Title

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Journal

Neurology: Genetics, 10(1)

ISSN

2376-7839

Authors

Morrison, Leslie

Liao, James

Gutierrez, Juan

et al.

Publication Date

2024-02-01

DOI

10.1212/NXG.0000000000200121

Peer reviewed

REVIEW OPEN ACCESS

Role of Rho-Associated Kinase in the Pathophysiology of Cerebral Cavernous Malformations

Cenk Ayata, MD, PhD, Helen Kim, MPH, PhD, Leslie Morrison, MD, James K. Liao, MD, Juan Gutierrez, MD, Miguel Lopez-Toledano, PhD, Enrique Carrazana, MD, Adrian L. Rabinowicz, MD, and Issam A. Awad, MD, MSc, FACS, MA

Correspondence
Dr. Awad
iawad@bsd.uchicago.edu

Neurol Genet 2024;10:e200121. doi:10.1212/NXG.0000000000200121

Abstract

Cerebral cavernous malformations (CCMs) are vascular lesions characterized by a porous endothelium. The lack of a sufficient endothelial barrier can result in microbleeds and frank intracerebral hemorrhage. A primary mechanism for lesion development is a sequence variant in at least 1 of the 3 CCM genes (*CCM1*, *CCM2*, and *CCM3*), which influence various signaling pathways that lead to the CCM phenotype. A common downstream process associated with *CCM* gene loss of function involves overactivation of RhoA and its effector Rho-associated kinase (ROCK). In this study, we review RhoA/ROCK–related mechanisms involved in CCM pathophysiology as potential therapeutic targets. Literature searches were conducted in PubMed using combinations of search terms related to RhoA/ROCK and CCMs. In endothelial cells, CCM1, CCM2, and CCM3 proteins normally associate to form the CCM protein complex, which regulates the functions of a wide variety of protein targets (e.g., MAP3K3, SMURF1, SOK-1, and ICAP-1) that directly or indirectly increase RhoA/ROCK activity. Loss of CCM complex function and increased RhoA/ROCK activity can lead to the formation of stress fibers that contribute to endothelial junction instability. Other RhoA/ROCK–mediated pathophysiologic outcomes include a shift to a senescence-associated secretory phenotype (primarily mediated by ROCK2), which is characterized by endothelial cell migration, cell cycle arrest, extracellular matrix degradation, leukocyte chemotaxis, and inflammation. ROCK represents a potential therapeutic target, and direct (fasudil, NRL-1049) and indirect (statins) ROCK inhibitors have demonstrated various levels of efficacy in reducing lesion burden in preclinical models of CCM. Current (atorvastatin) and planned (NRL-1049) clinical studies will determine the efficacy of ROCK inhibitors for CCM in humans, for which no US Food and Drug Administration–approved or EU-approved pharmacologic treatment exists.

Introduction

Cerebral cavernous malformations (CCMs) are vascular lesions of the brain,[1] characterized by a dysfunctional endothelium,[2] which can lead to microbleeds and intracerebral hemorrhage.[3,4] CCMs are relatively common, occurring in up to 1% of the population.[5] CCMs have been classified as familial or sporadic, with the sporadic form representing approximately 80% of cases with CCM.[6,7] Familial CCM is characterized by multiple lesions, whereas sporadic CCM is typically associated with a single lesion.[1] In vascular endothelial cells, Krev1 interaction trapped protein 1 (KRIT1, CCM1), CCM2, and programmed cell death protein 10 (PDCD10, CCM3) associate to form a protein (CCM) complex, and these proteins are essential for normal endothelial cell-cell junctions.[8-13] A loss of function in at least 1 of these proteins disrupts CCM-complex function, which is an underlying mechanism for lesion development.[1] The familial form of CCM is associated with germline and somatic sequence variants in *CCM1*, *CCM2*, or *CCM3* genes. Sporadic CCM results from 2 somatic sequence variants in a CCM gene (*CCM1*, *CCM2*, *CCM3*) or 1 somatic gain-of-function (GOF)

From the Neurovascular Research Unit (C.A.), Department of Radiology; Stroke Service, Department of Neurology (C.A.), Massachusetts General Hospital, Harvard Medical School, Boston; Center for Cerebrovascular Research (H.K.), Department of Anesthesia and Perioperative Care, University of California, San Francisco; University of New Mexico Health Sciences Center (L.M.), Albuquerque; University of Arizona (J.K.L.), College of Medicine, Tucson; Neurelis, Inc. (J.G., M.L.-T., E.C., A.L.R.), San Diego, CA; University of Hawaii John A. Burns School of Medicine (E.C.), Honolulu, HI; and University of Chicago Medicine and Biological Sciences (I.A.A.), Chicago, IL.

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing charge was funded by the authors.

