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Identification and Localization of Alternately Spliced mRNAs for Vascular Endothelial Growth Factor in Human Uterus and Estrogen Regulation in Endometrial Carcinoma Cell Lines[1]

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ABSTRACT

Repair of human endometrium after menstruation and preparation of the endometrium for implantation involves profound angiogenic changes. Vascular endothelial cell growth factor (VEGF) is a recently identified growth factor with significant angiogenic properties. Four species of mRNA encoding VEGFs were identified in human endometrium and myometrium. All species were present throughout the menstrual cycle. Two species, $VEGF_{165}$ and $VEGF_{121}$, were present in peripheral leukocytes, indicating tissue-specific splicing of the two other VEGF transcripts. In situ hybridization of mRNA encoding VEGF was not restricted to vascular smooth muscle but was present in epithelial and stromal cells of endometrium throughout the cycle, and the distribution changed during the course of the cycle. All four species of VEGF were expressed by the endometrial carcinoma cell lines Ishikawa, HEC 1-A, and HEC 1-B. Estradiol increased steady-state levels of mRNA encoding VEGF in a dose- and time-dependent manner in HEC 1-A cells. Conditioned medium from these cells possessed angiogenic activity that was depleted by passage through a heparin affinity column. None of the cell lines demonstrated mRNA for acidic or basic fibroblast growth factor (FGF), despite previous reports of the identification of immunoreactive basic FGF in HEC 1-A and HEC 1-B cells. These findings show that VEGFs, not FGFs, are the principal angiogenic growth factors secreted by these cells and that human endometrium expresses a secreted angiogenic growth factor whose site of expression changes during the menstrual cycle.

INTRODUCTION

Mammalian reproduction depends on the cyclical development of a receptive endometrium and involves the proliferation and differentiation of epithelial and stromal cells. Unlike other mammals, primates undergo shedding of the uterine surface at menstruation and must achieve rapid repair of the denuded tissue in order for successful implantation to occur. Ovarian steroids have been assumed to be the principal mediators of these events, but recent studies indicate that polypeptide growth factors, either alone [1] or in conjunction with steroids [2, 3], play an important role. One of the most profound changes that arises in the endometrium is the process of angiogenesis, involving the proliferation, maturation, and migration of endothelial cells [4]. Many angiogenic growth factors are now recognized [5]; but although angiogenic activity is present in human endometrium [6], the precise nature of this activity is unclear.

The best-characterized angiogenic growth factor, basic fibroblast growth factor (bFGF) [7], is an 18-kDa peptide, and the human cDNA has been isolated from kidney, fetal heart and liver, placenta, and breast carcinoma libraries [8]. Basic FGF-like immunoreactivity has been reported in human endometrium and three endometrial carcinoma cell lines, AN3CA, HEC 1-A, and HEC 1-B, and is apparently regulated by ovarian steroids [9]. Acidic FGF (aFGF), structurally similar to bFGF, is less widely distributed, being found in hypothalamus, retina, cartilage, and brain [10, 11].

Neither of these growth factors contains a secretory signal peptide sequence, being released from extracellular matrix only by specific peptidases [12]. However, the presence of angiogenic activity in conditioned medium indicates secretion of such a factor by transformed (mouse Sa 180) and untransformed (rat granulosa and porcine thyroid follicle) cells [13–15].

Recently, a specific vascular endothelial growth factor (VEGF) has been identified in various cell lines including bovine folliculostellate cells [16], rat glioma cells GS-9L [17], mouse Sa 180 cells [18], human HL60 cells [19], rat corpora lutea [20], and human fetal vascular smooth muscle cells [21]. Purified VEGF is a ~46-kDa dimer [16, 22]. The peptide is the product of mRNA derived from 8 exons, and analysis of genomic and cDNA clones predicts four forms, of 206, 189, 165, and 121 amino acid residues, arising from alternative splicing of exons 6 to 8 [21, 23]. These peptides contain hydrophobic secretory sequence at their NH_2 termini. Vascular endothelial growth factor, as its name suggests, is a highly specific mitogen for endothelial cells and

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is intimately involved in angiogenesis [24]. Variant 189 was isolated independently and is also called vascular permeability factor (VPF) [25, 26].

Having noted the striking angiogenesis that occurs in the endometrium, we sought to determine whether VEGF was present in this tissue. We used the polymerase chain reaction to identify transcripts for VEGF in fresh human uterine tissue and human endometrial carcinoma cell lines. We identify a new splice variant and demonstrate the specific expression of this growth factor in endometrial and myometrial cells. VEGF mRNA was present in glandular and stromal cells of the endometrium, and estradiol increased steady-state levels of the message in endometrial carcinoma cell lines.

