JEM Journal of Experimental Medicine

INSIGHTS

Beyond genes and transcription factors: A potential mechanism for the pathogenesis of cerebral cavernous malformations

William A. Muller

In this issue of *JEM*, Hong et al. (https://doi.org/10.1084/jem.20200140) identify a major step in the pathogenesis of cerebral cavernous malformations (CCMs), which at the same time offers insight into potential therapy for this disease.

Cerebral cavernous malformations (CCMs) are vascular lesions that develop in the white matter of the brain, producing dilated, blood-filled spaces up to several centimeters in diameter (Cavalcanti et al., 2012; Draheim et al., 2014). They appear to arise by proliferation of postcapillary venule endothelial cells expanding into tortuous, dilated, thin-walled, blood-filled channels lacking mural cells or elastic tissue, forming "mulberry-like" lesions (Cavalcanti et al., 2012; Draheim et al., 2014). These lesions are prone to rupture and often cause seizures. The familial type, accounting for ∼20% of the cases, is inherited in an autosomal dominant pattern and produces numerous lesions in the same individual. It is passed as a heterozygous mutation requiring a second hit later in life (Cavalcanti et al., 2012). Approximately 80% of the cases occur sporadically, generally later in life and with a single lesion, often discovered incidentally. However, these too can cause symptoms depending on their location within the brain. Compared with other congenital lesions, CCMs are relatively common (0.1–0.5% of live births; Cavalcanti et al., 2012; Draheim et al., 2014); however, compared with other vascular diseases, such as atherosclerosis, hemangiomas, and vasculitis, they are rare. CCMs may have received a disproportionate degree of attention in major journals because what we are learning about them is shedding light on normal vascular development.