Glossary

CCMs = cerebral cavernous malformations; **ECM** = extracellular matrix; **GOF** = gain of function; **HEG1** = heart of glass; **ICAP-1** = integrin cytoplasmic domain–associated protein–1; **KLF** = Kruppel-like factor; **LIMK** = LIM domain kinase 1; **MAP2K5** = mitogen-activated protein kinase kinase 5; **MEF** = myocyte enhancer factor; **MRLCs** = myosin regulatory light chains; **ROCK** = Rho-associated kinase; **tMCAO** = transient middle cerebral artery occlusion; **VEGF** = vascular endothelial growth factor.

sequence variant to *MAP3K3* (*MEKK3*).[1,14] Somatic sequence variants associated with sporadic CCM can develop after exposure to ionizing radiation,[1] although the underlying causes are not fully understood. In addition, *PIK3CA* somatic GOF sequence variants along with CCM LOF sequence variants have been detected in familial and sporadic CCMs[15] and can increase lesion growth and risk of hemorrhage.[16]

A common downstream process associated with *CCM* gene loss of function involves overactivation of RhoA and its effector Rho-associated kinase (ROCK). The objective of this narrative review was to describe pathologic endothelial mechanisms of CCM formation that involve RhoA/ROCK activation, including isoform-specific functions, as described in preclinical and clinical studies of CCM. ROCK inhibitors that have been used to ameliorate lesion burden in studies of CCM are also reviewed. Literature searches were conducted in PubMed using combinations of the following search terms: cerebral cavernous malformation, cavernoma, cavernous angioma, RhoA, Rho-associated protein kinase, Rho-associated kinase, Rho-associated coiled-coil containing kinase, and ROCK.

Rho-Associated Kinase

ROCK is a serine/threonine kinase and downstream effector of the GTPase RhoA.[17] In blood vessels, RhoA/ROCK can influence or stimulate contractility, migration, proliferation, differentiation, and the integrity of cell-cell junctions.[18-20] Rho-associated kinase is widely expressed across diverse tissue types; however, ROCK isoforms (ROCK1 and ROCK2) can display tissue-specific expression patterns and functions.[17,21] In endothelial cells, ROCK has been associated with the development of stress fibers, whereas ROCK2 directly influences the integrity of endothelial cell-cell junctions.[21,22] ROCK2 is the predominant isoform in the brain,[17] and increased expression or activation of ROCK2 has been associated with various neurodegenerative diseases (e.g., Alzheimer disease and Parkinson disease) and chronic cerebral ischemia.[23] Partial ablation of *Rock2* in a hemizygous CCM knockout mouse model ($Ccm3^{+/-}Rock2^{+/-}$) resulted in fewer mice with lesions compared with partial ablation of *Rock1* ($Ccm3^{+/-}Rock1^{+/-}$), suggesting that ROCK2 is a key isoform in the development of CCM lesions.[24]

Regulation of RhoA/ROCK Activity by CCM Complex Proteins

Normally, the CCM protein complex inhibits RhoA/ROCK signaling.[1,11,22,25,26] Loss of CCM1, CCM2, or CCM3 function leads to disinhibition of RhoA-dependent ROCK activity (Figure 1).[22,25,26] Protein-protein interactions have been identified between CCM2 and RhoA[27] as well as CCM1 and ROCK1/ROCK2.[12] Moreover, CCM2 associates with mitogen-activated protein kinase kinase kinase 3 (MAP3K3),[28,29] and a loss of CCM complex function leads to greater activation of MAP3K3.[1,2,28,30] Somatic *MAP3K3* GOF sequence variants can also occur independent of CCM protein function. The MAP3K3–mitogen-activated protein kinase kinase 5 (MAP2K5, MEK5)–extracellular signal–regulated kinase 5 (ERK5, MAPK7) pathway leads to activation of myocyte enhancer factor (MEF) 2A and MEF2C transcription factors that induce expression of Kruppel-like factor (KLF) 2 and KLF4 transcription factors,[28,31,32] which increase RhoA-dependent ROCK activation.[1,30]

Other proteins interact with the CCM complex to participate in MAP3K3-independent regulation of RhoA/ROCK activity. SMAD-specific E3 ubiquitin protein ligase 1 colocalizes with CCM2 and associated proteins (CCM complex),[33] where it degrades RhoA in a CCM2-dependent manner.[33,34] Localized degradation of RhoA may have physiologic relevance at sites of CCM complex localization and function.[34] Ste-20 oxidant stress response kinase 1 (SOK-1, STK25), a GCK-III serine/threonine kinase, associates with CCM3 to phosphorylate moesin, which reduces RhoA activity.[9,35] Loss of CCM3 and/or SOK-1 attenuates the moesin inhibitory action on RhoA, leading to RhoA activation.[35] CCM1, CCM2, and integrin cytoplasmic domain–associated protein–1 (ICAP-1) form a stable protein complex, which maintains β1-integrin inactivation through an ICAP-1–mediated protein interaction.[36] A loss of CCM1 and CCM2 leads to destabilization of ICAP-1, which leads to increased activation of β1 integrins and RhoA/ROCK activation.[36]

