Genes & Diseases 8 (2021) 798–813

Available online at www.sciencedirect.com

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REVIEW ARTICLE

The multifaceted *PDCD10/CCM3* gene

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Received 24 September 2020; received in revised form 10 December 2020; accepted 22 December 2020
Available online 30 December 2020

KEYWORDS
Cancer;
Cell signaling;
Cell-cycle;
Neurovascular unit;
PDCD10/CCM3

Abstract The programmed cell death 10 (*PDCD10*) gene was originally identified as an apoptosis-related gene, although it is now usually known as *CCM3*, as the third causative gene of cerebral cavernous malformation (CCM). CCM is a neurovascular disease that is characterized by vascular malformations and is associated with headaches, seizures, focal neurological deficits, and cerebral hemorrhage. The PDCD10/CCM3 protein has multiple subcellular localizations and interacts with several multi-protein complexes and signaling pathways. Thus *PDCD10/CCM3* governs many cellular functions, which include cell-to-cell junctions and cytoskeleton organization, cell proliferation and apoptosis, and exocytosis and angiogenesis. Given its central role in the maintenance of homeostasis of the cell, dysregulation of *PDCD10/CCM3* can result in a wide range of altered cell functions. This can lead to severe diseases, including CCM, cognitive disability, and several types of cancers. Here, we review the multifaceted roles of *PDCD10/CCM3* in physiology and pathology, with a focus on its functions beyond CCM.

Introduction

Although the programmed cell death 10 (*PDCD10*) gene was initially identified as an apoptosis-related gene,[1] soon after it was further defined as the third causative gene of cerebral cavernous malformation (CCM). Thus, it has the alternative name of *CCM3*,[2–4] and is here referred to as *PDCD10/CCM3*.

The disease CCM is defined by the presence of cavernous angiomas or cavernomas. These consist of capillary–venous

Abbreviations: CCM, cerebral cavernous malformation; CNS, central nervous system; CSC, CCM signaling complex; ECs, endothelial cells; GBM, glioblastoma multiforme; NVU, neurovascular unit; VEGF, vascular-endothelial growth factor.

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Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2020.12.008

malformations that are enlarged and irregular in structure, and almost exclusively affect the brain micro-circulation, in terms of the so-called neurovascular unit (NVU). The NVU is formed by the endothelial cells (ECs) when they are surrounded by pericytes and are in close contact with the neuroglia (astrocytes, oligodendroglia, microglia) and neurons. Overall, this structure forms the blood–brain barrier, which tightly regulates the exchange of oxygen, nutrients, neurotoxic plasma components, circulating inflammatory cells, and pathogens between the blood and the brain tissue.[5–9]

Cavernomas are leaky and are prone to microbleeds, which can lead to headaches, seizures, focal neurological deficits, and cerebral hemorrhage.[10–15] CCM affects 0.16%–5.0% of the general population,[16,17] and it can be either familial or sporadic.[11,18–21] Familial cases are caused by mutations in any one of the three CCM genes: *CCM1* (Krev/Rap1-interacting trapped; KRIT1),[22,23] *CCM2* (malcavernin or osmosensing scaffold for mitogen-activated protein kinase kinase-3/Osm),[24] and *PDCD10/CCM3*.[2] The corresponding proteins can be found within the same complex, known as the CCM signaling complex (CSC), which stabilizes cell-to-cell junctions and controls homeostasis of the blood–brain barrier. However, PDCD10/CCM3 can also act apart from its most known associations with CCM1 and CCM2, and it has been implicated in a number of biological processes that cover different roles, including regulation of the cell cycle,[25–27] tumorigenesis,[28,29] chemo resistance,[30] and neuronal cell migration.[31,32] In addition, the multiple functions of *PDCD10/CCM3* are regulated by micro (mi)RNAs and context dependent, which adds up an aura of complexity that is of significant clinical interest.

This review focuses on *PDCD10/CCM3*, to provide an updated overview of this multifaceted gene and to discuss its functions under both physiological and pathological conditions. In particular, we describe here the biological roles of *PDCD10/CCM3* that lie apart from its relationship with CCM, and which underlie its potential research and translational implications.

Discovery and characterization of *PDCD10/CCM3*

The *PDCD10/CCM3* gene is also known as TF-1 cell apoptosis related gene 15 (*TFAR-15*),[1] and it was first identified in 1999 through a screening for differentially expressed genes during apoptosis in the TF-1 human pre-myeloid cell line. Wang et al showed that *TFAR-15* is highly expressed upon deprivation of granulocyte macrophage colony-stimulating factor and demonstrated that a recombinant TFAR-15 protein expressed in the human embryonic kidney 293T cell line inhibited natural cell death. Moreover, another study showed that *TFAR-15* was expressed in a fibroblast cell line exposed to specific apoptosis inducers.[33] Taken together, these studies have revealed the involvement of *PDCD10/CCM3* in apoptosis.

Further studies then identified *PDCD10/CCM3* as one of the three genes responsible for CCM, which focused the attention on its roles in vessel development and maturation; for this reason, it is also known as *CCM3*.[2–4] *PDCD10/CCM3* is located on chromosome 3 (3q26.1), is expressed ubiquitously, and it encodes a 25-kDa protein composed of 212 amino acids. Many orthologs have been identified in both vertebrates and invertebrates, including *Mus musculus*, *Danio rerio*, *Drosophila melanogaster*, and *Caenorhabditis elegans*, which thus defines *PDCD10/CCM3* as a conserved gene from nematode to human.[2]

PDCD10/CCM3 is part of the *PDCD* gene family, which is composed of 12 genes where their main role is related to programmed cell death. This complex biological program is crucial during both physiological and pathological processes. The *PDCD* gene family members are highly conserved and widely expressed, and can be down-regulated and up-regulated in context-dependent manners. Apart from their relationship with cell death, the *PDCD* genes have further crucial roles related to developmental disorders, immune diseases, cancers, and other human diseases.[34]

The function of the PDCD10/CCM3 protein resides on two main regions: its dimerization domain at the N-terminal, and its carboxyl-terminal focal adhesion targeting (FAT) homology domain (Fig. 1A). The dimerization domain comprises four α-helices and is required for PDCD10/CCM3 homodimerization.[35] Moreover, this region has been hypothesized to include a serine/threonine kinase binding and phosphorylation domain that is responsible for the interaction of PDCD10/CCM3 with the germinal-center kinase (GCK)III proteins, which results in the formation of heterodimers.[36–38] The FAT homology domain is similarly composed of four α-helices, and it is responsible for the direct interactions between PDCD10/CCM3 and the phosphotyrosine-binding domain of CCM2/malcavernin.[39] In addition, the FAT homology domain has a surface region known as 'hydrophobic patch 1', through which PDCD10/CCM3 interacts with several protein partners, including the striatin component of striatin-interacting phosphatase and kinase (STRIPAK) complexes,[40] the phosphatidylinositides,[41] and paxillin,[42] through its recognition of leucine-rich motifs. Moreover, it has been suggested that through its C-terminal region, PDCD10/CCM3 interacts with, and stabilizes, vascular-endothelial growth factor (VEGF) receptor 2 signaling.[43]

