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Vascular endothelial growth factor-related protein: A ligand and specific activator of the tyrosine kinase receptor Flt4

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ABSTRACT **The tyrosine kinases Flt4, Flt1, and Flk1 (or KDR) constitute a family of endothelial cell-specific receptors with seven immunoglobulin-like domains and a split kinase domain. Flt1 and Flk1 have been shown to play key roles in vascular development; these two receptors bind and are activated by vascular endothelial growth factor (VEGF). No ligand has been identified for Flt4, whose expression becomes restricted during development to the lymphatic endothelium. We have identified cDNA clones from a human glioma cell line that encode a secreted protein with 32% amino acid identity to VEGF. This protein, designated VEGF-related protein (VRP), specifically binds to the extracellular domain of Flt4, stimulates the tyrosine phosphorylation of Flt4 expressed in mammalian cells, and promotes the mitogenesis of human lung endothelial cells. VRP fails to bind appreciably to the extracellular domain of Flt1 or Flk1. The protein contains a C-terminal, cysteine-rich region of about 180 amino acids that is not found in VEGF. A 2.4-kb VRP mRNA is found in several human tissues including adult heart, placenta, ovary, and small intestine and in fetal lung and kidney.**

The formation of new blood vessels either from differentiating endothelial cells during embryonic development (vasculogenesis) or from preexisting vessels during adult life (angiogenesis) is an essential feature of organ development, reproduction, and wound healing in higher organisms (1–3). Angiogenesis is also necessary for certain pathological processes including tumorigenesis (4) and retinopathy (5). While several growth factors can stimulate angiogenesis (6, 7), vascular endothelial growth factor (VEGF) (8) is a potent angiogenic factor that acts via the endothelial cell-specific, receptor tyrosine kinases Flt1 (9, 10) and Flk1 (also designated KDR) (11–16). These two VEGF receptors and a third, orphan receptor, Flt4 (17–19), constitute a subfamily of class III receptor tyrosine kinases that contain seven, extracellular, immunoglobulin-like domains and a split, intracellular tyrosine kinase domain (20). These three receptors have 31–36% amino acid identity in their extracellular, ligand-binding domains. Mice deficient in Flt1 (21) or Flk1 (22) (generated by gene targeting in embryonic stem cells) have severe defects in vasculogenesis and die *in utero* at embryonic day 8–9. Mice lacking Flt1 have a disorganized vascular endothelium that extends to the major vessels as well as to the microvasculature, while endothelial cell differentiation appears to be normal (21). Mice lacking Flk1 have a major defect in the development of mature endothelial cells as well as a severe reduction in hematopoietic cell progenitors (22). Thus, VEGF may act on endothelial cells at more than one stage of vasculogenesis.

Flt4 is also specifically expressed in endothelial cells; it is first observed in day 8.5 mouse embryos in endothelial cell precursors (23, 24). As development proceeds, Flt4 expression becomes confined to the venous and lymphatic endothelium and is finally restricted to the lymphatic vessels. Consistent with this finding, adult human tissues show Flt4 expression in lymphatic endothelia while there is a lack of expression in arteries, veins, and capillaries (23). Clones encoding human and mouse Flt4 have been isolated either by PCR with primers from conserved tyrosine kinase regions (19, 25) or by low stringency hybridization with a Flk2 probe (26). Alternative splicing of the Flt4 mRNA produces two variants of the protein differing by 65 amino acids at the C terminus (27). These variants migrate as bands of 170–190 kDa that are partially cleaved proteolytically in the extracellular domain to produce a form of about 125 kDa (27, 28). Expression of the longer spliced form of Flt4 as a chimera with the extracellular domain of the CSF-1 receptor shows that the Flt4 intracellular domain can signal a ligand-dependent, growth response in rodent fibroblasts (28, 29). Flt4 has been localized to human chromosome 5q34-q35 (25, 26); Flt1 and Flk1 are located at 13q12 (30) and 4q12 (31, 32).

