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EDITORIAL COMMENT

CCM Function in the Heart

Working From Outside-In Rather Than Inside-Out*

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As its name implies, the cerebral cavernous malformation (CCM) complex has been investigated almost exclusively from the perspective of its central role in CCM disease. CCMs are vascular malformations that arise specifically in the central nervous system, where their fragility often leads to hemorrhage and stroke. Human and mouse genetic studies performed over the past 15 years have identified mutations in 3 adaptor proteins that form a single complex, KRIT1 (aka CCM1), CCM2, and PDCD10 (aka CCM3), as the molecular and genetic basis of this disease. A convincing body of mouse and human data support a model in which the disease arises caused by loss of CCM complex function in brain vascular endothelial cells. The CCM complex negatively regulates signaling through the MEKK3 mitogen activated protein kinase and the KLF2 and KLF4 transcription factors. Loss of CCM function results in excess KLF2/4 activity and PI3K-mTOR signaling that drive cell proliferation, and can synergize with PI3K gain of function mutations to drive malformation.[1]

Although CCMs arise because of loss of complex function in endothelial cells, the CCM genes are broadly expressed. Thus, the complex may serve functions outside the vasculature and in cell types other than endothelial cells. To date, few studies have addressed the non-endothelial functions of the CCM complex, a fact that limits our broader understanding of this important pathway. Studies in other cell types are also likely to advance our understanding of CCM disease. In a study published in this issue of *JACC: Basic to Translational Science,* Wang et al[2] address this hole in the investigation of CCM biology by using genetic approaches in mice to define new CCM roles in the epicardium. Their findings provide new insight into both the known developmental roles for CCM function in heart development and previously unknown roles in the postnatal heart.

The authors use the mesothelial cell-specific WT1-Cre to delete CCM2, an essential member of the CCM complex that binds KRIT1, PDCD10, as well as the target effector MEKK3 kinase in endothelial cells. WT1-Cre mediated loss, which eliminates CCM2 in epicardial cells and not in endothelial or myocardial cells in the heart, and confers mid-late gestation lethality associated with myocardial thinning and epicardial cell defects in proliferation and migration over the surface of the heart. This cell nonautonomous effect is consistent with known roles of the epicardium during cardiac development, during which epicardial cells secrete growth factors and matrix proteins required for, and are a source of noncardiomyocytes in the heart such as fibroblasts and smooth muscle cells. The authors do not delve deeply into how the deficiency of epicardial cells affects the growing heart, but they demonstrate significant reductions in markers of fibroblasts and mesenchymal cells (Pdgfra, Desmin) as well as smooth muscle cells (Pdgfrb and Acta2). Thus, it appears that the effect on myocardial function can be attributed to both failure to generate critical cell types and secreted myocardial growth factors during cardiac development.

The authors dig more deeply into the cell autonomous defects of the CCM2-deficient epicardial cells, where their findings both help explain the epicardial

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cell phenotype and provide clues pertinent to CCM function more broadly. Elegant in vivo and ex vivo studies demonstrate that both the proliferation and polarity of epicardial cells are disturbed by CCM2 loss, consistent with the observation that CCM2-deficient epicardial cells fail to spread over the heart as rapidly and thoroughly as wild-type epicardial cells. These defects are associated with profound changes in the expression of matrix proteins that are known to play a critical role in epicardial cell function as well as myocardial cell growth and function. A particularly interesting finding is that loss of CCM2 in epicardial cells results in highly significant transcriptional changes. Many of these help explain the epicardial cellular phenotypes observed in vivo and ex vivo, eg, large changes in the expression of matrix proteins and cytokines. Other changes, eg, the marked increase in the Klf2 and Klf4 transcription factors, suggest how loss of CCM2 might have such a profound effect on gene expression.

A question that emerges from these findings is whether and to what extent the roles for the CCM pathway in epicardial cells reflect similar roles in endothelial cells, and therefore might yield new insight into CCM pathogenesis. Changes in endothelial cell matrix protein expression and polarity have also been observed in CCM-deficient endothelial cells ex vivo and with CCM formation in vivo,[3] but their contributions to disease pathogenesis remain poorly understood. Augmented expression of versican-degrading ADAMTS proteases are causal for defects in embryonic heart development and CCM formation in postnatal mice, but the more extensive matrix changes reported in epicardial cells in this study have not been demonstrated. The present study suggests that changes in matrix gene expression and cell polarity conferred by CCM loss may play more central roles than previously thought in the pathogenesis of CCM disease. Alternatively, it is possible that the CCM pathway is used in distinct ways in different cellular contexts.

The rise in KLF2 and KLF4 observed following CCM2 loss in epicardial cells is considered a central event in the endothelial changes associated with CCM pathogenesis, and suggests that many epicardial cell changes similarly arise because of augmented MEKK3-KLF2/4 signaling. If so, this would be the first genetic evidence that this mechanism operates in non-endothelial cells. Prior studies of CCM pathogenesis used genetic rescue of CCM-deficient phenotypes (eg, midgestation cardiac defects or postnatal CCM formation in the hindbrain) by partial loss of MEKK3 or KLF2/KLF4 function to demonstrate the critical role of this pathway in endothelial cell biology and disease.[4] A similar rescue strategy for the epicardial defects observed by Wang et al[2] would be a rigorous test to determine whether this molecular mechanism is conserved across cell types. If rescue is observed and an MEKK3-KLF2/4 pathway is demonstrated, the question of what might stimulate this pathway in epicardial cells becomes very interesting.

A final question addressed by the present study is whether CCM2 might function in the adult epicardium to preserve heart function after cardiac injury. The authors report no short-term defects in heart function in the undisturbed heart following loss of epicardial CCM2 in mature mice, but following experimental myocardial infarction (MI), they observe larger regions of myocardial injury and reduced ejection fractions in the absence of CCM2. These results are particularly intriguing in light of zebrafish studies of heart regeneration after injury. The fish epicardial cells contain a key source of new cells and secreted factors required for myocardial growth after injury.[5] Studies in mice have also implicated the epicardium in post-MI cellular and molecular responses. The present work provides exciting new functional support for an important epicardial role in cardiac responses to injury in mammals. Future studies that define the transcriptional and cellular responses are needed to define the role of the epicardium after MI and other common injuries such as pericarditis.

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