

# The Related *FLT4*, *FLT1*, and *KDR* Receptor Tyrosine Kinases Show Distinct Expression Patterns in Human Fetal Endothelial Cells

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## Summary

The growth factor receptors expressed on endothelial cells are of special interest because of their potential to program endothelial cell growth and differentiation during development and neovascularization in various pathological states, such as wound healing and angiogenesis associated with tumorigenesis. Vascular endothelial growth factor ([VEGF] also known as vascular permeability factor) is a potent mitogen and permeability factor, which has been suggested to play a role in embryonic and tumor angiogenesis. The newly cloned *FLT4* receptor tyrosine kinase gene encodes a protein related to the VEGF receptors *FLT1* and *KDR/FLK-1*. We have here studied the expression of *FLT4* and the other two members of this receptor family in human fetal tissues by Northern and in situ hybridization. These results were also compared with the sites of expression of VEGF and the related placenta growth factor (PlGF). Our results reveal *FLT4* mRNA expression in vascular endothelial cells in developing vessels of several organs. A comparison of *FLT4*, *FLT1* and *KDR/FLK-1* receptor mRNA signals shows overlapping, but distinct expression patterns in the tissues studied. Certain endothelia lack one or two of the three receptor mRNAs. These data suggest that the receptor tyrosine kinases encoded by the *FLT* gene family may have distinct functions in the regulation of the growth/differentiation of blood vessels.

Receptor tyrosine kinases (RTKs)<sup>1</sup> play a major role in the growth and differentiation of various cell types during development and in adult organisms. The intrinsic tyrosine kinase function of RTKs is activated upon binding of growth factors with diverse biological activities (1). Of special interest are growth factor receptors regulating the proliferation of blood vessels, which are necessary for the normal growth and development of many tissues and an integral part of the development of all organs in multicellular organisms. Two different mechanisms are involved in the formation of blood vessels, namely vasculogenesis, the development of blood vessels from in situ differentiating endothelial cells, and angiogenesis, the sprouting of capillaries from preexisting vessels (2, 3). Vasculogenesis may be unique to embryonic development, whereas angiogenesis can occur during the entire life span in both physiological and pathological

processes and is essential for e.g., reproduction, wound healing, and tumorigenesis. Common to the various processes of angiogenesis is the pattern of response of endothelial cells to angiogenic stimuli, which include a number of known growth factors (4, 5).

The vascular endothelial growth factor (VEGF) is a potent direct acting endothelial cell mitogen with angiogenic activity (6–8). The expression pattern of *VEGF* in embryonic tissues suggests that it may induce the growth of capillaries into tissues during mouse development (9). It has been shown that *VEGF* is also expressed in macrophages and in proliferating epidermal keratinocytes, as well as in several types of tumor cells, e.g., in glioblastomas (10–14). Thus VEGF may be involved in tumor angiogenesis and in the healing of skin wounds. As VEGF has been shown to increase capillary permeability and to stimulate migration of monocytes across the endothelial cell monolayer, it may also be responsible for tissue edema associated with inflammation (15–17). The VEGF-related placenta growth factor (PlGF) has not been found in other tissues besides placenta (18, 19).

<sup>1</sup> Abbreviations used in this paper: PDGFR, platelet-derived growth factor receptor; PlGF, placenta growth factor; RTK, receptor tyrosine kinase; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.

Recent studies have shown that the *FLT1* and *KDR* RTK genes encode endothelial cell receptors for VEGF (20–24). The related *fms*-like tyrosine kinase-4 (*FLT4*) cDNA was cloned from a human erythroleukemia cell cDNA library (25, 26) and from a human placental cDNA library (27, 28). The *FLT* gene family belongs to the class III RTKs (1), which also includes two protooncogenes (*c-fms* and *c-kit*), the  $\alpha$  and  $\beta$  chains of platelet-derived growth factor receptors (PDGFR) and the product of the *FLT3/FLK2* gene. Recently, *FLT4* has also been cloned from quail (29), and *KDR* from mouse (designated *FLK-1*), rat, and quail (29, 30–32). The *FLT4*, *FLT1*, and *KDR* RTKs differ from other members of class III RTKs by having seven instead of five Ig-like loops in their extracellular domains. Thus they constitute a novel subfamily of class III tyrosine kinases.

The *FLT4* gene maps to the long arm of chromosome 5, where many growth factor and growth factor receptor genes, including *PDGFRB*, *FMS*, *FGFR4*, and *FGFA* are known to be located (25, 27, 33). In analysis of RNA isolated from human adult tissues *FLT4* mRNA was found to be expressed in placenta, lung, kidney, heart, and liver in a decreasing order (25). *FLT4* transcripts were also detected in most fetal tissues studied (26). We have here analyzed *FLT4* mRNA in human fetal tissues by Northern and in situ hybridization and compared the expression pattern to those of *FLT1* and *KDR* receptor genes, *VEGF*, and to the endothelial cell marker von Willebrand factor (vWF) (34). Our results show *FLT4* mRNA in vascular endothelial cells in developing vessels. Distinct, but partially overlapping patterns of expression of the three receptors are found in various endothelia.

## Materials and Methods

**Tissues.** Tissues from 17- and 20-wk-old human fetuses were obtained from legal abortions induced with prostaglandins. The study was approved by the Ethical Committee of the Helsinki University Central Hospital. The gestational age was estimated from the fetal foot length (35). The fetal tissues were fixed with 4% paraformaldehyde for about 20 h before dehydration and paraffin embedding.

**Northern Analysis.** As templates for hybridization probes we used the inserts of the *FLT1* (20), *KDR* (23), *VEGF* (8), *PlGF* (18), and vWF (36) cDNA plasmids and a 2.5-kb insert of *FLT4* cDNA, encoding the extracellular domain of the receptor (26). These were labeled by the random priming method and hybridized to a multiple tissue Northern blot (Clontech, Palo Alto, CA), containing 2  $\mu$ g of poly(A)<sup>+</sup> RNA from several human fetal tissues. Hybridization was carried out in 50% formamide, 5 $\times$  Denhardt's solution (100 $\times$  Denhardt's solution is 2% Ficoll, polyvinylpyrrolidone, and BSA), 5 $\times$  SSPE (3 M NaCl, 200 mM NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, 20 mM EDTA, pH 7.0), 0.1% SDS, and 0.1 mg/ml of sonicated salmon sperm DNA at 42°C for 18–24 h. The filter was washed in standard conditions in 2 $\times$  SSC (300 mM sodium chloride, 30 mM sodium citrate) containing 0.05% SDS for 15 min followed by washes in 1 $\times$  SSC/0.1% SDS and exposed to Kodak XAR-5 film.