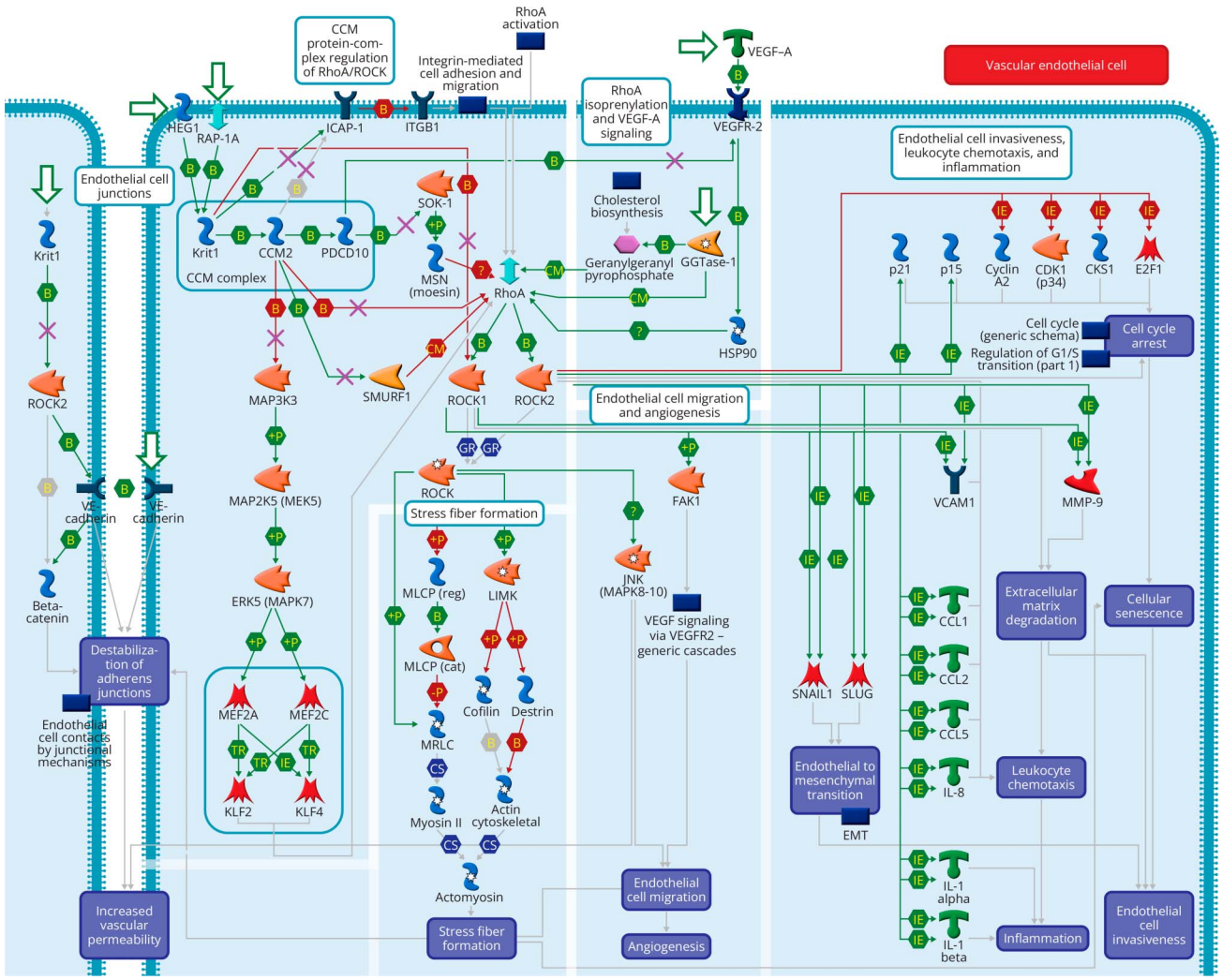
Role of ROCK in CCM Vascular Lesions

Formation of Stress Fibers

Stress fibers are composed of actin and myosin, anchored by focal adhesions to the extracellular matrix (ECM).[37,38] Stress fibers have the capacity to contract and disrupt cell-cell junctions,[37] which can increase cerebrovascular permeability and the risk of bleeding.[2]

Rho-associated kinase phosphorylates myosin regulatory light chains (MRLCs) of myosin II,[20,22,39] which leads to increased actomyosin contractility and the formation of stress fibers (Figure 1).[20,22,37,40] ROCK also phosphorylates myosin phosphatase target 1 subunit, a regulatory subunit of myosin

