tyrosine kinase [27]. A similar study recently identified the molecular and crystal structure of the CCM2 C-terminus, suggesting that there exists a folded region of the CCM2 C-terminus with structural similarity to scaffolding protein harmonin [18]. The mechanism by which the CCM2 harmonin domain mediates signaling to induce cell death in the endothelial cells still remains elusive. Recently, a putative CCM2-Like (CCM2L) protein was identified in the endothelial cells of mice and zebrafish during cardiovascular development [59, 84]. This protein is able to compete with its paralog CCM2 and binds to HEG-KRIT1 complex rather interacting with PDCD10. Moreover, Rosen et al. [59] predicted that the human homologue of CCM2L, C20ORF160 may be involved in the pathogenesis of CCM.

PDCD10

PDCD10 or alternatively named as TF-1 cell apoptosis-related protein (TFAR15) is a 212-amino acid protein consisting of a N-terminus dimerization domain and a C-terminus focal adhesion targeting homology domain (FAT-H) [12, 25, 42] (Fig. 1). It has been recently identified and known to be involved in apoptosis. Although studies have documented that PDCD10 exists in complex with KRIT1 and CCM2 [38, 67], there are lines of evidences suggesting PDCD10 might have roles distinct from that of KRIT1 and CCM2. For example, Chan et al. demonstrated that PDCD10 has a non-endothelial role in different developments from KRIT1 and CCM2 [7]. Besides, there exist separate binding partners for the three CCM proteins. Li et al. showed that the interaction between PDCD10 with CCM2 PTB domain and paxillin LD motifs is mediated through its FAT-H domain and CCM pathogenesis is