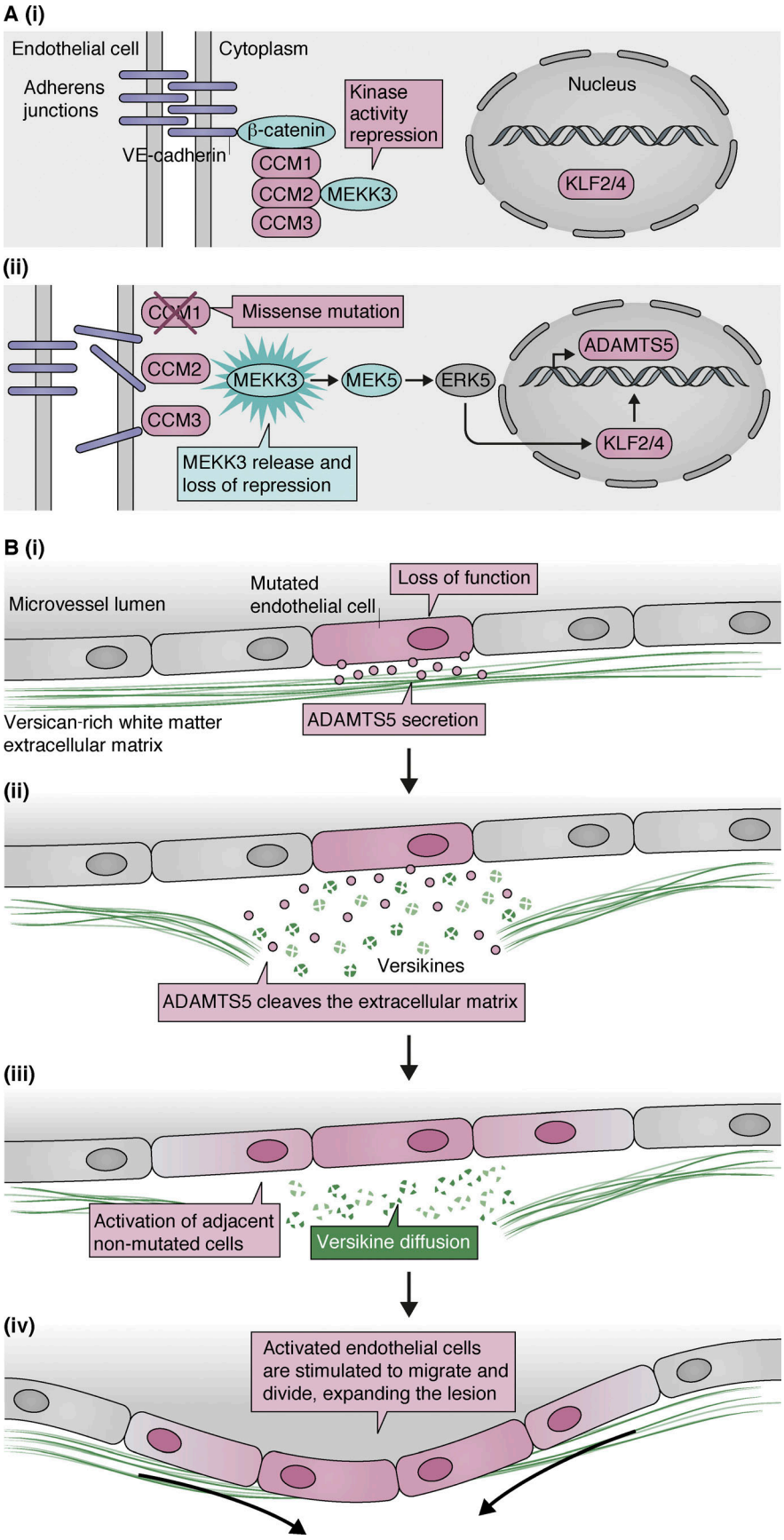
In virtually all of the familial type and the vast majority of the sporadic type, CCMs result from loss-of-function mutations in one of three genes: *CCM1/KRIT1*, *CCM2*, or *CCM3/PDCD10* (Boulday et al., 2011; Cavalcanti et al., 2012; Chan et al., 2011). The protein products of these genes, CCM1, CCM2, and CCM3, are scaffolding molecules normally involved in a variety of signaling pathways that influence junctional integrity, cell polarization and morphology, proliferation, and apoptosis (Draheim et al., 2014; Stockton et al., 2010). Due to the multiple roles of these three proteins, the pathogenesis of CCMs is not completely understood. One major clue derives from the fact that these molecules can form a ternary complex that, when disrupted by any one of these mutations, leads to a similar vascular phenotype (Cavalcanti et al., 2012; Draheim et al., 2014; Zhou et al., 2016). In previous studies, the Kahn lab and others have demonstrated that activation of the MEKK3 signaling pathway eventually leads to activation of Krüppel-like transcription factors 2 and 4 (KLF2/4; Parmar et al., 2006; Zhou et al., 2015; Zhou et al., 2016), which is required for lesion formation (see figure). Only CCM2 directly binds the endothelial cell–specific mitogen-activated protein kinase kinase kinase 3 (MEKK3/MAP3K3), hindering its activation (Cullere et al., 2015). Yet MEKK3 is abnormally activated in lesions resulting from mutation in any of the *CCM* genes (see figure).

Insights from William A. Muller.

The pathogenesis of CCMs is even more complex. While lesion initiation requires the loss-of-function mutation in affected endothelial cells, growth of the lesions involves a cell nonautonomous effect on neighboring nonmutated endothelial cells, which are incorporated into the lesion (Detter et al., 2018; Malinverno et al., 2019). Thus, while we have made significant progress understanding the genes and signaling pathways underlying CCM formation, the downstream targets of KLF2/4 and the mechanism(s) that account for the cell nonautonomous effects of the genetic defects were not known.

Northwestern University Feinberg School of Medicine, Chicago, IL.

William A. Muller: wamuller@northwestern.edu.



