Corrections

EVOLUTION, ANTHROPOLOGY. For the article “Genera of the human lineage,” by Camilo J. Cela-Conde and Francisco J. Ayala, which appeared in issue 13, June 24, 2003, of Proc. Natl. Acad. Sci. USA (100, 7684–7689; First Published June 6, 2003; 10.1073/pnas.0832372100), the authors note that, due to a printer’s error, the references in the heading of Table 1 on page 7688 should be 16 and 17 rather than 32 and 33. The reference in the footnote should be 17 rather than 32. The corrected table appears below.

Table 1. Modification of previous taxonomies (16, 17), including formal taxonomic designations and reference to geological and geographical ranges

<table>
<thead>
<tr>
<th>Family Hominidae Gray, 1825. Miocene to the present, worldwide.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus † Sahelanthropus Brunet et al., 2002, incertae sedis. Miocene, Central Africa.</td>
</tr>
<tr>
<td>Species † Sahelanthropus tchadensis Brunet et al., 2002. Miocene, Central Africa.</td>
</tr>
<tr>
<td>Genus † Praeanthropus Senyurek 1955 (includes Orrorin Senut et al., 2001). Miocene–Pliocene, Africa.</td>
</tr>
<tr>
<td>Species † Praeanthropus tugenensis* Senut et al., 2001. Miocene, East Africa.</td>
</tr>
<tr>
<td>Species † Praeanthropus bahrelghazali Brunet et al., 1996. Pliocene, Subsahara.</td>
</tr>
<tr>
<td>Species † Praeanthropus anamensis M. G. Leakey et al., 1995. Pliocene, East Africa.</td>
</tr>
<tr>
<td>Species † Praeanthropus garhi Asfaw et al., 1999. Pliocene, East Africa.</td>
</tr>
<tr>
<td>Genus † Ardipithecus White et al., 1995. Miocene–Pliocene, East Africa.</td>
</tr>
<tr>
<td>Species † Ardipithecus ramidus White et al., 1994. Miocene–Pliocene, East Africa.</td>
</tr>
<tr>
<td>Subfamily Australopithecinae Gregory and Hellman, 1939. Pliocene, Africa.</td>
</tr>
<tr>
<td>Species † Australopithecus africanus* Dart, 1925. Pliocene, Africa.</td>
</tr>
<tr>
<td>Species † Australopithecus euhominus Arambourg and Coppens, 1968. Pliocene, East Africa.</td>
</tr>
<tr>
<td>Species † Australopithecus robustus Broom, 1938. Pleistocene, southern Africa.</td>
</tr>
<tr>
<td>Subfamily Homininae Gray, 1825. Pliocene to the present, worldwide.</td>
</tr>
<tr>
<td>Genus Homo Linnaeus, 1758 (includes Pithecanthropus Dubois, 1894; Proconsulinae Haeckel, 1895; Sinanthropus Black, 1927; and Parasaurolophus Arambourg and Coppens, 1967). Pliocene to the present, worldwide.</td>
</tr>
<tr>
<td>Species † Homo platyops* M. G. Leakey et al., 2001. Pliocene, East Africa.</td>
</tr>
<tr>
<td>Species † Homo rudolfensis Alexeev, 1986. Pliocene, Africa.</td>
</tr>
<tr>
<td>Species † Homo habilis L. S. B. Leakey et al., 1964. Pliocene, Africa.</td>
</tr>
<tr>
<td>Species † Homo ergaster Groves and Mazák, 1975. Plio-Pleistocene, Africa and Eurasia.</td>
</tr>
<tr>
<td>Species † Homo erectus Dubois, 1892. Pleistocene, Africa and Eurasia.</td>
</tr>
<tr>
<td>Species † Homo heidelbergensis Schoetensack, 1908. Pleistocene, Africa and Eurasia.</td>
</tr>
<tr>
<td>Species † Homo neanderthalensis King, 1864. Pleistocene, western Eurasia.</td>
</tr>
<tr>
<td>Species Homo sapiens Linnaeus, 1758. Pleistocene to the present, worldwide.</td>
</tr>
</tbody>
</table>