**In Situ Hybridization.** The *FLT4* cDNA probes (antisense and sense) were synthesized from linearized pGEM-3Z(f+) plasmid (Promega Corp., Madison, WI), containing a *SacI* fragment from the 5' end of the *FLT4* cDNA (nucleotides 55–207) (26), using T7 and SP6 polymerases and [<sup>35</sup>S]UTP (Amersham Corp., Arling-

ton Heights, IL). The *FLT1* cRNA probe was generated by subcloning an *AvaI-SacI* fragment (nucleotides 706–2310) (20) into pGEM-3Z(f+). For *KDR*, an *EcoRI-HindIII* fragment covering base pairs 6–715 of the region coding for the extracellular part of the receptor was used (23). The *VEGF* probe was an *EcoRI* fragment containing base pairs 57–638 of the cDNA (8) cloned in different orientations for sense and antisense RNA synthesis. The *PlGF* probe was an *EcoRI* fragment containing base pairs 304–944 in pGEM-3Z(f+) (18) and the vWF probe was an *EcoRI-HindIII* fragment covering base pairs 1–2334 (36).

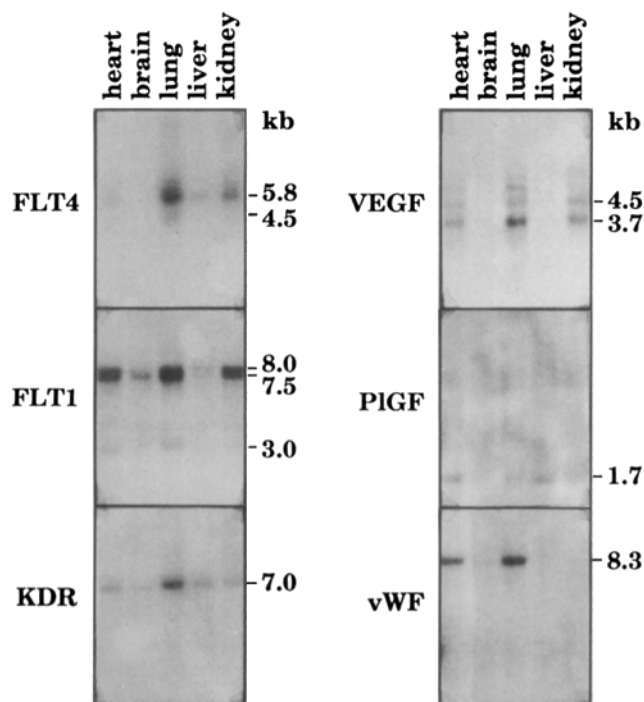
In situ hybridization of tissue sections was performed according to Wilkinson et al. (37, 38) with the following modifications: (a) instead of toluene, xylene was used before embedding in paraffin wax; (b) 6- $\mu$ m sections were cut, placed on a layer of diethyl pyrocarbonate-treated water on the surface of glass slides pretreated with 2% 2-aminopropyltriethoxysilane; (c) alkaline hydrolysis was omitted for the *FLT4* probe; (d) the hybridization mixture contained 50% deionized formamide; (e) the high stringency wash was for 90 min at 65°C in a solution containing 30 mM DTT and 1 $\times$  SSC. The sections were covered with NTB-2 emulsion (Eastman Kodak Co., Rochester, NY) and stored at 4°C. The slides were exposed for 2–8 wk, developed, and stained with hematoxylin. Control hybridizations with sense strand and RNase A-treated sections did not give a specific signal above background.

## Results

**Analysis of *FLT* Receptor *VEGF*, and *PlGF* mRNAs in Human Fetal Tissues.** For comparative studies of *FLT4*, *FLT1*, and *KDR* expression in human fetal tissues, a Northern blot containing polyadenylated RNA from fetal heart, brain, lung, liver, and kidney was hybridized with labeled cDNA inserts of plasmids representing each one of the three genes. Probes from the extracellular domains of the receptors were used in order to minimize crosshybridization, as these regions show only about 30% nucleic acid sequence homology with one another (26). The resulting autoradiograms are shown in Fig. 1.

As can be seen from Fig. 1, the *FLT4* gene is expressed in fetal lung, kidney, heart, and liver as a more abundant 5.8-kb mRNA and a minor 4.5-kb mRNA, that had a stable ratio in the different samples. These two mRNAs differ in their 3' regions as a result of differential RNA splicing and encode COOH-terminal-distinct polypeptides (39). However, they share the same 5' sequences, including the region of the probe. The *FLT1* gene is expressed as three mRNAs of about 8.0, 7.5, and 3.0 kb. The shortest mRNA form cannot be detected using probes from the region of cDNA encoding the cytoplasmic domain, and thus it is likely to represent a splicing variant encoding a truncated extracellular form of the receptor (20). When compared to the distribution of the *FLT4* mRNA, the *FLT1* mRNA is more abundant in the brain and heart samples. The *KDR* probe detects a single mRNA band of 7.0 kb. This is distributed similarly to the *FLT4* mRNA, except that a definitive *KDR* mRNA band can be seen also in the brain sample. From the Northern hybridization results it can be concluded that each one of the three receptors is expressed at high levels in fetal lung, kidney, and heart, and less in brain and liver.

Strikingly, the expression pattern of the three receptors



**Figure 1.** Analysis of FLT receptor, VEGF, and PLGF mRNAs in human fetal tissues. A Northern blot containing polyadenylated RNA from the indicated tissues of human fetuses was hybridized with the gene-specific cDNA fragments used as templates for cRNA synthesis for the in situ hybridization. The sizes of the transcripts are shown.

parallels the expression of the *VEGF* gene. Abundant 3.7 and 4.5 kb mRNA signals for VEGF are seen in the lung, kidney, and heart RNA, and none in the brain or liver at this time of development (Fig. 1). In contrast, weak signals were obtained for the PIGF mRNA from the liver, heart, lung, and kidney. The mRNA of the endothelial cell marker vWF was abundant only in the lung and heart samples and was considerably weaker in the brain. Surprisingly, no expression was detected in the kidney.

