Endothelial Growth Factor Receptors in Human Fetal Heart

Taina A. Partanen, MD; Taija Makinen, MSc; Johanna Arola, MD, PhD; Toshio Suda, MD, PhD; Herbert A. Weich, MD, PhD; Kari Alitalo, MD, PhD

Background—Endothelial receptor tyrosine kinases include 3 members of the vascular endothelial growth factor receptor (VEGFR) family and 2 members of the angiopoietin receptor (Tie) family. In addition, the VEGF_165 isoform binds to neuropilin-1 (NP-1), a receptor for collapsins/semaphorins. The importance of these receptors for vasculogenesis and angiogenesis has been shown in gene-targeted mice, but so far, little is known about their exact expression patterns in the human vasculature.

Methods and Results—Frozen sections of human fetal heart were stained immunohistochemically with receptor-specific monoclonal (VEGFR, Tie) or polyclonal (NP-1) antibodies. The following patterns were observed: The endocardium was positive for VEGFR-1, VEGFR-2, NP-1, Tie-1, and Tie-2 but negative for VEGFR-3. The coronary vessels were positive for Tie-1, Tie-2, VEGFR-1, and NP-1 and negative for VEGFR-2 and VEGFR-3. Myocardial capillaries and epicardial blood vessels stained for VEGFR-1, VEGFR-2, NP-1, and Tie-1; myocardial capillaries and epicardial veins weakly for Tie-2; and epicardial lymphatic vessels for VEGFR-2 and VEGFR-3, weakly for Tie-1 and Tie-2, but not for VEGFR-1 or NP-1.

Conclusions—The results demonstrate differential expression of the endothelial growth factor receptors in distinct types of vessels in the human heart. This information is useful for the understanding of their roles in physiological and pathological processes and for their diagnostic and therapeutic application in cardiovascular medicine. (Circulation. 1999;100:583-586.)

Key Words: angiogenesis ■ endothelium ■ endocardium ■ myocardium ■ growth substances

Angiogenesis is a growth factor–driven complex multistep process by which new blood vessels are formed from the preexisting vasculature. The failure of angiogenesis and insufficient growth of collateral vessels is a major problem in vascular diseases, such as arteriosclerosis and restenosis. In the normal adult vessels, growth factors angiogenic in early development serve vessel maintenance functions and regulate such things as vascular permeability. An interesting question of considerable importance for therapeutic purposes is how the growth factor signaling cascade can again be recruited to an angiogenic response in adults.

Known endothelial specific receptor tyrosine kinases include the vascular endothelial growth factor receptor (VEGFR) and tyrosine kinase with immunoglobulin and epidermal growth factor homology domains (Tie) families (for reviews, see References 1 and 2). Gene targeting of these receptors or their ligands usually leads to a failure to complete embryonic development because of abnormalities of vasculogenesis and/or angiogenesis. VEGF is the best-known and the most important ligand for the VEGFRs involved in angiogenic processes in physiological and pathological conditions. Other members include the placenta growth factor, VEGF-B, VEGF-C, and VEGF-D. At least 1 VEGF isoform (VEGF_165) binds to neuropilin-1 (NP-1), a receptor for the collapsins/semaphorins. Interestingly, overexpression of NP-1 in chimeric mice has been shown to cause lethal anomalies of the cardiovascular system.

Tie-1 and Tie-2/tunica interna endothelial cell kinase (Tek) constitute the Tie family. Angiopoietin-1 and angiopoietin-2 have been reported to be stimulatory and antagonistic ligands for Tie-2, respectively, whereas no ligands for Tie-1 have been identified as yet. Both of the Tie receptor tyrosine kinases are required during embryonic development for the integrity and survival of vascular endothelial cells, particularly in the regions undergoing angiogenic sprouting of capillaries, and later for endothelial cell maintenance.

Human VEGF is one of the most promising candidates to be used for therapeutic angiogenesis via recombinant protein or gene therapy. However, a single growth factor may be insufficient for therapeutic purposes, because the development of a functional vascular system requires a variety of factors and their receptors and signals.
define the molecular anatomy of the known endothelial growth factor receptors in the cardiovascular system, we studied their expression patterns in fetal heart by immunohistochemistry.