PDCD10/CCM3 shares a promoter with the nonhomologous *SERPINI1* gene, which encodes a serine protease inhibitor. These two genes are oriented head-to-head and separated by an evolutionarily conserved, exceptionally short intergenic region of 851 bp that functions as a bidirectional promoter. The short sequence from nucleotides 1-175 adjacent to *PDCD10/CCM3* functions as the minimal bidirectional promoter for both genes, while sequence 176–473 represents an enhancer element for *PDCD10/CCM3* and a repressive element for *SERPINI1*.[44]

Interestingly, SERPINI1 is predominantly expressed in the brain and is down-regulated in brain tumors, while PDCD10/CCM3 is ubiquitously expressed in all normal tissues, and its transcription is aberrant in different types of cancers. Five polymorphisms have been identified in the *PDCD10/SERPINI1* promoter, which are possibly related to down-regulation of *PDCD10/CCM3* expression in patients with CCM, without any reduction in *SERPINI1*.[45]

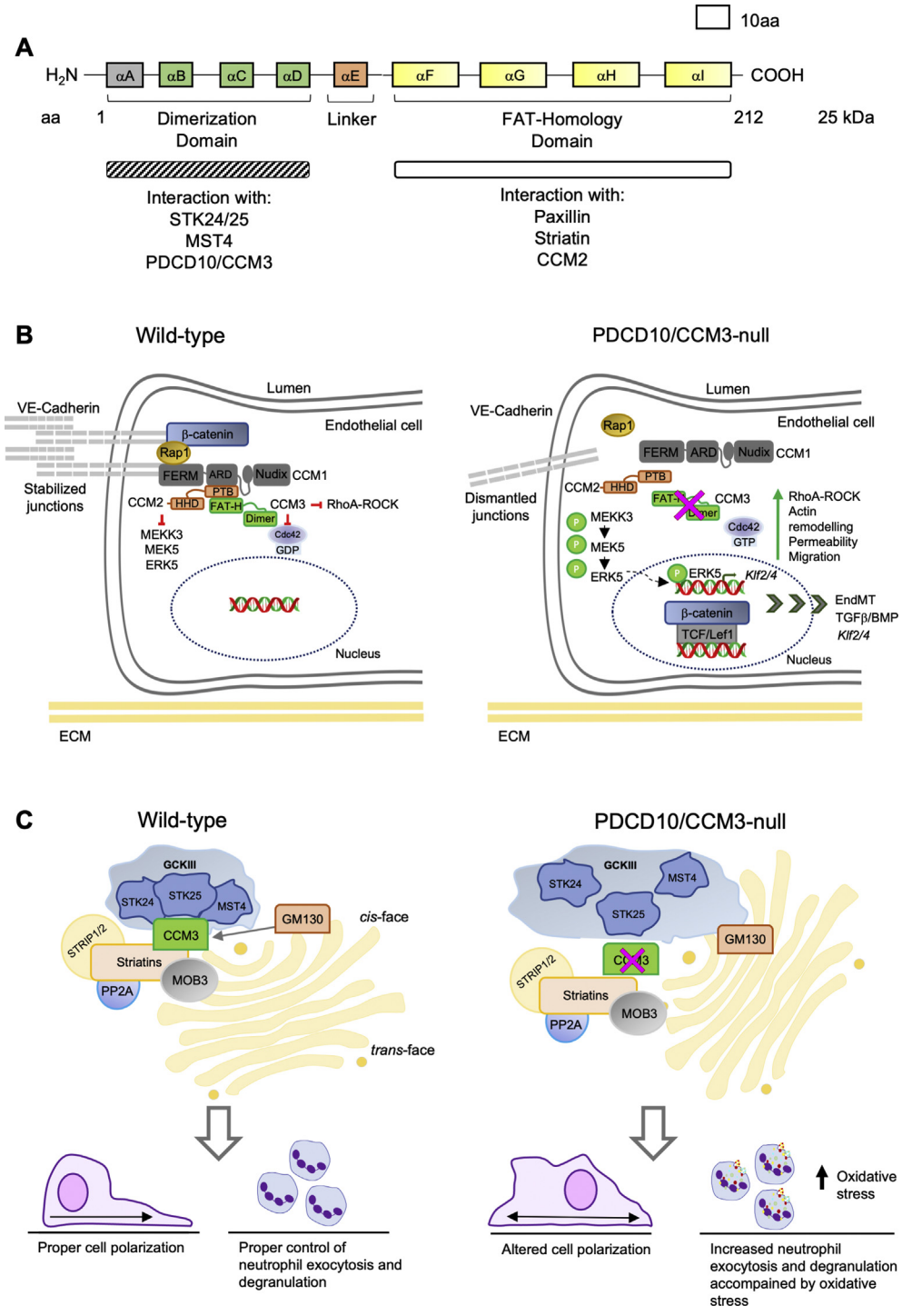


Figure 1 Structure and interactions of PDCD10/CCM3. (**A**) Schematic representation of the PDCD10/CCM3 domains and its interactors. The PDCD10/CCM3 dimerization domain at the N-terminal has four α-helices (green, α A-D) as well as the FAT-homology domain at the carboxy-terminal (yellow, α F—I), as shown. The different interactors that are responsible for the functions of PDCD10/CCM3 are listed under their relative domains. (**B**) Overview of the CCM signaling complex structure and its localization, interactions and functions under wild-type and PDCD10/CCM3-null conditions. (**C**) Beyond the CSC, PDCD10/CCM3 is a STRIPAK component and stabilizes GCKIII kinases through the binding to GM130, a Golgi-resident protein. Loss of PDCD10/CCM3 leads to GCKIII kinases destabilization together with an impaired cell migration and a dysregulated neutrophil exocytosis.

The CCM signaling complex and its functions

As a CCM protein, PDCD10/CCM3 associates with CCM2 and CCM1, and together these form the CSC.[46–48] The CSC is a structurally unrelated complex that folds at adherens junctions and acts as a regulator of EC biology (Fig. 1B). CCM1 is the largest of the three CCM proteins, and it anchors the CSC to cell-to-cell junctions by forming a complex with the RAP1 and β-catenin proteins, through its C-terminal FREM domain. This complex contributes to maintenance of the organization and stabilization of adherens junctions, and therefore to homeostasis of ECs.[49–52] Through its NPXY motifs, CCM1 also binds to the phosphotyrosine-binding domain of CCM2,[53–55] which in turn binds to CCM3,[39,56] thus acting as a bridge for the formation of the CSC complex.[47]

When mutated in any one of its components (i.e., CCM1–3), the CSC unfolds, which results in the formation of vascular lesions, are mainly localized in the central nervous system (CNS).[4,25,57–60] These lesions are formed by enlarged and irregular blood vessels that develop over time into complex structures that can lead to micro bleeds, epileptic seizures, and cerebral hemorrhage. The first direct event of this CSC unfolding is the dismantling of the adherens junctions and mislocalization of VE-cadherin,[4,57,58,61,62] followed by activation of multiple signaling pathways.