VEGF is a homodimeric, cysteine-rich protein that can occur in at least four forms due to alternative splicing of its mRNA (8). While VEGF is a high-affinity ligand for Flt1 and Flk1, it does not bind or activate Flt4 (28). The only other closely related member of the VEGF family is placental growth factor (PlGF) which has 47% amino acid identity with VEGF (33). PlGF also occurs in two alternatively spliced forms which differ in the presence or absence of a basic heparin binding domain of 21 amino acids (34, 35). PlGF binds to Flt1 but not to Flk1 (36); its binding to Flt4 has not been determined. PlGF fails to duplicate the capillary endothelial cell mitogenesis or vascular permeability activities of VEGF, suggesting that these activities are mediated by Flk1 (36).

In this work we present the characterization of a protein, designated VEGF-related protein (VRP), that binds and activates the receptor tyrosine kinase Flt4.‡

MATERIALS AND METHODS

Clones Encoding Human Flt4. To identify novel tyrosine kinase receptors, cDNA synthesized with mRNA from the human, megakaryocytic leukemia cell line CMK11-5 was amplified with redundant PCR primers based on conserved regions of tyrosine kinase receptors (37). One amplified fragment of about 180 bp with a novel DNA sequence (designated SAL-S1 or tk1) was used to screen (38) cDNA libraries from CMK11-5 and DAMI cells to obtain overlapping clones that encode Flt4, which has also been identified by other laboratories (19, 25, 26). The Flt4 sequence encoded by the assembled clones (1298 amino acids) matched that reported from an erythroleukemia

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Abbreviations: EST, expressed sequence tag; Flt4/IgG, Flt1/IgG, Flk1/IgG, and Htk/IgG, fusion proteins containing the extracellular domain of the indicated tyrosine kinase receptor fused to an immunoglobulin Fc domain; PlGF, placental growth factor; VEGF, vascular endothelial growth factor; VRP, VEGF-related protein.

‡The sequences reported in this paper have been deposited in the GenBank data base [accession nos. U43143 (Flt4) and U43142 (VRP)].

cell line (17); it encodes 8 amino acid differences from another reported Flt4 sequence (18). Clones encoding the long form of Flt4 (1363 amino acids) were constructed by synthesizing the differing 3′ DNA sequence of about 200 bp based on the published sequence (27).

Receptor IgG Fusion Proteins and Flt4/IgG Antiserum. Flt1/IgG (36), Flk1/IgG (36), and Htk/IgG (39) were produced as described. For Flt4/IgG, DNA encoding the extracellular domain of Flt4 (amino acids 1–775) was spliced to the Fc region of a human IgG heavy chain (40) and cloned into the vector pRK5 (41) to yield the plasmid pRK5.tk1ig1.1. Flt4/IgG was purified from the serum-free conditioned medium of transfected (38) 293 cells (ATCC CRL 1651) with protein A-agarose (Calbiochem). Flt4 antiserum was generated by injection of purified Flt4/IgG into rabbits.

Clones Encoding Human VRP. While we failed to identify ligands for Flt4 by the screening of cell supernatants or tissue extracts with the Flt4 phosphorylation assay (below) or by the expression cloning of putative membrane-bound ligands with labeled Flt4/IgG (data not shown), clones encoding VRP were isolated by screening a cDNA library made from the human glioma cell line G61 (42). This library was prepared with reagents from GIBCO/BRL (SuperScript) using poly(A)$^+$ RNA isolated as described (43, 44) and cloned in the plasmid pRK5B (45) digested with *Xho* I and *Not* I. The library was screened with probes based on an expressed sequence tag (EST) (GenBank locus HSC1WF111). Seven positives were identified and characterized from 650,000 clones screened. Clones vh1.4 (pRK.vh1.4.1) and vh1.6 included the full coding region (see Fig. 2) and were sequenced completely. They differ only in length and the lack of two Ts preceding the 3′ poly(A) sequence in vh1.6. Clone vh1.2 is collinear with vh1.4. Clones vh1.3, vh1.5, and vh1.7 are identical and have a 557-bp deletion when compared with vh1.4 (a deletion of bp 519–1075) and clone vh1.1 has a 152-bp deletion when compared with vh1.4 (deletion of bp 924–1075).