Figure 1 RhoA/ROCK Signaling in CCM



CCM protein complex regulation of RhoA/ROCK: Loss of function in CCM1, CCM2, or CCM3 proteins leads to activation of RhoA/ROCK. Stress fiber formation: ROCK activity increases phosphorylation status of MRLC, the formation of actomyosin, and stress fibers. RhoA isoprenylation and VEGF-A signaling: VEGF/VEGFR-2 enhances HSP90-dependent RhoA/ROCK activity, phosphorylation of JNK/FAK1, and endothelial cell migration and angiogenesis. Endothelial cell invasiveness, leukocyte chemotaxis, and inflammation: ROCK2 regulates the expression of cell cycle activators and inhibitors, leading to cell cycle arrest and senescence. RhoA/ROCK activity increases expression of SNAIL1 and SLUG to promote endothelial to mesenchymal transition and endothelial cell invasiveness, while cell adhesion proteins and chemokines are upregulated to promote leukocyte chemotaxis and inflammation. Endothelial cell junctions: Basal ROCK2 activity is required for normal endothelial intercellular junctions, and a loss of CCM1 at adherens junctions prevents ROCK2 recruitment, resulting in junctional instability. Abbreviations: B = binding; CCL = CC motif chemokine ligand; CCM = cerebral cavernous malformation; CDK1 = cyclin-dependent kinase 1; CKS1 = cyclin-dependent kinase regulatory subunit 1; CM = covalent modifications; CS = complex subunit; E2F1 = E2F transcription factor 1; ERK5 = extracellular signal–regulated protein kinase 5; FAK1 = focal adhesion kinase 1; GGTase-1 = geranylgeranyltransferase type 1; HEG1 = heart of glass; HSP90 = heat shock protein 90; ICAP-1 = integrin cytoplasmic domain–associated protein-1; IE = influence on expression; IL = interleukin; ITGB1 = β1 integrin; JNK = c-Jun N-terminal kinase; KLF2 = Kruppel-like factor 2; KLF4 = Kruppel-like factor 4; Krit1 = Krev1 interaction trapped protein 1; LIMK = LIM domain kinase 1; MAP2K5 = mitogen-activated protein kinase kinase 5; MAP3K3 = mitogen-activated protein kinase kinase kinase 3; MAPK8-10 = mitogen-activated protein kinases 8–10; MEF2A = myocyte enhancer factor 2A; MEF2C = myocyte enhancer factor 2C; MEK5 = mitogen/extracellular signal-regulated kinase kinase-5; MLCP = myosin light-chain phosphatase; MMP-9 = matrix metalloproteinase-9; MRLC = myosin regulatory light chain; PDCD10 = programmed cell death protein 10; RAP-1A = Ras-related protein Rap-1A; ROCK1 = Rho-associated kinase 1; ROCK2 = Rho-associated kinase 2; SLUG = zinc finger protein SNAI2; SMURF1 = SMAD specific E3 ubiquitin protein ligase 1; SNAIL = zinc finger protein SNAI1; SOK-1 = Ste-20 oxidant stress response kinase 1; TR = transcription regulation; VCAM1 = vascular cell adhesion molecule 1; VE-cadherin = vascular endothelial cadherin; VEGF = vascular endothelial growth factor; VEGFR-2 = vascular endothelial growth factor receptor 2. Symbols: +P, phosphorylation; -P, dephosphorylation; ?, unspecified interactions. Symbol colors: Green indicates positive/activation, red indicates negative/inhibition, and gray is unspecified. An X indicates disruption in disease. See eAppendix 1 (links.lww.com/NXG/A667) for full graphic key.

light-chain phosphatase, which reduces dephosphorylation of MRLC.[19,20,41-44] In addition, ROCK phosphorylates LIM domain kinase 1 (LIMK), which regulates cofilin-mediated and destrin-mediated actin depolymerization and filament turnover.[20,45] Phosphorylation of cofilin and destrin by ROCK-LIMK prevents cofilin-dependent and destrin-dependent actin cytoskeletal depolymerization, resulting in a greater number of actin filaments.[20,22,45] Myosin II cross-linking with actin results in the formation of actomyosin and stress fibers.[20,22]

Endothelial Cell Migration and Angiogenesis

Depending on the strength of cell adhesion, increased stress fiber formation can either reduce or promote endothelial cell migration.[20,37,46] In the presence of angiogenic factors, such as

vascular endothelial growth factor (VEGF), endothelial ROCK activation contributes to focal adhesion turnover, actin polymerization, and the development of stress fibers, leading to cell migration and angiogenesis.[46] Pathologic angiogenesis can contribute to the development and growth of vascular lesions in CCM.[1]

RhoA isoprenylation and VEGF-A/VEGF receptor 2 (VEGFR-2) signaling influences RhoA/ROCK function, which leads to endothelial cell migration during angiogenesis (Figure 1).[27,46] Cholesterol biosynthesis products, such as geranylgeranyl pyrophosphate, are required for isoprenylation and membrane localization of RhoA to exert its effects; isoprenylation is catalyzed by geranylgeranyltransferase type 1 (GGTase-1) via the transfer of the geranylgeranyl moiety from geranylgeranyl pyrophosphate to RhoA.[47-50] VEGF-A–stimulated VEGFR-2 interacts with heat shock protein 90 (HSP90) that activates RhoA/ROCK1, resulting in phosphorylation of focal adhesion kinase 1 (FAK1).[46,51,52] An interaction between CCM3 and VEGFR-2 has been described in which a loss of CCM3 attenuated VEGF-A/VEGFR-2 signaling.[53] However, other studies have either failed to detect a direct interaction between CCM3 and VEGF signaling or have detected an increase in VEGF signaling with a loss of CCM3.[54,55] This remains an area for further study.