†, Taxon is extinct; *, taxon considered species germinalis (17), i.e., a species originating a genus; ?, uncertainty about the taxon’s presence in that region.

www.pnas.org/cgi/doi/10.1073/pnas.1734099100
NEUROSCIENCE. For the article “Mifepristone (RU486) protects Purkinje cells from cell death in organotypic slice cultures of postnatal rat and mouse cerebellum,” by A. M. Ghoumari, I. Dusart, M. El-Etr, F. Tronche, C. Sotelo, M. Schumacher, and E.-E. Baulieu, which appeared in issue 13, June 24, 2003, of Proc. Natl. Acad. Sci. USA (100, 7953–7958; First Published June 16, 2003; 10.1073/pnas.1332667100), the authors note that “(suppress cultures)” should be removed from the Fig. 2 legend, and that “and mouse” should be removed from the first sentence of the Fig. 5 legend. The sentence should have read, “Purkinje cell death in organotypic slice cultures of postnatal rat cerebella does not result from oxidative stress.” The figures and their corrected legends appear below.

Fig. 2. Effects of RU486 on Purkinje cell survival in P5 and P7 rat cerebella. Organotypic slices of P5 and P7 rat cerebella (A and B, respectively) were cultured for 5 DIV in the absence (A and B) or presence (A’ and B’) of 20 μM RU486. At all ages, RU486 protects Purkinje neurons from cell death. (Scale bar = 250 μm.)

Fig. 5. Purkinje cell death in organotypic slice cultures of postnatal rat cerebella does not result from oxidative stress. P3 cerebellar slices were treated with different antioxidant agents. (A) VitE (50 μM), NAC (5 mM), and GSH were used alone or in combination. (B) MnTBAP (200 μM), a cell-permeable superoxide dismutase (SOD) mimetic and peroxynitrite scavenger, was also used.
MEDICAL SCIENCES. For the article “Blockade of vascular endothelial growth factor receptor-3 signaling inhibits fibroblast growth factor-2-induced lymphangiogenesis in mouse cornea,” by Hajime Kubo, Renhai Cao, Ebba Bräkenhielm, Taija Mäkinen, Yihai Cao, and Kari Alitalo, which appeared in issue 13, June 25, 2002, of Proc. Natl. Acad. Sci. USA (99, 8868–8873; First Published June 17, 2002; 10.1073/pnas.062040199), the authors note that the indication of black and white bars was inverted in the legend of Fig. 3. The legend should have read, “Black bars, angiogenic score; white bars, lymphangiogenic score.” The figure and its corrected legend appear below.

![Figure 3: Quantification of the corneal angiogenesis and lymphangiogenesis.](image-url)

**Fig. 3.** Quantification of the corneal angiogenesis and lymphangiogenesis. (a) Quantification of the angiogenesis and lymphangiogenesis induced by VEGF, VEGF-C, and FGF-2 (n = 3 each) analyzed by immunostaining as described in Materials and Methods. (b) Quantification of the angiogenesis and lymphangiogenesis in FGF-2-implanted corneas treated with anti-VEGFR3 (α-VEGFR-3) in comparison with the corneas of animals treated with nonblocking anti-VEGF-2 control antibodies (Control). Black bars, angiogenic score; white bars, lymphangiogenic score. The graphs represent mean values ± SEM.

