Role of ROCK I/II in vascular branching

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The highly similar Rho kinases ROCK I and II have drawn a lot of attention over the past decade as potential targets for novel treatment options for a variety of cardiovascular disorders such as (pulmonary) hypertension, ischemic stroke, and vascular leakage (27). Several previous in vivo studies suggested that inhibition of Rho kinase also could provide a potent strategy for inhibition of angiogenesis (9, 21, 22, 26, 28, 31, 37). Data obtained from in vitro and explant models, however, indicated that Rho kinase inhibitors do not only impair angiogenesis but also may enhance in vitro tube formation (19, 29). In their article, Kroll et al. (15) provide important in vivo evidence for a potentiating effect of Rho kinase inhibition on angiogenesis, supplying further fuel to this controversial issue (Fig. 1). Together with a recent report by Fischer et al. (5), these data shed new light on the role of Rho kinase in angiogenesis. Novel information on the contribution of disordered RhoA activity to excessive, but dysfunctional, angiogenesis in a common vascular dysplasia called cerebral cavernous malformation (CCM) underscores the importance of these findings (36).

The role of members of the Rho family of small GTPases, with its main members RhoA, Rac1, and Cdc42, as key regulators of angiogenesis has been well established [see Bryan and d’Amore (3) for review]. Rho GTPases modulate a diversity of cellular processes, including extracellular matrix (ECM) remodeling, migration, proliferation, morphogenesis, and survival. RhoA signaling plays an essential role in vascular endothelial growth factor (VEGF)-dependent in vivo angiogenesis and in initial steps of in vitro endothelial cord assembly (3, 11, 23, 34). RhoC has also been implicated (35). Other studies have suggested that RhoA plays a role in endothelial tube collapse and regression (4). Using a RNAi-mediated suppression approach, Cdc42 and Rac1 have been implicated in the process of endothelial lumen formation, whereas RhoA appears to play a minimal role here (14).

The effects of RhoA are mediated at least in part by its downstream target Rho kinase. Where previous studies with in vitro or explant and in vivo models indicated an essential role for Rho kinase in angiogenesis, we provided in 2003 the first evidence for a dual role of Rho kinase in angiogenesis (32); the Rho kinase inhibitor Y-27632 reduced the length of VEGF-induced tube-like structures in an in vitro model for angiogenesis, but Y-27632 also enhanced initial sprouting. These data suggested a role for Rho kinase in three-dimensional migration, which was confirmed in a two-dimensional wound healing model. However, the increased sprouting upon treatment with the Rho kinase inhibitor remained unexplained at that time.

The merit of the work of Kroll et al. is that they elucidate a putative inhibitory role of Rho kinase in VEGF-driven angiogenesis, providing in vivo evidence for an inhibitory role for Rho kinase in neovascularization. In a mouse retinal vascularization model, they show that a highly selective Rho kinase inhibitor enhanced angiogenesis by ~50%. Similarly, downregulation of ROCK I/II expression in cultured endothelial cells (ECs) enhanced tube formation in a human umbilical vein endothelial cell spheroid model. Furthermore, they provide a novel molecular explanation for the counteracting role of Rho kinase by demonstrating that Rho kinase inhibitors enhance activation of the VEGF receptor VEGFR2/KDR. It remains to be investigated whether this VEGFR2 hyperactivation acts synergistically with enhanced nitric oxide (NO) bioavailability, since inhibition of Rho kinase is known to induce expression of endothelial nitric oxide synthase and NO plays an integral role in development and maintenance of the microvascular network (25).

An important feature in determining the morphology of tubular systems such as the vascular bed is the frequency and geometry of branching. Hence, deciphering the molecular mechanisms underlying the sprouting of new branches is the key to understanding the formation of tubular systems (12). In recent years, much attention has been directed toward the role played by tip cells in the growth of blood vessels. Much of the knowledge of endothelial tip vs. stalk cell specification in the vascular system was derived from observations of angiogenesis in the postnatal mouse retina (12). An important molecular mechanism is induction and expression of delta-like ligand 4 (dll4) by VEGFR activation in individual cells conferring a cell-tip phenotype and activating Notch in adjacent ECs. Notch activation suppresses VEGFR expression and prevents these cells from conversion into tip cells (10, 17). Interestingly, dll4+/− mice have a phenotype highly reminiscent to that induced by inhibition of Rho kinase as described by Fischer
et al. (5), showing an increased number of sprouting vessels. Data from keratinocytes and vascular smooth muscle cells indicate that Notch signaling interferes both with ROCK II expression and activity (2, 16), indeed suggesting a link between Notch signaling and Rho kinase activity.