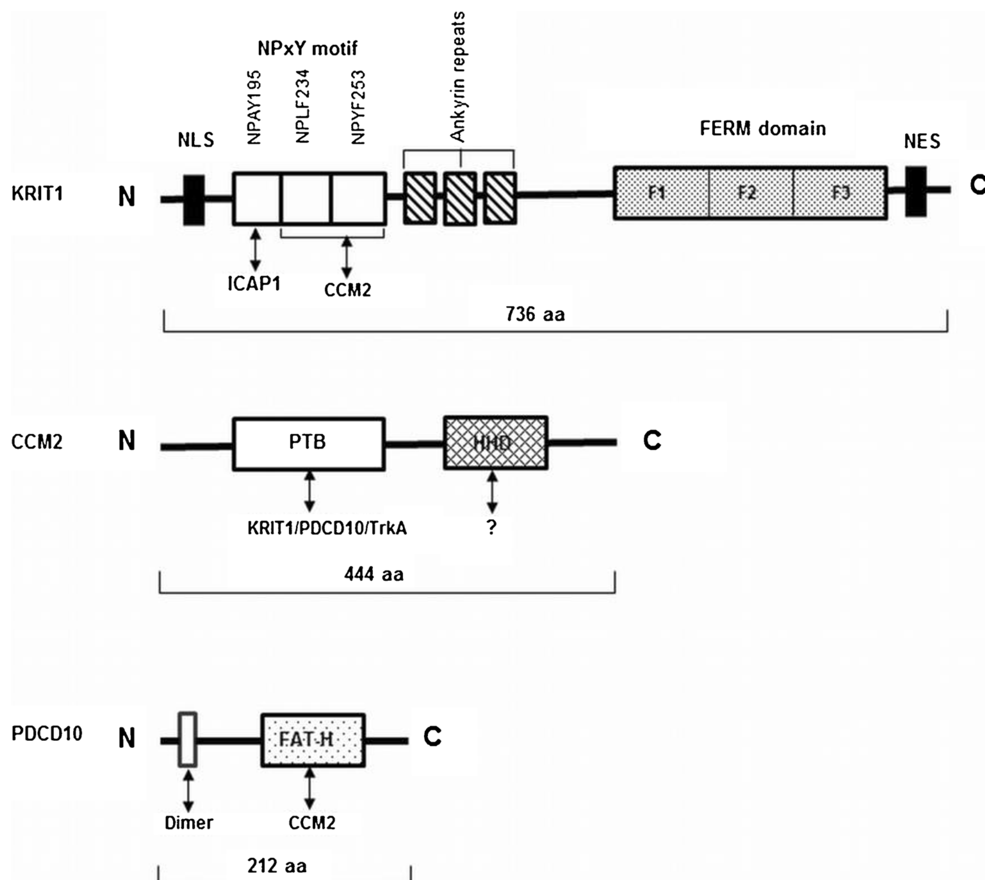

Fig. 1 Structural assembly of CCM proteins. KRIT1 consists of a C-terminal FERM domain (F for 4.1 protein, ezrin, radixin, moesin), an N-terminal region consisting of three NPxY motif (NPAY195, NPLF234, NPYF253) and 3 ANK domain. The NPAY195 domain binds to ICAP1, while the NPLF234 and NPYF253 domain interacts with the CCM2 PTB domain. The F3 subunit of FERM domain has a PTB-like domain and binds to multiple proteins. Additionally, KRIT1 has an N-terminal NLS and a C-terminal NES region to help shuttle from cytoplasm to nucleus. CCM2 consist a PTB domain and a C-terminal HHD. The PTB domain shares similarity to the ICAP1 and binds to KRIT1, PDCD10, and TrkA. The PDCD10 has a much simpler structure and consists of an N-terminal dimerization domain and a C-terminus FAT-H domain. PDCD10 binds to a variety of proteins including CCM2, germinal center kinases III subfamily, GCKIII (STK24, STK25, and MST4), paxillin, and VEGFR. *ANK* ankyrin repeat domain; *FERM*, F for 4.1 protein, ezrin, radixin, moesin; *NLS* nuclear localization signal; *NES* nuclear export signal; *PTB* phosphotyrosine-binding domain; *HHD* harmonin-homology domain; *TrkA* tyrosine kinase A; *FAT-H* focal adhesion targeting homology domain; *VEGFR* vascular endothelial growth factor receptor

related to point mutations in the PDCD10 FAT-H domain [42]. Moreover, substantial evidences implicate the critical role of germinal center kinases III subfamily, GCKIII (STK24, STK25, and MST4), in PDCD10 function and its contribution to CCM pathogenesis [31, 69, 75, 76]. Fidalgo et al. showed that PDCD10 localizes in the Golgi body and forms a complex with GCKIII kinases and GM130, a Golgi protein that is important for Golgi assembly and orientation [15]. Other partners known to interact with PDCD10 are protocadherin [44] important for neuronal survival and VEGFR2, crucial for vascular development and angiogenesis [28].

Any other additional genes?

Besides the three known CCM genes, previous studies speculate the existence of an additional CCM locus.

Bergametti et al. observed low frequency mutation of *PDCD10* in 8 of 20 families that were negative for *KRIT1* and *CCM2* [2]. In another study, Liquori et al. reported that the sequence analysis of *PDCD10* in 29 probands lacking *KRIT1* and *CCM2* mutations revealed only three mutations [46]. The probable explanations for such lower number of mutation in CCM affected families might be the following: (a) the existence of a fourth unidentified CCM gene which could have gone undetected by the exon sequencing, (b) hypermethylation of CpG sites in the promoter regions of the *KRIT1* and *PDCD10* genes that carry CpG islands, and (c) mutations in regulatory elements of the *KRIT1*, *CCM2*, and *PDCD10* genes like enhancers distant to the gene locus. Further linkage and high throughput mutational screening might be interesting to shed light into the structure and function of this hypothesized CCM locus.

PTEN structure

The successful identification of homozygous deletion in human chromosome 10q23 in diverse human cancer types led to the isolation and characterization of the tumor suppressor gene *PTEN*/*MMAC1* (phosphatase and tensin homologue deleted on chromosome ten) [41, 68]. PTEN, a 403-amino acid protein consists of a short N-terminal phosphatidylinositol-4-5-bisphosphate (Ptdlns (4,5) P_2)-interacting motif (PBD), a catalytic signature motif, HCXXGXXR located in the active sites of protein tyrosine phosphatases (PTPs) [40, 66]. Located beyond the PTPs domain, is a C2 domain, a carboxy-terminal tail, and a PDZ-binding motif [56, 66]. The carboxy-terminal tail is known to stabilize PTEN, and mutations in this domain induce tumor growth and proliferation [19]. A host of other mutations in the phosphatase and C2 domain also cause protein misfolding and results in uncontrolled cellular process. An essential function of PTEN is to oppose the activity of phosphatidylinositol 3-kinases (PI3K)/Akt pathway by hydrolyzing phosphatidylinositol-3,4,5-triphosphate (PIP3) to phosphatidylinositol −4,5-biphosphate (PIP2) [49].