Isoprenylation of RhoA is necessary for RhoA-dependent phosphorylation of c-Jun N-terminal kinase (JNK, MAPK8-10) and FAK1 (Figure 1).[27,47] Activated JNK and FAK1 increase vascular permeability and promote endothelial cell migration[27,46,51,52,56]

Endothelial Cell Invasiveness, Leukocyte Chemotaxis, and Inflammation

Cell senescence underlies various disease states, including cardiovascular disease and neurologic disorders.[57,58] Increases in ROCK1 and ROCK2 activity due to a loss of CCM2 function lead to reprogramming of endothelial cells into a senescence-associated secretory phenotype.[59] The phenotype is characterized by the production of factors including proinflammatory cytokines, chemokines, and matrix metalloproteinases.[59,60]

Endothelial cell reprogramming involves the following mechanisms that depend on ROCK activity.[59] A ROCK-mediated increase in stress fiber formation results in premature senescence of CCM2-deficient endothelial cells. ROCK2 is more effective than ROCK1 in upregulating the expression of cell cycle inhibitors (such as p21 and p15) and downregulating the expression of cell cycle activators (such as cyclin A2, cyclin-dependent kinase 1 [CDK1, p34], cyclin-dependent kinase regulatory subunit 1B [CKS1B], and E2F transcription factor 1 [E2F1]),[59] leading to cell cycle arrest and subsequent cellular senescence (Figure 1).[59,60,e1] ROCK1 and ROCK2 increase gene expression for proteins (zinc finger protein SNAI1 [SNAIL] and zinc finger protein SNAI2 [SLUG]) that promote endothelial to mesenchymal transition (EndMT) (Figure 1).[59,e2] EndMT may contribute to vascular lesions in CCM as well as endothelial cell invasiveness.[59,e3-e5] ROCK1 and ROCK2 upregulate gene expression associated with cell adhesion proteins (such as vascular cell adhesion molecule 1 [VCAM1]) and chemokines (such as CC motif chemokine ligand [CCL] 1, CCL2, CCL5, and interleukin [IL]–8[e6]) that are involved in leukocyte chemotaxis (Figure 1).[59,e7] ROCK2 has been shown to be the main ROCK isoform that increases leukocyte and endothelial cell chemotaxis (chemoattraction).[59] Leukocyte chemotaxis then promotes inflammation that contributes to vascular lesions in CCM.[59,e6-e8] ROCK1 and ROCK2 also increase the expression of cytokines (such as IL-1 alpha and IL-1 beta) that are involved in inflammation and are characteristic of the senescence-associated secretory phenotype.[59,60,e9] ROCK1 has been shown to be the main isoform that contributes to ECM degradation,[59] which is associated with increased activity of matrix metalloproteinases (MMPs), and ECM degradation can further promote leukocyte chemotaxis.[e10] In addition, ROCK2 can influence the expression of MMP-9,[e11] which has a role in ECM degradation.[e12] ECM degradation and cellular senescence also support the invasiveness of endothelial cells in CCM.[59]

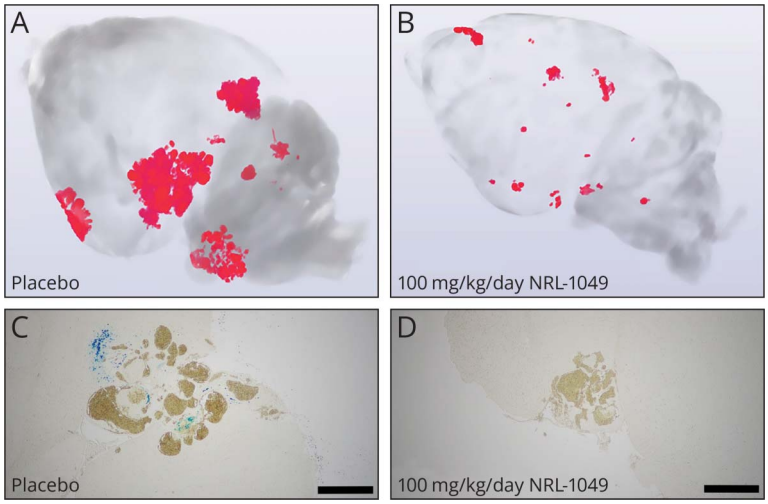
Endothelial Cell Junctions

Tight junctions and adherens junctions are specialized protein complexes that partly form interendothelial junctions and contribute to the blood-brain barrier.[e13] CCM1 binds to ROCK2 and recruits ROCK2 to the vascular endothelial (VE)–cadherin/beta-catenin complex of adherens junctions, where ROCK2 interacts with vascular endothelial cadherin (VE-cadherin) and beta-catenin, promoting VE-cadherin-VE-cadherin interendothelial junctions (Figure 1). Loss of CCM1 may prevent ROCK2 recruitment to the VE-cadherin/beta-catenin complex, attenuating stabilization of adherens junctions and increasing vascular permeability.[12] The formation of stress fibers leads to increased focal adhesions and destabilization of adherens junctions, which further increases vascular permeability.[37] In turn, this increased vascular permeability is associated with bleeding, a hallmark of CCM disease.[1] Heart of glass (HEG1), a transmembrane protein, binds to CCM1 and recruits the CCM complex to the cell membrane to control junctional stability.[11,e14,e15] Ras-related protein Rap-1A (RAP-1A) also binds to CCM1 and relocalizes CCM1 from microtubules to the cell membrane to stabilize interendothelial junctions by inhibiting the RhoA/ROCK signaling pathway.[e16,e17] Thus, CCM1 sequence variants may disrupt HEG1-mediated and RAP-1A–mediated stability of interendothelial junctions.