**In Situ Hybridization Analysis of Human Fetal Lung.** In the present and previous Northern blotting analysis, abundant FLT4 mRNA was found in human fetal and adult lungs. Lung tissue was therefore chosen for the first in situ hybridization analysis with the FLT4 cRNA probe. Comparison was made with the FLT1 and KDR mRNAs, which also are highly expressed in the lung. Fig. 2 shows the darkfield and lightfield photographs of lung tissue from a 17-wk-old human fetus hybridized with the three probes.

As seen in Fig. 2, A and B, the FLT4 signal decorates mainly the borders of interlobular septa and groups of cells in the connective tissue between the lobuli, whereas only little specific hybridization signal is obtained in the lung parenchyma. Proliferation of the mesenchyme occurs in human lung between the 16th and 26th weeks of gestation, and during this period a rich blood supply develops within the mesenchyme (40, 41). The pattern and distribution of the FLT4 mRNA signal as well as its comparison with the hybridization signal of vWF mRNA (Fig. 2, A and B and J and K) suggest that

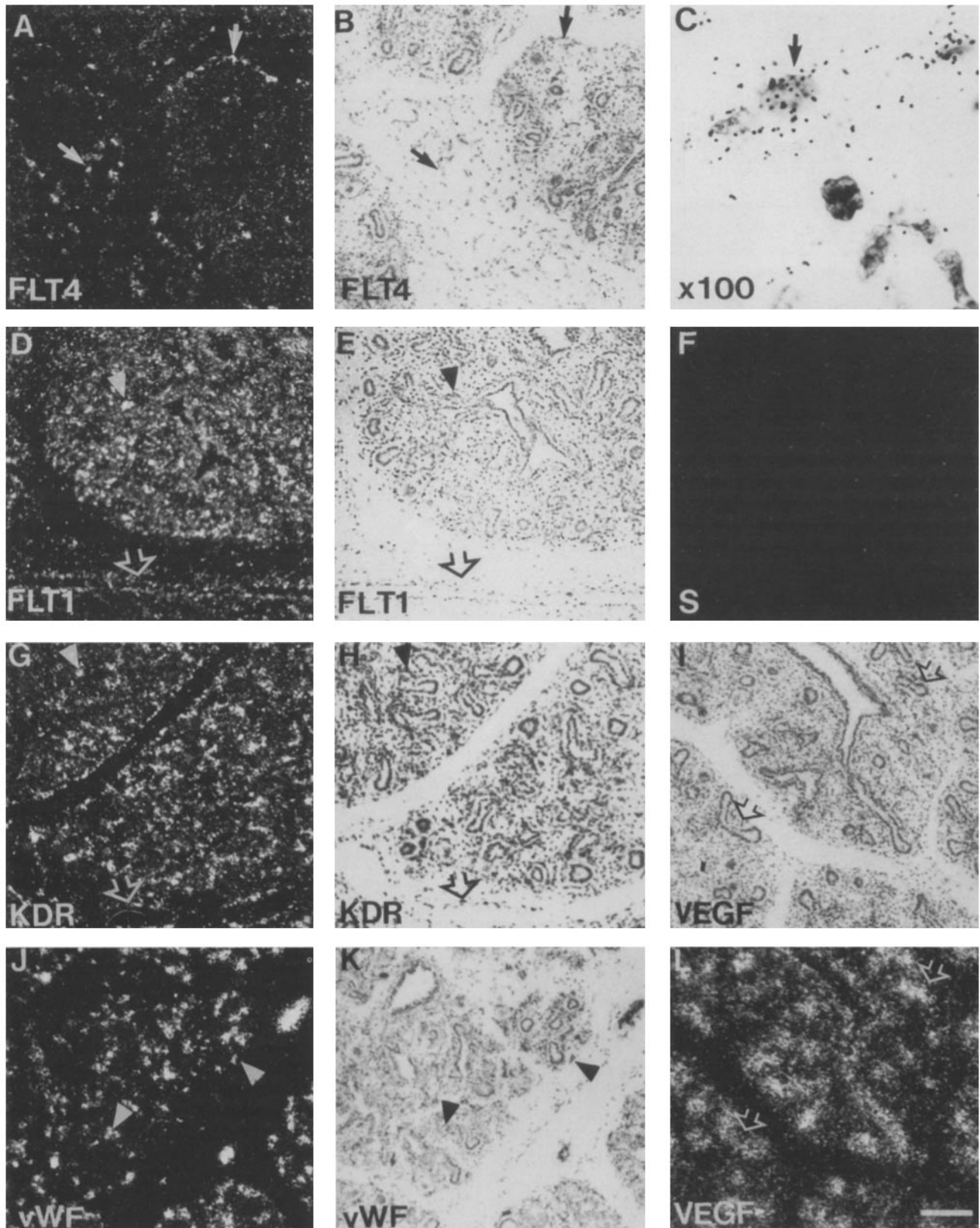
these cells are endothelial cells, although their definitive organization into a continuous endothelium of a vessel cannot be discerned even at higher magnification (Fig. 2 C). However, many preformed endothelia of vWF positive vessels in the lung parenchyma were negative for FLT4. The FLT4 mRNA signal in the interlobular septa appears to be more prominent than the signal for vWF (compare Fig. 2, A and J). This may depend on a difference in the timing of maximal expression of the two mRNAs, which has been shown in a comparison with vWF and *tek*, a novel endothelial RTK (42). Control hybridization with the FLT4 sense probe is shown in Fig. 2 F. No specific signal can be detected, despite a prolonged exposure of this in situ autoradiograph.

In contrast to FLT4, FLT1 expression was seen primarily in lung parenchyma, mainly in-between the primitive respiratory bronchioles and alveolar ducts (Fig. 2, D and E). Most of this signal apparently comes from endothelial cells in developing capillaries (arrowheads), which also are positive for vWF mRNA (Fig. 2, J and K). In addition, as pointed out by the open arrows, certain large vessels in the interlobular septa are positive for FLT1.

