**Editorial**

**Born to Die**

**Blood Vessel Regression Research Coming of Age**

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Blood vessel network formation is a pivotal process of physiological development and pathological conditions such as tumor growth, diabetic retinopathy, and cardiovascular diseases. During neovascularization, vasculogenesis is followed by the well-described process of sprouting angiogenesis, including tip cell induction and selection, sprout elongation, and stalk formation, leading to the formation of a dense primary vascular network. To match vessel perfusion with the local metabolic demand of the tissue, initial sprouting angiogenesis is followed by vessel pruning and maturation to form a hierarchically defined vascular network of large arteries and veins branching into smaller capillaries. Although the phenomenon of vessel regression was described by Rouget in 1873 and further characterized by Ashton in 1960, the mechanisms of vessel pruning and the signaling pathways controlling this process are still largely undefined.

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Controlled regression of redundant vessels and stabilization of other vessels by mural cells and extracellular matrix occur during vessel remodeling. The process of vessel regression starts with vessel narrowing and cessation of blood flow, which leads to luminal occlusion and retraction. The absence of blood flow may lead to endothelial cell apoptosis and pericyte dropout, leaving behind avascular empty basement membrane sleeves.

The initiating triggers of vessel regression are largely unknown. Conceptually speaking, the question of whether regression is induced by active signaling pathways or merely results from the withdrawal of survival factors (or a combination of both) remains unresolved. Changes in vessel perfusion have been described as regulators of vessel regression. Yet, it is not known whether shear stress, oxygen, or angiogenic factors are the primary mediators of flow-induced vessel regression. Early studies have suggested that regression is dependent on the status of tissue oxygenation.

After the formation of the first primary vascular network, the resulting high tissue oxygen tension induces downregulation of the survival factor vascular endothelial growth factor (VEGF) and subsequent controlled endothelial cell apoptosis.

Beyond VEGF, Notch and Wnt signaling have been described to orchestrate vessel remodeling. Dll4/Notch signaling induces Nrp expression, thereby promoting canonical Wnt signaling and subsequently inducing vessel regression. Overexpression of endothelial β-catenin during development resulted in embryonic lethality at embryonic day 11.5 as a consequence of induced Notch signaling and subsequent remodeling, branching and arteriovenous specification defects, and an increase in vessel lumen. Studies of the postnatal hyaloid vessels in the eye have shown that Angiopoietin-2 stimulates Wnt7b expression in macrophages and that these 2 factors then synergistically inhibit endothelial cell survival and induce vessel regression via the canonical Wnt pathway. Oxygen-induced Dll4/Notch signaling was recently shown to control vessel remodeling and regression by shifting the expression of vasoactive genes toward a vasoconstrictive phenotype, thereby inducing vessel occlusion and subsequent endothelial cell apoptosis.

Pruning of immature vessels is an important mechanism of antiangiogenic vessel-normalizing therapies. The identification of the factors and mechanisms controlling this process is consequently a very timely and important topic of contemporary vascular biology research. In this issue of Circulation, Cheng and coworkers report the identification of FYVE, Rho guanine exchange factor, and pleckstrin homology domain containing 5 (FGD5) as an important novel regulator of vessel regression in the postnatal retina. The FGD family of guanine exchange factors comprises 7 members: FGD1 through FGD-6 and FRG. The FGD proteins contain a Dbl homology domain and FYVE and pleckstrin homology domains and regulate inactive GDP to active GTP exchange of small GTPases such as Cdc42, RhoA, and Rac1. Whereas the function of FGD5 is unknown, FGD1, FGD4, and FRG have been described to control cdc42 activation and subsequent actin cytoskeleton reorganization, filopodia formation, and Jun N-terminal kinase pathway activation.

In the present study, FGD5 was shown to be specifically expressed by endothelial precursor cells during different stages of mouse embryonic development, primary human endothelial cells, and zebrafish larvae. Furthermore, the FGD family members, only FGD5 was enriched in adult mouse aorta compared with other tissues. Similar results have been reported recently by Kurogane et al, who identified a particularly strong FGD5 expression in highly vascularized organs such as lung, kidney, and ovary and in primary human endothelial cells.