One of these is the MEKK3–MEK5–ERK5 signaling pathway, which is involved in several physiological processes, including cell proliferation and early cardiovascular development.[63–65] Through its C-terminal helical harmonin domain, CCM2 binds the N-terminal region of MEKK3, and thus prevents MEKK3 activation.[54,66,67] Therefore, dismantling of the CSC triggers the pathway that elicits activation of the downstream effectors Kruppel-like factor 2/4 (KLF2/KLF4), which are two pivotal players in the initiation of CCM pathogenesis.[68,69] As KLF2/4 activation has been reported in murine models and human patients with mutations in any one of the three *CCM* genes,[25,57,68,69] as well as in sporadic cavernomas,[58] this underlines the central role of the disruption of the CSC in the onset of CCM.

Another well-known pathway controlled by the CCM proteins is RhoA–ROCK signaling. The CSC prevents activation of RhoA and its effector ROCK,[70,71] thus controlling cell migration and junction integrity. Activation of RhoA–ROCK signaling has been shown following mutations in any one of the three *CCM* genes.[3,51,70–72] In addition, loss-of-function of PDCD10/CCM3, and also of CCM1,[49] results in activation of β-catenin–driven transcription through a ligand-independent mechanism. Without PDCD10/CCM3, β-catenin levels at junctions decrease, and β-catenin is seen to be concentrated in the nucleus, where it activates transcription and thus contributes to CCM pathogenesis.[4] Transcriptional activation of β-catenin along with KLF4, which activates the TGFβ/BMP signaling pathway in turn, induces the so-called endothelial-to-mesenchymal transition of the ECs that line vascular lesions. These thus undergo a process of de-differentiation, which is one of the major hallmarks of the onset and progression of CCM.[4,25,57,69]

Among several binding interactions of PDCD10/CCM3, the one with CDC42 has been shown to occur within the CSC complex. CDC42 is a small GTPase that regulates diverse cell functions in a variety of tissues and cell types, such as cytoskeletal and junctional rearrangements,[73] formation of membrane protrusions,[74] apico–basal polarity and lumen formation,[75] and cell migration.[76] Interestingly, mutation of CDC42 reproduces the phenotype and the molecular hallmark of CCM without affecting the levels of the CCM proteins.[77] The landscape of interactions and signaling pathways controlled by the CSC is very complex, and its detailed description goes beyond the focus of this review. However, the structure and functions of the CSC have been thoroughly reviewed elsewhere.[62,78–80]

Cellular functions beyond the CSC that are mediated by *PDCD10/CCM3*

Beyond the CSC, PDCD10/CCM3 can be defined as a multi-talented protein, because it regulates a variety of cell functions separately from CCM1 and CCM2. Its unique roles could be the reason why, once mutated, CCM3 gives rise to a more aggressive form of CCM.[3] This review now focuses in particular on these multiple roles of PDCD10/CCM3, to present this protein in all of its pleiotropic aspects.

The STRIPAK component

The striatin-interacting phosphatase and kinase (STRIPAK) complex is a multiprotein assembly that was initially identified in 2009 using an iterative affinity purification–mass spectrometry approach.[81] STRIPAK contains the protein phosphatase 2A (PP2A) catalytic and scaffolding subunits, the striatins, the striatin-associated protein MOB3, striatin-interacting proteins (STRIP) 1 and 2, the GCKIII subfamily of Ste20 protein kinases (i.e., STK24/MST3, STK25/SOK1, MST4/MASK), and PDCD10/CCM3 (Fig. 1C).[56,82,83] PP2A has been implicated in the control of cell growth, proliferation and differentiation, and the GCKIII kinases have been shown to be involved in modulation of cell proliferation, migration and cell death.[81,84,85]

The best known role and function mediated by PDCD10/CCM3 outside of the CSC is based on it being a component of the STRIPAK complex.[86,87] Indeed, within the STRIPAK complex, PDCD10/CCM3 interacts with the members of the MST4, STK24, and STK25 GCKIII kinase subfamily through hetero-dimerization.[37,38,88] In addition, PDCD10/CCM3 binds striatins directly, to function as a linker between GCKIII kinases and PP2A phosphatase.[81]

The most important interaction here is the binding of PDCD10/CCM3–GCKIII to GM130, a Golgi-resident protein. Through this interaction, PDCD10/CCM3 localizes to the *cis*-face of the Golgi apparatus, and stabilizes the GCKIII kinases and protects them from ubiquitin ligation. Therefore, loss of PDCD10/CCM3 leads to STK25 kinase down-regulation and destabilization, and impaired cell migration that is correlated with the loss of repositioning of both the Golgi apparatus and the centrosome towards the leading edge of the cell.[38,89] These effects of PDCD10/CCM3 on Golgi assembly are mediated, at least in part,

through phosphorylation of the 14.3.3ζ protein, which is targeted by STK25. Also, the overexpression of PDCD10/CCM3 (or PDCD10/CCM3—MST4) or the expression of the whole subfamily of GCKIII kinases leads to rescue of cell migration and Golgi assembly. Interestingly, clinically relevant mutants of PDCD10/CCM3 cannot bind and stabilize STK25 efficiently, and therefore fail to restore the defects of the Golgi apparatus. This suggests that full functionality of PDCD10/CCM3 is essential for Golgi assembly.[90]

As a STRIPAK component, PDCD10/CCM3 is also involved in the regulation of neutrophil degranulation, through its interaction with STK24. STK24 is localized to neutrophil granules and it inhibits neutrophil vesicle exocytosis. In addition, STK24 competes with the vesicle fusion regulator UNC13D for the binding to lipids, therefore leading to further inhibition of exocytosis. PDCD10/CCM3 functions as a dual regulator in maintenance of the equilibrium of neutrophil exocytosis: it binds and stabilizes STK24, which decreases neutrophil exocytosis, and at the same time, counteracts STK24-mediated inhibition of exocytosis, which increases the binding of UNC13D to liposomes through its C2B domain. Hence, loss of PDCD10/CCM3 increases exocytosis of granules in neutrophils, and leads to increased oxidative damage. This mechanism was shown in a renal ischemia reperfusion injury model, where reperfusion resulted in increased damage, which highlights the importance of the role of PDCD10/CCM3—STK24 in neutrophil exocytosis.[90]

The molecular machinery that drives trafficking of exocytic vesicles that includes PDCD10/CCM3 and the GCKIII sub-family members has a broad expression pattern across tissues, which suggests that PDCD10/CCM3 is essential in the regulation of exocytosis in cells other than neutrophils.[91] This is the case for ECs, which contain the Weibel—Palade body, a type of secretory vesicle that releases von Willebrand factor, P-selectin and ANGPT2.[92] Here, PDCD10/CCM3 binds STK24 and UNC13B, which is the primary isoform of the UNC13 family that is expressed in vascular ECs, and prevents exocytosis of the Weibel—Palade body. Consequently, EC loss of PDCD10/CCM3 leads to uncontrolled secretion of ANGPT2, which exacerbates the dismantling of the adherens junctions and vascular instability.[93] As increased exocytosis of ANGPT2 is not associated to loss of CCM1 or CCM2, this indicates that this mechanism is particular to mutations of PDCD10/CCM3, and it can partially explain why PDCD10/CCM3 loss results in more severe disease in humans and mice.