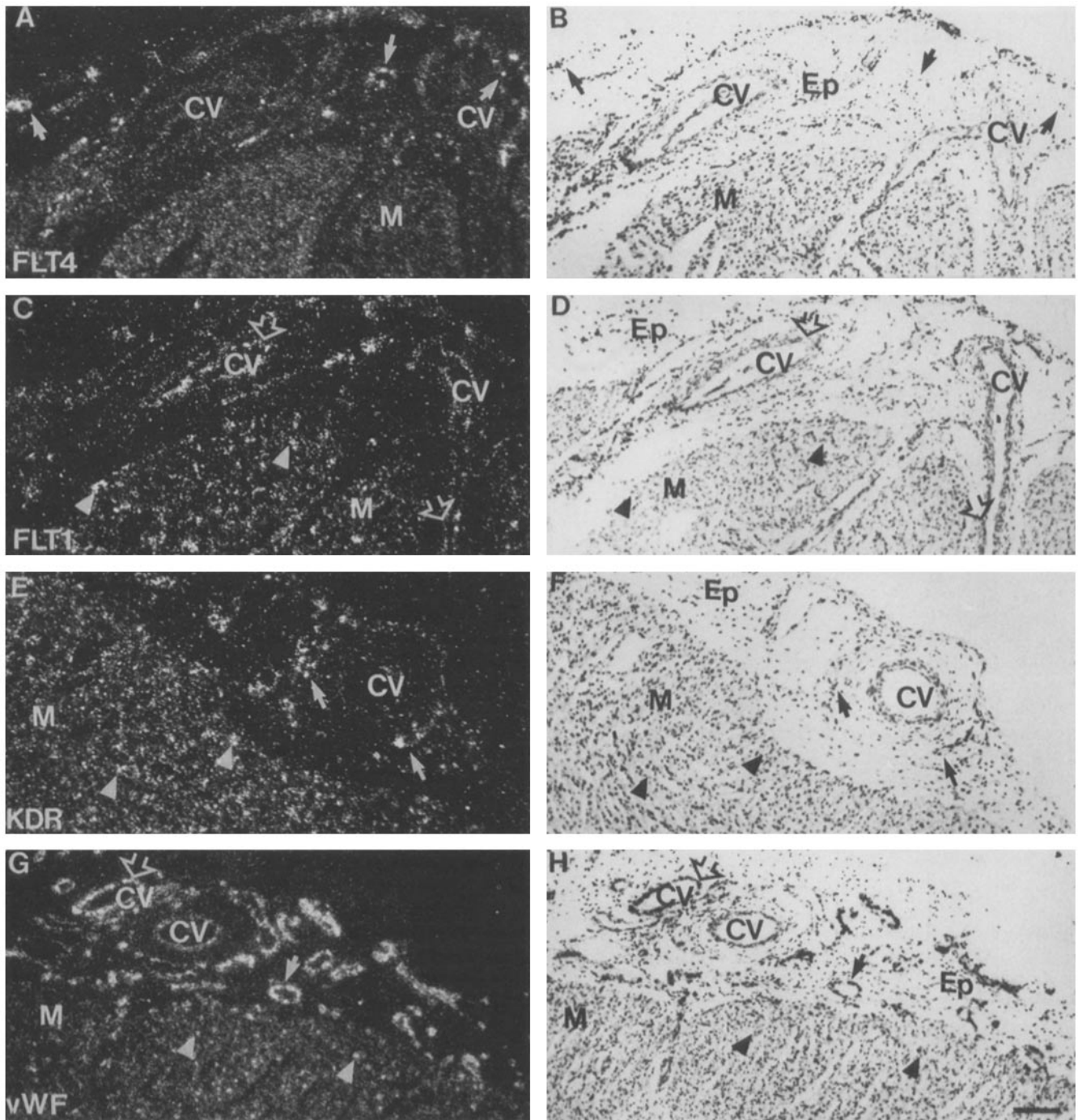
Of the three genes, the strongest hybridization signals were obtained for KDR (Fig. 2, G). The distribution of KDR mRNA overlapped with those of FLT4 and FLT1. A similar spotlike hybridization pattern was obtained in the lung parenchyma as with the FLT1 probe (Fig. 2, D and G), but a more continuous pattern was detected in the capillaries of interlobular septae. Control sense probes for FLT1 and KDR gave also negative results (data not shown). For comparison, the mRNA for VEGF, which is the ligand for these two receptors (22–24), was expressed in the epithelia of primitive respiratory bronchioles and alveolar ducts (Fig. 2, L and I). This agrees with the data of Berse et al. (10), who reported VEGF expression in rat lung alveolar epithelial cells.

**FLT Receptor mRNA Expression in the Fetal Heart.** Figs. 3 and 4 show that FLT4, FLT1, and KDR hybridization results in the fetal heart and comparison with vWF. The epicardium consists of a thin superficial layer of simple squamous epithelial cells overlying a layer of connective tissue containing small blood and lymphatic vessels and nerve fibers. Deeper in this layer are larger blood vessels, such as the coronaries (43). FLT4 mRNA was present in a spotlike pattern in the epicardium; also few small epicardial vessels were positive (arrows, Fig. 3, A and B). However, no association of this signal with the major coronary vessels was seen. Both FLT1 and KDR were expressed in many smaller vessels in the epicardium (arrows in Fig. 3, C and E). Interestingly, FLT1 mRNA was present also in the coronary endothelium (Fig. 3, open arrows), whereas KDR mRNA was not. The myocardial capillaries were negative for FLT4, although the myocardium appears pale in Fig. 3 A because of some unspecific background and overexposure of the photomicrograph. In contrast, the FLT1 and KDR probes decorated small capillaries in the myocardium (arrowheads, Fig. 3, C, D and E, F, respectively). The vWF probe gave a strong signal from both epicardial and myocardial vessels, suggesting that its mRNA is more abundant than the receptor mRNAs investigated (Fig. 1, G and H).

In the endocardium, equally interesting differences were



**Figure 2.** Localization of FLT4, FLT1, KDR, and VEGF transcripts in lung tissue of a 17-wk-old human fetus. Shown are darkfield and lightfield photomicrographs of in situ autoradiograms. Note that *FLT4* (A–C) is mainly expressed in endothelial cells of interlobular septae in a spotlike pattern (arrows). *FLT1* and *KDR* expression is found in lung tissue (arrowheads, D, E and G, H, respectively) and in the vessels in between the lobuli (open arrows). Hybridization with the sense probe is shown in (F). VEGF transcripts are seen in the epithelial cells of the primitive respiratory bronchioles and alveolar ducts (I and L). Control hybridization with the vWF probe is shown (J and K). Scale bar, 100  $\mu$ m.