Receptor IgG Precipitation of ^{35}S-Labeled VRP. The VRP expression plasmid, pRK.vh1.4.2, was constructed by deleting about 360 bp of 5′ untranslated sequence [5′ of the *Age* I site (see Fig. 1*A*)] from vh1.4 (above). This DNA and plasmids encoding VEGF$_{165}$ (46), PlGF$_{152}$ (36), or the vector alone (pRK5) (41) were transfected into 293 cells by electroporation (38) and pulse-labeled for 5 h with [^{35}S]methionine and [^{35}S]cysteine. Ninety microliters of 8-fold concentrated (Centricon-10, Amicon) conditioned medium were incubated with 5 nM receptor IgG and a 40 μl of a 50% slurry of protein A-agarose (Calbiochem) in binding buffer (below) overnight at 4°C. The precipitates were washed in the same buffer, boiled in SDS sample buffer, and electrophoresed on polyacrylamide gels (Novex).

Flt4 Tyrosine Phosphorylation. Flt4 was stably expressed in 293 cells (above) by transfection of the plasmid pRK.tk1-3.1 which encodes the long form of Flt4 (above) in the vector pRK5 (41). One million Flt4 expressing cells in 100 μl of phosphate-buffered saline (PBS)/0.1% bovine serum albumin were mixed with 100 μl of sample and incubated at 37°C for 15 min. The cells were then collected by centrifugation and lysed in 250 μl of 0.15 NaCl/10% glycerol/1% Triton X-100/50 mM Hepes, pH 7.3/4 μg of phenylmethanesulfonyl fluoride per ml/0.02 unit of aprotinin per ml (Sigma)/20 mM sodium orthovanadate. Flt4 was immunoprecipitated by the addition of 8 μl of rabbit Flt4/IgG antiserum (above) and 30 μl of protein A-agarose (above). Washed precipitates were boiled in SDS sample buffer, electrophoresed on polyacrylamide gels (Novex), transferred to nitrocellulose (38), and probed with an anti-phosphotyrosine monoclonal antibody (Upstate Biotechnology, Lake Placid, NY) and an alkaline phosphatase detection system (Promega).

Samples containing VRP or VEGF were prepared by the electroporation of expression plasmids encoding vh1.4 (pRK.vh1.4.2, above) or VEGF (46) into 293 cells and 20-fold concentration (Centricon-10, Amicon) of the 3-day serum-free conditioned medium. In the receptor IgG competition experiments, the concentrated, conditioned media were preincubated 1 h at 4°C with receptor IgG.

Purification of VRP and Binding to Labeled Flt4/IgG. The reading frame encoding the N-terminal secretion signal sequence and about 30 amino acids of the herpes glycoprotein D (47, 48) were fused with a short linker sequence to the putative mature sequence of VRP (vh1.4) and cloned in the vector pRK5 (above) to give the plasmid pRK.vh1.4.5. VRP fusion protein was purified from serum-free conditioned medium of transfected (38) 293 cells (above) by anti-glycoprotein D monoclonal antibody (5B6) affinity chromatography and quantitated by colorimetric assay (Bio-Rad).

Flt4/IgG was iodinated to a specific activity of 1000–1500 Ci/mmol (1 Ci = 37 GBq) with Iodobeads (Pierce). Binding was performed with ≈20,000 cpm of ^{125}I-Flt4/IgG and 12 ng of VRP/glycoprotein D fusion protein in PBS, 0.5% bovine serum albumin, 0.02% Tween 20, 1 μg of heparin per ml containing 20 μl of a 50% slurry of glass beads conjugated to ≈30 μg of anti-gD monoclonal antibody (5B6) in a final volume of 100 μl for 4–6 h at 22°C. Beads were collected by filtration (Millipore Multiscreen-HV), washed five times with 200 μl of the same buffer, and counted. For binding at increasing concentrations of Flt4/IgG (Fig. 5*B*), the buffer was DMEM (low glucose)/F12 (50:50), 20 mM sodium Hepes, pH 7.2, 10% fetal bovine serum, 0.2% gelatin, and 1 μg of heparin per ml (binding buffer).