A recent report indicated the importance of the association between PDCD10/CCM3 and MST4 in a model of subarachnoid hemorrhage. The primary cause of high rates of mortality and morbidity in individuals suffering from subarachnoid hemorrhage is the early brain injury. This causes exacerbation of the phenotype, as it gives rise to inflammation, oxidative stress excitotoxicity, and impaired ion homeostasis.[94] An important pathway that participates in early brain injury after subarachnoid hemorrhage acts via tumor necrosis factor (TNF) receptor-associated factor (TRAF6), which is controlled at both the transcriptional and post-transcriptional levels. This is seen by TRAF6 phosphorylation that is mediated by MST4, which inhibits its oligomerization and auto-ubiquitination, along with activation of the related inflammatory responses. The biological function of MST4 depends mainly on its interaction with PDCD10/CCM3: in this model, the expression of PDCD10/CCM3 is reduced 6 h after subarachnoid hemorrhage, which is accompanied by an increase in nuclear factor-κB (*NF-κB*) gene binding after 24 h. This inverse correlation is accompanied by increased blood levels of the proinflammatory cytokines TNF-α and interleukin-1β. In addition, overexpression of PDCD10/CCM3 ameliorates neuronal necrosis and neurobehavioral responses after subarachnoid hemorrhage. This suggests that the interaction between PDCD10/CCM3 and MST4 guides TRAF6-mediated regulation of the NF-κB signaling pathway under conditions of inflammation and oxidative stress.[95]

Cell proliferation, apoptosis, and senescence

As already mentioned, *PDCD10/CCM3* was initially identified as an apoptosis-related gene, and further studies were carried out to investigate its role during apoptotic responses. Chen et al (2009) used different *in vitro* assays to investigate the effects of *PDCD10/CCM3* knock-down and overexpression, through an analysis of the well-known apoptosis marker of activated caspase-3.[96] They showed that overexpression of PDCD10/CCM3 in HeLa cells resulted in increased numbers of apoptotic cells compared to control. Conversely, human umbilical vein ECs cultured in low-serum medium showed up-regulation of PDCD10/CCM3 expression that was associated with activation of caspase-3, which indicated active apoptosis. Under the same conditions, knock-down of PDCD10/CCM3 decreased cell death, which correlated with reduced caspase-3 activation. Together, these *in vitro* data demonstrate that PDCD10/CCM3 is both necessary and sufficient to induce apoptosis[97] (Fig. 2).

Oxidative stress increases the expression of PDCD10/CCM3 and its interactor STK25, and their association promotes apoptosis. Indeed, although overexpression of either PDCD10/CCM3 or STK25 induces apoptosis upon H_2O_2 exposure, overexpression of both PDCD10/CCM3 and STK25 gives rise to a synergistic effect, which suggests their powerful cooperation for activation of the apoptotic pathway. This promoting effect was suggested to be mediated through activation of ERK kinase.[98] This pro-apoptotic function has also been shown to be micro (mi)-RNA dependent, as Wu et al (2016) showed that miR-613 targets, and down-regulates, PDCD10/CCM3 expression to suppress cardiomyocyte apoptosis induced by ischemia-reperfusion.[99]

As well as being a pro-apoptotic gene, *PDCD10/CCM3* directly regulates the cell cycle and cell proliferation (Fig. 2). *PDCD10/CCM3*-null ECs showed increased proliferation in brain lesions in an *in vivo* murine model.[4] Subsequently, Malinverno et al (2019) showed *in vitro* that the depletion of PDCD10/CCM3 is sufficient to increase EC proliferation and to drive entrance into S-phase, as 5-

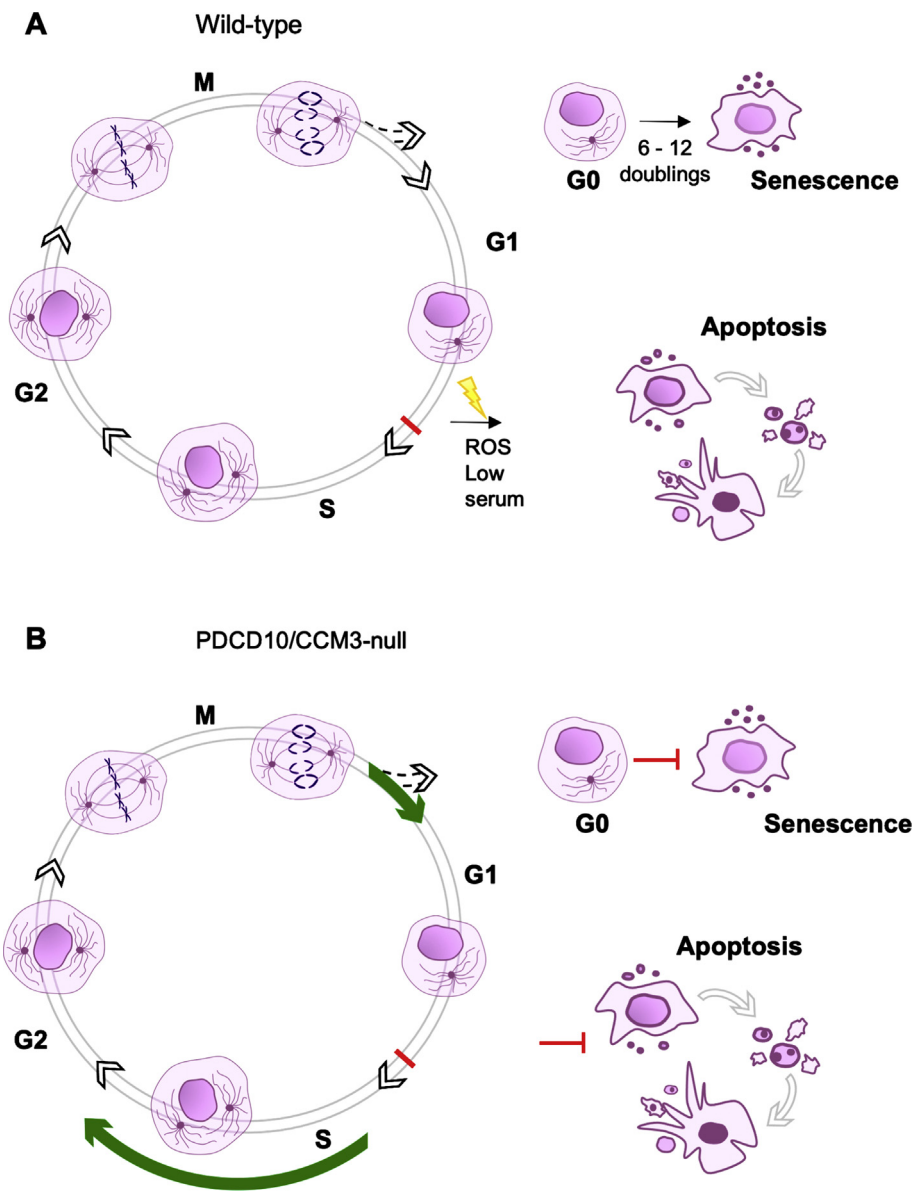


Figure 2 PDCD10/CCM3 regulates the cell-cycle. (**A**) Under physiological condition the cell cycle is tightly regulated: the cells enter into senescence after an excessive number of *in vitro* cell doublings or into apoptosis in response to stressful events such as elevated levels of ROS species or low levels of serum. (**B**) PDCD10/CCM3 is a pivotal regulator of these events as its depletion leads to impaired entrance into senescence (G1 phase — green arrow) and apoptosis. In addition, the loss of PDCD10/CCM3 alters the proliferative behavior of the cell driving its aberrant entrance into S phase (S phase — green arrow).

