Developmental expression and regulation of basic fibroblast growth factor and vascular endothelial growth factor in rat decidua and in a decidual cell line

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ABSTRACT

During pregnancy, the decidua is comprised of two separate tissues located either mesometrially or antimesometrially in the uterus. Trophoblast invasion takes place only in the mesometrial decidua, where extensive angiogenesis, essential for successful implantation, occurs. Both basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) have been implicated in this phenomenon.

The aim of this study was to determine whether the expression of both growth factors is intrinsic to decidua and occurs in the absence of conceptuses, whether their genes are expressed specifically in the mesometrial decidua, the site of angiogenesis, and whether both growth factors are developmentally and hormonally regulated.

Decidual tissue was dissected from pseudo-pregnant rats and levels of both bFGF and VEGF mRNA were examined in mesometrial and antimesometrial decidua by semi-quantitative RT-PCR at different stages of pseudopregnancy. Although induction of decidualization triggered the mRNA expression of bFGF, VEGF mRNA expression remained unchanged. VEGF mRNA level was similar in both antimesometrial and mesometrial decidua, and remained constant throughout pseudopregnancy. In sharp contrast, bFGF mRNA was

highly expressed in the mesometrial decidua at a time when extensive angiogenesis takes place in this tissue. Very little signal was observed in the antimesometrial decidua.

To examine the regulation of these growth factors, we used a temperature-sensitive decidual cell line developed by transforming antimesometrial decidual cells with SV-40 tsA 209 mutant virus. These cells express both bFGF and VEGF mRNA. Because progesterone is necessary for decidualization and decidua secretes prolactin (PRL)-related hormones, we examined the role of these hormones on VEGF and bFGF mRNA expressions. Neither progesterone nor PRL had any effect on VEGF mRNA levels. However, bFGF mRNA expression was greatly stimulated by PRL.

In conclusion, results of this investigation have revealed that bFGF, but not VEGF, mRNA becomes highly expressed in the mesometrial decidua, where angiogenesis occurs, and where trophoblasts, by invading decidual cells, may promote the release of bFGF. In addition, these results indicate that the locally secreted PRL-like hormone up-regulates the mRNA expression of bFGF.

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INTRODUCTION

Decidualization in pregnant or pseudopregnant rat is marked by extensive proliferation and differentiation of endometrial stromal cells in response to either blastocyst implantation or artificial stimuli. Morphological changes that occur during decidualization have been extensively studied (De Feo 1967,

O'Shea *et al.* 1983). Decidualization in the rat gives rise to two different cell populations, located on opposite sides of the uterine lumen (Bell 1983, Gu & Gibori 1995). The cells that decidualize in the antimesometrial site are highly differentiated and secrete several hormones and growth factors (Jayatilak *et al.* 1985, Gu *et al.* 1994, 1995, Gu & Gibori 1995), whereas the mesometrial decidua where blood

vessels gain access is the site of extensive angiogenesis. Angiogenesis is a fundamental process by which new blood vessels are formed (Folkman & Shing 1992) and it is essential in reproduction, development and wound repair. Decidualization is accompanied by extensive angiogenesis, and these two processes are interdependent for successful fetal survival. Angiogenesis, involving proliferation, maturation and migration of endothelial cells is not only regulated by ovarian steroids but also by angiogenic growth factors. The cells comprising the mesometrial region secrete α_2 -macroglobulin (Gu *et al.* 1992), which is a potent protease inhibitor and, therefore, limits the invasion of trophoblast. α_2 -Macroglobulin is also known to bind these growth factors (Dennis *et al.* 1989, Soker *et al.* 1993). The fibroblast growth factors (FGFs) are a distinct class of potent mitogens (Folkman & Klagsbrun 1987, Thomas 1987). These factors bind to heparin sulfate proteoglycans (Vlodavsky *et al.* 1987) and stimulate endothelial cells to migrate and form tubes (Montesano *et al.* 1986). Vascular endothelial growth factor (VEGF) also acts as mitogen specific to endothelial cells and it increases the vascular permeability in these cells (Ferrara & Henzel 1989, Gospodarowicz *et al.* 1989). VEGF mRNA expression is observed in the mouse uterus at the site of implantation (Chakraborty *et al.* 1995), and in artificially induced mouse decidual cells (Shweiki *et al.* 1993), whereas non-decidualized stromal cells fail to express this gene.

In this investigation, we examined the mRNA expression of these two potent angiogenic factors (bFGF and VEGF) in both the antimesometrial and mesometrial decidua of rats, throughout the course of their development. Because progesterone is also implicated in regulating the process of angiogenesis and the expression of these angiogenic factors *in vivo* (Cullinan-Bove & Koos 1993, Koos & Olson 1989, Rider & Psychoyos 1994), we examined the role of progesterone on the mRNA expression of bFGF and VEGF in a decidual cell line. Because decidual cells secrete decidual prolactin (PRL)-like hormones and possess the receptors for PRL (Gu *et al.* 1996) we also investigated the role of PRL in the expression of bFGF and VEGF mRNA.