Mitogenic Assay. Human lung microvascular endothelial cells (HMVEC-L, Clonetics, San Diego) were seeded at 6500 cells per well in 48-well plates (Costar) with the growth medium recommended by the supplier (EGM-MV with 5% fetal calf serum). After overnight incubation, the medium was removed, and the cells were cultured in the growth medium (2% fetal calf serum) without bovine brain extract and supplemented with VEGF$_{165}$ (Genentech) or VRP/glycoprotein D fusion protein (above). After 4 days, the cells were trypsinized and counted with a Coulter Counter (Hialeah, FL).

RNA Blots. Blots containing poly(A)$^+$ human RNA were from Clontech. For the G61 glioma cell line, 5 μg of poly(A)$^+$ and of poly(A)$^-$ RNA (above) were electrophoresed on a 1% agarose/2.2 M formaldehyde gel and transferred to nitrocellulose (38). Blots were hybridized with ^{32}P-labeled probes encoding amino acids 141–156 and 221–236 and washed in 30 mM NaCl/3 mM trisodium citrate at 55°C.

RESULTS AND DISCUSSION

cDNA Clones Encoding VRP. In the course of attempts to identify ligands for the tyrosine kinase receptor Flt4, one EST of 299 bp (GenBank locus HSC1WF111) was found that encodes an amino acid sequence that is 36% identical with VEGF over 50 residues, including an 11- of 13-residue match beginning at VEGF amino acid 56 (Fig. 1*B*). Full-length cDNA clones encoding this protein, designated VRP, were identified from a human glioma cDNA library (*Materials and Methods*). The DNA sequences of two of the clones, vh1.4 and vh1.6, contain an open reading frame of 419 amino acids beginning with an ATG codon preceded by a purine residue at position −3 as expected for a translation initiation site (50). About 250 bp 5′ of this ATG are two in-frame ATG codons followed in 4 or 10 amino acids by a stop codon. Both of these ATGs have a pyrimidine at position −3 and would not be expected to function as a strong translation initiation site (50). The encoded amino acid sequence immediately following the start of the 419-amino acid reading frame is hydrophobic, indicative of an N-terminal, secretion signal sequence (51) (Fig. 1*A*). The most likely cleavage site for this sequence would be after amino acid 20, although cleavage following residues 15 or 16 cannot