MATERIALS AND METHODS

Tissue Collection and Culture of Cell Lines

Endometrial tissue was removed at dilatation and curettage or hysterectomy, performed for benign gynecological conditions. The majority of patients were undergoing dilatation and curettage for the investigation of subjective mennorhagia. The tissue obtained at this time was shown to be histologically normal. It has been reported that at least 50% of women complaining of mennoragia in fact have blood loss values within the normal range [27]; therefore tissue obtained from such women would be expected to be normal. Informed consent was obtained from the patients and the study was approved by the ethical committee of the Cambridge District Health Authority. Myometrium was obtained from hysterectomy specimens. Tissue was rinsed in sterile PBS and snap-frozen in liquid N_2. To determine whether mRNA for VEGF was present in contaminating blood cells, circulating leukocytes were isolated from peripheral blood and purified using lymphoprep (Nycomed, Sheldon, UK) as described by Boyum [28]. Platelets were prepared by filtering leukocyte preparations through 0.45-μm Sepacell filters (Kimal Scientific Products, Uxbridge, UK) The preparation of RNA from leukocytes and platelets has been described previously [29].

Endometrial adenocarcinoma cell lines were obtained from the following sources: Ishikawa, Prof. M. Nishide, University of Tsukuba, Japan; HEC 1-A and HEC 1-B, American Type Culture Collection, Rockville, MD. Cells were grown in McCoy's medium (ICN Flow Laboratories, Irvine, CA) with 10% fetal calf serum (FCS; ICN Flow) plus 2 mM L-glutamine (ICN Flow) and 50 U/ml and 50 mg/ml penicillin/streptomycin (ICN Flow). Conditioned medium was prepared by growing HEC 1-A cells in 175-cm^2 flasks to 70–80% confluence. Cells were then washed in PBS and the medium was replaced with serum-free medium containing Dulbecco's Modified Eagle's Medium: Ham's F-12 nutrient medium (vol 1:1; ICN Flow), 20 mM Hepes buffer (ICN Flow), 1% nonessential amino acids (Sigma Chemicals, Poole, UK), 2 mM L-glutamine (ICN Flow), 10 μg/ml insulin (Sigma), 100 ng/ml hydrocortisone (Sigma), 25 ng/ml selenite (Sigma), 10 μg/ml transferrin (Sigma), 50 U/ml penicillin, and 50 mg/ml streptomycin. This medium was collected after 18 h; cells were removed by centrifugation and filtration through a 0.2-μm filter. The conditioned medium was assayed for angiogenic activity by determining the stimulation of ^{3}H-thymidine incorporation in human umbilical vein endothelial cells (HUVEC).

RNA Extraction and Polymerase Chain Reaction (PCR)

RNA was prepared from frozen tissue by the method of Chirgwin [30] and as described [29]. Briefly, tissue was homogenized in a guanidinium isothiocyante (Gibco, Uxbridge, UK)-containing buffer and RNA purified by cesium chloride (Boehringer, Mannheim, Germany) density gradient centrifugation.

Complementary DNA was synthesized using AMV reverse transcriptase (Super RT, Anglian Biotechnology, Colchester, UK) and 10 μg of total RNA primed with oligo(dT). Amplification was performed in two stages. In the first stage, primers A and F were used through 35 cycles (30 sec at 95°C, 30 sec at 58°C, 30 sec at 72°C). One microliter was then transferred to a fresh 50-μl reaction mix containing primers C and B and reamplified using the above conditions but for only 20 cycles. One tenth of the product was then analyzed by agarose gel electrophoresis. To clone full-length cDNAs as template for generation of RNA probes for in situ hybridization, cDNA derived from placental total RNA was first amplified in two stages as described above except that primers I and J were used for the first stage (30 sec at 95°C, 30 sec at 60°C, 30 sec at 72°C) for 35 cycles, and primers G and J were used for the second stage, under the same conditions but for 20 cycles only. The primers used in this study were:

A 5′ TGCGATGCGGGGGCTGC 3′
F 5′ TTTCCTGGTGAGAGATCTGG 3′
C 5′ GAGTGTGTGCCCACTGAGGA 3′
B 5′ CTCGGCTTGTCACATCT 3′
G 5′ GGGCTCTAGAATGAACTTTCTGCTGTCTTGGGT '3
I 5′ GGGCTCTAGATCGGGCCTCCGAAACCAT 3′
J 5′ GGGCTCTAGAGCGCAGAGTCTCCTCTTC 3′

Primers A, B, C, and F were chosen because they amplify exons 5–8 and thus can be used to resolve the relatively small differences in length that arise due to alternative splicing of these exons. They also span exon/intron boundaries; thus amplification of contaminating genomic DNA either is prevented (because the introns are too large to amplify) or would be readily detected.

The PCR products were end-repaired by the addition of T4 DNA polymerase (5 units) and $MgCl_2$ to a final concentration of 10 mmol/L directly to the reaction tube after amplification; they were incubated at 37°C for 10 min. This was followed by phenol and chloroform extraction and ethanol precipitation. The full-length cDNA encoding $VEGF_{165}$

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was gel purified from products of PCR amplification with primers G and J, digested with Xba 1 (New England Biolabs, Beverly, MA). PCR products were ligated into suitable prepared Bluescript II KS^+ plasmid vector (Stratagene, La Jolla, CA) and sequenced using the dideoxy chain-termination method [31].