The PI3K/Akt signaling

The PI3K/Akt signaling pathway is well established and targeted by PTEN through its lipid phosphatase activity [78]. The identification of this pathway dates back to 1980s with the characterization of insulin receptor signaling that subsequently led to the recognition of the components of PI3K/Akt pathway [5, 29]. The PI3K/Akt pathway is highly conserved and is regulated by a multistep signaling process. Based on their structural and functional attributes, the PI3K are divided into three separate classes: class I, II, and III [33, 66], of which class I is the best characterized and is involved in regulating cell survival and growth [6]. The class IA PI3Ks consists of p110α, p110β, and p110δ catalytic subunits and are activated by receptor tyrosine kinases (RTKs), whereas the class IB PI3Ks (PI3K) are activated by G protein-coupled receptors (GPCRs) [14]. The class II PI3Ks (PIK3C2α, PIK3C2β, and PIK3C2) lack regulatory subunits and comprises a common C-terminus C2 motif [33]. Additionally, the class III PI3Ks uses phosphatidylinositol (Ptdlns) as their substrate and bears a catalytic as well as an adaptor subunit [74]. The protein-serine-threonine kinase AKT (protein kinase B) is a crucial downstream regulator of the PI3Ks pathway and consists of three isoforms: AKT1, AKT2, and AKT3 [63]. These isoforms have a common pleckstrin homology (PH) domain, a catalytic, and a C-terminal hydrophobic motif which when phosphorylated regulates downstream signaling process such as cell survival, growth, and metabolism. A major role of PTEN is to negatively regulate the PI3K/Akt signaling. Following functional loss of PTEN, PIP3 accumulation in the cell activates the downstream Akt signaling, resulting in excessive cell growth, decreased apoptosis, and angiogenesis.

Role of PTEN/PI3K/VEGF signaling in angiogenesis

Here, we will briefly discuss the interplay of PTEN, PI3K, VEGF, and hypoxia in angiogenesis and then proceed to define their role in CCM pathogenesis. Previous studies have identified the central role of *PTEN* mutation in angiogenesis [72, 77]. However, Kini et al. demonstrated the potential role of PTEN in inducing angiogenesis in endothelial cells [36]. Although the exact mechanism is not clear, further studies are required to delineate the role of PTEN activation in endothelial cells. Angiogenesis can be triggered by growth factors, by PI3K pathway alterations, or by mutations in the *PTEN* gene. The vascular endothelial growth factor (VEGF), a common proangiogenic factor, is crucial in regulating vasculogenesis and angiogenesis via the PI3K pathway. Mammalian VEGF are classified into five related growth factors: VEGF-A, VEGF-B, VEGF-C, and VEGF-D and placental growth factor (PLGF) [33]. Among them, VEGF-A is crucial in regulating angiogenesis in the endothelial cells [33]. VEGF-B role in angiogenesis is not clear while VEGF-C and -D are predicted to stimulate lymphangiogenesis. Studies showed that PTEN loss promotes VEGF-mediated angiogenesis during normal vascular development and tumor formation both in vitro and in vivo [8, 26, 30]. Apart from the role of VEGF in triggering angiogenesis, angiopoietins (ANG) also possess angiogenic property and mediate signaling through the endothelial membrane receptor (TIE2). ANG-1 and −2 are solely expressed in the endothelial cells and may have antagonistic properties [10, 50]. ANG-1 can activate the PI3K pathway by interacting with the p85 subunit of PI3K in a phosphotyrosine-dependent manner [35], whereas ANG-2 can be downregulated following the activation of PI3K/Akt pathway [73]. Tsigkos et al. also demonstrated that PTEN acts as an agonist for ANG-2 release in the endothelial cells. The potent role of PTEN, PI3K, VEGF, and ANG under hypoxic conditions [85, 90] has also been well documented. Hypoxia, a condition of decreased oxygen concentration in the cells, is reported to be a key regulator of angiogenesis. Hypoxia increases the VEGF expression by binding to the hypoxia-responsive element (HRE) and results in the formation of hypoxia-inducible factor 1 (HIF-1), a heterodimer consisting of HIF-1α and HIF-1β subunits [65]. HIF-1 is predicted to regulate several genes involved in cell survival, proliferation, migration, and angiogenesis [65]. The role of p53 tumor suppressor protein, PTEN, and HIF-1 in tumor angiogenesis has been previously reported [55]. The oncoprotein, MDM2 (mammalian double minute 2), is phosphorylated upstream by the Akt molecule, and its activation results in the inhibition of p53 protein post transcriptionally (Fig. 2).