ROCK as a Therapeutic Target

Rho-associated kinase inhibition to reduce lesion burden (e.g., size, number) has been tested using a specific but isoform-nonselective ROCK inhibitor (fasudil); statins (simvastatin, atorvastatin), which have pleiotropic effects that include ROCK inhibition; and a selective ROCK2 inhibitor (NRL-1049, formerly BA-1049).[24,e18-e20] In heterozygous CCM1-knockout mice ($Ccm1^{+/-}Msh2^{-/-}$), fasudil treatment (100 mg/kg/d) that began at weaning and continued until

Figure 2 Effect of Rho-Associated Kinase (ROCK) Inhibition on Cavernous Cerebral Malformation Lesion and Bleeding



(A–B): Representative microcomputed tomography images illustrating the effect of treatment with placebo (A) or NRL-1049 (B), a selective ROCK2 inhibitor, on CCM lesions in *Ccm3*$^{+/-}$*Trp53*$^{-/-}$ mice. (C–D): Representative Perls' Prussian blue staining, which detects nonheme iron, illustrating the effect of placebo (C) or NRL-1049 (D) on lesional bleeding. Bar, 500 μm. Adapted with permission from McKerracher, et al.[24]

4–5 months of age reduced the prevalence of CCM lesions compared with placebo, with greater effects noted on the prevalence of multicavernous stage 2 lesions.[e18,e20] Lesion size was smaller with fasudil treatment, and there were lower rates of inflammation and endothelial cell proliferation.[e20] In heterozygous CCM3-knockout mice (*Ccm3*$^{+/-}$*Trp53*$^{-/-}$), lesion volume was lower with fasudil treatment (100 mg/kg/d) compared with placebo.[e19] In addition, lesional bleeding was lower in *Ccm1*$^{+/-}$*Msh2*$^{-/-}$ and *Ccm3*$^{+/-}$*Trp53*$^{-/-}$ mice treated with fasudil.[e18-e20] Fasudil was not associated with a negative influence on survival in *Ccm1*$^{+/-}$*Msh2*$^{-/-}$, *Ccm2*$^{+/-}$*Trp53*$^{-/-}$, or *Ccm3*$^{+/-}$*Trp53*$^{-/-}$ mice, indicating that the dosage used was well tolerated in these models. Fasudil is approved in Japan for treatment of cerebral vasospasm with intracranial hemorrhage;[e21] however, it is not clinically approved for any indication in the United States.

Atorvastatin and simvastatin have been examined for their potential to reduce lesion burden in animal models of CCM. In *Ccm3*$^{+/-}$*Trp53*$^{-/-}$ and *Ccm3*$^{+/-}$*Msh2*$^{-/-}$ mice, atorvastatin (80 mg/kg/d, treated from weaning to age 5 months) attenuated lesion volume and bleeding compared with placebo.[e19] Simvastatin (40 mg/kg/d, treated from weaning until age 4–5 months) did not decrease lesion number or volume in *Ccm1*$^{+/-}$*Msh2*$^{-/-}$, *Ccm2*$^{+/-}$*Trp53*$^{-/-}$, or *Ccm3*$^{+/-}$(in *Trp53*$^{-/-}$ and *Msh2*$^{-/-}$ sensitized backgrounds) mice, although it was effective at reducing lesion bleeding.[e18,e19] A phase 1/2 randomized, double-blind, placebo-controlled trial (NCT02603328) is currently being conducted to investigate atorvastatin (40–80 mg/d) in patients with CCM who experienced symptomatic bleeding within 1 year of enrollment.[e22] A randomized controlled pilot study that examined simvastatin treatment (20–40 mg/d) in patients with familial CCM did not report a difference in CCM permeability (percentage change between first [baseline] and second [3 months after treatment] dynamic contrast-enhanced perfusion magnetic resonance images, with and without normalizing to white matter) compared with the control arm.[e23]