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Blockade of vascular endothelial growth factor receptor-3 signaling inhibits fibroblast growth factor-2-induced lymphangiogenesis in mouse cornea

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Vascular endothelial growth factor receptor-3 (VEGFR-3) is a major mediator of lymphangiogenesis. Recently, VEGFR-3 ligands, VEGF-C, and VEGF-D were reported to promote tumor lymphangiogenesis and lymphatic metastasis, and these processes were inhibited by blocking of the VEGFR-3-signaling pathway. Here, we have adapted the mouse corneal angiogenesis assay to study potential lymphangiogenic factors and inhibitors. Immunohistochemical analysis with lymphatic endothelial markers showed that VEGF-C induces lymphatic as well as blood vessel growth in the cornea. By contrast, VEGF induced angiogenesis but not lymphangiogenesis. Fibroblast growth factor-2 (FGF-2) stimulated both angiogenesis and lymphangiogenesis. FGF-2 up-regulated VEGF-C expression in vascular endothelial and perivascular cells. Furthermore, administration of blocking anti-VEGFR-3 antibodies inhibited the FGF-2-induced lymphangiogenesis. These findings show that VEGFR-3 can mediate lymphangiogenesis induced by other growth factors. Because increased expression of FGF-2 and VEGF-C has been associated with lymphatic metastasis, our results provide a potential strategy for the inhibition of lymphatic metastasis in cancer therapy.

Metastasis to the regional lymph nodes by the lymphatic vessels is a common step in the progression of cancer, an important prognostic factor in many types of cancer, and the basis for surgical and radiation treatment of local lymph nodes. Recent evidence suggests that tumor lymphangiogenesis, the growth of tumor-associated lymphatic vessels, promotes lymphatic metastasis (1–4) and that the inhibition of lymphangiogenesis may provide a new strategy to block lymph node metastasis in cancer therapy (5).

Vascular endothelial growth factor (VEGF)-C, fibroblast growth factor-2 (FGF-2), and LYVE-1 are considered as lymphatic endothelial markers. In the mouse corneal angiogenesis assay, LYVE-1 (22, 23), a new homologue of the CD44 glycoprotein, LYVE-1 (22, 23), a new homologue of the CD44 glycoprotein, fibroblast growth factor-2; LYVE-1, lymphatic vessel endothelial hyaluronan receptor-1.

Materials and Methods

Animals and Reagents. Male 5- to 6-week-old C57BL6/J mice were housed in groups of four or fewer. For all procedures, the mice were anesthetized by using a mixture of dormicium and hypnorm (1:1), and killed with a lethal dose of CO2. All animal studies were reviewed and approved by the animal care and use committee of the Stockholm Animal Board. A recombinant, mature form of human VEGF-C was expressed in Pichia pastoris and purified as described (34). Recombinant human VEGF165 was obtained from R&D Systems, and FGF-2 from Pharmacia and UpJohn (Milan, Italy). Rat monoclonal antibodies against FGF-2 were purchased from Pharmacia and UpJohn (Milan, Italy).

Abbreviations: VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; FGF-2, fibroblast growth factor-2; LYVE-1, lymphatic vessel endothelial hyaluronan receptor-1.

*H.K. and R.C. contributed equally to this work.

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mouse VEGFR-3 and rabbit polyclonal antibodies against mouse LYVE-1 were used as described (23, 35).

**Mouse Corneal Micropocket Assay.** The mouse corneal assay was performed according to procedures described (19). Corneal micropockets were created with a modified von Graefe cataract knife in both eyes of each mouse. A micropellet (0.35 × 0.35 mm) of sucrose aluminum sulfate (Bukh Meditec, Copenhagen, Denmark) coated with hydron polymer type NCC (IFN Science, New Brunswick, NJ) containing 160 ng of VEGF-C or VEGF, or 80 ng of FGF-2, was implanted into each pocket. The pellet was positioned 0.6–0.8 mm from the limbus. After implantation, erythromycin ophthalmic ointment was applied to the eyes. The eyes were examined by a slit-lamp biomicroscope at the indicated days. Vessel length and clock hours of circumferential neovascularization were measured. For the inhibition studies, mice that received corneal implants containing FGF-2 were randomized into two groups and given i.p. injections of neutralizing anti-VEGFR-3 antibodies or nonblocking anti-VEGFR-2 antibodies (35) (600 μg per mouse) on postoperative days 0, 2, and 4. The corneas were photographed on day 5 by a slit-lamp biomicroscope, and the immunohistochemical analysis was performed as described below.