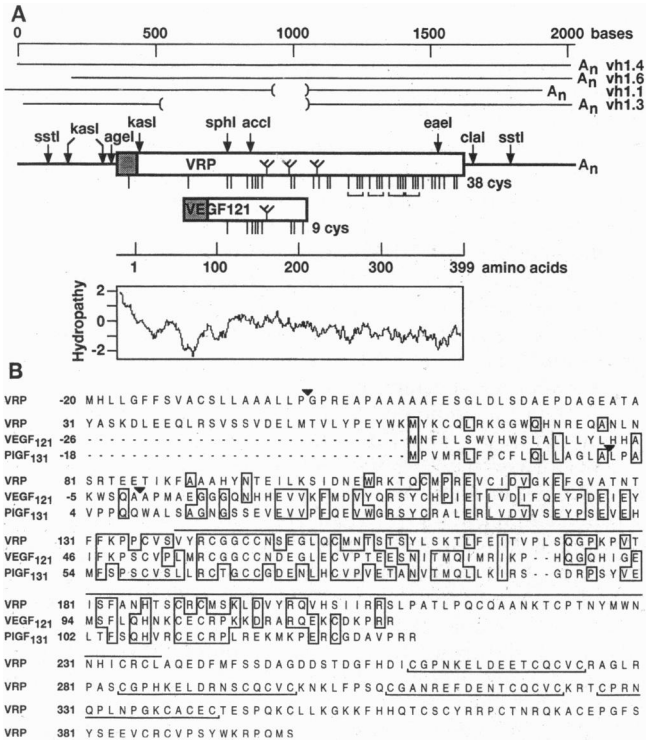

FIG. 1. Map of cDNA clones encoding human VRP and an alignment of the protein sequence. (*A*) Map of the DNA and encoded protein sequence. The extent of four VRP cDNA clones is shown (vh1.4, vh1.6, vh1.1, vh1.3); parentheses indicate the missing portions of vh1.1 and vh1.3. Restriction enzyme sites are indicated by arrows; shaded box, putative secretion signal sequence; open box, mature protein; potential N-linked glycosylation sites are indicated in the open box; vertical lines, cysteine residues; brackets show the four $CX_{10}CXCXC$ repeats. A diagram of $VEGF_{121}$ is shown for comparison. The hydropathy plot (49) is for VRP. (*B*) Encoded amino acid sequence of human VRP aligned with that of $VEGF_{121}$ and $PlGF_{131}$. Triangles indicate the putative secretion peptidase cleavage sites; overlining indicates the region encoded by expressed sequence tag (EST) HSC1WF111; brackets show the four $CX_{10}CXCXC$ repeats.

be excluded (52). The open reading frame is preceded by a GC-rich 5′ untranslated region of about 380 bp and followed by a 3′ untranslated region of about 400 bp.

The predicted mature amino acid sequence of VRP contains 399 amino acids (translated molecular mass = 44.8 kDa), of which 37 (9.3%) are cysteine residues; there are three potential N-linked glycosylation sites (Fig. 1*A*). An alignment of the amino acid sequence of VRP with the six forms of VEGF and PlGF shows that it is most similar to $VEGF_{121}$ (32% identical) and $PlGF_{131}$ (27% identical) (Fig. 1*B*); the locations of 8 of the 9 cysteine residues are conserved. While VRP does not contain the regions of basic amino acids found in some forms of VEGF and PlGF, it is considerably larger than VEGF and contains a cysteine-rich C-terminal half of the molecule that is not found in VEGF. This cysteine-rich domain has four copies of the pattern Cys-Xaa_{10}-Cys-Xaa-Cys-Xaa-Cys (Fig. 1), a repeat found more than 50 times in a diptran Balbiani ring 3 protein (53). Flt4/IgG binds to the surface of the glioma cell line from which the VRP encoding clones were isolated (data not shown). Perhaps, VRP interacts with other membrane-bound proteins on these cells via the cysteine residues; such an intermolecular interaction has been proposed for the Balbiani protein (53).

Two of the cDNA clones (vh1.1 and vh1.3) contain a 152- or 557-bp deletion when compared with vh1.4 (Fig. 1*A*). Both of these deletions end at the same nucleotide and are presumed to be the result of alternative splicing. Both deletions would be

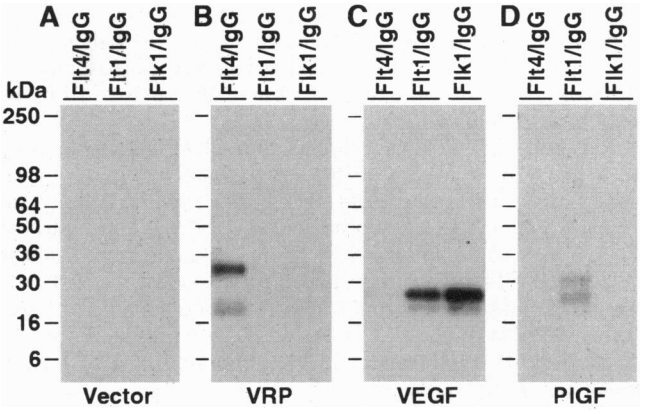

FIG. 2. Precipitation of ^{35}S-labeled VRP, VEGF, and PlGF with Flt4/IgG, Flt1/IgG, and Flk1/IgG. Labeled conditioned medium with VRP, VEGF, PlGF, or vector transfected cells was precipitated with the indicated receptor IgG fusion protein as described in the text.