In Situ *Hybridization*

In situ hybridization was carried out according to the method of Kanzaki et al. [32] and as described previously [33]. Cryostat sections were collected onto slides coated with poly-L-lysine (Sigma), air dried, and fixed in 4% paraformaldehyde in PBS for 20 min at 4°C. Slides were dehydrated through alcohols and stored at −70°C. Plasmids linearized with *Bam*H1 and with *Eag* 1 (New England Biolabs) were used as templates for the generation of sense and antisense RNA probes, respectively. Approximately 2×10^6 cpm of ^{35}S-UTP were incorporated per microgram of plasmid template. Following acetylation, the air-dried slides were prehybridized in 50% formamide, single-strength Denhardt's, 20 mM Tris-HCl (pH 8), 0.3 M NaCl, 5 mM EDTA, 10 mM sodium phosphate, and 0.5 mg/ml yeast tRNA at 50°C for 1 h. The prehybridization buffer was removed and hybridization buffer was added, containing—in addition to the above 10% dextran sulfate (Pharmacia, Milton Keynes, UK)—50 mM dithiothreitol, and 1×10^5 cpm ^{35}S-labeled sense or antisense RNA probe. Hybridization was carried out at 50°C for 16 h. The slides were washed twice in double-strength saline sodium citrate (SSC)/14 mM β-mercaptoethanol at 50°C for 20 min, rinsed in double-strength SSC, and treated with RNAse A (10 μg/ml) for 30 min at 37°C in 0.5 M NaCl, 10 mM Tris, pH 7.0, 1 mM EDTA. The slides were washed twice in double-strength SSC at room temperature for 15 min followed by 0.1-strength SSC for 60 min at 65°C, then dehydrated in an ethanol series. Autoradiography was carried out at 4°C for 2–3 wk by coating the slides in Ilford K5 emulsion. The slides were developed with Kodak D19 (Rochester, NY), fixed, and counterstained with haemalum.

Northern Blot Hybridization

To determine whether the steady-state VEGF mRNA concentration was influenced by estradiol, Northern blot analysis was performed using RNA from steroid-treated cells. HEC 1-A cells were grown as described above in 175-cm^2 flasks. This medium was then replaced with serum- and phenol red-free medium and the cells were incubated for a further 24 h prior to the addition of 10^{-8}M estradiol-17β. Cells were harvested at varying intervals up to 46 h later. RNA was prepared as described by Chomsninzski and Sarchi [34]; Northern blot analysis of HEC 1-A total RNA was performed using formaldehyde-containing denaturing agarose gels [35] and capillary blotting onto Hybond N (Amersham International PLC, Aylesbury, Bucks., UK). Blots were UV fixed and prehybridized in roller bottles (Techne, Cambridge, UK) in 6-strength SSC, 5-strength Denhardt's solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA at 65°C for 1–3 h. The probe used was the entire plasmid containing exons 5–8 of the VEGF cDNA, labeled via the random hexamer method [36]. Following overnight hybridization the blots were washed twice in double-strength SSC, 0.1% SDS and twice in 0.1-strength SSC, 0.1% SDS. All washes were for 15 min at 65°C. Autoradiography was carried out for 3–7 days at −70°C with intensifying screens. Blots probed for VEGF mRNA were stripped and re-probed for actin in a similar manner in order to permit loading and blotting differences between lanes to be compensated for. Autoradiographs were analyzed through use of a molecular dynamics scanning densitometer.

Measurement of Stimulation of ^{3}H-Thymidine Incorporation in HEC 1-A-Conditioned Media

HUVECs were isolated from fresh umbilical cords collected from the delivery suite [37]. The veins in cords at least 15 cm long were cannulated and washed with 30–60 ml of PBS to remove blood clots. The PBS was replaced by 10 ml of collagenase (Sigma; 1 mg/ml in PBS) and veins were incubated at room temperature for 10 min. The supernatant was collected and veins were rinsed with 10 ml of M199 (ICN Flow). The cells were collected by centrifugation and resuspended in 5 ml of M199 (ICN Flow) with 15% FCS plus L-glutamine (1%) and penicillin/streptomycin (50 U/ml/50 mg/ml) and plated in 25-cm^2 Falcon tissue culture flasks (Becton-Dickinson, Plymouth, UK). The culture medium was changed after 48–72 h and cultures not close to confluence by this time were discarded. To measure ^{3}H-thymidine incorporation, the HUVECs were plated at a density of 15 000/well in 24-well plates that had been pre-wet with 0.2% gelatin (Sigma). The medium was changed after 48 h and the culture was used after 72 h. The monolayers were washed with PBS, and the test media and ^{3}H-thymidine (110 000 dpm/well) were added and incubated. After 30 h, media were removed and cells were fixed by the addition of 1 ml of 10% cold trichloroacetic acid (TCA; BDH, Poole, UK). After incubation at 4°C for 15 min, TCA was removed and the wells were washed with 1 ml ethanol/ether (3:1). After air drying, the precipitated material was redissolved in 0.2 M NaOH and radioactivity was determined by liquid scintillation counting.