**Immunohistochemistry.** Mice were killed between days 5 and 13 after the implantation of the pellets. Enucleated eyes were fixed in 3% paraformaldehyde, dehydrated, and embedded in paraffin and sectioned radially in parallel to the growing limbal vessels (see Fig. 2 c–e). Sections (8 μm) were immunostained by using monoclonal antibodies against CD31 (1:800, PharMingen) and VEGFR-3 (1 μg/ml, clone AFL4) (35), as well as polyclonal antibodies against mouse LYVE-1 (23), FGF receptor-1 (FGFR-1) (1:200, Santa Cruz Biotechnology) and VEGF-C (1:400, R&D Systems; 1:400, Santa Cruz Biotechnology, A-18). Peroxidase activity was developed with 3-amino-9-ethyl carbazole (Sigma) and the sections were counterstained with hematoxylin. The tyramide signal amplification (NEL Life Science) was used to enhance staining. In negative-control stainings the primary antibodies were omitted. The specificity of anti-VEGFR-C antibodies was checked by blocking with a 10-fold molar excess of recombinant VEGF-C.

**Quantification of Corneal Neovascularization.** The angiogenic or lymphangiogenic grading scores were based on immunohistochemical analysis of the distance of vessel growth from the limbus. By staining several 8-μm sections with hematoxylin and eosin, sections including corneal vessels or pellets were found. At least five series of three sequential sections through the eyes were immunostained for CD31, LYVE-1, and VEGFR-3. The grading was: 0, no corneal lymphatic vessels, including the cases with growth of lymphatic vessels in the limbus only; 5, corneal lymphatic vessels penetrating into the pellet; 1–4, the corneal area between limbus and the pellet was divided into equal segments and scored from 1 to 4 in the order of distance from the limbus.

**Cell Stimulation Assay.** Isolation of primary lymphatic and blood vascular endothelial cells from cultures of human dermal microvascular endothelial cells was performed as described (36). The endothelial cells, and smooth muscle cells derived from human coronary artery (HCASMC, Promo Cell, Heidelberg) were grown to subconfluence, starved for 48 h in serum- and growth factor-free medium, and then incubated with or without FGF-2 (5 ng/ml) for 24 h. RNA was isolated with RNeasy Mini (Qiagen, Hilden, Germany), electrophoresed, blotted, hybridized with the VEGF-C and glyceraldehyde3-phosphate dehydrogenase cDNA probes, and subjected to phosphoimaging.

**Results**

**Lymphatic Vessels in the Eye.** The distribution of lymphatic vessels in the mouse eye was analyzed by immunostaining for the lymphatic-specific markers LYVE-1 and VEGFR-3 and by comparison with the pan-endothelial marker CD31. In the limbal region, several vessels were positive for CD31 (Fig. 1a and b). The vessels that stained weakly for CD31 (Fig. 1b, arrow) were also immunoreactive for VEGFR-3 (Fig. 1c) and LYVE-1 (Fig. 1d). These vessels had thin walls lined by endothelial cells with an attenuated cytoplasm and a protruding nucleus, and they were thus identified as lymphatic vessels. No immunostaining occurred in the cornea, consistent with the fact that the cornea represents an avascular tissue (data not shown).