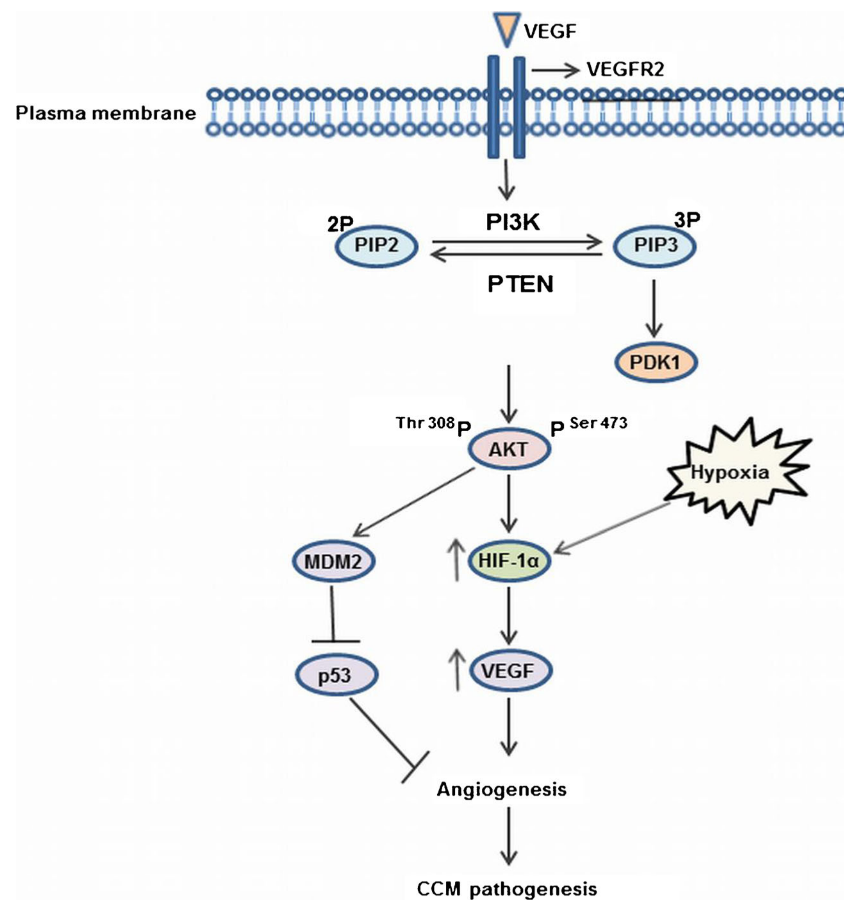

Fig. 2 Predicted role of PTEN/PI3K/Akt/VEGF signaling in CCM pathogenesis. PI3K activation is induced by the binding of VEGF to its receptor VEGFR2. PTEN, a tumor suppressor protein, has a crucial role in regulating angiogenesis by hydrolyzing PIP3 to PIP2, thus negatively regulating the PI3K signaling pathway. When PTEN protein is lost, PIP3 accumulation in the cells activates the downstream Akt signaling by increasing MDM2, HIF-1α, or VEGF expression. CCM angiogenesis may be mediated by phosphorylation of MDM2 which binds to p53 and inhibits its post transcriptional regulation. Such inhibition results in increased angiogenic activity. Therefore, a major strategy to check angiogenesis in endothelial cells might be to target the PTEN/PI3K/Akt or the MDM2-p53 interface by the application of inhibitors. *P* phosphate, *VEGF* vascular endothelial growth factor, *VEGFR2* vascular endothelial growth factor receptor 2, *PI3K* phosphatidylinositol 3-kinases, *PTEN* phosphatase and tensin homologue, *PDK1* phosphoinositide-dependent kinase 1, *Akt* protein-serine-threonine kinase, *HIF-1α* hypoxia-inducible factor-1α, *MDM2* mammalian double minute 2, *p53* tumor suppressor