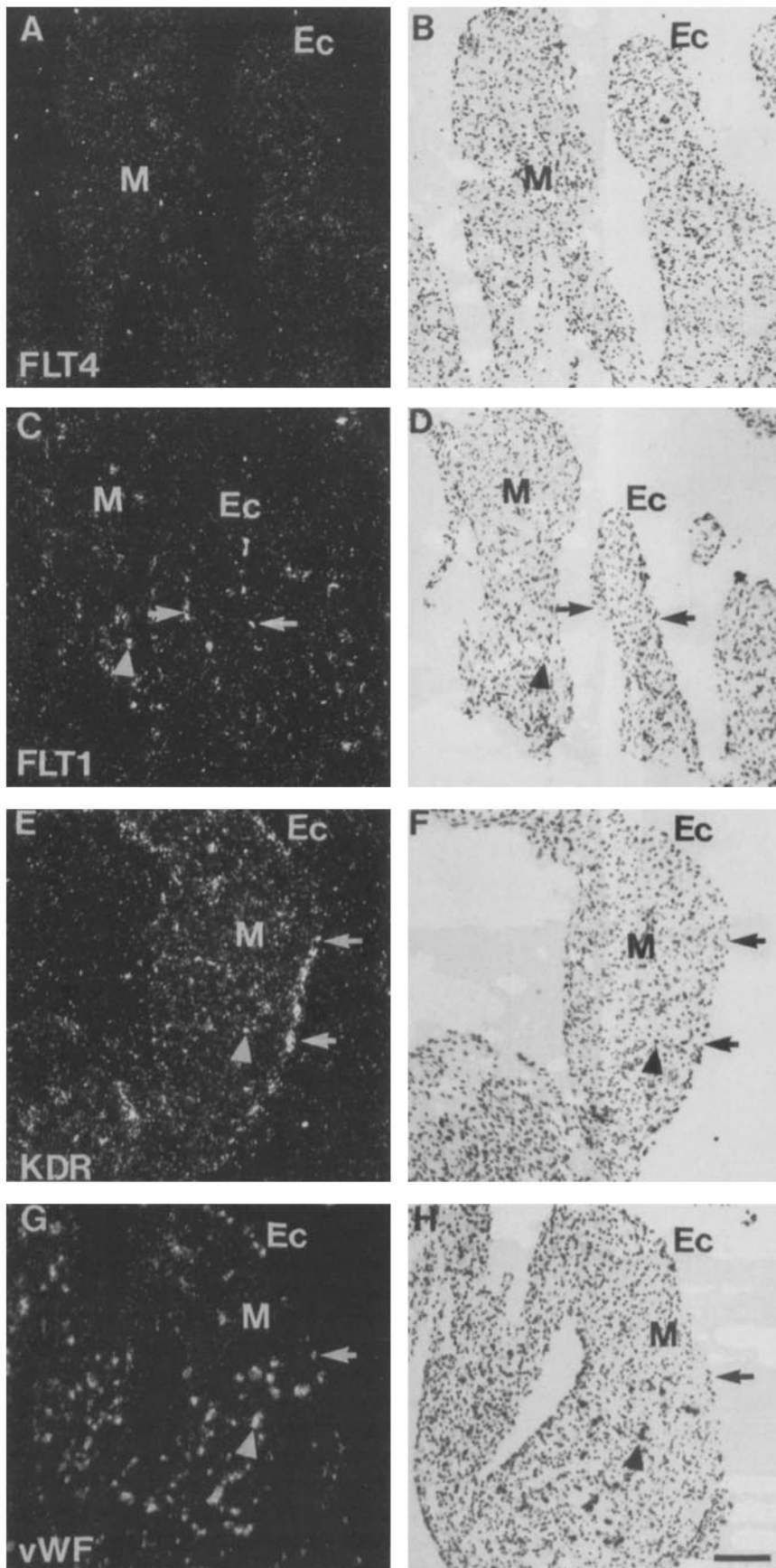


**Figure 3.** Expression of *FLT4*, *FLT1*, and *KDR* in fetal heart. *FLT4* mRNA is expressed in a spotlike pattern in certain endothelial cells of the epicardium (A and B). Arrows indicate some *FLT4* positive epicardial vessels (A). *FLT1* and *KDR* probes decorate small myocardial (M) capillaries (arrowheads, C, D and E, F, respectively). *FLT1* transcripts are also seen in the endothelium of coronary vessels (CV) (open arrows, C and D). Both *FLT1* and *KDR* expression is detected in many smaller vessels in the epicardium (arrows, C and E). (Ep) Epicardium. The vWF control is shown (G and H). Scale bar, 100  $\mu$ m.

