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Gyrate: CCM3 Dances with a Different Angiogenic Partner

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Abstract

A healthy vasculature is an essential component of development and is regulated by different signaling pathways. One of the most critical pathways involved is the vascular endothelial growth factor (VEGF) pathway. Components of this pathway serve as the first marker of primitive endothelial cells and are instrumental in inducing the initial differentiation of endothelial cells and later refining them into either arteries or veins. However, the regulation of VEGF signaling remains a mystery, with most studies focusing on the downstream components of this signaling cascade. New evidence shows that the protein cerebral cavernous malformation 3 (CCM3) is a key regulator of the VEGF pathway, bringing to light a previously unknown component of the VEGF signaling axis and opening the door to an exciting new era of vasculogenic research.

The transition from simple single-celled organisms to large complex organisms requires a vascular system that can deliver nutrients and oxygen as well as remove waste products, keeping individual cells and the whole organism healthy. Severe vascular malformations are incompatible with life, and less severe (where “less” is a relative term) vascular malformations result in diseases such as coronary artery disease, stroke, and aneurysms. Thus, understanding how signaling pathways are coordinated to form a healthy vasculature is essential. One of the major signaling pathways involved in vascular development is the vascular endothelial growth factor (VEGF) cascade. This pathway influences nearly every aspect of vasculogenesis and is one of the most studied signaling pathways in vertebrate development. However, despite considerable effort, we are still unraveling the intricacies of this complex pathway. He *et al*. identified and characterized the recently discovered cerebral cavernous malformation 3 (CCM3) protein as a crucial regulator of VEGF signaling during vascular development.

To truly appreciate the significance of He *et al*.’s findings, we must first understand just how vital VEGF signaling is during development. During the initial steps of vasculogenesis, VEGF receptor 2 (VEGFR2) (also known as Flk1) is the earliest definitive marker of endothelial cells (1–4). VEGF-A induces these VEGFR2-containing endothelial precursors to form clusters of blood islands: the first site of vascular development in the embryo, located in the yolk sac and comprising primitive erythroblasts surrounded by a layer of endothelial cells. These blood islands eventually give rise to both endothelial and hematopoietic cells, of which only the definitive endothelial cells retain VEGFR2 labeling (5–7). During the later stages of angiogenesis, the amount of VEGF signaling determines whether vessels commit to an arterial or venous fate (8–11): VEGF signaling remains high in endothelial cells that will commit to an arterial fate, whereas low VEGF signaling results in a venous commitment (8, 9). Many factors that increase VEGF abundance have been identified; however, the regulation of this signaling pathway remains largely unknown. Most

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diagrams of the VEGF signaling cascade show VEGF at the top of the cascade, with any regulation occurring downstream of the initial binding of VEGF to its receptor. However, according to the findings of He *et al*. (12), there is a new regulator in town, and it starts at the top of the VEGF signaling cascade.

He *et al*.'s surprising discovery of CCM3's role in regulating VEGF signaling arose from a study to characterize the molecular role of CCM3 in vascular development. CCMs are a common vascular malformation, affecting up to 0.5% of the population (13). These malformations include abnormally enlarged vessels that may leak and are susceptible to hemorrhage. Although many patients are asymptomatic, the presence of CCMs may lead to strokes, seizures, and death (13). Mutations in genes encoding the three proteins CCM1, CCM2, and CCM3 account for approximately 80% of familial CCM patients. There are no known phenotypic distinctions between patients with defects in any of these three CCM proteins, which has led to the hypothesis that the proteins work in concert (13). Indeed, when substoichiometric amounts of CCM3 are present, CCM3 interacts directly with CCM2, which then interacts with CCM1 (Fig. 1) (14). This CCM1-CCM2-CCM3 complex phosphorylates the mitogen-activated protein kinase p38, leading to apoptosis (15). Truncated mutant forms of CCM3 found in some CCM patients, however, are unable to phosphorylate p38, resulting in inhibited apoptosis (15).

To better understand the molecular role of CCM3 in vascular development, He *et al*. generated both a global and an endothelial cell–specific conditional CCM3 knockout mouse. Both the global and the endothelial cell–restricted conditional CCM3 knockout mouse were embryonic lethal due to vascular defects, with the global knockout dying by embryonic day 9.5 (E9.5) and the endothelial cell–restricted conditional knockout dying by E10.5. Based on these severe cardiovascular defects, the authors thus examined how known markers of vascular development are affected by the deletion of CCM3. They observed that VEGFR2 mRNA abundance and protein phosphorylation were reduced in the knockout mice and that the entire VEGF signaling cascade was disrupted, seemingly from the initial point of VEGF stimulation of VEGFR2 (12). It is worth noting that the global CCM3 knockout mouse is phenotypically reminiscent of the VEGFR2-null mouse, which completely lacks blood islands and vessels and is embryonic lethal between E8.5 and E9.5 (6). The authors hypothesized that CCM3 stabilizes VEGFR2, thus promoting VEGF signaling. In the absence of CCM3, VEGRF2 was destabilized, and VEGF was unable to stimulate the phospholipase C–γ (PLC-γ), Akt, and extracellular signal–regulated kinase (ERK) pathways (12). In matrigel assays, VEGF-stimulated CCM3-deficient endothelial cells formed fewer, larger vessels that exhibited reduced branching than did cells with endogenous CCM3. The authors went on to generate mutations of the CCM3 protein that are consistent with the CCM3 mutations that have been reported in some CCM patients. These N-terminally truncated CCM3 mutants were unable to bind and hence stabilize VEGFR2, lending credence to the hypothesis that a deficiency in the CCM3-VEGFR interaction may be a leading cause of CCM.