bromo-2′-deoxyuridine–positive cells were significantly increased upon *PDCD10/CCM3* deletion. Consistent with this, re-expression of PDCD10/CCM3 rescued the proliferative phenotype, which demonstrated a novel role for *PDCD10/CCM3* as a regulator of the cell cycle and cell proliferation in ECs.[25]

Transcriptome analysis of lesion-derived NVU microdissected from mice with brain endothelial specific deletion of *PDCD10/CCM3* showed that the most enriched gene ontology pathways were related to cell division processes.[27] Among the dysregulated genes here, network analyses identified polo-like kinase 1 and cyclin B, which are both important cell-cycle regulators.[100,101] This supports the concept that the rapid development and high burden of these lesions *in vivo* are sustained by the active proliferation of mutant ECs.[27,102] As a related point, Guerrero et al (2015) showed that loss of *PDCD10/CCM3* in primary ECs altered their entrance into senescence induced by an excessive number of *in vitro* cell doublings. Indeed, while control ECs stopped cell proliferation after 6–12 doublings, thus showing the typical morphology of senescent cells, this was not the case for the PDCD10/CCM3-depleted counterpart, which continued to divide for the same number of population doublings, and did not acquire any

characteristic of senescent cells nor accumulate senescence-associated β-galactosidase.[26]

On the other hand, PDCD10/CCM3 deficiency results in accumulation of DNA damage, as demonstrated by the presence of γH2AX, a known marker of DNA double-strand breaks.[103] Re-expression of PDCD10/CCM3 recovered this defect in terms of senescence, as the cells started to accumulate β-galactosidase activity. Transcriptomic analysis revealed two sets of genes that were especially down-regulated upon *PDCD10/CCM3* knockdown, which were related to cytokine—cytokine receptor interactions and lysosomes. Among the regulatory pathways that control cytokines during senescence, C/EPBβ activation was impaired in PDCD10/CCM3-depleted ECs. The enforced C/EPBβ expression rescued the senescence bypass, thus suggesting that this senescence bypass is related to impaired induction of the transcription factor C/EPBβ.[26]

The neurovascular unit

Among the different studies that have focused on the roles of *PDCD10/CCM3*, those that have explored its functionalities in the NVU are of particular interest. As already mentioned, the EC-specific loss of *PDCD10/CCM3* gives rise to CCM, a neurovascular disease that causes the appearance of mulberry-like lesions throughout the CNS. Given the importance of the communication between the vasculature and the other components of the NVU for correct development and function of the CNS, it becomes pivotal to understand the interactions between several molecular factors, and the multiple roles that each component has within the NVU.[32] Among these, PDCD10/CCM3 has been shown to be expressed in the NVU both in developing mouse brain and human tissue, and therefore, it has been hypothesized to have a role in the cross-talk between neural cells (neurons and neuroglia), pericytes and the endothelium[104] (Fig. 3).

With the aim of exploring *PDCD10/CCM3* functions in the neural context, Louvi and colleagues (2011) generated neural-cell-specific knockout murine models of *PDCD10/CCM3* using three Cre lines that drive recombination in different populations of neural cells: *Nestin*-Cre, *Gfap*-Cre, and *empty spiracles homolog 1* (*Emx1*)-Cre. All of these three mutants showed increased brain size and abnormal cytoarchitecture, which suggested a neural-cell-specific autonomous role of PDCD10/CCM3. Indeed, mutated neurons showed altered morphology, with impaired neurite growth due to remodeling of the actin and microtubule cytoskeleton (Fig. 3A—D). Neurons also showed defects in cell migration, which was mostly due to non-autonomous cellular effects of PDCD10/CCM3 deletion in radial glia (Fig. 3E—F). All of these phenotypes have been linked to increased activity of the RhoA signaling pathway.[31] Even though PDCD10/CCM3 deficiency altered the development of radial glia processes and neuronal cell migration, this did not affect proliferation of neural cell progenitors and neurogenesis.

In contrast, primary cortical astrocytes isolated from *Gfap*-Cre;*Ccm3*$^{lox/lox}$ (*Gfap-Ccm3*) mice at P3 were highly proliferative and resistant to cycloheximide-induced apoptosis, while showing lower levels of activated caspase-3. This thus suggested a pro-survival phenotype upon PDCD10/CCM3 loss. This pro-survival phenotype was demonstrated to be dependent on activation of Akt and FoxO1, which are part of a signaling pathway with documented effects on cell proliferation and survival.[105] The *Gfap-Ccm3* and *Emx1*-Cre;*Ccm3*$^{lox/lox}$ (*Emx1-Ccm3*) neural mutants also showed a CCM-like phenotype (Fig. 3A—D), with cerebrovascular lesions that resembled cavernomas, which suggested that this neural-cell-specific *PDCD10/CCM3* deletion has non-autonomous cellular effects in the cerebral vasculature.[32] Moreover, transcriptomic analysis carried out on samples derived from these lesions demonstrated the major involvement of cytoskeletal remodeling pathways, with the activation of Rho GTPases. This finding is consistent with RhoA activation upon EC-specific deletion of the *CCM* genes.[3,51,70—72] Interestingly, neural-cell-specific depletion of CCM2 did not give rise to cerebrovascular lesions,[70,106] which suggested that the role of PDCD10/CCM3 in neural cells does not involve the CSC. While PDCD10/CCM3 has been shown to be mainly associated to the Golgi apparatus in nonneuronal cells,[38,89] in cultured neurons PDCD10/CCM3 is found throughout the cell body and processes, and not enriched in the Golgi apparatus.[107] Here, PDCD10/CCM3 directly interacts with the protocadherin (PCDH)-γ isoform, which sequesters PDCD10/CCM3 at the membrane, thus preventing its pro-apoptotic activity. Coherent with this, depletion of PCDH-γ leads to neuronal cell death due to PDCD10/CCM3-induced apoptosis.[107—109] Moreover, while *PDCD10/CCM3* overexpression is sufficient to induce neuronal cell apoptosis, its knockdown is not effective against neuronal cell survival on its own. This thus demonstrates the role of the PDCD10/CCM3—PCDH-γ interaction in the regulation of this biological process.[107]

The other major components of the NVU are pericytes, which surround the endothelium with a 1:1 ratio[110] and control the formation and maintenance of the blood—brain barrier. The work of Zhou et al (2016) showed that the increased secretion of ANGPT2 upon EC-specific depletion of *PDCD10/CCM3* causes impaired pericyte coverage within cavernomas, through a cell non-autonomous mechanism. On the other side, pericyte-specific depletion of *PDCD10/CCM3* induces the formation of cavernomas by reducing cell migration and EC-pericyte association.[111] Even though a direct influence of *PDCD10/CCM3* depleted pericytes on ECs phenotype has still to be assessed, this would be reasonable given the close cell-to-cell cross talk between these two cell types.[112,113]

Therefore, deleting *PDCD10/CCM3* in either EC, pericytes or astrocytes leads to the formation of vascular malformations through cell autonomous and non-autonomous mechanisms. This highlights the central role of *PDCD10/CCM3* in the homeostasis of the NVU, and can explain why cavernomas form preferentially in the brain despite the ubiquitous mutation of *PDCD10/CCM3* in patients with CCM.

