The Role of Vascular Endothelial Growth Factor in Restenosis
The Controversy Continues
Ichiro Shiojima, MD, PhD; Kenneth Walsh, PhD

In 1996, the late Jeffrey Isner proposed the use of vascular endothelial growth factor (VEGF) gene delivery in patients undergoing percutaneous transluminal angioplasty as a strategy to limit the risk of restenosis. VEGF promotes endothelial cell function and is a potent stimulator of endothelial cell migration and survival. Because normal endothelium inhibits smooth muscle cell proliferation, it was hypothesized that accelerating the reendothelialization of the balloon-injured site would diminish the severity of restenosis. Indeed, reports in animal models demonstrate that local administration of VEGF-A or VEGF-C leads to accelerated reendothelialization and a reduction in intimal thickening.

However, the utility of VEGF or other proangiogenic factors for the treatment of restenosis is questioned by reports that have documented extensive vascular networks in atherosclerotic plaques and balloon-injured coronary arteries. Thus, the delivery of proangiogenic agents might exacerbate the growth of vascular lesions, analogous to the concept that angiogenesis contributes to tumor growth. These concerns are supported by studies showing that treatment of apolipoprotein E (ApoE)–deficient mice with an angiogenesis inhibitor reduced intimal neovascularization and plaque growth, and administration of VEGF enhanced atherosclerotic plaque progression, which was associated with increased capillary density.

VEGF-Traps have been shown to effectively neutralize endogenous VEGF bioactivity and to inhibit angiogenesis occurring “VEGF-Trap,” several modified versions of the naturally occurring soluble form of VEGFR1, and systemically injected VEGF-TrapR1R2, which contains the second Ig domain of VEGFR1, the third Ig domain of VEGFR2, and the Fc portion of human IgG, and overexpressed the VEGF-TrapR1R2 protein in the liver by systemic intravenous injection of the adenovirus. Ohtani et al used plasmid vector of sFlt-1, the naturally occurring soluble form of VEGFR1, and systemically overexpressed sFlt-1 protein by intramuscular injection of the plasmid. Khurana et al used soluble chimeric VEGFR1 protein Flt(1-3)IgG, which contains the first 3 Ig domains of VEGFR1 fused to a mouse IgG Fc fragment, and locally applied the protein around the injured vessels.

Although the authors of the 3 studies basically used the same strategy to block endogenous VEGF signaling, their results are quite divergent. Hutter et al used a wire injury model of mouse femoral artery and systemically injected adenovirus vectors encoding VEGF or VEGF-TrapR1R2 1 day before injury. In their study, the authors showed that VEGF treatment resulted in enhanced reendothelialization and attenuation of neointima formation after vascular injury. Moreover, treatment with VEGF-Trap resulted in reduced reendothelialization and enhanced neointima formation when...
compared with control animals. These data suggest that both exogenous and endogenous VEGF signaling contribute to attenuation of restenosis by promoting endothelial recovery after vascular injury. Ohtani et al\textsuperscript{12} examined the effects of systemic delivery of sFlt-1 on neointima formation in mice, rats, and rabbits, and found that sFlt-1 attenuates neointima formation without affecting luminal reendothelialization. They also showed that adenovirus-mediated intraluminal delivery of VEGF to balloon-injured rabbit carotid artery resulted in increased adventitial angiogenesis without affecting neointima formation. These results suggest that endogenous VEGF signaling accelerates neointimal formation after vascular injury and that exogenous and endogenous VEGF may have differential effects on restenosis. Khurana et al\textsuperscript{11} examined the effects of angiogenic growth factors or angiogenesis inhibitors including Flt(1-3)IgG applied to the periadventitial area of injured vessels (ie, from outside of the vessels). They demonstrated that angiogenic growth factors promote adventitial angiogenesis and neointima formation, whereas angiogenesis inhibitors by themselves had no effect on adventitial angiogenesis or neointima formation. Because essentially the same results were obtained both in silicone collar–induced and in balloon injury–induced neointima formation, these data suggest that periadventitial angiogenesis promotes restenosis independent of the integrity of the luminal endothelium. As summarized in the Table, some of the results of these studies are contradictory to each other. Then how can we reconcile these apparently paradoxical findings?

