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**The role of CCM1 loss-of-function induced endothelial-to-mesenchymal transition
in the development of cavernous malformations**

Jian Guan, MD, and William T. Couldwell, MD, PhD

Department of Neurosurgery, Clinical Neurosciences Center, University of Utah, Salt
Lake City, Utah

Corresponding author: William T. Couldwell, MD, PhD

Department of Neurosurgery

Clinical Neurosciences Center

University of Utah

175 N. Medical Drive East

Salt Lake City, UT 84132

Phone: 801-581-6908

Fax: 801-581-4138

Email: neuropub@hsc.utah.edu

Cerebral cavernous malformations (CCM) occur in two variants: sporadic and familial. Mutations in three genes—CCM1, CCM2, and CCM3—play a role in both subtypes, with mouse models showing the development of multiple cavernous malformations in animals with loss of function in any of these three genes. Identification of these genes has already allowed for improved screening of family members at risk for familial cavernous malformations, but deeper knowledge about the underlying physiology of these lesions could open up new avenues for treatment.

A recent paper by Maddaluno et al. examines the role of endothelial-to-mesenchymal transition (EndMT) in the development of CCMs (1). EndMT is a set of irregularities in which endothelial tissues begin to exhibit mesenchymal tissue characteristics such as loss of cellular polarity, increased proliferative and migratory activity, and junctional disorganization. Genetic abnormalities leading to EndMT have already been implicated in a number of conditions, and the authors sought to examine the role of loss-of-function mutations of CCM1 in CCM pathology.

The authors bred mice with an endothelial-specific, tamoxifen-inducible CCM1 loss-of-function mutation. These mice subsequently developed multiple central nervous system vascular malformations that shared many characteristics with human CCMs. Maddaluno et al. examined the endothelial cells of these lesions, which were found to have significantly elevated mesenchymal markers such as SLUG, inhibitor of DNA binding 1, and α -smooth muscle actin. The abnormal cells also had significantly elevated N-cadherin and disorganized VE-cadherin compared with nearby normal vascular structures.

To investigate why CCM1 loss-of-function induces EndMT, the authors began by examining the TGF- β pathway—a known inducer of EndMT. They found that phosphorylation of SMAD1 and SMAD3—two mediators of TGF- β signaling—was dramatically increased in CCM1 knockout mice. In addition, inhibition of the TGF- β pathway using LY-364947 (a TGF- β receptor and phosphorylated SMAD signaling inhibitor) inhibited EndMT, while further inhibition using a combination of LY-364947 and SB-431542 (a second phosphorylated pSMAD inhibitor) actually decreased number and size of vascular lesions (Figure 1). These data, combined with the observation that animals with CCM1 loss of function did not have inherently higher TGF- β levels or more TGF- β receptors, suggested that an increased sensitivity to TGF- β is one of the effects of CCM1 mutation.

Gene products involved in the TGF- β signaling pathway were tested to identify what could mediate this increased sensitivity to TGF- β . The factor that appeared most significantly elevated in abnormal endothelial cells was bone morphogenic protein 6 (BMP6), a protein that facilitates SMAD phosphorylation. Supporting the role of BMP6 in EndMT, Maddaluno et al. found that EndMT markers were significantly decreased in cells where BMP signaling was inhibited using the BMP inhibitor DMH1 and in animals who had BMP loss-of-function mutations. Previous studies had shown that CCM1 acts as a Notch signaling pathway activator, one of the downstream effects of which is inhibition of BMP6 expression. The authors therefore concluded that CCM1 loss of function leads to inhibition of Notch signaling, which allows for increased BMP6 activity leading to increased sensitivity to TGF- β and ultimately EndMT.

Although this study was conducted using a mouse model, Maddaluno et al. observed that lesions obtained from human patients with CCM1 and CCM2 mutations demonstrated similar pSMAD3 upregulation, increased N-cadherin expressivity, and VE-cadherin disorganization. They believe that further elucidation of mechanisms of EndMT in cavernous malformations could lead to future therapeutic targets and a better understanding of the development and growth of CCMs. Such innovations could greatly enhance our ability to manage CCMs, especially those in high-risk surgical locations or in patients who are poor surgical candidates.

References

1. Maddaluno L, Rudini N, Cuttano R, Bravi L, Giampietro C, Corada M, Ferrarini L, Orsenigo F, Papa E, Boulday G, Tournier-Lasserre E, Chapon F, Richichi C, Retta SF, Lampugnani MG, Dejana E: EndMT contributes to the onset and progression of cerebral cavernous malformations. Nature advance online publication, 2013.

Figure 1: TGF- β signaling inhibition reduces number and size of lesions and vessel leakage. a, Wild-type, iCCM1 vehicle-treated and LY-364947/SB-431542-treated mouse brains after dissection (left) and quantification of number and size of lesions (right) ($n = 4$ in each group from three different litters). b, Whole brains photographed after fluorescent cadaverine injection (left) and quantification of the recovered fluorescence (right). Data are mean \pm s.d. c, Confocal microscopy of astrocytes (GFAP staining) in CCM lesions (dotted areas) of iCCM1 vehicle- and LY-364947/SB-431542-treated animals.

Arrowheads denote normal astrocyte coverage in a LY-364947/SB-431542-treated mouse (red magnification). Arrows denote astrocyte detachment from a vascular lesion of a vehicle treated mouse (yellow magnification). Data are mean \pm s.d. Scale bars, 500 μ m (a, b); 60 μ m (c); 20 μ m (magnifications in c). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(Figure reproduced with permission from Maddaluno L, Rudini N, Cuttano R, Bravi L, Giampietro C, Corada M, Ferrarini L, Orsenigo F, Papa E, Boulday G, Tournier-Lasserre E, Chapon F, Richichi C, Retta SF, Lampugnani MG, Dejana E: EndMT contributes to the onset and progression of cerebral cavernous malformations. Nature advance online publication, 2013 (1).)