RESULTS

Amplification of a variety of endometrial and myometrial cDNA with VEGF-specific nested PCR primers consistently produced four products (Fig. 1), while amplification of cDNA prepared from peripheral leukocytes revealed two products and that from platelets, no products. Amplification of genomic DNA with these primers also failed to yield a product, presumably due to the length of the introns be-

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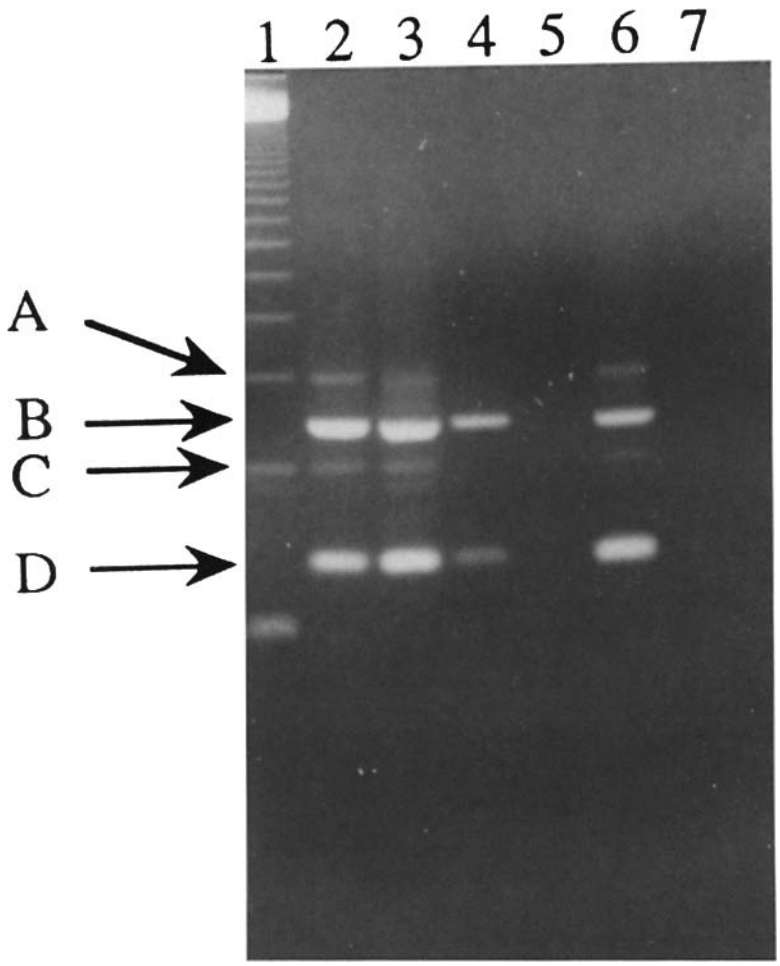

FIG. 1. Agarose gel showing PCR products from the amplification of cDNA from proliferative endometrium, secretory endometrium, peripheral leukocytes, platelets, myometrium, and negative control (2–7, respectively). Nested PCR with the primers specific to VPF/VEGF described in the text were used. Bands labeled A–D correspond to $VEGF_{189}$, $VEGF_{165}$, $VEGF_{145}$, and $VEGF_{121}$, respectively. Lane 1 shows molecular weight markers (123-bp ladder; BRL, Gaithersburg, MD).

tween the primers (data not shown). The nucleotide sequence of each PCR product confirmed that they were all derived from mRNAs encoding VEGF. Three of the products (A, B, and D) corresponded exactly to those already described and were derived from exons 1–5, 6, 7, and 8; 1–5, 7 and 8, and 1–5 and 8, respectively [21]. However, in the endometrial samples an additional splice variant was seen (C). The sequence of this cDNA indicated that it contained exons 1–5, 6, and 8. This would be predicted to encode a protein of 145 amino acids. This splice variant has not been described previously, and as it was not observed in cDNA derived from peripheral leukocytes, it probably is a tissue-specific splice.

Endometrial carcinoma cell lines (Ishikawa, HEC 1-A, and HEC 1-B) were also examined for the presence of cDNAs encoding VEGF. All four variants were found in the three cell lines studied. HEC 1-A and HEC 1-B cells were postulated to produce the heparin-binding growth factor basic FGF [9]. However, when nested PCR with specific primers for both acidic or basic FGF were used, no products could be found, whereas cDNA encoding VEGF was readily detectable in the same cDNA preparations (Fig. 2). The FGF-specific primers effectively demonstrated the presence of both acidic and basic FGF in normal endometrium and myometrium.