expected to encode the same frameshifted protein 3′ of the deletion which terminates at a stop codon within 15 amino acids. The protein encoded by clone vh1.3 would include none of the core cysteine region similar with VEGF and, thus, would not be a protein in the VEGF family. Clone vh1.1 encodes much of the region that is similar to VEGF; its deletion, however, is not analogous to the various known forms of VEGF or PlGF (8, 34, 35). Given the various active forms of VEGF and PlGF that have been characterized, perhaps additional forms of VRP remain to be identified.

VRP Binding to Flt4/IgG. To determine whether VRP is a ligand for Flt4, plasmids containing cDNA clone vh1.4 as well as control plasmids encoding VEGF, PlGF, or the expression vector alone were transfected into mammalian cells and the proteins were labeled with ^{35}S amino acids. Conditioned media from these cells were precipitated with Flt4/IgG, Flt1/IgG, and Flk1/IgG. Two specific bands of 33 and 19 kDa were precipitated from the VRP transfection by the Flt4/IgG (Fig. 2*B*); these bands were absent in the vector transfection (Fig. 2*A*). Little or no specific precipitation of these two bands was found with Flt1/IgG or Flk1/IgG.§ Transfection with a VEGF-expressing plasmid showed the expected precipitation of a strong band of about 23 kDa with Flt1/IgG and Flk1/IgG (10–12, 14) but no precipitation with Flt4/IgG (Fig. 2*C*). For PlGF, no precipitation was found for Flt4/IgG, but the expected precipitation by Flt1/IgG, but not by Flk1/IgG was found (Fig. 2*D*) (36). These data indicate that VRP binds to the extracellular domain of Flt4 but does not interact (or does so much more weakly) with the VEGF receptors Flt1 or Flk1. They also confirm the lack of an interaction of VEGF with Flt4 (28) and indicate that PlGF is also not a ligand for this receptor.

Flt4 Tyrosine Phosphorylation Assay. In order to assay Flt4 tyrosine phosphorylation (presumably via Flt4 activation and autophosphorylation), Flt4 was expressed in 293 cells and Flt4 phosphorylation was monitored by phosphotyrosine immunoblot (Fig. 3*A*). Without stimulation, 293 cells expressing (lane 2) or not expressing (lane 1) Flt4 showed little or no Flt4 tyrosine phosphorylation. Stimulation of the Flt4-expressing cells by Flt4/IgG antiserum (lane 4) showed the tyrosine phosphorylation of two bands of 180 and 120 kDa. No increase above basal phosphorylation was observed with preimmune serum (data not shown), and no bands were found with Flt4/IgG antiserum stimulation of nonexpressing cells (lane 3). Two Flt4 bands of about this size have been reported as being expressed by DAMI and HEL cells (27). In addition,

§At times, some VRP precipitation is detected with Flk1/IgG, suggesting that VRP may have a low-affinity interaction with Flk1.

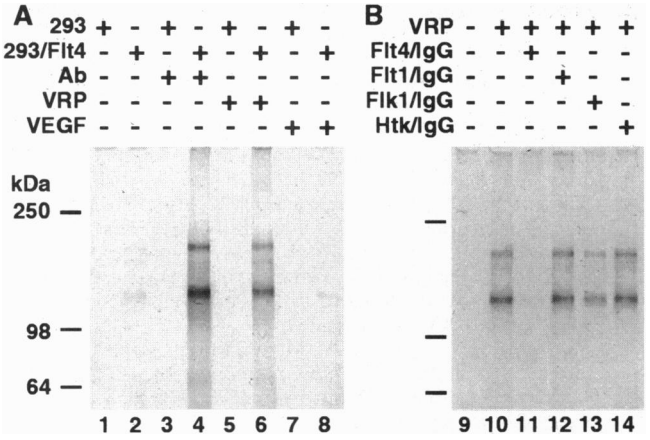

FIG. 3. Flt4 tyrosine phosphorylation. The stimulation of Flt4 tyrosine phosphorylation was performed as described in the text. (*A*) Assay performed with Flt4 expressing cells (293/Flt4) or with parental cells (293). Stimulation is with Flt4/IgG antiserum (Ab) or with VRP or VEGF conditioned medium as indicated. (*B*) Assay performed with Flt4 expressing cells. Stimulation as indicated with VRP conditioned medium preincubated with 135 nM of the indicated receptor IgG fusion protein.