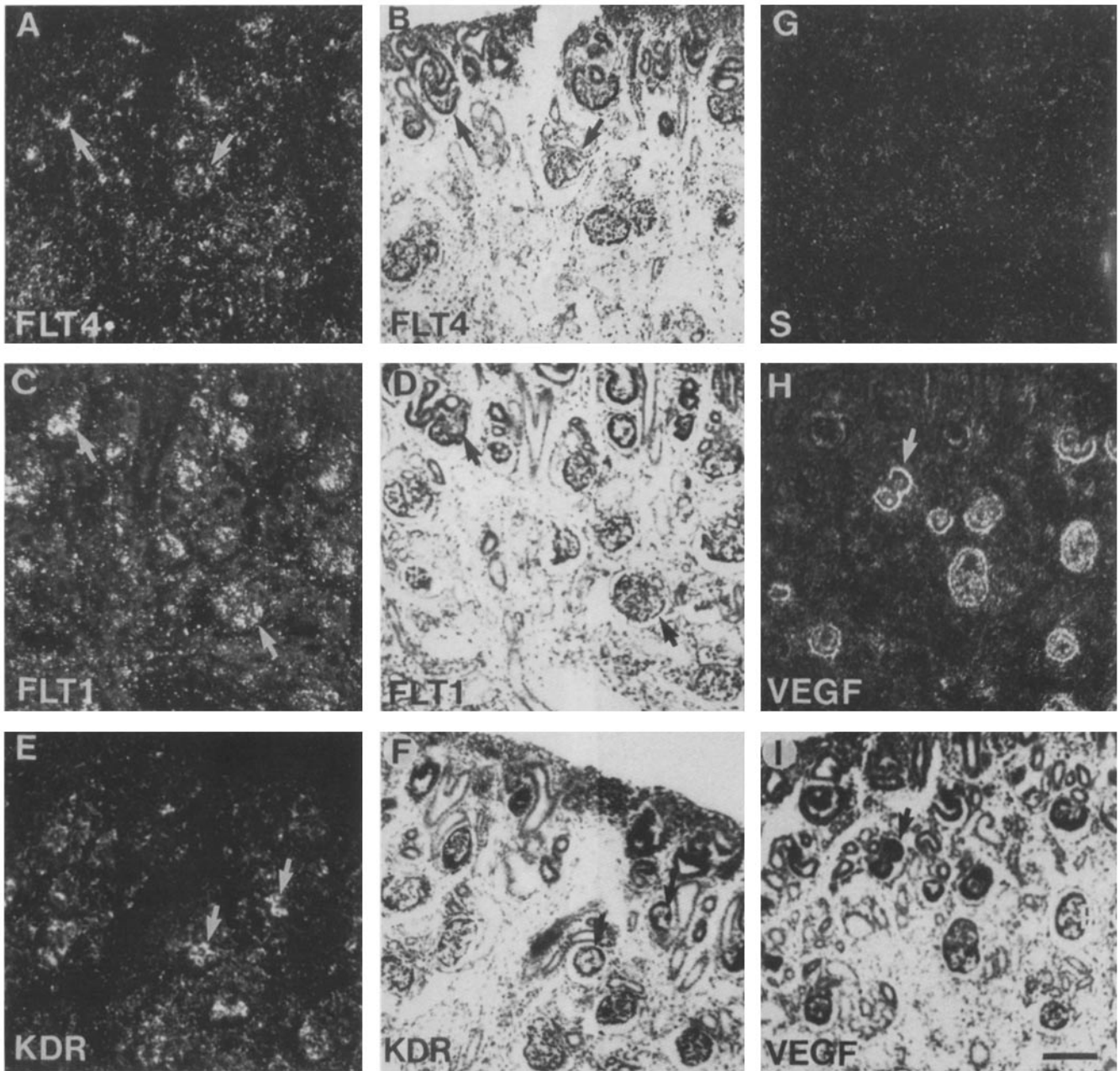
seen in the expression patterns of the three receptor mRNAs. No *FLT4* mRNA was detected in the endocardial cells (Fig. 4, A and B). *FLT1* was present predominantly in the intramyocardial capillaries (Fig. 4, arrowheads), and hybridized weakly in a spotlike manner in the endocardial cells (arrows

in Fig. 4, C and D), whereas more abundant amounts of *KDR* mRNA and high amounts of vWF mRNA were expressed both in the endocardial cells and in the myocardial capillaries (Fig. 4, E-H).

*In Situ Analysis of Fetal Kidney.* *FLT4* gave a weak, but



**Figure 4.** mRNA expression in fetal endocardium. No *FLT4* expression is detected (*A* and *B*). *FLT1* is expressed predominantly in the myocardial capillaries (*arrowheads*, *C* and *D*), and in some endocardial (*Ec*) cells (*arrows*). *KDR* is expressed more abundantly both in the endocardium (*arrows*, *E* and *F*) and in the myocardial capillaries (*arrowheads*, *E* and *F*). *vWF* expression is shown as a control (*G* and *H*). Scale bar, 100  $\mu$ m.