PTEN/PI3K/VEGF signaling in CCM pathogenesis

Endothelial cells in the cerebral vasculature are vital in maintaining brain homeostasis. However, disruption of these cells under pathological condition results in abnormal angiogenesis, leading to CCM and other vascular malformations [39, 71, 83]. Previous studies have demonstrated the role of *PTEN* mutation in various harmatomas, such as Cowden syndrome, Proteus-like syndrome, and Bannayan-Riley-Ruvalcava syndrome [43, 51, 86]; however, the role of *PTEN* deletion in CCM have only recently been identified. The involvement of *PTEN* promoter methylation in CCM via PI3K/Akt-independent pathway was first reported by Zhu et al. [86]. The authors demonstrated the activation of Erk 1,2 of the MAPK/Erk (mitogen-activated protein kinase/extracellular signal-related kinases) signaling pathway but not the PI3K/Akt pathway, suggesting the regulation of *PTEN* promoter methylation in a tissue- and disease specific manner. Interestingly, Zhu et al. were able to show that following addition of the PI3K inhibitor, wortmannin to the endothelial cells, there was no change in the increased endothelial proliferation induced by *PTEN* promoter silencing. How *PTEN* promoter methylation resulted in Erk1, 2 activation and not PI3K/Akt needs further study. The role of VEGF-PI3K signaling in CCM pathogenesis has also been reported. VEGF acts upstream in the PI3K signaling and regulates vascular development by promoting VEGFR2 binding to the C-terminal domain of PDCD10 [28]. Through computational modeling, Dibble et al. demonstrated the involvement of PDCD10 in the PI3K signaling pathway [11]. Using site-directed mutagenesis, the authors constructed a three-dimensional model of PDCD10 and were able to co-localize the wild type PDCD10 with the membrane-bound active PI3K (p110-CAAX) in the plasma membrane. Further studies are needed to rule out the role of PDCD10 in VEGF-

PI3K signaling and their contribution to CCM pathogenesis. Recent reports show that following *PDCD10* gene silencing, VEGF signaling was activated in the endothelial cells, implying its importance in angiogenesis [80, 88]. Moreover, the role of hypoxia has been documented not only in cancer biology but also in noncancerous vascular disease [54, 70, 88].

Conclusion

Despite the significant advances in understanding PTEN/PI3K/Akt/VEGF signaling in angiogenesis, much remains to be explored how these pathways simultaneously regulate endothelial cell function in patients suffering from CCM. It is difficult to figure out whether PTEN insufficiency alone or other molecular factors in the PI3K/Akt/VEGF signaling contribute to CCM pathogenesis. Preferably, the current available treatment for CCM suffering patients is surgical intervention, but not all the lesions located in the eloquent areas (brainstem, pons) can be successfully removed. Therefore, generation of suitable CCM gene knock-out animal models mimicking human CCM lesion would be helpful in understanding the role of PTEN in PI3K/Akt/VEGF signaling. Moreover, in order to develop therapeutic targets for CCM treatment, extensive linkage and mutational studies in humans are required to delineate the existence of the hypothesized fourth CCM locus, its interaction with other CCM genes and their contribution to the above mentioned signaling pathways.

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Comments

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The insights in the underlying molecular mechanisms driving cerebral cavernous malformations dramatically increased over the last few years,

shifting our understanding to a point that even the name of the lesion has to be critically reviewed. The first big step was the identification of mutations in the genes CCM1, CCM2, and CCM3 in the vast majority of patients with familial cerebral cavernous malformations who develop multiple lesions [1–5]. Nearly all mutations in the CCM1-3 genes resulted in a premature terminated protein with a loss of function as consequence (reviewed in [6]). Such pattern indicated a tumor suppressor gene "two hit" mechanism and, indeed, a first early report reported the case of an identical biallelic germline and somatic CCM1 mutation [7]. However, the next big step was the systematical analysis of this biallelic "two hit" knock-out mechanism by Amy Akers and coworkers who identified by using a sophisticated strategy for all three CCM genes in many cases a germline mutation in all cells of the body and a second, so-called somatic mutation solely in a fraction of the endothelial cells of a specific cerebral cavernous malformation [8]. Furthermore, they identified the somatic mutations only in endothelial cells, thereby delineating this tissue component of cerebral cavernous malformations to be the driving one. In summary, all these data bring up the question if cerebral cavernous lesions are "malformations" or if it would be more appropriate to move them to the group of benign vascular tumors—and include them in the upcoming WHO classification of brain tumors.