SDS gel analysis of purified Flt4/IgG shows that it is composed of peptides of 150, 80, and 70 kDa (data not shown). N-terminal amino acid sequence of the Flt4/IgG peptides shows that the 150- and 70-kDa bands have the amino acid sequence YSMTPPTL (matching the Flt4 sequence starting at residue 25) and that the 80-kDa band has the sequence SLRRRQQQD (matching the Flt4 sequence beginning at residue 473). Thus, both the Flt4/IgG and the full-length Flt4 appear to be partially cleaved in the extracellular domain, and the tyrosine phosphorylated bands of 180 and 120 kDa observed in the Flt4 phosphorylation assays (Fig. 3) would correspond to the 150- and 80-kDa peptides of Flt4/IgG. These data show that polyclonal antibodies generated to the extracellular domain of Flt4 are capable activating Flt4 tyrosine phosphorylation.

To determine whether VRP activates the tyrosine phosphorylation of Flt4, we assayed conditioned medium from mammalian cells transfected with the VRP expression plasmid (Fig. 3*A*). This conditioned medium stimulated the tyrosine phosphorylation of the same 180- and 120-kDa bands (lane 6) found with the agonist polyclonal antibodies, demonstrating that VRP can activate and as well as bind to Flt4. Conditioned medium from a VEGF transfection failed to activate Flt4 tyrosine phosphorylation (lane 8). To confirm the specificity of VRP binding to the receptors of the VEGF family, Flt4/IgG, Flt1/IgG, and Flk1/IgG were tested for their ability to compete for VRP-stimulated Flt4 phosphorylation (lanes 10–13). As expected if VRP is a ligand for Flt4, Flt4/IgG prevented the VRP-stimulated phosphorylation (lane 11); while Flt1/IgG (lane 12), Flk1/IgG (lane 13), and Htk/IgG, a fusion protein from an unrelated tyrosine kinase receptor (lane 14), had little or no effect. These data show that VRP is able to activate the tyrosine phosphorylation of Flt4.

Binding of Purified VRP to the Flt4 Extracellular Domain. In order to show the binding of VRP to the extracellular domain of Flt4 as purified components, VRP was expressed with an epitope tag at the N terminus and purified by antibody affinity chromatography (*Materials and Methods*). Purified VRP specifically bound to ^{125}I-Flt4/IgG as is indicated by the competition with unlabeled Flt4/IgG (Fig. 4*A*). The ^{125}I-Flt4/IgG binding was not competitively inhibited by unlabeled Flt1/IgG or Flk1/IgG. Binding competition with increasing concentrations of unlabeled Flt4/IgG (Fig. 4*B*) gave an EC_{50} for this interaction of ≈0.7 nM, suggesting that the binding of

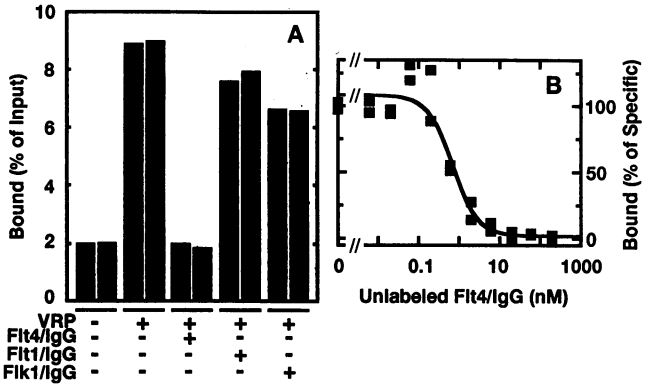

FIG. 4. Binding of ^{125}I-Flt4/IgG to purified VRP. Binding was performed as described in the text in the absence (−) or presence (+) of 100 nM receptor IgG fusion protein (*A*) or with increasing concentrations of Flt4/IgG (*B*).