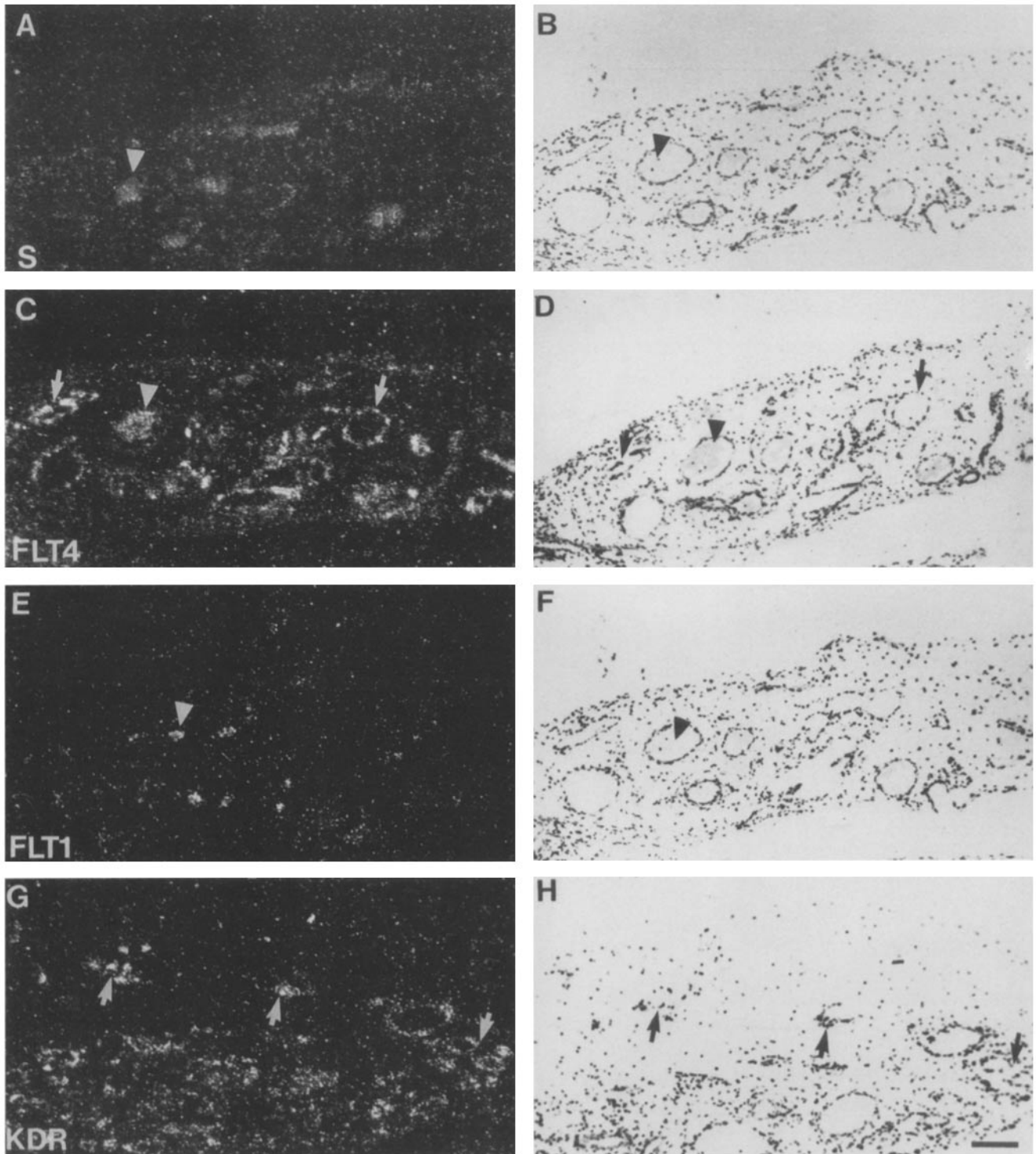


**Figure 5.** Expression of FLT4, FLT1, KDR, and VEGF transcripts in fetal kidney. *FLT4* is expressed weakly in several glomeruli (arrows) and in cells interspersed in the mesenchymal tissue of the cortical region of the kidney (A and B). Strong *FLT1* mRNA expression is detected in the glomerular endothelial cells (arrows, C and D). *KDR* is found in the glomerular endothelium in the more medullary part of the kidney (arrows, E and F). Hybridization with the sense probe is shown (G). VEGF mRNA is present in the glomerular epithelial cells (arrows, H and I). Scale bar, 100  $\mu$ m.

definite signal in several glomeruli in the cortex (Fig. 5, arrows) and in cells interspersed in the mesenchymal tissue of the cortical region of the kidney (Fig. 5, A and B). Strong *FLT1* expression was seen in the glomerular endothelial cells (Fig. 5, C and D) and some in the peritubular capillaries in the medullary parts (data not shown), whereas *KDR* was specific to the endothelium of glomerular capillaries in the more medullary regions of the kidney cortex (Fig. 5, E and F). No specific signal was evident with the sense probe (Fig. 5

G). Also, as reported previously for adult rats (10), VEGF mRNA was produced by the glomerular epithelial cells (Fig. 5, H and I).

*Analysis of Fetal Mesenterium and Gut.* Fig. 6 shows a comparison of receptor expression in the mesenterium. The mesenterium carries the blood supply to and from the gut. In addition, it contains abundant lymphatic vessels. In tissue sections, the blood vessels frequently contained remaining RBC, which gave a false positive reflection of light in darkfield micros-

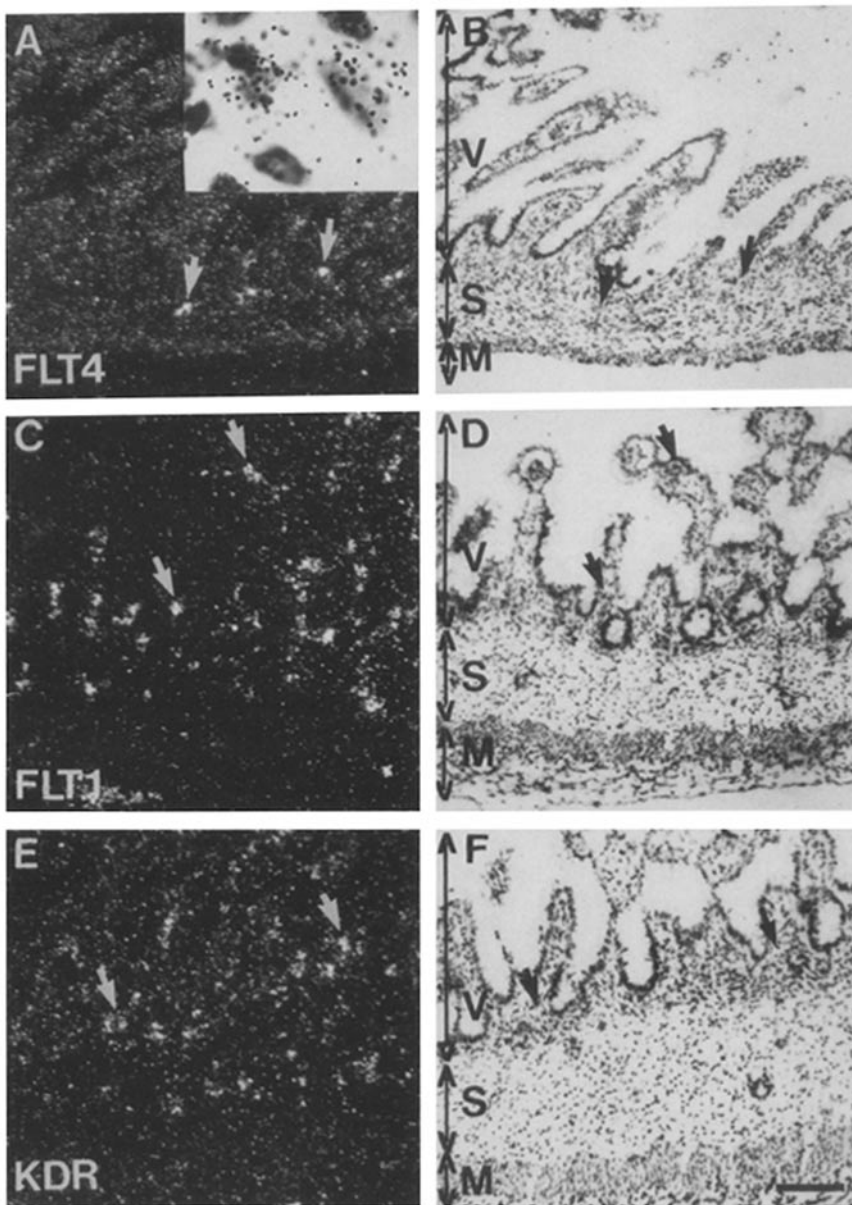


**Figure 6.** Localization of transcripts in fetal mesenterium. Hybridization with sense probe (*A* and *B*) shows a false positive reflection of light due to RBC (arrowheads, *A-F*). Strong *FLT4* (arrows, *C* and *D*) and *KDR* (arrows, *G* and *H*) expression is found in most vessels of the mesenterium. In contrast, no *FLT1* expression is detected (*E* and *F*). Scale bar, 100  $\mu$ m.

copy even in sections hybridized with the sense probe (arrowheads in Fig. 6, *A-F*). Interestingly, strong *FLT4* (Fig. 6, *C* and *D*) and *KDR* (Fig. 6, *G* and *H*) signals were present in most vessels of the mesenterium (arrows), whereas *FLT1* appeared negative in these sections (Fig. 6, *E* and *F*).

