**The Role of Vascular Endothelial Growth Factor in Restenosis**

**The Controversy Continues**

Ichiro Shiojima, MD, PhD; Kenneth Walsh, PhD

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**Editorial**

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.

The VEGF–VEGF receptor (VEGFR) system includes at least 5 members of the VEGF family and 3 members of the VEGFR family (Figure), and it is critically involved in both physiological and pathological blood vessel formation. VEGF-A binds to VEGFR1 (Flt-1) and VEGFR2 (Flk-1/KDR); VEGF-B and placental growth factor (PIGF) bind to VEGFR1; and VEGF-C and VEGF-D bind to VEGFR2 and VEGFR3. VEGF-C and VEGF-D seem to have major roles in lymphangiogenesis via VEGFR3. Two receptors for VEGF-A, VEGFR1 (Flt-1) and VEGFR2 (Flk-1/KDR), are highly related transmembrane proteins with 7 immunoglobulin-like (Ig) domains in the extracellular portion, although VEGF-A binds to VEGFR1 with higher affinity than VEGFR2. Alternative splicing of the same gene also produces soluble VEGFR1 (sFlt-1), which contains amino-terminal 6 Ig domains but lacks transmembrane and intracellular kinase domains and functions as a decoy VEGF receptor to inhibit VEGF signaling. In addition to this naturally occurring “VEGF-Trap,” several modified versions of VEGF-Traps have been shown to effectively neutralize endogenous VEGF bioactivity and to inhibit angiogenesis (Figure). The 3 papers discussed here all used one of these VEGF-Traps as an inhibitor of endogenous VEGF signaling. Hutter et al used an adenovirus vector–encoding 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

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The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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This controversy has been rekindled by 3 articles appearing in the present issue of *Circulation*,. Although many of the previous studies focused on the effects of exogenously administered angiogenic growth factors on vascular lesions, these new studies analyze the role of endogenous angiogenic growth factor signaling in neointima formation after acute vascular injury. Specifically, these studies tested whether VEGF-Traps (Figure), inhibitors of endogenous VEGF signaling, have a positive or negative effect on the process of intimal hyperplasia. On the basis of these new studies, we can conclude that VEGF inhibits restenosis, VEGF promotes restenosis, and exogenous VEGF accelerates restenosis whereas endogenous VEGF does not.

The VEGF–VEGF receptor (VEGFR) system includes at least 5 members of the VEGF family and 3 members of the VEGFR family (Figure), and it is critically involved in both physiological and pathological blood vessel formation. VEGF-A binds to VEGFR1 (Flt-1) and VEGFR2 (Flk-1/KDR); VEGF-B and placental growth factor (PIGF) bind to VEGFR1; and VEGF-C and VEGF-D bind to VEGFR2 and VEGFR3. VEGF-C and VEGF-D seem to have major roles in lymphangiogenesis via VEGFR3. Two receptors for VEGF-A, VEGFR1 (Flt-1) and VEGFR2 (Flk-1/KDR), are highly related transmembrane proteins with 7 immunoglobulin-like (Ig) domains in the extracellular portion, although VEGF-A binds to VEGFR1 with higher affinity than VEGFR2. Alternative splicing of the same gene also produces soluble VEGFR1 (sFlt-1), which contains amino-terminal 6 Ig domains but lacks transmembrane and intracellular kinase domains and functions as a decoy VEGF receptor to inhibit VEGF signaling. In addition to this naturally occurring “VEGF-Trap,” several modified versions of VEGF-Traps have been shown to effectively neutralize endogenous VEGF bioactivity and to inhibit angiogenesis (Figure). The 3 papers discussed here all used one of these VEGF-Traps as an inhibitor of endogenous VEGF signaling. Hutter et al used an adenovirus vector–encoding 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\(^{12}\) 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\(^{11}\) 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\(^{10}\) are consistent with the notion that both exogenous and endogenous VEGF attenuate restenosis. The results of Ohtani et al\(^{12}\) 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\(^{10}\) (VEGF-Traps attenuates endothelial recov-

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

<table>
<thead>
<tr>
<th>Vascular Injury</th>
<th>Delivery Method</th>
<th>Neointima Formation</th>
<th>Luminal Endothelium</th>
<th>Adventitial Angiogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artery</td>
<td>Injury Type</td>
<td>Species</td>
<td>Vector or Protein</td>
<td>Day</td>
</tr>
<tr>
<td>Hutter et al(^{10})</td>
<td>Femoral artery Wire injury Mouse Ad-VEGF</td>
<td>IV injection Day (−1)</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Femoral artery Wire injury Mouse Ad-VEGF-TrapR1R2</td>
<td>IV injection Day (−1)</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Ohtani et al(^{12})</td>
<td>Femoral artery Wire injury Mouse sFlt-1 plasmid</td>
<td>IM injection Day (−3)</td>
<td>↓</td>
<td>→</td>
</tr>
<tr>
<td>Carotid artery Balloon injury Rabbit rat sFlt-1 plasmid</td>
<td>IM injection Day (−3)</td>
<td>↓</td>
<td>→</td>
<td>↓</td>
</tr>
<tr>
<td>Carotid artery Balloon injury Rabbit rat Ad-VEGF</td>
<td>Local delivery (balloon catheter) Day (0)</td>
<td>→</td>
<td>...</td>
<td>↑</td>
</tr>
<tr>
<td>Khurana et al(^{11})</td>
<td>Carotid artery Silicone collar Rabbit Ad-VEGF or Ad-PR39</td>
<td>Local delivery (periadventitial) Day (5)</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Carotid artery Silicone collar Rabbit Flt(1-3)IgG protein or Ad-dnFGFR</td>
<td>Local delivery (periadventitial) Day (5)</td>
<td>→</td>
<td>...</td>
<td>→</td>
</tr>
<tr>
<td>Carotid artery Silicone collar Rabbit Ad-PR39 + Flk(1-3)IgG protein or Ad-dnFGFR</td>
<td>Local delivery (periadventitial) Day (5)</td>
<td>→</td>
<td>...</td>
<td>→</td>
</tr>
<tr>
<td>Carotid artery Balloon injury Rat PR11 peptide</td>
<td>Local delivery (periadventitial) Day (0)</td>
<td>↑</td>
<td>→</td>
<td>↓</td>
</tr>
<tr>
<td>Carotid artery Balloon injury Rat Flt(1-3)IgG protein or Ad-dnFGFR</td>
<td>Local delivery (periadventitial) Day (0)</td>
<td>→</td>
<td>...</td>
<td>→</td>
</tr>
</tbody>
</table>
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. 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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