### Methods

#### Fetal Tissue

The fetal hearts were obtained from one 5- to 6-week, two 13-week, two 15-week, and one 20-week legal abortions of healthy women induced with prostaglandins. One 27- to 30-week fetal heart was obtained from spontaneous abortion. The gestational age was estimated from the foot length. The study was approved by the Ethical Committee of the Helsinki University Central Hospital. Three hearts were snap-frozen, and 4 were fixed with 4% paraformaldehyde for 20 hours, dehydrated, and paraffin-embedded for sectioning.

#### Immunohistochemistry

Sections 4 μm thick were immunostained with mouse monoclonal antibodies against human VEGFR-3 as described earlier, except that the paraffin sections were stained by use of the TSA kit (New England Nuclear Life Science Products, Inc). The other monoclonal antibodies used were against CD31 (platelet/endothelial cell adhesion molecule-1; DAKO Immunoglobulins), von Willebrand factor/factor VIII–related antigen (6.3 μg/mL; DAKO), desmoplakin 1 and 2 (5 μg/mL; Progen Biotechnik GmbH), α-smooth muscle actin (0.5 mg/mL; Sigma, clone 19), an as yet molecularly undefined blood vascular endothelial antigen (PAL-E; Sanbio), VEGFR-1 (1:200 dilution of the supernatant of clone 19), VEGFR-2 (1:800 dilution), Tie-2 (1.32 μg/mL), and Tie-1 (8 μg/mL). Immunohistochemistry for NP-1 was carried out according to Kitsukawa et al using affinity-purified rabbit IgG against mouse NP-1 (1:200, 450 μg/mL; a kind gift from Dr H. Fujisawa). After the staining

### Expression of Endothelial Growth Factor Receptors in the Human Fetal Heart Vasculature

<table>
<thead>
<tr>
<th>Structure</th>
<th>Receptor</th>
<th>VEGFR-1</th>
<th>VEGFR-2</th>
<th>VEGFR-3</th>
<th>NP-1</th>
<th>Tie-1</th>
<th>Tie-2</th>
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<tbody>
<tr>
<td>Epicardium</td>
<td>Coronaries</td>
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<td>+/++</td>
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<tr>
<td></td>
<td>Capillaries</td>
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<tr>
<td></td>
<td>Arteries</td>
<td>++</td>
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<td>–</td>
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<td>++</td>
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</tr>
<tr>
<td></td>
<td>Veins</td>
<td>++</td>
<td>++</td>
<td>–</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Lymphatics</td>
<td>–</td>
<td>++</td>
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<td>++</td>
</tr>
<tr>
<td>Myocardium</td>
<td>Capillaries</td>
<td>++</td>
<td>++</td>
<td>–/++</td>
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<td>+/++</td>
<td>+/++</td>
</tr>
<tr>
<td></td>
<td>Arteries</td>
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<tr>
<td></td>
<td>Endocardium</td>
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<tr>
<td>Blood vessels</td>
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Gestational ages 5 to 30 weeks.

* Fetuses of 13 to 30 weeks; ++ for the 5-week embryo.

Figure 1. Comparison of adjacent sections from a 15-week human fetal heart immunostained for VEGFR-1 (A), VEGFR-2 (B), VEGFR-3 (C), NP-1 (D), Tie-1 (E), Tie-2 (F), CD31 (G), PAL-E (H), and von Willebrand factor (vWF) (I). Negative control (without primary antibody) is shown in inset of (I). In epicardium, coronary artery (CA) and vein (V) were negative for VEGFR-2 (B) and VEGFR-3 (C), whereas lymphatic vessels (L) stained strongly for both of these receptors. Arrowheads identify capillaries in epicardium, which react with antibodies against VEGFR-1 (A) and VEGFR-2 (B). Staining with antigen-blocked anti–NP-1 antiserum is shown in inset in (D). Bar = 68 μm.
procedures, all samples were examined by a trained pathologist (J.A.). Positive control slides (e.g., placenta for Tie-1 and Tie-2), negative controls involving nonimmune IgG, and controls that used PBS instead of the primary antibodies were included in the stainings for each antigen.