Potential mechanism to explain the non–cell autonomous growth of CCMs. (A) Initiation of CCM lesions. (A i) In normal endothelial cells, CCM1, CCM2, and CCM3 form a complex that is often in association with adherens junctions due to interactions between CCM1 and β-catenin, which in turn is bound to the cytoplasmic tail of VE-cadherin (purple bars). The CCM complex binds MEKK3 and represses its kinase activity. (A ii) A missense mutation in CCM1 (shown), CCM2, or CCM3 that impairs their ability to form a ternary complex results in release and loss of repression of MEKK3, initiating a kinase cascade that results in activation of the transcription factors KLF2 and -4 and expression of ADAMTS5. (B) Non–cell autonomous growth of CCM lesions. (B i) In an involved microvessel, an endothelial cell carrying a loss-of-function mutation in one of the CCM genes (pink cell) secretes inappropriate ADAMTS5 into the versican-rich extracellular matrix of the adjacent white matter. (B ii) ADAMTS5 cleaves the extracellular matrix, producing bioactive fragments of versican (versikines, green particles). (B iii) As the versikines diffuse away from the mutant cell, they activate adjacent nonmutated endothelial cells in the vicinity. (B iv) The activated endothelial cells are stimulated to migrate and divide, expanding the lesion as they do so.

Much of what we know about the molecular basis of CCMs comes from work in zebrafish and mice with genetically induced absence of one of the *CCM* genes. Congenital knockout of these genes is lethal due to defects on the developing heart. Thus, the development of endothelial cell–specific inducible knockout mice was instrumental to this field. Nonetheless, knowledge gained by this group from studies in the developing mouse heart were key to the research described in this paper. In this work, the authors follow up on observations they previously made studying cardiac development in congenital *CCM1* knockout mice in which MEKK3 activation led to increased expression of the protease ADAMTS5, which prematurely cleaved versican in the cardiac jelly to impair normal cardiogenesis (Zhou et al., 2015). Since the white matter of the brain is rich in versican, they investigated whether a similar mechanism could be at work in the formation of CCMs.

Using a series of genetically altered mice, they show that ADAMTS5 is expressed and functional in mice with CCM1 deletion, that endothelial-specific ADAMTS5 overexpression is synergistic with CCM1 loss, and that endothelial-specific deletion of ADAMTS5 reduces CCM formation (Zhou et al., 2015). Proteolysis of versican could be pathogenic either by removing versican from its role as a matrix protein and/or the generation of bioactive fragments (versikines; Binder et al., 2017; Hope et al., 2016). To distinguish these possibilities, they performed an intellectually satisfying experiment comparing endothelial cell–specific inducible *CCM1*-deficient mice expressing wild-type or reduced levels of versican. If CCMs were due to loss of versican in the matrix, the lesions in the mice with reduced versican expression should be worse. Conversely, if CCMs were promoted by versican proteolytic fragments, the mice expressing lower levels of versican should be protected. The latter was clearly the case. They show that ADAMTS5 cleavage products of versican are present in the white matter immediately surrounding the lesions in vivo and that they are capable of inducing sprouting of normal endothelial cells in vitro (Hong et al., 2020). This provides a plausible explanation for the cell nonautonomous effects of *CCM1* gene mutation on wild-type neighboring endothelial cells in the developing mouse brain (see figure).

Overexpression of ADAMTS5 by endothelial cells also leads to loss of pericyte coverage on white matter venules, another feature of CCM that could be downstream of the MEKK3–KLF2/4 signaling axis (Hong et al., 2020). However, whether versikines, proteolytic fragments of some other ADAMTS5 substrate, or a completely unrelated mechanism are responsible is not clear. As the authors point out, since ADAMTS5 overexpression in the absence of KLF2/4 activation does not reproduce the full lesional effect, there are likely other yet-to-be discovered targets of KLF2/4 that are relevant to the pathogenesis of CCMs.