The results of Hutter et al\textsuperscript{10} are consistent with the notion that both exogenous and endogenous VEGF attenuate restenosis. The results of Ohtani et al\textsuperscript{12} on the other hand, suggest that endogenous VEGF actually promotes restenosis. Although it is difficult to reconcile these apparently paradoxical findings, one possible explanation is that the extent of injury may have some effect on the outcome. The predominant target of VEGF seems to be endothelial cells in the study by Hutter et al\textsuperscript{10} (VEGF-Trap\textsubscript{RIR2} attenuates endothelial recov-

### Summary of Vascular Injuries, Treatments, and Responses to Injury

<table>
<thead>
<tr>
<th>Vascular Injury</th>
<th>Artery</th>
<th>Injury Type</th>
<th>Species</th>
<th>Vector or Protein</th>
<th>Delivery Method</th>
<th>Day</th>
<th>Neointima Formation</th>
<th>Luminal Endothelium</th>
<th>Adventitial Angiogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hutter et al\textsuperscript{10}</td>
<td>Femoral artery</td>
<td>Wire injury</td>
<td>Mouse</td>
<td>Ad-VEGF</td>
<td>IV injection</td>
<td>Day (−1)</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>Wire injury</td>
<td>Mouse</td>
<td>Ad-VEGF-TrappRC</td>
<td>IV injection</td>
<td>Day (−1)</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>…</td>
</tr>
<tr>
<td>Ohtani et al\textsuperscript{12}</td>
<td>Femoral artery</td>
<td>Wire injury</td>
<td>Mouse</td>
<td>sFlt-1 plasmid</td>
<td>IM injection</td>
<td>Day (−3)</td>
<td>↓</td>
<td>→</td>
<td>↓</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>Balloon injury</td>
<td>Rabbit</td>
<td>Flt(1-3)IgG</td>
<td>IM injection</td>
<td>Day (−3)</td>
<td>↓</td>
<td>→</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>Carotid artery</td>
<td>Balloon injury</td>
<td>Rabbit</td>
<td>sFlt-1</td>
<td>Ad-VEGF</td>
<td>Local delivery (balloon catheter)</td>
<td>Day (0)</td>
<td>→</td>
<td>…</td>
<td>↑</td>
</tr>
<tr>
<td>Khurana et al\textsuperscript{11}</td>
<td>Carotid artery</td>
<td>Silicone collar</td>
<td>Rabbit</td>
<td>Ad-VEGF or Ad-PR39</td>
<td>Local delivery (periadventitial)</td>
<td>Day (5)</td>
<td>↑</td>
<td>…</td>
<td>↑</td>
</tr>
<tr>
<td>Carotid artery</td>
<td>Silicone collar</td>
<td>Rabbit</td>
<td>Flt(1–3)IgG protein or Ad-dnFGFR</td>
<td>Local delivery (periadventitial)</td>
<td>Day (5)</td>
<td>→</td>
<td>…</td>
<td>→</td>
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<tr>
<td>Carotid artery</td>
<td>Silicone collar</td>
<td>Rabbit</td>
<td>Ad-PR39 + Flk(1–3)IgG protein or Ad-dnFGFR</td>
<td>Local delivery (periadventitial)</td>
<td>Day (5)</td>
<td>→</td>
<td>…</td>
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<tr>
<td>Carotid artery</td>
<td>Balloon injury</td>
<td>Rat</td>
<td>PR11 peptide</td>
<td>Local delivery (periadventitial)</td>
<td>Day (0)</td>
<td>↑</td>
<td>→</td>
<td>↑</td>
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<tr>
<td>Carotid artery</td>
<td>Balloon injury</td>
<td>Rat</td>
<td>Flt(1–3)IgG protein or Ad-dnFGFR</td>
<td>Local delivery (periadventitial)</td>
<td>Day (0)</td>
<td>→</td>
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tery), whereas the VEGF target in the study by Ohtani et al(21) seems to be other cell types (sFlt-1 attenuates smooth muscle cell proliferation). It is possible that Hutter et al(10) used relatively moderate injury that partly removed endothelial cells, whereas Ohtani et al(21) used severe vascular injury that completely removed all endothelial cells and some of the medial smooth muscle cells. In moderate injury, VEGF might preferentially work on residual endothelial cells to enhance reendothelialization and therefore inhibit intimal formation. On the other hand, in severe injury, VEGF might preferentially work on other cell types to promote smooth muscle proliferation and enhance neointima formation.