**Induction of Corneal Lymphangiogenesis by VEGF-C.** Implantation of micropellets of aluminum sulfate coated with a slow-release polymer hydron containing VEGF-C induced an angiogenic response in the cornea, which was intensive on day 5 after implantation but started to regress on day 13 (Fig. 1a; data not shown). Because the lymphatic vessels were not visible by intravital observation, histologic examination was performed. Because lymphatic vessel growth has been reported to follow wound healing (20, 37), the mice were killed and their eyes were analyzed between days 5 and 13 after implantation. Light microscopic studies of sections stained with hematoxylin and eosin disclosed no infiltration of inflammatory cells into the corneal stroma, suggesting that an inflammatory response is not a prerequisite for neovascularization in this model. Immunohistochemical analysis revealed that CD31-, VEGFR-3-, and LYVE-1-positive lymphatic vessels penetrated into the cornea and reached the pellet (Fig. 2c–e). Blood vessels could be clearly distinguished from the lymphatic vessels by their thick endothelial cells, strong CD31 staining, and blood cells within the vessels, and by the lack of VEGFR-3 and LYVE-1 staining (Fig. 2f–h). The lymphatic vessels were located adjacent to the blood vessels in the corneal stroma, generally within the...
VEGF Induces Angiogenesis but No Lymphangiogenesis. VEGF has been considered to induce angiogenesis without affecting the lymphatic vessels (10). To confirm that the corneal assay is suitable for the differential assessment of angiogenic and lymphangiogenic factors, pellets containing VEGF 

VEGF-C is stained for CD31 (f), VEGFR-3 (g), and LYVE-1 (h) in adjacent sections of the corneal neovasculature induced by VEGF-C shows the presence of corneal lymphatic vessels (arrows). The blood vessels indicated by the arrowheads (f–h) are positive only for CD31. The corneal neovasculature induced by VEGF is stained for CD31 (i), VEGFR-3 (j), and LYVE-1 (k) in adjacent, non-counterstained sections of the limbal (arrow) and corneal tissue. Note that several CD31-positive but LYVE-1-negative blood vessels are also stained for VEGF-C in the corneal area. A few LYVE-1-positive limbal lymphatic vessels (arrows) are present close to the induced capillaries. (Magnifications: c–e, ×100; f–h, ×400; i–k, ×200).

FGF-2 Induces both Angiogenesis and Lymphangiogenesis in the Corneal Assay. To investigate the ability of FGF-2 to induce lymphangiogenesis in vivo, hydro pellets containing FGF-2 were implanted into the corneas. Strong angiogenesis and lymphangiogenesis was stimulated by FGF-2 (Fig. 3a). CD31-, LYVE-1-, and VEGFR-3-positive lymphatic vessels were distributed in the same manner as in the VEGF-C-implemented corneas (Fig. 4a–c, arrows). The FGF2-induced corneal blood vessels were negative for VEGFR-3, except at the tips of the vessel sprouts (Fig. 4d; data not shown). FGF-1 (23), the major receptor for FGF-2 in the vascular endothelium, was expressed by vascular endothelial cells and perivascular cells (Fig. 4d, small arrows and arrowheads).

FGF-2-Induced Lymphangiogenesis Is Mediated by VEGFR-3 Ligand. To test the possibility that VEGF-C was induced in the corneas stimulated by FGF-2, immunostaining for VEGFR-3 was performed. No immunoreactivity for VEGF-C was detected in the normal corneas or limbus, or in the VEGF-implanted corneas (data not shown). In sections of the eyes containing FGF-2 pellets, VEGF-C staining showed a pattern similar to the staining for FGF1. The endothelium and perivascular cells of some newly formed capillaries in the corneas and pre-existing limbal vessels were stained with the anti-VEGF-C antibodies (Fig. 4e). The VEGF-C staining also decorated the endothelial cells in the dense conjunctival lymphatic capillary network, whereas no staining was observed on the side opposite to the pellet (data not shown).

To test the effect of FGF-2 on VEGF-C expression by endothelial cells and smooth muscle cells in vitro, isolated human primary blood vascular and lymphatic endothelial cells and smooth muscle cells were stimulated with 5 ng/ml of FGF-2 in serum-free medium for 24 h. Northern blot analysis revealed that VEGF-C mRNA expression was up-regulated by FGF-2 in the blood vascular endothelial cells (Fig. 4f). The FGF2-treated lymphatic endothelial cells showed essentially no VEGF-C mRNA. Both endothelial cell types expressed FGFR-1 (data not shown). In contrast, FGF-2 stimulation did not affect the relatively high endogenous levels of VEGF-C mRNA in the smooth muscle cells (Fig. 4g).