**Results**

The localizations of the growth factor receptors in hearts of different gestational ages were similar and are summarized in Table. In the epicardial area, the coronary artery and vein as well as some capillaries were weakly positive for VEGFR-1 (Figure 1A). The coronary arteries were VEGFR-2-negative, whereas the coronary veins and adjacent capillaries were weakly positive (Figure 1B). The epicardial lymphatic vessels were negative for VEGFR-1 but strongly positive for both VEGFR-2 (Figure 1B) and VEGFR-3 (Figure 1C). These thin-walled vessels were defined as lymphatic vessels with a CD31-positive, PAL-E antigen–negative endothelium (Figure 1G and 1H), presence of desmoplakins, and lack of red cells and desmin- or smooth muscle actin–positive pericytes/smooth muscle cells. The myocardial small arterioles and capillaries and the endocardium stained strongly for VEGFR-1 and VEGFR-2 (Figure 2A and 2B). Very weak VEGFR-3 signals were also observed in some myocardial capillaries (Figure 2C) but not in the endocardium (e in Figure 2C).

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**Figure 2.** Expression of VEGFR-1 (A), VEGFR-2 (B), VEGFR-3 (C), NP-1 (D), Tie-1 (E), and Tie-2 (F) in 15-week fetal myocardium and endocardium. Note that all receptors except VEGFR-3 are expressed in endocardium (e). In myocardium, some capillaries are weakly positive for VEGFR-3 (C, arrowheads). Arrows indicate myocardial (m) capillaries. C, p represents a paraffin section used to confirm weak staining obtained from frozen (f) section.
The NP-1 immunostaining pattern was similar to that of VEGFR-1 (Figure 1D). Antigen-blocking experiments indicated that the staining for NP-1 was specific (Figure 1D). The Tie-1 and Tie-2 proteins were coexpressed in the endothelium of large and small blood vessels and in the atrial and ventricular endothelia (Figures 1E, 1F, 2E, and 2F). Tie-2 was more prominent in the venous than the arterial endothelium. The pericardial thin-walled VEGFR-3–positive vessels expressed Tie-1 and Tie-2 weakly.

Discussion

Our results constitute a survey of the distribution of known endothelial growth factor receptors in the developing human heart, and they are consistent with previous localization of receptor mRNAs except that in the present study, a minor population of the myocardial capillaries also contained VEGF-R-3 in 13- to 30-week fetuses. The more intense staining of VEGFR-3 in 5-week embryos (Table) suggests that the expression is downregulated in the blood vessels during the first trimester. Interestingly, a particularly strong coexpression of VEGFR-1, VEGFR-2, NP-1, Tie-1, and Tie-2 was associated with the endocardial endothelium, which is subject to a considerable degree of hemodynamic stress. In contrast, VEGFR-3 was not detected in the endocardium or other endothelia subject to shear stress. The lymphatic vessels at this stage of development seem to contain both the Tie tyrosine kinase receptors and VEGFR-2 and VEGFR-3, which they may use for signal transduction. VEGFR-2 is the only known receptor that binds both VEGF and VEGF-C. It has been speculated that the ability of VEGF-C to stimulate the migration of capillary endothelial cells and the permeability of blood vessels are mediated via VEGFR-2.

In summary, our findings suggest that the developing vasculature of the heart requires a variety of endothelial growth factor receptors. Although the expression of VEGFR-1 and VEGFR-2 overlapped, only the former was detected in coronary arteries and myocardial capillaries and no VEGFR-3 was detected in the blood vessels or in the endocardium, where all the other receptors were coexpressed. This suggests that although VEGFR-3 is needed for early cardiovascular development, it later serves a more specialized biological function mainly in lymphatic endothelia. In preliminary experiments, frozen sections of adult heart tissue gave qualitatively similar results, but further studies are needed to clarify the expression of endothelial growth factor receptors in ischemic adult heart.

Acknowledgments

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References

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