VRP to Flt4 is of high affinity as would be expected if VRP is a biologically relevant ligand for Flt4.

Mitogenic Activity of VRP. To test whether VRP has mitogenic activity like that found for VEGF, the growth of human lung endothelial cells was determined at increasing concentrations of VRP or VEGF (Fig. 5). VRP promoted the growth of these endothelial cells and, thus, shares this mitogenic activity with VEGF. This is in contrast to PlGF, which has been reported to lack such mitogenic activity (up to 35 nM) (36). While an effective mitogenic agent, VRP was about 100-fold less potent than VEGF in this assay. Although the intracellular domain of Flt4 has been shown to be capable of stimulating mitogenesis in cells such as fibroblasts (28, 29), additional experiments will be required to establish that the mitogenic effects of VRP on endothelial cells are due to activation of Flt4. VRP could act via some as yet identified receptor or receptor hybrid.

VRP Expression Pattern. The G61 glioma cell line used in the cloning of VRP expresses a major VRP mRNA band of about 2.4 kb (Fig. 6). A minor band of about 2.2 kb may also be present. A 2.4-kb band was expressed in adult human tissues from heart, placenta, ovary, and small intestine; a weaker band was found in lung, skeletal muscle, spleen, prostate, testis, and colon (Fig. 6). Expression of a 2.4-kb mRNA was also found in fetal lung and kidney.

In conclusion, we have identified a secreted protein, VRP, that promotes the mitogenesis of vascular endothelial cells. VRP binds and activates the receptor tyrosine kinase Flt4 and could prove to be an important endothelial cell growth factor that promotes vasculogenesis and the development of lymphatic vessels via this receptor. VRP is a third member of the VEGF family and has about 30% amino acid identity with

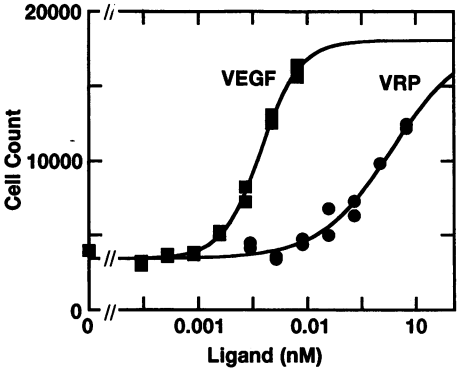

FIG. 5. Mitogenic activity of VRP. The growth of human lung endothelial cells was performed as described in the text.

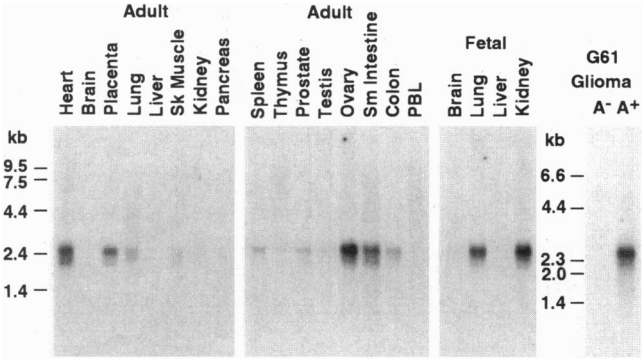

FIG. 6. Expression of RNA encoding VRP. Hybridization to blots of human RNA was performed as described in the text.

VEGF and PlGF. In addition to the VEGF-like domain, VRP contains an ≈180-amino acid C-terminal, cysteine-rich domain not found in other members of the VEGF family. VRP fails to interact appreciably (at least under the conditions tested) with the extracellular domain of the VEGF receptors Flt1 and Flk1.

David V. Goeddel is a consultant to Genentech.

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