In oncology, the next step after establishing the genetic background is to functionally characterize the consequences of the driving forces. In line with this notion, the review presented here by Souvik Kar and coworkers sheds light on current knowledge regarding the CCM1-3 proteins and their potential cross talk with the PTEN/PI3K/Akt/VEGF signaling pathway that has been well characterized in many neoplasms. The reviewed protein functions open up various opportunities for upcoming research projects.

Still the cerebral cavernous malformation research is hampered by the very low number of "neoplastic" cells within such lesions. Conventional approaches fail in many ways to go ahead. Presumably massive parallel sequencing will allow determining the somatic mutational status of the CCM1-3 genes in a much faster ways than by methods the group by Amy Akers used [8]. Such next generation sequencing approach may even help to identify CCM1-3 biallelic mutations in sporadic cases of cerebral cavernous malformations. The currently available CCM1/2 knock-out mouse models unfortunately generated not too many functional insights in the disease [9, 10]. Maybe it would instead be worth using one of the established endothelial cell system models to knock-in biallelic CCM1-3 mutations. This way simple expression array studies should generate data about up- and downregulated genes due to the functional loss of CCM1-3 gene products. Furthermore, the chances are given to identify specifically upregulated genes so—in a best setting—the particular CCM1, CCM2, or CCM3 subtype might become determinable by simple immunohistochemistry-based surrogate markers. Having such simple assay correlation studies between the particular genotype and morphology and/or clinical phenotype should become achievable. Next, such CCM1-3 knock-in endothelial cell system model would allow studies of altered protein-protein interactions and transformed signaling pathways. Finally, understanding of deregulated signaling pathways might lead to drug therapies for those patients suffering from cerebral cavernous malformations that cannot be cured by surgery alone.

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Cerebral cavernous malformation (CCM), classified as sporadic and familial (inherited) forms, is one of the most common cerebral vascular anomalies involving aberrant angiogenesis. Although familial CCM (fCCM) accounts for only around 20 % of CCM, a recent study by Spiegler et al. (Mol Genetics & Genomic Med 2014) has shown that the mutation detection rate of CCM1 (60 %), CCM2 (18 %), and CCM3 (22 %) in fCCM is much higher than previous thought; moreover, increasing evidence indicates more aggressive features, e.g., often presence of multiple lesions, earlier onset, and increased hemorrhage rate, in fCCM than in sporadic CCM. In view of the literature, researchers have put much attention to study the angiogenic function and the underlying signaling pathways of CCM proteins during the last decade, which has significantly improved our understanding on the pathogenesis of CCM. The present review has outlined the structure and the function of three CCM proteins, highlighting the protein-protein interaction at the molecular structure basis. Differently from other recent review articles, Kar and his colleagues have identified not only the key advances of current understandings on the signaling pathways related to the common or distinct functions of three CCM proteins but further emphasized the PTEN/PI3k/Akt/VEGF signaling in CCM. PTEN deficiency due to its mutation or epigenetic alterations leads to the activation of PI3k/Akt signaling, which in turn activates VEGF pathway and subsequently stimulates angiogenesis. Despite of the well-defined central role of this pathway in the angiogenesis of various human cancers, it is just recently implicated in CCM and it is an obviously important part of the pathomechanism of CCM. PTEN DNA promoter methylation is rarely seen in normal tissues, but PTEN deficiency due to its promoter methylation was detected by our group in 15.9 % of a series of 69 CCM, in 5 of 6 fCCM and in 46.7 % of CCMs with multiple lesions (Zhu et al.; Stroke 2009). These data point out that it is worthwhile to further characterize PTEN DNA promoter methylation and the downstream pathways in a large series of fCCM. As concluded by Kar and his colleagues in this review, PTEN/PI3k/Akt/VEGF signaling together with other identified pathways may simultaneously contribute to the pathomechanisms of CCM.

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