Conditioned serum-free medium from these endometrial carcinoma cell lines stimulated ^{3}H-thymidine uptake in HUVECs in a dose-dependent manner. This stimulation was partially abolished by passing the medium through a heparin affinity column (Fig. 3), indicating that the cell lines release a heparin-binding angiogenic factor into the medium.

Northern blot analysis of RNA from these cells also demonstrated the presence of mRNA hybridizing, under high stringency, to the VEGF cDNA probe. The treatment of these cells in serum- and phenol red-free medium with estradiol

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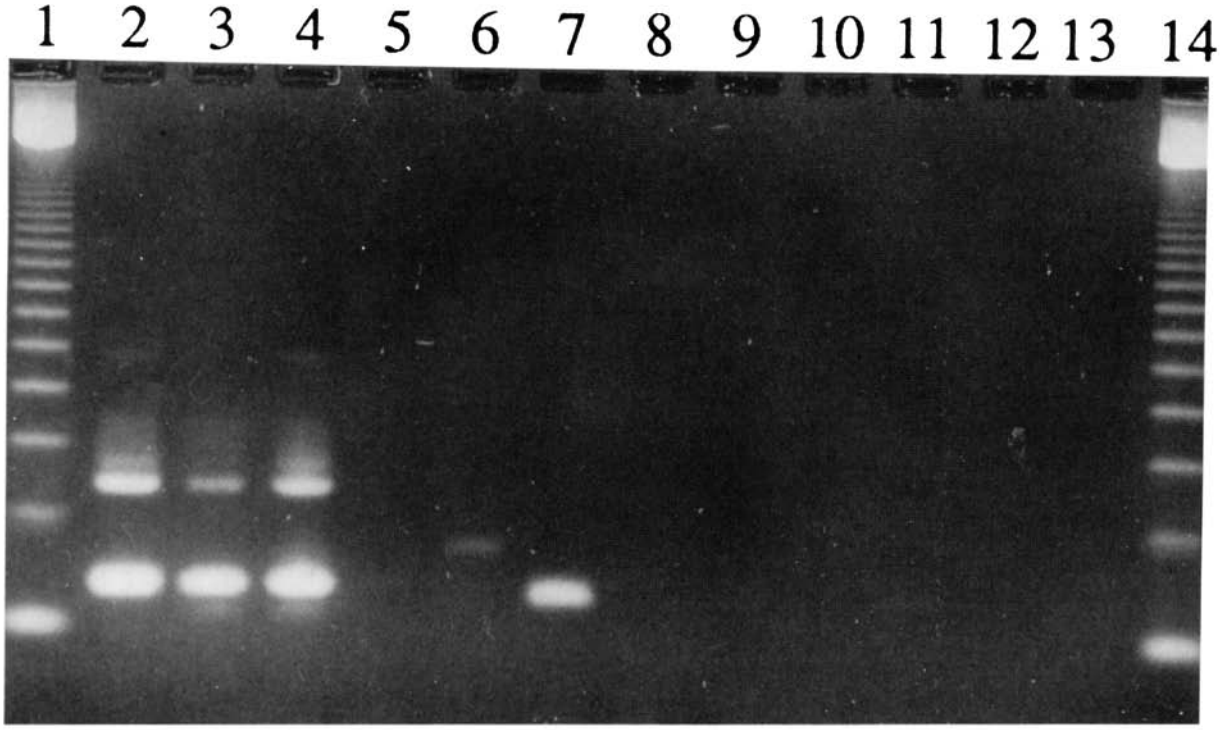

FIG. 2. Agarose gel showing PCR products from the amplification of cDNA from the proliferative endometrium (lanes 2, 6, and 7), HEC 1-A cell line (lanes 3, 8, and 9), HEC 1-B cell line (lanes 4, 10, and 11); and control (no template) (lanes 5, 12, and 13) with nested primers specific to VEGF (lanes 2, 3, 4, and 5), acidic FGF (lanes 6, 8, 10, and 12), and basic FGF (lanes 7, 9, 11, and 13). Lanes 1 and 14 are molecular weight markers (123-bp ladder; BRL).