These surprising data beg the question: How does a protein that has known roles in moderating apoptosis, actin dynamics, and c-Jun N-terminal kinase (JNK) signaling (Fig. 1) affect the most critical pathway of vascular development? Part of this answer may depend on whether CCM3 is acting alone to regulate VEGF signaling or whether one of the CCM complexes already identified (Fig. 1) is also involved. Although the He *et al*. study suggests that CCM2 cannot stabilize VEGFR2, there are other CCM3-binding proteins that could be involved. If CCM3 is acting alone, this newly discovered protein may have an even more important in vivo role than previously suspected.

Two studies have demonstrated elevated VEGF signaling in three patients with CCM (16, 17). However, no current studies link either the absence of VEGF or VEGFR2 or mutations in genes encoding these proteins with CCM. CCMs have also been associated with abnormal p38 and ERK signaling (15, 18), which are downstream of both VEGF and the CCM1-CCM2-CCM3 complex, but other downstream components of the VEGF pathway have not been implicated in CCM. These studies suggest that the CCM proteins, not VEGF, are the primary factor in causing CCMs. Even so, if the truncated CCM3 proteins analyzed in this study (which are also found in some CCM patients) are unable to stabilize VEGFR2, how do these patients survive to adulthood? Do other pathways provide redundant regulation in vivo? Clearly, the CCM3-VEGF story is just unfolding. Even in these early stages, though, this discovery of CCM3's involvement has brought to life a new aspect of VEGF regulation and reminds us that there is much to learn about this complex vasculogenic pathway.

The study by He *et al*. demonstrates that CCM3 regulates VEGFR2 stability and, therefore, VEGF signaling in cultured endothelial cells. In light of the fact that the loss of VEGF signaling has not been directly implicated in CCM patients, one must wonder what other pathways also regulate VEGF. Can other proteins stabilize VEGFR2 in vivo in the absence of CCM3? Would these other proteins be restricted to the endothelium, or can other tissues secrete proteins that affect VEGFR2 stability? Altogether, these unanswered questions highlight the ever-growing complexity of the VEGF signaling pathway and suggest that VEGFR2, as the primary receptor of this pathway, may have quite a few more partners on its dance card.

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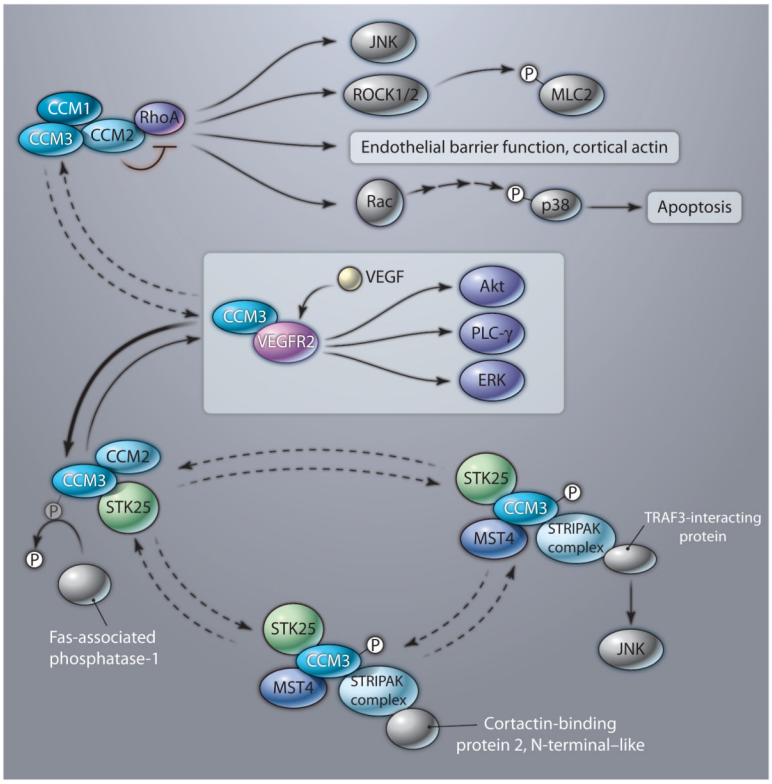

Fig. 1.

CCM3 interacts with multiple signaling complexes. The pathway identified by He *et al*. is highlighted. At low stoichiometric amounts, CCM3 preferentially interacts with CCM1 and CCM2, which inhibits RhoA signaling. In addition, CCM3 also promotes VEGF signaling, and it is unknown whether it associates with other proteins while complexed with VEGFR2. At high stoichiometric amounts, CCM3 binds to serine-threonine kinase 25 (STK25) and then to either CCM2 or the striatin-interacting phosphatase and kinase (STRIPAK) complex. However, the equilibrium between all of these pathways is currently unknown. Dashed arrows, unknown equilibrium; P, phosphate group. ROCK1/2, Rho-associated, coiled-coil–containing protein kinase 1/2; MLC2, myosin light chain 2; TRAF3, tumor necrosis factor receptor–associated factor 3. [Adapted from (12, 14, 15, 19)]