Fine-Tuning the Angiogenic Response to Vascular Endothelial Growth Factor

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Vascular endothelial growth factor (VEGF) is an important mediator of vascular development and postnatal angiogenesis. Homozygous deletion of VEGF gene leads to impaired vascular formation and early embryonic lethality. Interestingly, deletion of just one VEGF allele is sufficient to produce similar vascular abnormalities, suggesting that the strength and integrity of the VEGF signaling pathway are critical for the spatial-temporal formation of blood vessels in utero. These actions of VEGF are mediated predominantly by 2 tyrosine kinase receptors: VEGFR-1 (flt-1) and VEGFR-2 (flk-1/KDR). Mice with homozygous mutations that inactivate either receptor die in utero with a similar phenotype to mice with VEGF deletion, indicating that both receptors are obligatory for the function of VEGF. Although the vascular phenotype between these VEGFR mutant mice are overlapping to some degree, they do differ in terms of showing that VEGFR-2 plays a greater role in vascular organization, whereas VEGFR-1 appears to be more important in mediating hemangioblast commitment and endothelial cell replication. Nevertheless, both VEGFR-1 and VEGFR-2 are essential in coordinating endothelial cell assembly and vascular formation during embryonic development.

The upregulation of VEGF is also necessary for physiological and pathological neovascularization in response to hypoxia and for tumor growth. The precise role of VEGFR-1 and VEGFR-2 in mediating the postnatal angiogenic response to VEGF, however, is less well understood, and in some cases, somewhat controversial. For example, most, if not all, of the angiogenic response to VEGF is mediated through VEGFR-2 rather than VEGFR-1 because mutant mice lacking the tyrosine kinase domain of VEGFR-1 exhibit normal vascular development and angiogenic response. However, direct activation of VEGFR-1 by placental growth factor leads to increased angiogenesis and synergism with VEGF, suggesting potential positive crosstalk or cross-coupling between VEGFR-1 and VEGFR-2. In contrast, other studies suggest that VEGFR-1, either membrane-bound or in soluble form, acts as an inert decoy of VEGF, which negatively modulates VEGFR-2 signaling and endothelial cell proliferation. Thus, depending on the circumstances, VEGFR-1 could positively or negatively modulate the angiogenic response to VEGF.

To further define the mechanism underlying the potential cross-talk between VEGFR-1 and VEGFR-2 in ischemia- or VEGF-induced angiogenesis, Nishi et al., in this issue of Circulation Research, investigated the role of VEGFR-1 in endothelial cell culture in vitro using small interfering RNA approach and in vivo using heterozygous VEGFR-1 mice subjected to hindlimb ischemia. They found that angiogenesis and blood flow recovery was mildly impaired in VEGFR-1+/− mice compared to wild-type (WT) littermates, despite comparable protein expression of VEGF and VEGFR-2 in both groups of mice. Surprisingly, phosphorylation of Akt was higher in the aorta of VEGFR-1−/− mice, and this finding was confirmed in vitro when VEGFR-1 was knocked down in endothelial cells using retroviral small interfering RNA for VEGFR-1 but not VEGFR-2. Interestingly, the phosphorylation of endothelial NO synthase (eNOS) was higher, but cGMP level was lower in VEGFR-1 knocked down endothelial cells, suggesting decreased NO bioavailability. Unfortunately, eNOS activity and NO production were not directly measured in these cells, and additional studies were not performed with NO donors or eNOS inhibitors. These studies would have been interesting and could have provided further insights into the mechanism involved because eNOS is an important downstream mediator of ischemia-induced angiogenesis. Furthermore, previous studies have shown that VEGF can activate eNOS via VEGFR-1 and that the NO produced then negatively regulates endothelial cell proliferation by VEGFR-2 while promoting endothelial cell tubular formation and differentiation into capillary networks. Knocked down or deletion of VEGFR-2, however, leads to decrease in Akt and eNOS phosphorylation and, presumably, subsequent loss of angiogenic response to VEGF.

The authors hypothesize that excessive activation of Akt via VEGFR-2 may be responsible for the impaired angiogenic response in VEGFR-1−/− mice. To test this, they generated double heterozygous VEGFR-1+/− Akt−/− mutant mice and showed that the impaired angiogenic response to hindlimb ischemia was restored to a level that was comparable to what was observed in WT mice. These findings are consistent with a previous study showing that Akt−/− mice have increased vascular permeability and augmented angiogenic response. Interestingly, Akt−/− mice in this study did not show a greater angiogenic response to hindlimb ischemia compared to WT mice, despite having lower levels of phosphorylated Akt. This is puzzling and somewhat contradictory to the hypothesis of the authors. Perhaps, there is a threshold level of phosphorylated Akt that is required for

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(Circ Res 2008;103:229-230.)

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Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/CIRCRESAHA.108.181628
enhancing angiogenesis. Nevertheless, the authors conclude that VEGFR-1 facilitates ischemia-induced angiogenesis by fine-tuning and preventing excessive Akt activation by VEGF through VEGFR-2. Whether this modulation of VEGFR-2 signaling is attributable to a direct crosstalk or an indirect decay effect of VEGFR-1 remains to be determined. These findings, therefore, underscore the importance of having just the right amount of VEGF signaling at the right time, when deficient or excessive Akt activation via VEGFR-2 could lead to impaired angiogenic response to VEGF.

In agreement with the notion that excessive Akt activation mitigates the angiogenic response to VEGF, the authors have previously shown that Akt increases senescence, negatively regulates endothelial cell lifespan, and inhibits endothelial tubular formation via a p53/p21-dependent pathway. These findings, however, appear to be contradictory to other studies showing that Akt1 is critical for ischemia-induced and VEGF-mediated angiogenesis. Perhaps, the discrepancies regarding the role of Akt in VEGF-induced angiogenesis could be explained, in part, by the differences in the duration of Akt activation. Indeed, short-term activation of endothelial Akt leads to increase NO production, re-endothelialization, of Akt activation. In agreement with the notion that excessive Akt activation negatively regulates the in vitro lifespan of human endothelial cells via a p53/p21-dependent pathway.

As the study by Nishi et al. has demonstrated, having too much Akt activation may not necessary be beneficial. The precise mechanisms of how Akt activation is regulated under physiological and pathological conditions still requires further investigation, but, perhaps, VEGFR-1 could serve as an important factor that balances and fine-tunes the angiogenic response to VEGF.

Sources of Funding

This work was supported by NIH grants HL052233 and HL080187.

Disclosures

None.

References


Key Words: VEGF ■ angiogenesis ■ protein kinase Akt ■ ischemia
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Circ Res. 2008;103:229-230
doi: 10.1161/CIRCRESAHA.108.181628

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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World Wide Web at:
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