MATERIALS AND METHODS

Materials

Cell culture supplies were purchased from Mediatech (Washington, DC, USA) unless otherwise indicated. Trypan blue and Hanks' balanced salt solution (HBSS; without Ca^{2+} and Mg^{2+}) were obtained from Sigma Chemical Co. (St Louis,

MO, USA). Taq DNA polymerase was procured from Perkin-Elmer Corp. (Foster City, CA, USA). Collagenase (type I) was obtained from Worthington Biochemical Corp. (Freehold, NJ, USA) and dispase (type II) and deoxyribonuclease were purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN, USA). Fetal bovine serum (FBS) was purchased from HyClone (Logan, UT, USA), and [$\alpha^{32}\text{P}$]deoxy CTP (dCTP) obtained from Amersham (Arlington Heights, IL, USA).

Decidualization of animals

All experiments were conducted in accordance with the principles and procedures of the NIH Guide for the Care and Use of Laboratory Animals and approved by the institutional care and use committee. Pseudopregnancy was induced in Sprague-Dawley female rats by mating them with vasectomized male rats at the Harlan facilities (Madison, WI, USA). Rats were housed in a controlled temperature (20–22 °C) and photoperiod (14 h light : 10 h darkness). Purina rat chow (Ralston Purina, St Louis, MO, USA) and water were available *ad libitum*. Decidualization of the uterine endometrium was induced by scratching the antimesometrial surface of both uterine horns with a hooked needle on day 5 of pseudopregnancy under ether anesthesia. Rats were killed on days 6–14 of pseudopregnancy by an overdose of ether. The uterine horns were dissected out, freed from adjoining tissues and washed repeatedly in ice-cold PBS to remove excess blood. The antimesometrial and mesometrial decidua was dissected out as described previously (Gu & Gibori 1995).

Cell culture

Decidual cells (antimesometrial and mesometrial) were isolated and cultured as described earlier (Gu & Gibori 1995). The regulation of bFGF and VEGF mRNA was studied in an SV-40-transformed decidual cell line, termed GG-AD cells. The development and characterization of GG-AD cells has been described earlier (Srivastava *et al.* 1995). These cells were cultured at the permissive temperature (33 °C) in RPMI-1640 medium supplemented with 10% FBS, 2 × antibiotic–antimycotic solution, 1 mM sodium pyruvate, 1 × L-glutamine, 1 × non-essential amino acids and 0.5% D-glucose until they were 75% confluent. Cells were then transferred to non-permissive temperature (39 °C) for 3 days. All the experiments were conducted at 39 °C.

RNA extraction and reverse-transcription (RT)-PCR assay

Total RNA from dissected antimesometrial and mesometrial tissues were purified by homogenization in guanidinium thiocyanate and centrifugation through a cesium chloride cushion (Chirgwin *et al.* 1979), whereas total RNA from cultured GG-AD cells were isolated by a one-step guanidinium thiocyanate-phenol-chloroform extraction procedure (Chomczynski & Sacchi 1987).

For detecting bFGF mRNA, total RNA (1–3 µg) was reverse transcribed at 42 °C in the presence of random hexamer primers (100 pmol) and Moloney murine leukemia virus reverse transcriptase (Life Technologies, Gaithersburg, MD, USA) in a 20-µl reaction mixture. Oligonucleotide primers specific to rat bFGF (5'-GGC TTC TTC CTG GCG ATC CA-3' and 5' AGA AGG TTA CAG ACG ATT CTC G-3') were used for PCR amplification. A pair of oligonucleotide primers specific for a rat ribosomal protein L 19 (5'-CTG AAG GTC AAA GGG AAT GTG-3' and 5'-CTC TAG TAG TTC TGA GAC AGG-3') was also included in each reaction as an internal control. A mixture containing the oligonucleotide primers (500 ng each), [α^{32} P] dCTP (2 µCi; 3000 Ci/mmol), dNTP (2 mM), MgCl₂ (1.5 mM) and Taq DNA polymerase (2.5 U) was added to each reaction to a total volume of 90 µl. Amplification was carried out for 30 cycles using a 60 °C annealing temperature in a Perkin-Elmer/Cetus thermal cycler (Norwalk, CT, USA). The conditions were such that amplification of the product was in the exponential phase and the assay was linear with respect to the amount of input RNA. For detecting VEGF mRNA, total RNA (1–5 µg) was reverse transcribed at 42 °C using Moloney murine leukemia virus reverse transcriptase and 100 pmol random hexamers in 20 µl reaction mixture. Primer pair specific for the rat VEGF sequence (5'-CAC CGC CTT GGC TTC TCA CAT-3' and 5'-GGT CAC GTG GGT TCT CTC CTC GTC-3') was used for PCR amplification. A pair of L19 primers was also included in the reaction and the amplification was performed under conditions similar to those described for bFGF. Amplification was carried out for 30 cycles. The samples were separated on an 8% polyacrylamide non-denaturing gel. After autoradiography, the gel was dried and analyzed by a phosphorImager using Image Quant version software (Molecular Dynamics, Sunnyvale, CA, USA). The intensity of signals was normalized by using L19 as internal standard. The VEGF-specific primers used for PCR amplification gave three different PCR products (434, 564 and 631 bp), whereas L19 appeared at 194 bp. Two kinds of negative controls were prepared: 1) the reverse

transcription was performed with total RNA without reverse transcriptase to detect the possible contamination in RNA samples by genomic DNA; 2) total RNA was omitted in reverse transcription. These controls confirmed that no contamination occurred during the course of the RT-PCR procedure.