The identification of downstream effectors involved in the pathogenesis of CCM is important for more than academic reasons. This study identifies a potential therapeutic target for treating CCMs, particularly the sporadic type, which represent the vast majority of these lesions and develop later in life. As mentioned earlier, while most of these are asymptomatic incidental findings, they can bleed and cause symptoms such as headaches and seizures. While MEKK3, KLF2, and KLF4 are much too ubiquitous to serve as drug targets, selective inhibition of a specific protease is reasonable. Due to the leakiness of the CCM lesions, the usual obstacle to delivering drugs to the brain—the blood–brain barrier—is not a problem. In fact, it is an asset. ADAMTS5, secreted by endothelial cells carrying a mutant *CCM* gene, would leak into the local white matter surrounding the lesions, but so would any inhibitor. This drug would likely be prevented from crossing the healthy blood–brain barrier in unaffected areas, enhancing specificity.

The first step is to identify whether there is a similar role for ADAMTS5 in human CCM. This is not a trivial question, since there is still debate over whether ADAMTS5 or ADAMTS4 (ADAMTS4 had no role in the mouse CCM model; Hong et al., 2020) is the critical matrix metalloprotease in osteoarthritis, a disease where proteolysis of aggrecan, another ADAMTS substrate, is clearly involved in the pathogenesis (Dancevic and McCulloch, 2014).

However, assuming that a similar role for this family of proteases is relevant for human CCM, small molecule inhibitors of ADAMTS4 and ADAMTS5 are already in clinical trials for osteoarthritis (Dancevic and McCulloch, 2014). AGG-523, a small molecule inhibitor of ADAMTS4 and -5, is in clinical trials (trial no. NCT00454298). GLPG1972, a small molecule inhibitor of ADAMTS5, is in clinical trials for osteoarthritis scheduled to finish at the end of 2020 (trial no. NCT03595618). It is possible that even if these compounds and others are not adequate to treat severe osteoarthritis, if they prove safe and specific enough, they could find a role in treatment of CCM. Future basic research into this fascinating condition will provide a fuller understanding of normal vasculogenesis and vascular homeostasis. That knowledge will certainly translate into therapeutic options for a condition where the only therapeutic option currently is surgery.

References

Binder, M.J., et al. 2017. *Cancer Lett.* https://doi.org/10.1016/j.canlet.2016.11.001

Boulday, G., et al. 2011. *J. Exp. Med.* https://doi.org/10.1084/jem.20110571

Cavalcanti, D.D., et al. 2012. *J. Neurosurg.* https://doi.org/10.3171/2011.8.JNS101241

Chan, A.C., et al. 2011. *J. Clin. Invest.* https://doi.org/10.1172/JCI44393

Cullere, X., et al. 2015. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.1510495112

Dancevic, C.M., and D.R. McCulloch. 2014. *Arthritis Res. Ther.* https://doi.org/10.1186/s13075-014-0429-9

Detter, M.R., et al. 2018. *Circ. Res.* https://doi.org/10.1161/CIRCRESAHA.118.313970

Draheim, K.M., et al. 2014. *J. Cell Sci.* https://doi.org/10.1242/jcs.138388

Hong, C.C., et al. 2020. *J. Exp. Med.* https://doi.org/10.1084/jem.20200140

Hope, C., et al. 2016. *Blood.* https://doi.org/10.1182/blood-2016-03-705780

Malinverno, M., et al. 2019. *Nat. Commun.* https://doi.org/10.1038/s41467-019-10707-x

Parmar, K.M., et al. 2006. *J. Clin. Invest.* https://doi.org/10.1172/JCI24787

Stockton, R.A., et al. 2010. *J. Exp. Med.* https://doi.org/10.1084/jem.20091258

Zhou, Z., et al. 2015. *Dev. Cell.* https://doi.org/10.1016/j.devcel.2014.12.009

Zhou, Z., et al. 2016. *Nature.* https://doi.org/10.1038/nature17178