A comparison of the distribution of these three receptor mRNAs in the gut (Fig. 7) shows also distinct differences. *FLT4* is positive only in a few endothelial cells in the submucosal (*S*) layer (Fig. 7, *A* and *B*). *FLT1* is strongly positive in the endothelium of small vessels extending to the intes-





**Figure 7.** FLT4, FLT1, and KDR, mRNA in fetal gut. FLT4 expression is detected only in some interspersed endothelial cells (inset, A) and in small vessels in the submucosal layer (arrows, A and B). FLT1 is expressed in the endothelium of capillaries extending into the intestinal villi (arrows, C and D). KDR mRNA is present both in the submucosa and in the capillaries of the villi (arrows, E and F). (V) villi; (S) submucosa; (M) mucosa. Scale bar, 100  $\mu$ m.

tinal villi in (Fig. 7, C and D). Much weaker signal is seen for KDR mRNA (Fig. 7, E and F), which is present both in the submucosa and in the capillaries of the villi (arrows).

### Discussion

Our results revealed distinct but overlapping patterns of expression of three RTK genes of the FLT family in the human fetus. The results showed that the FLT4 mRNA, like the FLT1 and KDR mRNAs, is expressed in certain endothelia. The wide distribution of all three mRNAs in the different organs suggests that these growth receptors have general and important roles in endothelial cell growth and/or differentiation.

Our Northern hybridization results indicated similar patterns of distribution of the three receptor mRNAs in major fetal tissues. The highly vascularized tissues, lung, kidney,

and heart had the highest levels of all three receptor mRNAs. However, lower amounts of these mRNAs are widely distributed, as shown by previous Northern blotting and hybridization analyses of many fetal and adult tissues (20, 25, 26, 28, 30–32). Furthermore, like the receptor mRNAs, VEGF mRNA was also highly expressed in the lung, kidney, and heart, whereas small amounts of PlGF mRNA were mainly expressed in the heart and liver. Our previous data have indicated that abundant PlGF mRNA is present in placental tissue (19) and apparently the amounts are much smaller in fetal tissues.

Interestingly, our in situ hybridization studies revealed differences in the expression patterns of the three RTK mRNAs in all tissues. A good example of the differences was seen in endocardial cells, where no FLT4 mRNA could be detected, but a few cells showed positivity for FLT1 mRNA and KDR mRNA was expressed quite prominently. According to a re-

cent report (29) the avian homologues of *FLT4* and *KDR*, *Quek2* and *Quek1*, respectively, are expressed in endothelial cells during early development. In a 9-d quail embryo, these genes are switched off in various compartments of the vascular network. For example, the endocardium, except for the cardiac valves, becomes negative for *Quek2* and *Quek1*. Of particular interest is that the genes are not switched off simultaneously in the same endothelial cells (29). Also, *FLT1* and *FLK1* mRNA expression differ from each other during mouse development. Both receptors are expressed in endothelial cells during early development. During the fetal period (E14-E16.5) *FLT1* is undetectable, whereas *KDR* mRNA continues to be expressed in all endothelia (24, 44). In adult mice, the *FLT1* receptor mRNA is again detected in endothelia.

The origin of the endothelial cells of the glomerulus and the mode of development of the kidney vasculature have been controversial subjects for a century. Most likely, a pair of vessels sprout from the dorsal aorta and invade the metanephric blastema in a close association with the ingrowing ureteric bud. When the latter branches and induces pretubular aggregates around its tips, the migrating endothelial cells follow the border between these condensed areas and the stroma. When the S-shaped bodies are formed, this directs the endothelial cells into the developing glomerulus (45). *FLT4* signal from the cells interspersed in the upper mesenchyme are most probably derived from endothelial cells, this being in agreement with the model of development of the kidney vasculature. However, no vWF could be detected in the kidney. It thus seems that all three receptors are early markers of endothelial cells. In addition, *FLK-1/KDR* mRNA expression has been detected in fetal liver cells of 12-day mouse embryos, which are enriched for hematopoietic stem cells, and in the mesodermal layer of the yolk sac (24, 31). Also, *FLT4* is expressed in undifferentiated teratocarcinoma cells, and down-regulated upon their induced differentiation (26). All three receptors of this class are also expressed in cultured human leukemia cell lines in vitro (unpublished data of Pertovaara,

L. and the authors). These observations suggest that *FLT4* may play a role not only in the growth and differentiation of endothelia, but in earlier progenitors of endothelial and hematopoietic cells as well.

Some endothelia appeared to entirely lack one or two of the three receptor mRNAs. Major coronary vessels appeared positive only for *FLT1*. *FLT4* mRNA was absent from the capillaries inside lung parenchyma, myocardium, and villi of the fetal gut. In the mesenterium, both *FLT4* and *KDR* expression was detected in vessels of middle size, although no *FLT1* expression was seen. This suggests that these three related receptors perform overlapping, but distinct functions in the fetal vessels. From other RTK families it is known that coexpressed receptor polypeptides may be activated by ligand-induced heterodimerization (1). The presence of multiple VEGF receptor forms thus has a potential to greatly diversify the VEGF-induced signals in the responding cells. Because multiple forms of VEGF and the related PlGF are known (17, 19), the three receptors may allow for fine-tuning of signals communicated through this ligand-receptor system. This is made more likely by the dimeric nature of the respective ligands. For example, dimeric complexes between VEGF and PlGF-related PDGF A and B chains are capable of binding homo- and heterodimeric complexes of PDGFR  $\alpha$  and  $\beta$  chains with different affinities (46, 47).

Although we have studied only a certain period of human development, interesting differences in the distribution of *FLT* receptor mRNAs were observed. These observations suggest that the expression of the three receptors is modulated in a dynamic manner during development. Endothelial cells play important roles in the regulation of coagulation and fibrinolysis, cellular growth and differentiation, immune and inflammatory responses and vascular tone (48-50). The different distribution of *FLT* family receptors in fetal endothelial cells indicates a specificity of their biological functions in the ontogeny and functions of the vascular system.

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