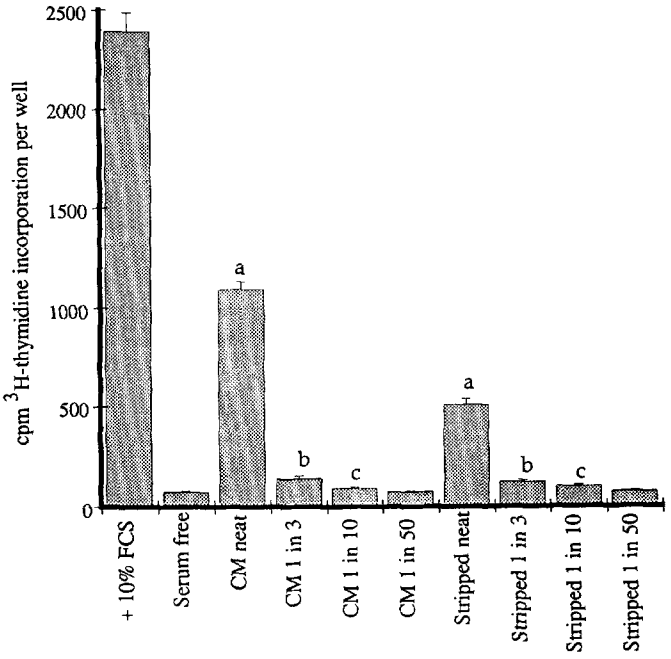

FIG. 3. Tritiated thymidine incorporation in subconfluent HUVEC. Acid-precipitable label was measured after 30-h incubation with various dilutions of HEC 1-A-conditioned medium. All assays were done in quintuplicate in the absence of serum (except for the positive control). Lanes labeled a, b, and c are significantly different from the serum-free negative control ($p < 0.0001$, $p < 0.01$, and $p < 0.05$, a–c, respectively). "Stripped" refers to conditioned media that had been passed through a heparin affinity column (Hitrap; Pharmacia).

(10^{-8}M) increased the steady-state concentration of VEGF mRNA in a time-dependent manner. As can be seen from Figure 4, the VEGF-specific hybridization rose approximately 6-fold after 20 h after correction for lane-to-lane loading differences. Through comparison with the mobility of the ribosomal RNA, the hybridizing band was estimated to have a size of approximately 3.7 kb.

To identify the site of expression of VEGF mRNA, we carried out in situ hybridization on samples of endometrium throughout the menstrual cycle. Cryostat sections were probed with a ^{35}S-labeled antisense RNA probe. A probe transcribed from the sense strand was used as a control for nonspecific hybridization.

VEGF mRNA expression was seen in the stroma of proliferative endometrium, with a subset of cells expressing strongly, leading to a distinct punctate appearance (Fig. 5, A and B). A lower level of expression was seen in the glandular epithelium. Expression was also seen in the myometrium and was stronger at the myometrial/endometrial boundary (data not shown). During the secretory phase this pattern altered (Fig. 5, C and D). The punctate distribution of expression in the stroma disappeared, and the expression in the glandular epithelium increased, culminating in very intense hybridization in the glands in menstrual tissue (Fig. 5, E and F). Within necrotic areas of menstrual tissue there were also high levels of expression by isolated groups of cells that may have been infiltrating macrophages.

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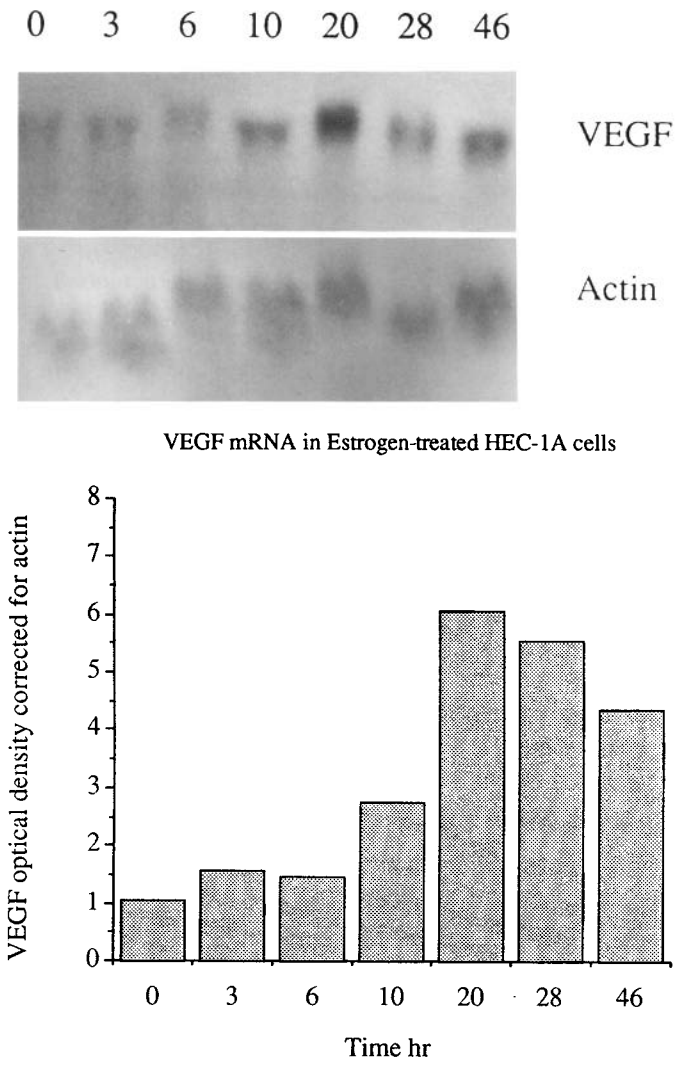

FIG. 4. Northern blot analysis (top) of RNA from estrogen-treated HEC 1-A cells probed with both VEGF-specific or actin-specific cDNA probes and described in the *Materials and Methods* section. Densitometric analysis (bottom) of VEGF-specific signal after correction for loading differences showing an approximately 6-fold increase in VEGF mRNA concentration after 20 h of 10^{-8}M estradiol-17β treatment.