To investigate the functional significance of the up-regulation
demonstrate that FGF-2 stimulates lymphangiogenesis indirectly through corneal lymphatic vessels induced by FGF-2. These results suggest that the angiogenic growth factor FGF-2 can also induce lymphangiogenesis in the cornea. The corneal assay could be especially advantageous for further studies of the mechanisms involved, given the possibility to observe lymphatic vessels intravitaly by way of dye injection or fluorescent imaging.

To date, numerous activators of blood vessel growth have been identified (39), and it is possible that various factors are also involved in lymphangiogenesis. Our results raise the possibility that FGF-2 is also involved in the regulation of physiological and pathological lymphatic vessel growth. The results of immunostaining showed that FGF-2 increases the amount of VEGF-C in vascular endothelial cells. VEGF-C was also up-regulated due in part to the stimulation of vascular permeability by VEGF and in part to the lack of associated lymphangiogenesis. In VEGF-C-implanted corneas the lymphatic vessels were present along with the blood vessels. Because the mature form of VEGF-C used in the experiments binds to and activates both VEGFR-2 and VEGFR-3, VEGF-C should directly stimulate the endothelium of both the blood and lymphatic vessels in these experiments (34).

To investigate whether lymphatic vessels can penetrate into the cornea without a concurrent blood vessel growth, the VEGF-C-specific mutant factor VEGF-C156S (38) was used in the corneal assay. However, VEGF-C156S could induce neither lymphangiogenesis nor angiogenesis (H.K., R.C., E.B., Y.C., and K.A., unpublished data). Our work has shown that transgenic mice overexpressing VEGF-C156S in the skin have hyperplastic cutaneous lymphatic vessels but normal blood vessels (14). The lack of lymphangiogenesis by VEGF-C156S suggests that factors produced during angiogenesis, such as proteolytic enzymes, may be essential to support lymphatic vessel sprouting into the avascular cornea. In fact, the corneal assay could be especially advantageous for further studies of the mechanisms involved, given the possibility to observe lymphatic vessels intravitaly by way of dye injection or fluorescent imaging.

In this study, we show that VEGF-C induces not only angiogenesis but also lymphangiogenesis in the cornea. The corneal assay revealed that the angiogenic growth factor FGF-2 can also induce lymphangiogenesis and that inhibition of VEGFR-3 signaling by anti-VEGFR-3 antibodies suppresses the growth of corneal lymphatic vessels induced by FGF-2. These results demonstrate that FGF-2 stimulates lymphangiogenesis indirectly and that, in general, the mouse corneal assay is valuable for the investigation of pro- and antilymphangiogenic factors.

Lymphangiogenesis has been reported in vascularized corneas in several clinical and pathological states (21). Here, we applied purified exogenous factors in the mouse cornea to examine their lymphangiogenic potential by immunohistochemical analysis using the newly identified lymphatic marker, LYVE-1. Although VEGF-C induced both corneal lymphangiogenesis and angiogenesis, VEGF induced only angiogenesis. VEGF caused the previously noted perilimbal edematous swelling, which may be due in part to the stimulation of vascular permeability by VEGF and in part to the lack of associated lymphangiogenesis. In VEGF-C-implanted corneas the lymphatic vessels were present along with the blood vessels. Because the mature form of VEGF-C used in the experiments binds to and activates both VEGFR-2 and VEGFR-3, VEGF-C should directly stimulate the endothelium of both the blood and lymphatic vessels in these experiments (34).

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important effector of FGF-2-induced lymphangiogenesis. Some VEGF-C staining occurred in the limbic lymphatic vessels, possibly as a result of VEGF-C binding to its receptors in these cells. The fact that anti-VEGFR-3 antibodies inhibited the FGF-2-induced lymphangiogenesis indicates that VEGF-C (and/or VEGF-D) is a major downstream mediator of the lymphangiogenic activity of FGF-2.

