Collateral Development

The Quest Continues

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The promise of stimulating collateral blood vessel growth in the heart, brain, or peripheral circulation remains a holy grail in the field of vascular biology. For decades, clinicians and researchers have observed some individuals remain asymptomatic, despite highly obstructive atherosclerotic disease because they possess well-developed innate collateral vessels.1-4 To date, we stand on a mountain of evidence supporting their existence, their potential to be modified by various stimuli (eg, shear stress and ischemia), and their clinical benefits.5-7 Yet, we incompletely understand how much of collateral development depends on the genetic composition of a patient, and how much it is related to the environment in which these vessels are exposed.

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A functional collateral circulation is the end result of 2 different processes: collaterogenesis and arteriogenesis. During embryogenesis, arterial–arterial connections at the level of the microcirculation form within the heart, brain, and skeletal muscle. In the brain, this mechanism occurs through the direct sprouting and proliferation of endothelial cells to form tubes that directly attach to a neighboring arteriole.8 Vascular endothelial growth factor (VEGF) seems to mediate this endothelial migration and proliferation through its receptor, flk-1, and Notch signal pathway.9 VEGF levels were directly related to the extent of native collateral growth in the brain and skeletal muscles when measured in different mouse strains.10,11 Interestingly, an expression quantitative trait locus (QTL) on chromosome 17 near vegfa was identified as a possible cause for the strain-dependent variation in VEGF expression.12 This first phase of collateral development is the establishment of arterial connections; however, these vessels are incapable of conducting sufficient blood flow to meet the tissue’s metabolic needs.

The postnatal remodeling of pre-existing arteriolar anastomoses is the hallmark of arteriogenesis. In the heart, arterial–arterial anastomoses (20–200-μm diameter) are incapable of conducting sufficient blood flow to support cellular metabolism because of their small caliber and lack of muscularity.12 Environmenal stimuli, such as changes in physical forces (eg, shear stress) or ischemia because of vascular occlusion from atherosclerotic disease, lead to increased growth factor expression.13,14 The endothelium, in response to these stimuli, recruits immune cells and endothelial progenitor cells, which secrete more growth factors to stimulate smooth muscle cell recruitment.5 Moreover, genotype analysis from patients with acute and chronic coronary disease identified significant association between VEGF mutations and collateral size.15 Within the VEGF signaling pathway, a glu298arg allele within the endothelial nitric oxide synthase gene leads to its impaired expression and poor collateral remodeling.16 Alleles for hypoxia-inducible factor-1α, a transcription factor capable of inducing VEGF, have been associated with collateral development.17,18 These results highlight the complexity of vascular remodeling and the multiple layers of regulation, genetic, and environmental, which are necessary in this process.

In this issue of Circulation Research, Sealock et al19 further our understanding of the genetic basis of collateralization in the brain and peripheral circulation through congenic mapping of a 27 Mb region on mouse chromosome 7 (Candq1) attributed to strain-dependent differences in innate collateral vessel diameter and number. Candq1 is located within a region containing other QTLs (Civq-5, Lsq-1) associated with differences in hindlimb perfusion and cerebral infarct volumes but does not share candidate gene targets with these loci.19 This divergence identified a need to refine and reconcile QTLs identified indirectly by ischemia with those identified by structural characteristics to the same physiological process (ie, collateralization). Congenic lines were developed by introgressing sections of Candq1 from c57bl/6 mice, a collateral-rich strain, to the background of BalbC, a collateral-poor strain. Phenotypic analysis, by measuring native collateral number, vessel size, infarct volume, and femoral blood flow, resulted in the direct characterization of a candidate region to 737 kb and included 28 genes. These candidate genes do not overlap with those in the previous identified QTLs. Because endothelial cell sprouting is required for collaterogenesis, first-line candidates were directed toward endothelial cell expressing genes.

In silico analysis of single nucleotide polymorphisms and miRNA targets within the Dce-J QTL highlighted a membrane trafficking protein rabep2 as the potential master controller of collaterogenesis. What makes Rabep2 an intriguing candidate for control of collaterogenesis is that it crosses paths with VEGF signaling. The VEGF receptor, KDR/flk-1, is responsible for mediating VEGF-stimulated proliferation and migration in collateral development.20 Rabep2 binds to Rab5 and the Rab5 exchange factor on the endosomal membrane to form the early endosome (Figure).21-23 In vitro, Rabep2 is necessary for the activation of Rab5, thus Rabep2 has the potential to regulate collateral development through endocytic trafficking of the activated...
VEGF receptor KDR/flk-1. Whether this is the master controller of native collateral development remains to be seen because genetic knockouts are in the process of development. Previous genetic studies where the extent of native collateral development was measured in VEGF overexpressing mice indicated the VEGF receptor 1, or flt-1, was a key mediator in the hindlimb, whereas flk-1 did not affect the extent of collaterogenesis. In the leptomeningeal collateral circulation, VEGF receptor 2 is found to be critical for collateral extent through Notch1 (Figure). This previous finding could conflict with Rabep2 and VEGF receptor trafficking as a mechanism for regulating native collateral growth. It would be interesting to know whether VEGF protein or receptor expression is altered in these congenic lines.

There is no obvious gene or miRNA, which could be directly attributed to the observed increases in collateral size and number. This highlights the frequent dilemma presented in genetic-based research. How can the results be interpreted and studied? Some candidates are dismissed because the transgenic knockout did not abolish collateralization (eg, jnjid5). Some candidates have unknown function. Some are expressed outside of the vasculature. This raises the question; can collateral development be defined by the absence of a single gene? I would argue the hypothesis that a single gene is responsible for native collateral extent must be met with skepticism. Collateral development occurs in diverse vascular beds and is subject to varying environmental factors. Phenotyping of the congenic lines used in this study were mainly focused on leptomeningeal collateral formation. This is evidenced by the inability of the intragrotesed collateral-rich strain to restore collateralization fully in the skeletal muscle of the collateral-poor strain. This may also explain the previous observation that flt-1 receptor signaling is important to hindlimb collateral development. Rabep2 might be the master controller in leptomeningeal collaterals but what about Rabep2’s function in other vascular beds?

As a vascular biology community, we continue on our quest to understand collateral development with the hope of 1 day using them as a practical therapy. I think that Sealock et al have made a significant advancement by shifting our focus in a promising new direction toward membrane trafficking. I look forward to the next chapter in this collateral saga.

Acknowledgment

I thank Dorothee Weihrauch, DVM, PhD for her thoughtful and careful review of this article.

Disclosures

None.

References


**Key Words:** Editorials ■ cerebral infarction ■ collateral circulation ■ rab G-proteins
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Circ Res. 2014;114:591-593
doi: 10.1161/CIRCRESAHA.114.303402

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