DISCUSSION

In the present paper, the cDNA sequences of four isoforms of VEGF in human endometrium and myometrium are described. The largest cDNA corresponds to VPF and contains the transcripts of 8 exons [25] encoding the mature peptide of 189 amino acids. The second largest cDNA is similar to that described as VEGF in HL 60 leukemia cells [19] and lacks the sequence encoding amino acids 115 to 139 corresponding to exon 6 [21]. The smallest PCR product has a splice site between amino acids 114 and 183 and corresponds to the removal of exons 6 and 7 [21]. However, in addition to these three previously described cDNAs, a fourth cDNA is presented here. This retains the 72-base sequence encoding the 24 amino acid fragment encoded by the mRNA from exon 6 but lacks the 44 amino acids derived from exon 7. This would be expected to encode a peptide with 145 amino acids. The largest molecular form of VEGF, $VEGF_{206}$, was not identified in the current study but was found previously only in a human fetal liver library [23].

All four cDNAs are present in proliferative and secretory endometrium, suggesting that differential expression is not grossly altered by progesterone; they all are also present in myometrium. Peripheral leukocytes contain only two isoforms: the smallest one, $VEGF_{121}$, and the one corresponding to $VEGF_{165}$. This is consistent with the description of T lymphocyte production of a vascular permeability factor [38], although it is not the $VEGF_{189}$ species originally described as VPF [25]. The fact that human vascular smooth muscle cells express only three isoforms [21] suggests that mRNA for $VEGF_{145}$, the new isoform, is specifically expressed in uterine tissue. It was not possible from the present experiments to determine the cellular source or to ascertain whether this variant has biological activity.

$VEGF_{189}$ contains sixteen cysteine residues, eight encoded by exons 3 and 4, seven by exon 7, and one by exon 8. Both exons 6 and 7 contain basic amino acids, so the different splice variants would be expected to have different structure and different isoelectric point changes. Loss of the sequence encoded by exon 6 either alone, as occurs in $VEGF_{165}$, or with exon 7, as in $VEGF_{121}$, does not result in loss of biological activity [19, 23, 25].

The predicted amino acid sequences of VPF and VEGF have 18 and 15% sequence homology with the A and B chains of platelet-derived growth factor (PDGF), and all of the sixteen cysteines of PDGF are conserved in VPF and VEGF. Despite this homology, PDGF is mitogenic in several cell types including fibroblasts and vascular smooth muscle cells [39], while VEGF has been shown to act as a mitogen only on endothelial cells [16]. Exon 6, found in VEGFs 206, 189, and 145, contains highly conserved residues also encoded by exon 6 of the A chain of PDGF [40], which imparts nuclear localization [41]. However, immunohistochemical staining of $VEGF_{189}$, expressed in 293 cells [23], showed no nuclear localization, suggesting that VEGFs 206 and 189, although not secreted, were probably retained within the cytoplasm of transfected cells. Since $VEGF_{145}$ retains exon 6 it is probable that this variant will be retained by the cell as are the other members of the family that contain this exon.

Localization by in situ hybridization suggests VEGF expression by both stromal and glandular cells within the endometrium during the proliferative phase, with generally increased expression in the glands in secretory tissue. It seems clear that both the level and site of expression vary through the cycle. The unusual cell population expressing VEGF in the proliferative phase may be macrophages, which make up 2–5% of the stromal cell population [42]. However, the expression in the stroma appears to be down-