From the physiological and rheological point of view, lymphatic vessels function as a drainage system and they are thus very different from blood vessels in vivo. In contrast to the in vitro assays, in vivo models permit the assessment of systemic host factors that influence the growth of the lymphatic vessels. For example, sprouting of lymphatic vessels into tumor stroma may not occur because these vessels lack sufficient pressure to penetrate into the stroma which has a high interstitial pressure (40). Results with isolated lymphatic endothelial cells could also be misleading because many cultured endothelial cells and pericytes/smooth muscle cells located close to the lymphatic vessels in vivo express VEGF-C constitutively (13, 41). Endogenous VEGF-C mRNA was not down-regulated in the cultured vascular smooth muscle cells by serum starvation, nor was it stimulated by FGF-2. However, VEGF-C mRNA expression increased after FGF-2 stimulation in blood vascular, but not lymphatic endothelial cells. This finding might explain, in part, the phenomenon of concurrent blood vessel growth during lymphangiogenesis in vivo.

In addition to being expressed in the lymphatics, VEGFR-3 is also up-regulated in angiogenic blood vessels in many types of cancers (42, 43). The present result that VEGF-induced angiogenic blood vessels may partly explain such a phenomenon. In a previous report, anti-VEGFR-3 antibodies destabilized blood vessels in C6 glioblastomas in nude mice, suggesting that VEGFR-3 could be involved in tumor angiogenesis (35). In the MCF-7 breast carcinoma model, angiogenesis was only inhibited. Indeed, anti-VEGFR-3 therapy could influence angiogenesis in some tumors, as well as lymphangiogenesis, but one also needs to determine whether the inhibition of VEGFR-3 signaling has effects on normal tissues. Transgenic mice expressing soluble VEGFR-3 were largely devoid of lymphatic vessels for the first 4 weeks postnatal (44). Nevertheless, the lymphatics regenerated after this point despite continuous VEGFR-3 inhibition (44). So far, anti-VEGFR-3 antibodies and adenoviral administration of soluble VEGFR-3 have had no detectable side effects on adult mice in our studies (unpublished data). However, in the present experiments tissue swelling was observed in the VEGF-implanted eyes and in FGF-2-implanted eyes treated with anti-VEGFR-3, where lymphangiogenesis was significantly reduced, suggesting that an imbalance between blood vessel and lymphatic vessel regeneration influences the interstitial fluid balance during neovascularization. This aspect should be addressed in future studies.

In conclusion, we have adapted the mouse corneal assay for simultaneous studies of pro- and antiangiogenic factors. This assay may be useful to address several questions about lymphatic growth, such as what is the origin of the lymphatic endothelial cells in lymphangiogenesis? Do they sprout out from preexisting lymphatic vessels? Do they originate from bone marrow-derived lymphatic endothelial precursors or from endothelial cells of angiogenic blood vessels? Furthermore, FGF-2 was a pro-lymphangiogenic factor in this assay, and blockade of VEGFR-3 signaling by neutralizing antibodies inhibited FGF-2-induced lymphangiogenesis. Because tumor lymphangiogenesis has been associated with lymphatic metastasis, these results suggest that antiangiogenic therapy by inhibition of VEGFR-3 function may be attempted for the inhibition of lymphatic metastasis in various cancers.

**Note Added in Proof.** FGF-2-induced lymphangiogenesis via the VEGF-C/VEGFR-3 pathway in the corneal model has been independently discovered by L. K. Chang, G. Garcia-Cardena, F. Farnebo, R. C. Mulligan, J. Folkman, and A. Kispaien (personal communication).

We thank Dr. Steven A. Stacker and Dr. Marc G. Achen for their critical comments on this manuscript, David Jackson and Erikki Ruohoshi for anti-LYVE-1 antibodies, and T. Tainola, R. Kivirikko, and A. Parsons for excellent technical assistance. This study was supported by grants from the Finnish Academy of Sciences, the Novo Nordisk Foundation, the Sigrid Juselius Foundation, the Human Frontier Science Program, and Swedish Cancer Foundation.