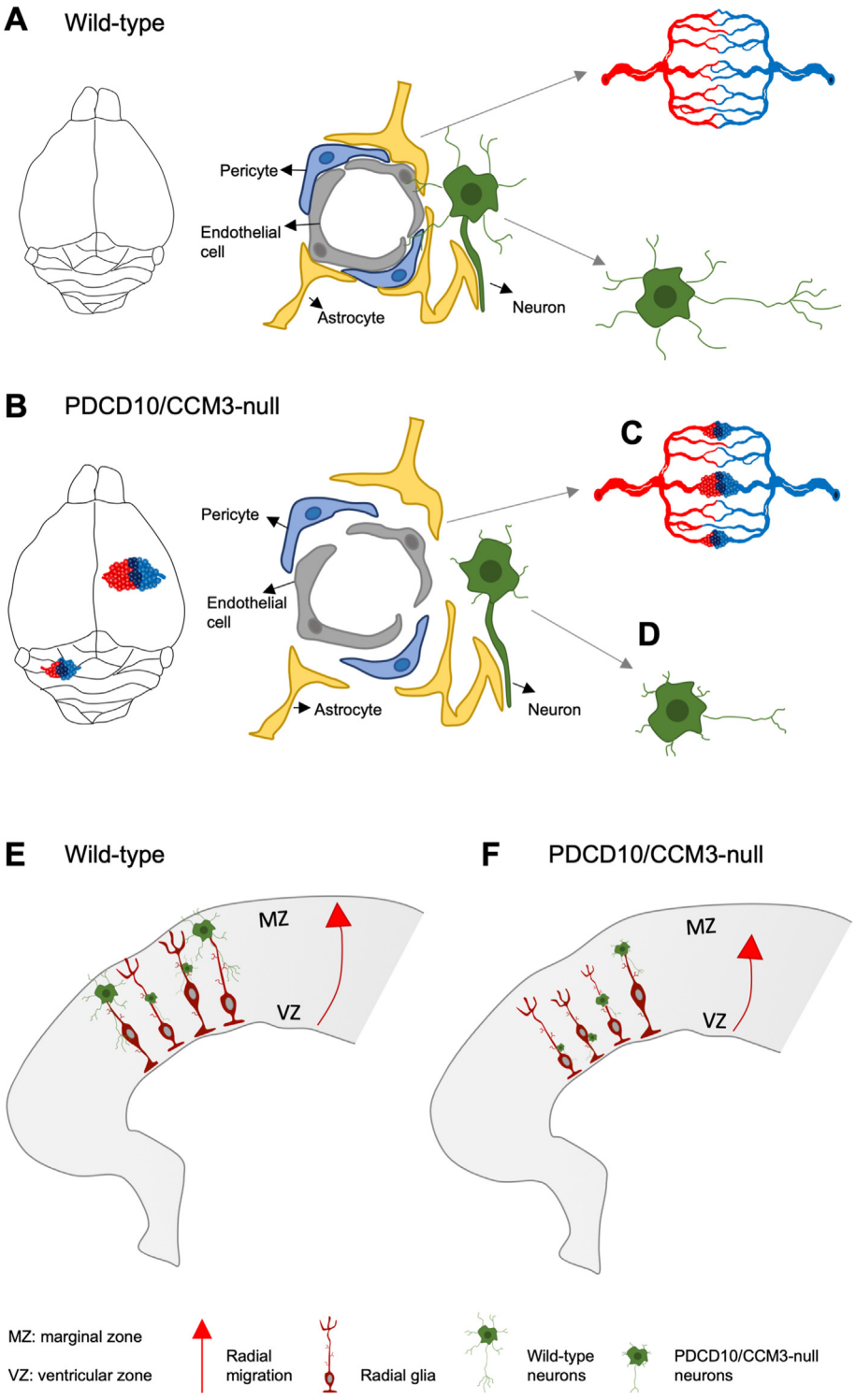


Figure 3 Cell-autonomous and non-autonomous roles of PDCD10/CCM3 within the neurovascular unit. (**A**) PDCD10/CCM3 plays multiple roles across the different components of the neurovascular unit and controls the proper formation of blood vessels and the maturation of neurons. (**B**) Depletion of PDCD10/CCM3 in neural cells causes an increased brain size and an impaired neurite growth in neurons (**D**), highlighting a cell-autonomous role. (**C**) PDCD10/CCM3 neural loss gives rise to cerebrovascular lesions, suggesting a non-autonomous cellular effect within the cerebral vasculature. (**E**) PDCD10/CCM3 has a major role during the regulation of radial migration, a process taking place in the cerebral cortex where neurons migrate along radial glia guides from the ventricular (VZ) to the marginal zone (MZ). (**F**) Loss of PDCD10/CCM3 in radial glia causes both cell autonomous and non-autonomous effects, respectively, and an impaired development of radial glia processes and altered migration and morphology of the neurons.

Cancers

Considering the multiple roles of PDCD10/CCM3 in pivotal mechanisms such as cell apoptosis and survival, and in angiogenesis, different studies have tried to determine its role in cancers. Among the cancer models that have been analyzed, glioblastoma multiforme (GBM) is the most aggressive primary tumor in the CNS, with its main features being microvascular hyperplasia and necrotic foci.[114–116] Of note, different case reports have highlighted the coexistence (although rare) of cavernomas with tumors of the CNS, including Schwannomas, neurofibromas, and gliomas,[117,118] which suggests that cerebral cavernomas have tumorigenic potential. Moreover, around one in four CCM patients with mutations in *PDCD10/CCM3* develops a brain tumor,[3] which are in most cases multiple dural-based meningiomas.[119–122]

In line with this evidence, *PDCD10/CCM3* was shown to be strongly down-regulated at both the mRNA and protein levels in human GBM, which was paralleled by activation of the Akt signaling pathway. This therefore suggests the involvement of *PDCD10/CCM3* in the pathogenesis of GBM.[28]

The typical morphological features of GBM are necrosis and microvascular proliferation, two events that are controlled by the cell proliferation/apoptosis balance. In human GBM, *PDCD10/CCM3* is absent in proliferating (i.e., proliferating cell nuclear antigen-positive) tumor cells and ECs of the infiltrating zone, while it is co-expressed with the active apoptotic protein caspase-3 in the hypoxic pseudopalisading cells that surround necrotic centers.[28] EC proliferation results in increased microvascular density in GBM, which is regulated by PDCD10/CCM3; indeed, lower PDCD10/CCM3 expression is associated with higher microvascular density and brain edema. Taken together, these data show that PDCD10/CCM3 is involved in the control of the apoptotic and proliferative state of GBMs and ECs, as well as the microvascular density, which suggests an additional role for PDCD10/CCM3 in GBM pathogenesis.