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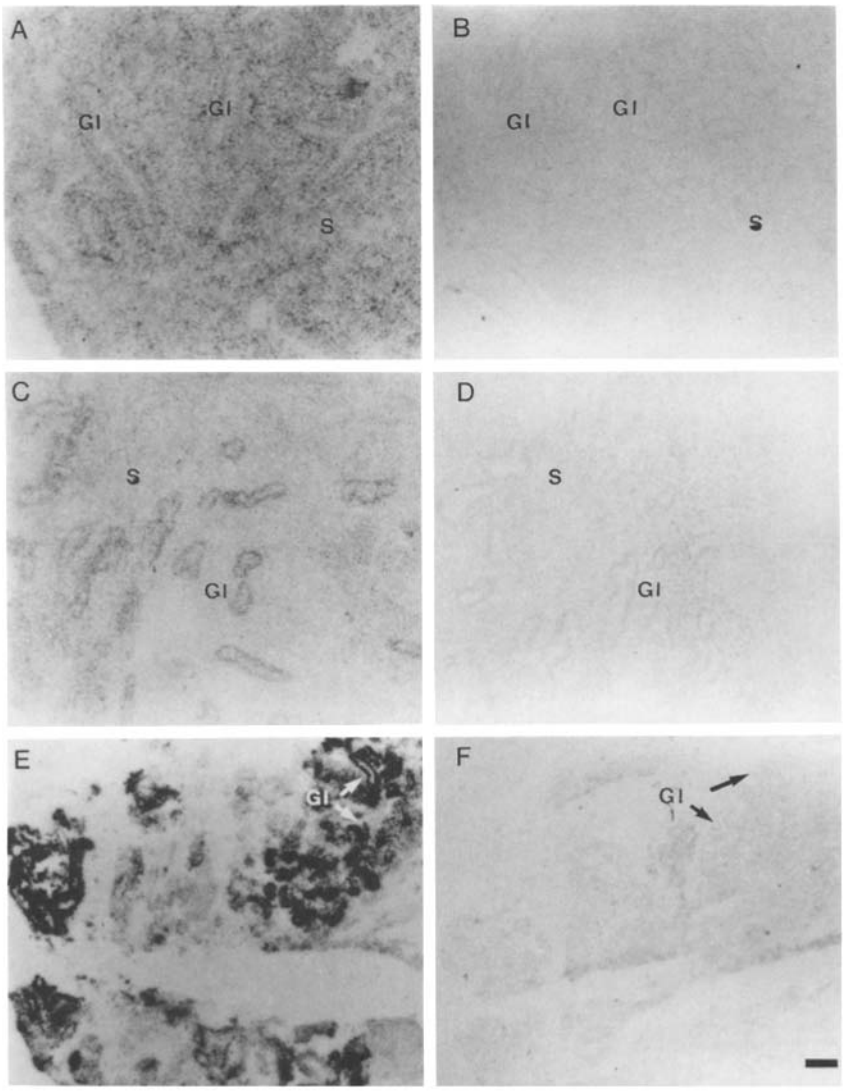

FIG. 5. In situ localization of VEGF mRNA in endometrial tissue. Bright-field photomicrographs of sections of endometrium after in situ hybridization with antisense VEGF RNA probe (A, C, E). Equivalent sections hybridized with sense probe are shown for comparison (B, D, F). Sections are counterstained with haemalum. A, B) proliferative phase endometrium. C, D) secretory phase endometrium. E, F) menstrual tissue. Endometrial glands (Gl) and stroma (S) are indicated. Scale bar: 250 μm.

regulated in the secretory phase while in the glands it is increased. This suggests different control mechanisms in the two cell types.

The extremely strong hybridization seen in the glands of menstrual endometrium suggests that very high levels of VEGF-encoding mRNA are present in this tissue. Since at menstruation the endometrium becomes severely hypoxic, this is probably another example of hypoxia-induced VEGF expression as recently found in glioblastoma cells [43, 44].

We have shown that estradiol at physiological concentrations (10^{-8}M) stimulated an increase in steady-state levels of mRNA encoding VEGF. The highest levels were achieved after 20-h exposure to estradiol. Lower doses of estradiol resulted in less stimulation (data not shown). Thus steroids are probably involved in this regulation.

The endometrial carcinoma cell lines Ishikawa, HEC 1-A, and HEC 1-B all express the four cDNAs described for normal endometrium. None of the cells were found to have cDNAs for acidic or basic FGF. As acidic and basic FGFs do not contain a consensus secretory sequence and as the conditioned medium from the cell lines contained angiogenic activity, it is unlikely that acidic or basic FGFs are the principal secreted angiogenic growth factors expressed by endometrial carcinoma cell lines. The present findings are in direct contradiction to those of Presta [9], who demonstrated immunoreactive basic FGF in HEC 1-A and HEC 1-B cells. The four pairs of primers used for acidic and basic FGFs successfully identified cDNA encoding both peptides in endometrium, and the cDNA prepared from the cell lines

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was used to identify the mRNA for VEGF. It is possible that these frequently passaged cells lost the ability to express FGFs, though this seems unlikely, or that the antibody used by Presta [9] was not binding to basic FGF. Alternatively, the antibody used by Presta could have bound to FGF derived from the fetal calf serum used in the culture of the cells and bound to specific cell-surface FGF receptors or to surface proteoglycans [46].

In addition to the endothelial mitogenic capacity of the VEGF family, its members are known to induce fluid and protein extravasation from blood vessels [23, 24]. The site of implantation in rodents is characterized by local edema, increased vascular permeability, and vasodilation [47]. The ability of endometrium to express VEGFs raises the possibility of its involvement in this process.

The present study demonstrates for the first time that RNAs for the VEGF family of growth factors are present in normal human endometrium and that they, not FGFs, are expressed by endometrial carcinoma cell lines. They are widely expressed throughout the endometrium by both glandular and stromal cells. This expression is almost certainly modulated by sex steroids in vivo.

The process of angiogenesis is essential for the repair of endometrium after menstruation. In addition, implantation and placentation involve profound angiogenesis. Along with other growth factors, the VEGFs are likely to be involved in the complicated interaction between steroids, growth factors, cell-surface molecules, and extracellular matrix [47] that regulates local vascular permeability and angiogenesis in endometrium and placenta.

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