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Albeit partly in conflict with the study of Kurogane et al., Cheng and coworkers demonstrated convincingly in a series of loss-of-function and gain-of-function studies (tube formation assay, mouse aortic ring assay, human umbilical vein endothelial cell–coated Cytodex bead assay) important functions of FGD5 as a negative regulator of angiogenesis. Moreover, the authors established in a Matrigel plug assay an inhibitory role of FGD5 on capillary network formation in vivo. Intravitreal injection of adenoviral FGD5 into P3 mice reduced retinal vascularization at postnatal day 8 and 11 as assessed by decreased total vascular tube length, number of junctions and neocapillaries, and increased mean tube length. Complementary results were observed on injection of FGD5 siRNA, supporting the antiangiogenic function of FGD5. The inhibitory effects of FGD5 on vascular network formation could be explained by an active pruning mechanism of pre-established vessels because collagen IV/isolectin IB4 staining of the retina revealed increased vessel regression on FGD5 overexpression. These findings were further corroborated in FGD5 silencing experiments.

FGD5-induced vessel regression was linked to increased apoptosis of Flk1+ and CD31+ cells in the retina, as assessed by PI/annexin V fluorescence-activated cell sorter analysis. Further in vitro experiments supported the apoptosis-inducing function of FGD5 and showed an upregulation of p53, p21, VEGF receptor 1 (VEGFR1), and Notch signaling components and the downregulation of VEGFR2 on FGD5 overexpression. The shift of the VEGFR1 versus VEGFR2 expression ratio supported the concept of an antiangiogenic function of FGD5 (the Figure). Intravitreal injection of soluble active VEGFR1 could correspondingly interfere with excessive vessel network formation in siFGD5-transduced retinas, indicating that the relative VEGFR1 to VEGFR2 availability regulated vascular pruning. By reducing the available VEGF, the increased VEGFR1 versus VEGFR2 ratio could either reduce vascular sprouting in angiogenic vessels or promote vessel regression by sequestering VEGF as a capillary survival factor.

Cheng and coworkers completed their elegant study by showing in loss- and gain-of-function experiments and in rescue experiments that a Hey-1/p53/p21 signaling cascade controlling endothelial apoptosis may be responsible for vessel regression in vivo. In line with these findings, Hey-1–dependent p53 and p21 activation has previously been reported. The authors of the present study further demonstrate that FGD5 interacted directly with and activated cdc42 as has been reported in parallel studies by Kurogane et al. In contrast, the activity of other GTPases such as RhO and Rac1 was only indirectly regulated by FGD5. Similar indirect mechanisms have previously been reported for FG4-dependent Rac activation.

So far, Cdc42 and Rac1 activation has been linked to VEGF-induced vessel sprouting by triggering filopodia and lamellipodia formation, respectively. Along these lines, Kurogane et al proposed a role for FGD5 in promoting endothelial tube formation in response to VEGF stimulation. Yet, the more extensive experiments by Cheng and coworkers strongly support an antiangiogenic function of FGD5 on vessel formation. To account for these apparent discrepancies, additional factors activating different upstream signaling pathways may fine-tune the FGD5 response of endothelial cells. As observed for Notch and VEGF signaling, the presence of a specific factor in the angiogenic or postangiogenic region might influence the net outcome of its downstream signaling. Because FGD5 was shown to interact with VEGF and Notch signaling pathways, the outcome of FGD5 signaling may be different in regions of active sprouting angiogenesis compared with regions of vessel pruning and regression. FGD5/Cdc42 signaling in angiogenic vessels may promote sprouting by filopodia formation, whereas in postangiogenic regions, different mechanisms, including FGD5/Cdc42-dependent VEGF sequestration and Hey-1/p53/p21–
dependent endothelial cell apoptosis, may favor vessel regression. Because Cdc42 and FGD5 act downstream of multiple signaling pathways, the net outcome of FGD5-induced signaling may vary, depending on which upstream signals integrate on these proteins. In general, FGD5 upstream pathways need to be studied in more molecular detail to better understand the mechanisms of vessel regression. Endothelial Cdc42 has been reported to increase cortical actin polymerization, PI3K and Akt activation, and matrix metalloproteinase activation and to control lumen formation.\(^{25,26}\) VEGF/VEGFR2 signaling via Cdc42 has further been shown to regulate endothelial cell polarization through microtubule organization via p38 activation.\(^{27}\) These different Cdc42-mediated processes may similarly play a role during vessel formation and during vessel regression.

In conclusion, the identification FGD5 as novel endothelial cell–specific regulator of vessel pruning is of major importance for the understanding of physiological vessel regression. The better molecular understanding of vessel pruning and regression mechanisms does not just contribute to basic vascular biology research but may hold significant translational potential because such knowledge may in the long run contribute to the optimization of antiangiogenic therapies. Similarly, building on the authors’ finding of upregulated FGD5 expression during ageing, the better understanding of the mechanisms of vessel regression may even contribute to the understanding of vessel remodeling processes during ageing.

**Disclosures**

None.

**References**


**KEY WORDS:** Editorials angiogenesis inducing agents