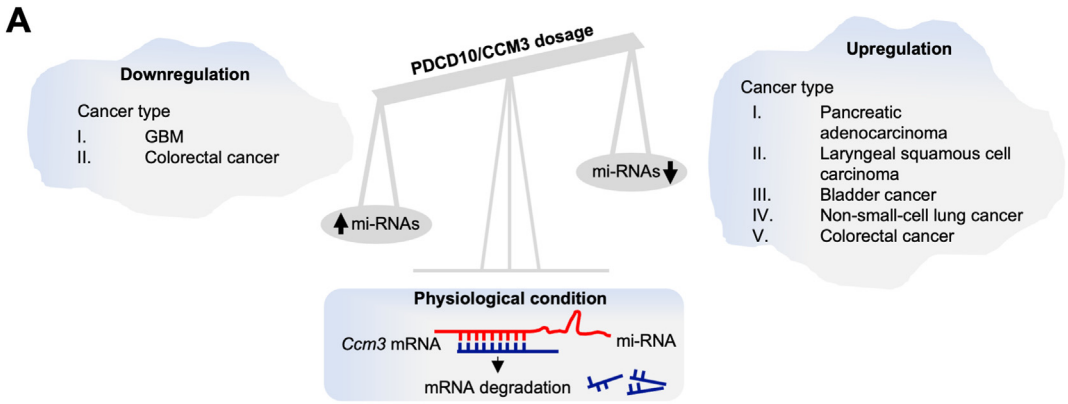
Neo-angiogenesis is a powerful tool through which tumors disseminate, and it is a hallmark of GBM. This neoangiogenesis is sustained by both autocrine and paracrine signals that are released by neoplastic and nonneoplastic cells.[123] Direct co-culture of PDCD10/CCM3-deficient ECs and GBM cells has demonstrated that EC loss of PDCD10/CCM3 can activate GBM cell proliferation, migration, and invasiveness. In contrast, the treatment of GBM cells with conditioned medium from PDCD10/CCM3-deficient ECs reduced the apoptotic response of the GBM cells. In parallel, EC deletion of PDCD10/CCM3 in a GBM xenograft model highlighted increased tumor growth and microvascular density only in the GBM cells, together with activation of the survival pathways via Erk1/2 and Akt. These pathways can be activated through a paracrine mechanism mediated by the EC counterpart, as was demonstrated by analysis of the conditioned medium of human umbilical vein ECs silenced for PDCD10/CCM3; this revealed the secretion of soluble factors that activated both the Erk1/2 and Akt pathways, including VEGF.[29]

PDCD10/CCM3 has also been implicated in the apoptotic response of GBM cells to treatment with temozolomide, the first-line drug for chemotherapy of GBM. Temozolomide impairs DNA repair, which blocks the cell cycle and promotes apoptosis, as well as senescence and autophagy.[124,125] Of note, GBMs are often resistant to temozolomide treatment.[125]

The knock-down of *PDCD10/CCM3* in GBM cells accelerates tumor growth and induces chemoresistance *in vitro* and in mice treated with temozolomide, through inhibition of apoptosis.[30] However, the role of *PDCD10/CCM3* in cancers is much more complex and is strictly context dependent, in terms of its expression patterns and mechanism of action. Indeed, *PDCD10/CCM3* is overexpressed in pancreatic adenocarcinoma,[126] laryngeal squamous cell carcinoma,[127,128] bladder cancer,[129] and non-small cell lung cancer.[130] Interestingly, in colorectal cancer, *PDCD10/CCM3* is either overexpressed[131,132] or down-regulated in metastatic cells resistant to chemotherapy.[133] PDCD10/CCM3 has also been shown to induce cell proliferation and to inhibit apoptosis in breast cancer[134,135] and in malignant T-cells in cutaneous T-cell lymphomas.[136] Taken together, these data suggest that PCDC10/CCM3 has a crucial role in the fine-tuning of cell proliferation and apoptosis, as its down-regulation and up-regulation both lead to aberrant cellular growth in cancer contexts. These apparently conflicting functions of PDCD10/CCM3 might be due to its interactions with different signaling pathways specifically in certain cancer contexts. For instance, in metastatic cells of breast cancer, up-regulation of tri-partite motif (TRIM) 59 stabilizes PDCD10/CCM3 through suppression of its ubiquitination-dependent autophagic degradation, and induces downstream suppression of the RhoA/ROCK and KLF2/4 signaling pathways. In contrast with what happens in CCM, the gain of function of PDCD10/CCM3 and suppression of RhoA/ROCK and KLF2/4 promote cancer-cell proliferation, mesenchymal migration, and tumor growth.[134,135]

In prostate cancer (PC-3) cells, PDCD10/CCM3 also interacts with MST4, a member of Ste-20-related kinases, to induce cell proliferation and cell transformation through the modulation of the ERK pathway during prostate cancer progression.[82] Of interest, MST4 has also been shown to be up-regulated in prostate cancer compared to benign prostatic hyperplasia, which demonstrates that MST4 has a potential role as a marker or target for the most aggressive forms of prostate carcinoma.[137]

The multifaceted role of PDCD10/CCM3 implies regulatory mechanisms that are particular to different cancer types and include micro (mi)RNAs. These miRNAs are small noncoding RNAs that regulate gene expression post-transcriptionally, and hence can have crucial roles in a wide range of biological processes, such as cell metabolism and proliferation, stress responses and apoptosis.[138,139] Recently, miRNAs have been shown to be dysregulated in human cancers, and can acquire either oncogenic or tumor suppressive functions depending on their target genes.[140–142] This is indeed also the case for the association between miRNAs and PDCD10/CCM3 (Fig. 4). In colorectal cancer, the expression of miR-425-



B

Disease	PDCD10/CCM3 expression	mi-RNA	mi-RNA expression	Pathological outcome	Reference
Colorectal cancer	Inhibited	miR-425-5p	Expressed	Chemoresistance	Zhang et al. (2016)
Bladder cancer	Up-regulated	miR-26a/b-5p	Down-regulated	Aberrant proliferation	Wu et al. (2018)
Non–small-cell lung cancer	Up-regulated	miR-103	Down-regulated	Cell migration and tumor proliferation	Fu et al. (2016)
Prostate cancer	Up-regulated	miR-103	Down-regulated	Cell migration and tumor proliferation	Fu et al. (2016)
Retinoblastoma	Inhibited	miR-181b	Expressed	Enhanced angiogenesis	Xu et al. (2015)

Figure 4 PDCD10/CCM3 expression in cancer and its regulation by mi-RNAs. (**A**) Under physiological conditions PDCD10/CCM3 is epigenetically-regulated by different mi-RNAs, while in cancer, mi-RNAs can act either as enhancers or silencers in a context-dependent manner, giving rise to different pathological conditions. (**B**) The context-dependent correlations between expression of *PDCD10/CCM3* and the indicated mi-RNA regulators are shown, along with their associated pathological phenotypes.