NRL-1049 is a novel selective inhibitor of ROCK2, the predominant isoform in the CNS and a key isoform in the development of CCM lesions.[24] The effectiveness of NRL-1049 in reducing lesion burden and bleeding was investigated in hemizygous CCM1 (*Ccm1*$^{+/-}$*Msh2*$^{-/-}$) and CCM3 (*Ccm3*$^{+/-}$*Trp53*$^{-/-}$) knockout mice. NRL-1049 (100 mg/kg/d) or placebo treatment was initiated at weaning and continued until 3 (*Ccm3*$^{+/-}$*Trp53*$^{-/-}$) or 4 (*Ccm1*$^{+/-}$*Msh2*$^{-/-}$) months of age. In both *Ccm1*$^{+/-}$*Msh2*$^{-/-}$ and *Ccm3*$^{+/-}$*Trp53*$^{-/-}$ knockout mice, lesion volume was reduced with NRL-1049 compared with placebo (Figure 2, A and B). In *Ccm3*$^{+/-}$*Trp53*$^{-/-}$ mice, the mutant model with greater lesion burden, NRL-1049 also reduced lesion volume at the 10-mg/kg/d dose level. The effect of NRL-1049 on lesion volume was most conspicuous on multicavernous stage 2 lesions. Significant attenuation of lesional bleeding (Figure 2, C and D) was detected at all doses tested (1, 10, and 100 mg/kg/d) compared with placebo. Survival in these animal models was not influenced by treatment.[24] An investigational new drug application with the US Food and Drug Administration was filed for NRL-1049,[e24] and a clinical trial to examine the safety, dosing tolerability, and pharmacokinetics in healthy volunteers began in 2023.[e25]

In all, these data suggest that ROCK inhibition may be an effective strategy to reduce CCM lesion burden. In addition, ROCK inhibitors, such as fasudil and NRL-1049, have reduced lesion burden in multiple CCM genotypes. Although different ROCK pathways have been associated with specific CCM proteins in preclinical studies, the common downstream RhoA/ROCK effect is significant and commensurate with the severity of disease irrespective of the causative CCM protein.

ROCK Isoforms and Vascular Dysfunction

Rho-associated kinase 2 has been characterized as the primary ROCK isoform that underlies vascular dysfunction (contractility, morphology) in murine models.[e26,e27] In a preclinical study, pharmacologically induced changes in vascular stiffness and morphology were examined in *Rock1*$^{+/-}$ and *Rock2*$^{+/-}$ mice.[e26] Compared with wild-type control mice, increases in blood pressure as well as vascular stiffening and remodeling after a 4-week treatment with angiotensin II (500 ng/kg/min) plus L-N$^{\omega}$-nitroarginine methyl ester (L-NAME, 0.5 g/L) were attenuated more in *Rock2*$^{+/-}$ than *Rock1*$^{+/-}$ mice. Treatment-mediated increases in collagen fibers and hypertrophy of the aorta were decreased in *Rock2*$^{+/-}$ mice, whereas elastic fibers were preserved.[e26] In a separate study, the role of ROCK2 in neuroprotection was evaluated in a model of cerebral ischemia (transient middle cerebral artery occlusion [tMCAO]).[e27] In brain and heart endothelial cells isolated from endothelial-specific *Rock2*$^{-/-}$ and/or constitutive *Rock2*$^{+/-}$ mice, endothelial nitric oxide synthase expression and nitric oxide production were greater compared with control mice following tMCAO. Similarly, endothelium-dependent relaxation of the aorta was also greater in *Rock2*$^{+/-}$ mice compared with wild-type control.[e27]

Rho-associated kinase 1 and ROCK2 are essential for normal development; however, ablation of these isoforms yields different phenotypes, underscoring isoform-specific functions of ROCK. Homozygous ROCK1 knockout mice (*Rock1*$^{-/-}$) are born with ventral wall deformities (omphalocele) and eyelid dysfunction (eyes open at birth), and most die shortly after birth.[e28] By contrast, most *Rock2*$^{-/-}$ mice die in utero likely because of vascular dysfunction (e.g., thrombus formation) in the labyrinth layer of the placenta.[e29] Hemorrhage of the hind limb has also been observed in *Rock2*$^{-/-}$ embryos.[e29] In this review, mechanistic studies that examined ROCK in CCM used mammalian and nonmammalian models as well as various cell lines. In some cases, ROCK1 and ROCK2 were characterized in specific signaling pathways of CCM; as such, both ROCK1[12] and ROCK2[24,e18] have been considered as potential therapeutic targets. However, ROCK2 is the primary isoform expressed in human brain,[17] and ROCK2 ablation leads to a greater reduction in lesion burden of CCM knockout mice.[24] In addition, ROCK2 inhibition avoids toxicities (e.g., abnormal hepatic function, intracranial hemorrhage, and hypotension) associated with nonselective ROCK inhibition.[e30] Taken together, selective ROCK2 inhibition may hold greater therapeutic value for vascular diseases, such as CCM.