Another possibility is that the different strategies to block endogenous VEGF signaling resulted in different outcomes. VEGFR1 binds to VEGF-A with at least 10-fold higher affinity than VEGFR2. In addition, VEGFR1-knockout mice exhibit endothelial cell overgrowth and disorganized blood vessel formation,20 VEGFR2 deletion results in a defect in vascular formation with very few endothelial cells,21 and homozygous deletion of the tyrosine kinase domain of VEGFR1 results in normal embryonic development with normal blood vessel formation.22 On the basis of these findings, it has been postulated that VEGFR1 is a nonfunctional decoy receptor for VEGF-A and negatively regulates VEGF-A/VEGFR2 signaling. However, several recent studies have suggested that this is not the case. For example, PIGF, a specific ligand for VEGFR1, is involved in pathological but not physiological angiogenesis,23,24 an antibody against VEGFR1 reduces atherosclerotic plaque growth without changing adventitial vessel density in ApoE-deficient mice, and PIGF/VEGFR1 signaling promotes recruitment of smooth muscle and/or inflammatory cells.25 These data suggest that VEGFR1-mediated signals stimulate angiogenesis and inflammation by predominantly working on nonendothelial cell types. Because sFlt-1 may form a heterodimer with full-length VEGFR1 and thereby inhibit VEGFR1 signaling in a dominant-negative fashion,26 it is possible that sFlt-1, in contrast to VEGF-TrapR1 or VEGF-TrapR2, preferentially inhibits VEGFR1 signaling. According to this reasoning, smooth muscle cell proliferation and progenitor cell recruitment will be more inhibited relative to VEGFR2-mediated endothelial recovery by sFlt-1 treatment. Furthermore, the relative importance of VEGFR1- versus VEGFR2-mediated signals may be dependent on the relative expression levels of VEGF-A, PIGF, VEGFR1, VEGFR2, and sFlt-1 in the different models.

The study by Khurana et al(11) differs from the other 2 studies in that angiogenic growth factors or angiogenesis inhibitors were applied from outside the injured vessels. Although recombinant Flt(1-3)IgG protein did not attenuate neointima formation or adventitial angiogenesis by itself, Flt(1-3)IgG was able to inhibit growth factor–induced acceleration of neointima formation and adventitial angiogenesis. The lack of an inhibitory effect of Flt(1-3)IgG on neointima formation by itself suggests that initial neointima formation is independent of endogenous VEGF signaling. However, the correlation between adventitial angiogenesis and neointima formation supports a model in which some forms of angiogenesis promote restenosis after vascular injury.7

In summary, the contradictory results of the 3 studies discussed here indicate the complex relationship between angiogenesis and restenosis. Future studies with tissue-specific VEGFR1- or VEGFR2-knockout mice could provide further insight into this complicated and controversial issue. It would also be of interest to extend these studies with balloon-injured porcine coronary arteries because this model better mimics the consequences of percutaneous transluminal angioplasty in patients. In this regard, 2 clinical trials have shown that VEGF treatment after angioplasty is safe and feasible, although VEGF neither reduced nor increased the frequency of restenosis.27,28 In contrast, Genentech recently disclosed that Avastin (bevacizumab), the anti-VEGF reagent used to treat metastatic colorectal cancer, increases the risk of serious arterial thromboembolic events. These findings suggest that endogenous VEGF has an essential protective action on the vascular endothelium. Therefore, further experiments utilizing VEGF-Trap technology are warranted to develop a better understanding of the roles of endogenous VEGF in the adult cardiovascular system.

References


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