Fischer et al. (5) now identify ROCK-mediated myosin II activity and stiffness of the ECM as two important cues inhibiting endothelial pseudopodial branch initiation, i.e., inhibition of ROCK II or reduced ECM stiffness promote branching. Myosin II is dynamically localized to the endothelial cortex and is partially released under conditions that promote branching. Local downregulation of myosin II-mediated cortical contraction allows pseudopodium initiation to mediate endothelial branching and hence guide directional migration and angiogenesis. Two recent studies indicate that aberrant RhoA/Rho kinase signaling contributes to pathological forms of angiogenesis (7, 36). Targeted disruption of the gene that causes cerebral cavernous malformation, Ccm2, hyperactivates RhoA, resulting in excessive but dysfunctional angiogenic sprouts form, which are unable to develop stable lumens to allow functional circulation. Normalization of RhoA activity with statins rescues the vasculature. 2 Tumor capillary ECs more readily form capillary networks in vitro because of enhanced Rho kinase activity (7). These cells exert greater traction force and display an enhanced ability to retract flexible ECM substrates. Moreover, decreasing Rho-mediated tension by the ROCK inhibitor Y-27632 can reprogram the tumor capillary ECs so that they normalize their ability to form tubular networks on ECM gels.

It is increasingly appreciated that the GTP-binding-GTP-hydrolysis cycle of Rho GTPases is highly coordinated in a spatiotemporally controlled manner for effective signaling output (30). During angiogenesis, specific guanine exchange factors (RhoGEFs) and GTPase-activating proteins (RhoGAPs) are dedicated to regulate RhoA activity. Loss-of-function studies in the zebrafish and mouse point to a specific role for the RhoGEF Syx in angiogenic sprouting in the developing vascular bed (6). Importantly, vascuogenesis and angioblast differentiation steps were unaffected. A vascular cell-restricted RhoGAP, p73-RhoGAP, plays a key role in angiogenesis (29), and, recently, p190B-RhoGAP also has been implicated in regulation of angiogenesis (8). Taken together, these data suggest that localized RhoA/Rho kinase activities determine branching sites of tip cells by regulating cortical contraction, whereas RhoA activity localized to the trailing edge facilitates migration of stalk cells by promoting tail retraction.

In one of their previous studies using the same sphereoid model, Nacak et al. (20) implicated the RhoGEF ECT-2 in regulation of angiogenesis, demonstrating that VEGF-induced activation of RhoA is indispensable for angiogenesis. It remains hard to reconcile these data with the current study, where they find enhanced angiogenesis when Rho kinase activity is abolished. It suggests that at least part of the effect of RhoA is mediated by downstream targets of RhoA other than ROCK II. RhoA-independent activation of ROCK, however, cannot be excluded, since Rho kinase also can be activated by other Rho proteins such as RhoB and RhoC, by arachidonic acid, by proteolytic cleavage by caspases, by phosphorylation by polo-like kinase-1, or even by methylation or altered expression such as induced by Notch (18, 24). An attractive alternative explanation might be that, in the models used by Kroll et al., the angiogenesis-inhibitory ROCK activities outweigh the angiogenesis-promoting ROCK activities. It would be interesting to investigate whether these angiogenesis-promoting and -inhibiting activities of Rho kinase could be attributed to specific ROCK isoforms, since studies are accumulating that show that ROCK I and ROCK II regulate different aspects of myosin II activity, resulting in distinct biological functions of ROCK I and II (33, 38).

Although the finding that ROCK I/II inhibitors promote angiogenesis fits with the general idea that inhibition of Rho kinase has positive effects on the vasculature, from a therapeutic point of view, this raises concern to an unlimited use of ROCK I/II inhibitors, especially in the eye. Rho kinase has been proposed as a unique therapeutic target in the treatment of proliferative vitreoretinal diseases (13) and diabetic retinopathy (1). Because disordered angiogenesis causes blindness, careful clinical investigation is warranted. Perhaps isoform-selective inhibitors may offer a solution here.

GRANTS

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REFERENCES