5p inhibits PDCD10/CCM3, and consequently induces chemoresistance to 5-fluorouracil and oxaliplatin, two chemotherapeutic agents used in combination in the clinic for patients with colorectal cancer. MiR-26a-5p and miR-26b-5p have well known antitumor roles and are down-regulated in multiple cancers, including bladder cancer.[143] In bladder cancer cells, miR-26a-5p and miR-26b-5p directly target and inhibit PDCD10/CCM3, which is overexpressed compared to healthy bladder tissue, and thus induce cell proliferation. *In vivo* xenograft experiments and clinical evidence have confirmed improved prognosis for patients with bladder cancer with high expression of miR-26a-5p and miR-26b-5p, and low expression of PDCD10/CCM3, over patients with low miR-26-5p and high PDCD10 expression.[129] Also, miR-103 has both oncogenic and tumor-suppression roles in various cancers,[144–147] and it has been described as an inhibitor of PDCD10/CCM3 expression. In non-small cell lung cancer tissue and cells, miR-103 levels are reduced compared to the corresponding nontumor lung tissues, which is paralleled by increased expression of PDCD10/CCM3. In addition, higher miR-103 expression correlated with longer overall survival for these patients. PDCD10/CCM3 has also been shown to be a direct target of miR-103 both *in vitro* and *in vivo*.[130] The same mechanisms have been described for prostate cancer, with miR-103 down-regulation and PDCD10/CCM3 up-regulation associated with tumor cell proliferation and migration.[148] Finally, PDCD10/CCM3 is targeted by miR-181b in retinoblastoma cells under hypoxic conditions.[149]

Interestingly, miRNAs are not only up-stream regulators of the expression of PDCD10/CCM3, as they have also been shown to be modulated themselves by PDCD10/CCM3, thus also acting as effectors of the PDCD10/CCM3 biological activity. Three recent studies investigated miRNome alterations due to the loss of PDCD10/CCM3 in ECs. Schwefel

and colleagues (2019) performed CRISPR/Cas9 genome editing in human umbilical vein ECs, and upon inactivation of PDCD10/CCM3, they identified five miRNAs that were down-regulated (i.e., miR-335-3p, miR-217, miR-493-3p, miR-493-5p, miR-216a-3p) and one that was up-regulated (i.e., miR-139-3p).[150] These miRNAs were associated with aging and vascular development. Then, a circulating miRNome analysis on sera from PDCD10/CCM3 heterozygous mice showed that miR-3472a was strongly down-regulated compared to wild-type mice.[27] Although miR-3472a has Cand2 as its putative target, which is generally dysregulated in the transcriptomes of other CCM models, its involvement in the pathogenesis of CCM remains to be clarified. Further, a genome-wide analysis performed on three patients with cavernomas and three healthy donors revealed a set of five miRNAs that were down-regulated in the patients with cavernomas: let-7b-5p, miR-361-5p, miR-370-3p, miR-181a-2-3p, and miR-95-3p.[151] These studies have thus confirmed that dysregulation of the miRNA profile is a common feature associated with CCM.

Final remarks

The PDCD10/CCM3 protein is ubiquitously expressed and has multiple functions in the cell. Being part of the CSC, it stabilizes cell-to-cell junctions and prevents the activation of important signaling pathways which include MEKK3-MEK5-ERK5-KLF2/4, RhoA-ROCK, β-catenin and CDC42 pathways (Fig. 1). PDCD10/CCM3 is a pro-apoptotic gene and controls the cell cycle as well as senescence entrance and apoptotic response to oxidative stress, inflammation, and DNA damage (Fig. 2). It also regulates cell migration, vascular permeability, Golgi assembly and release of exocytic vesicles. PDCD10/CCM3 acts through both cell autonomous and non-autonomous mechanisms being able, therefore, to influence the behaviour of surrounding cells. This is the case of several types of cancers of the NVU, where the deletion of PDCD10/CCM3 in each of its components (EC, pericytes or astrocytes) leads to alterations of the morphology and function of the other cell types (Fig. 3).

This plethora of functions is explained in part by the multiple subcellular localizations of PDCD10/CCM3, which can be found not only within the CSC clustered at cell-to-cell junctions, but also associated with the Golgi apparatus and exocytic vesicles, and within the STRIPAK complex. In addition, the multiple roles played by PDCD10/CCM3 are explained by its different interactors and by the various intracellular signalling in which it takes part. This makes *PDCD10/CCM3* a crucial gene for correct the functioning of the cell, and therefore its homozygous loss of function is incompatible with life in experimental animal models as well as in humans. Also, haplo-insufficiency of *PDCD10/CCM3* is a life-threatening condition that can cause the most aggressive, early-onset, forms of CCM that are associated with brain tumors, scoliosis, skin lesions, and cognitive disability.[3] A case study reported a 8-month-old child suffering from CCM associated with neutropenia and thrombocytopenia, with later development of B-cell acute lymphoblastic leukemia.[152] Somatic dysregulation of PDCD10/CCM3 expression is linked to several cancers, including GBM, breast cancer, colorectal cancer, lung cancer, and others. Interestingly, both its down- and up-regulation can seriously impact on the behavior of cancer cells, which suggests that its expression levels must be finely tuned for the correct functioning of cell. In fact, the expression of PDCD10/CCM3 is differentially regulated by mi-RNAs depending on the cancer context analysed: this kind of epigenetic regulation needs further studies as it could be a powerful tool to manage PDCD10/CCM3 expression levels through the design of novel mi-RNA-based therapeutics. Also, the controversial effects of PDCD10/CCM3 dysregulation in cancers could be related to the activation of cell autonomous and non-autonomous mechanisms that can have a differential impact on cancer cells' behaviour.

This central role of PDCD10/CCM3 in physiology and pathology needs further investigation to provide deeper and more comprehensive knowledge of its functions.

This knowledge would also help to better define PDCD10/CCM3 as a 'druggable' therapeutic target, either directly or indirectly through the various regulatory miRNAs that control its expression in a disease-dependent fashion. The definition of PDCD10/CCM3 as a "druggable" therapeutic target could be of pivotal importance in GBM cancer, as the down-regulation of PDCD10/CCM3 exacerbates the phenotype and induces chemoresistance. In addition, there seems to be similarities in the functioning of PDCD10/CCM3-deficient ECs in cerebral cavernomas and GBM, i.e., these mutant ECs influence the surrounding cells increasing proliferation, migration and invasiveness and inhibiting response to apoptosis. Shedding light on the tumorigenic potential of cerebral cavernomas and on the tumour suppressive role of PDCD10/CCM3 could raise new questions and open newsworthy research directions. Hence, the possibility of targeting PDCD10/CCM3 is intriguing in different contexts, ranging from the neurovascular unit to the tumour microenvironment, although further studies are needed to identify the best method to be used. Future research directions could be the design of a gene therapy strategy or the use of new RNA-based therapies such as SINEUPs.[153–155] In this perspective, we reviewed the correlation between the structure, the function and the context where PDCD10/CCM3 acts, highlighting its multifaceted activities under both physiological and pathological conditions.

Authors contribution

MV and MM conceived, wrote and edited the manuscript; ED raised funds and edited the manuscript.

Conflict of Interests

The authors declare no competing financial interests.

Acknowledgements

The lab of ED is supported by: The Swedish Research Council (contract No. 2013-9279), the Knut and Alice Wallenberg Foundation (contract No. 2015-0030), the European Research Council (project EC-ERC-VEPC, contract 74292), Associazione Italiana per la Ricerca sul Cancro (AIRC IG 23223), AIRC 5x1000 call "Metastatic disease: the key unmet need in oncology" to the MYNERVA Project (MYeloid NEoplasms Research Venture AIRC 21267), Telethon (New insight on the pathogenesis of hereditary Cerebral Cavernous Malformation, contract GGP19202); AIFA (a multicellular randomized clinical trial on propranolol in familial Cerebral Cavernous Malformation, contract AIFA-2016-02364593). Be Brave for Life Foundation Micro Grant 2019.

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