CCM: A Paradigm Disease

The pathophysiology of CCM shares common mechanisms with other disease states and with aging. Observations from studies of pharmacologic treatments for CCM may serve as proof of concept for future studies in other therapeutic areas. In a neuronal injury model (optic nerve crush), knockdown of ROCK2 reduced cell death and axonal degeneration and increased axon outgrowth.[e31] The degree of axon outgrowth rescued with ROCK2 knockdown was similar to previous reports using nonselective ROCK inhibitors, suggesting that ROCK2 is the primary ROCK isoform involved.[e31] ROCK mechanisms are involved in eye diseases and disorders, such as glaucoma, Fuchs' dystrophy, and diabetic retinopathy.[e32] ROCK-mediated mechanisms that lead to increases in intraocular pressure, endothelial apoptosis, and leukocyte adhesion, as well as reductions in endothelial proliferation, might be attenuated with ROCK inhibition.[e32] In addition, ROCK inhibition attenuated dopaminergic cell loss in a mouse model of Parkinson disease and preserved dopaminergic nerve terminals in culture.[e33] In the context of Alzheimer disease, ROCK inhibition has demonstrated effectiveness to attenuate Aβ levels, tau accumulation/phosphorylation, dendritic spine loss, and inflammatory responses.[50] In a mouse model of amyotrophic lateral sclerosis, ROCK inhibition maintained neuromuscular junctions, partly through reductions in microgliosis and proinflammatory cytokines/chemokines.[50]

In a transcriptomic analysis of brain tissue from patients with CCM, 320 genes (inflammation and extracellular matrix pathways) common to aging and CCM were dysregulated.[e34] Plasma levels of C-reactive protein (CRP) and angiopoietin 2 were higher with age, independent of CCM status (old non-CCM [50–79 years] vs young non-CCM [18–49 years]). Young patients with CCM (young sporadic CCM or young familial CCM) had higher levels of CRP and angiopoietin 2 compared with young patients without CCM (young non-CCM). Differences in plasma VEGF levels according to age and CCM mirrored those described for CRP and angiopoietin 2 with an exception for young sporadic CCM, which had VEGF levels similar to young non-CCM. Brain white matter permeability was greater with age and in those with familial CCM, whereas total iron deposition (bleeding) in frontal, parietal, and temporal lobes was elevated with age.[e34]

The pathophysiology of CCM is complex but not exclusive, with similar pathologic mechanisms (e.g., involving ROCK) described across various disease states and with aging. Therefore, studies of pharmacologic treatments of CCM could lay the foundation for future studies in other therapeutic areas.

Conclusions

Overactivation of RhoA-ROCK signaling is a significant mechanism that underlies the development of CCMs. Cellular signaling and function in CCM is dynamic and complex, and ROCK isoforms exhibit varying degrees of control on pathologic processes that contribute to lesion burden and bleeding. Specific inhibition of ROCK isoforms could be an effective treatment strategy for CCMs, addressing an important and currently unmet need for pharmacologic treatment.

Acknowledgment

Editorial support was provided by Kirk W. Evanson, PhD, of The Curry Rockefeller Group, LLC (Tarrytown, NY), and was funded by Neurelis, Inc. (San Diego, CA).

Study Funding

This manuscript was funded by Neurelis, Inc. (San Diego, CA).

Disclosure

C. Ayata serves on the scientific advisory board of Neurelis, Inc.; receives research support from the NINDS, the American Heart Association, and the Leducq Foundation; reports sponsored research agreements with Takeda Pharmaceuticals, Praxis Precision Medicines, and Neurelis, Inc.; and other research support from Hovid Berhad. H. Kim is a consultant for Recursion Pharmaceuticals; serves on the DSMB for Neurelis, Inc.; and receives research support from the NINDS. L. Morrison is a consultant for Neurelis, Inc. J.K. Liao is a consultant for Esperion. J. Gutierrez and M. Lopez-Toledano are employees of and have received stock options from Neurelis, Inc. E. Carrazana is an employee of and has received stock and stock options from Neurelis, Inc. A.L. Rabinowicz is an employee of and has received stock options from Neurelis, Inc. I.A. Awad is a consultant to Neurelis, Inc.; is chairman (unpaid) of the scientific advisory board for the Alliance to Cure Cavernous Malformation; and receives research support from the NINDS. Go to Neurology.org/NG for full disclosures.

Publication History

Received by *Neurology: Genetics* August 31, 2023. Accepted in final form November 20, 2023. Submitted and externally peer reviewed. The handling editor was Editor Stefan M. Pulst, MD, Dr med, FAAN.

Appendix Authors

Name	Location	Contribution
Cenk Ayata, MD, PhD	Neurovascular Research Unit, Department of Radiology; Stroke Service, Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA	Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data
Helen Kim, MPH, PhD	Center for Cerebrovascular Research, Department of Anesthesia and Perioperative Care, University of California, San Francisco, CA	Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data
Leslie Morrison, MD	University of New Mexico Health Sciences Center, Albuquerque, NM	Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data
James K. Liao, MD	University of Arizona, College of Medicine, Tucson, AZ	Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data

Appendix *(continued)*

Name	Location	Contribution
Juan Gutierrez, MD	Neurelis, Inc., San Diego, CA	Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data
Miguel Lopez-Toledano, PhD	Neurelis, Inc., San Diego, CA	Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data
Enrique Carrazana, MD	Neurelis, Inc., San Diego, CA; University of Hawaii John A. Burns School of Medicine, Honolulu, HI	Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data
Adrian L. Rabinowicz, MD	Neurelis, Inc., San Diego, CA	Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data
Issam A. Awad, MD, MSc, FACS, MA	University of Chicago Medicine and Biological Sciences, Chicago, IL	Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data

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