Immunocytochemistry

Isolated antimesometrial and mesometrial cells were cultured on 12-mm glass coverslips that were coated with FBS and then washed three times with ice-cold PBS and fixed with 3% paraformaldehyde in PBS for 1 h. Fixed cells were again washed three times in PBS, then treated for 15 min with 0.1 M glycine in PBS. The cells were permeabilized with 1% Triton X-100 in PBS for 4 min and washed three times with PBS. Non-specific antigenic sites were blocked with 1% BSA in PBS for 1 h at room temperature. Cells were then incubated with bFGF anti-rabbit IgG (Santacruz Biotechnology Inc., Santacruz, CA, USA) at 1 : 50 dilution and then washed with PBS. Cells were incubated with secondary antibody (anti-rabbit IgG-FITC) at 1 : 50 dilution from Santacruz Biotechnology Inc. for 1 h at room temperature and then washed to remove any non-specific bindings. Cells were mounted and immunostaining was visualized by fluorescence microscopy. For negative control, all the conditions remained the same, except that cells were not incubated with primary antibody.

RESULTS

We first examined the expression of VEGF mRNA by RT-PCR in the antimesometrial and mesometrial decidual tissues of rats from days 8 to 14 of pseudopregnancy, using VEGF-specific primers. Results revealed one major PCR product corresponding to 564 bp (Fig. 1B). In sharp contrast, bFGF mRNA levels were greater in the mesometrial decidua and its greatest expression was observed on day 11 of pseudopregnancy. bFGF mRNA declined thereafter, and totally disappeared from both antimesometrial and mesometrial decidua by day 15 of pseudopregnancy (Fig. 1A).

To elucidate whether decidualization of the endometrial stroma induces a rapid expression of bFGF and VEGF mRNA, total RNA was obtained from uteri of pseudopregnant rats in which decidualization was induced on day 5 in one uterine horn, the other uterine horn serving as control. As shown in Fig. 2A, bFGF mRNA was barely detectable in the non-decidualized uteri on day 7, but in the decidualized uteri, there was a rapid

FIGURE 1. Developmental expression of the bFGF and VEGF mRNA in rat decidua. Total RNA was isolated from both the antimesometrial (A) and the mesometrial (M) decidua of pseudopregnant rats. RNA was subjected to RT-PCR using (A) bFGF- and (B) VEGF-specific primers as described in Materials and Methods. Ribosomal protein L19 mRNA (L19) was used in each reaction as an internal control. Gels were exposed to X-ray film for 24 h.

induction of bFGF mRNA. Nevertheless, VEGF mRNA was detectable equally both in the non-decidualized and the decidualized uteri (Fig. 2B).

To determine the expression of bFGF protein in the two populations of decidual cells, antimesometrial and mesometrial decidual cells were separated by elutriation and subjected to immunocytochemistry using the specific antibody to bFGF. Although bFGF was localized both in antimesometrial and in mesometrial cells, the signal was more intense in the mesometrial cells (Fig. 3). This observation corroborates the findings concerning bFGF mRNA expression, which was also more abundant in mesometrial decidua.

We recently developed an SV-40-transformed temperature-sensitive rat decidual cell line (Srivastava *et al.* 1995). To determine that this cell line indeed expresses bFGF and VEGF mRNA, we cultured the cells until confluence at permissive temperature (33 °C) and total RNA was isolated. Meanwhile, another group of cells were cultured at 33 °C until 75% confluence and then at a non-

FIGURE 2. Expression of bFGF and VEGF mRNA in decidualized and non-decidualized uterus. Total RNA was isolated from both non-decidualized (–) and decidualized (+) uterine horns and subjected to RT-PCR using (A) bFGF-specific primers and (B) VEGF-specific primers. One transcript of size 354 bp was detected for bFGF mRNA, whereas two transcripts of sizes 434 and 564 bp were discernible for VEGF mRNA. The lower panel represents the quantification of the data.

FIGURE 3. Immunocytochemical localization of bFGF in the antimesometrial and mesometrial decidual cells. Antimesometrial and mesometrial decidual cells were separated from the day 9 pseudopregnant rat decidua by elutriation (Gu & Gibori 1995) and subjected to immunocytochemistry using bFGF-specific antibody as described in Materials and Methods. Note the high intensity of bFGF localization in the mesometrial cells compared with that in antimesometrial cells.

permissive temperature (39 °C). Total RNA was obtained from these cells after they were cultured at 39 °C for 1, 2, 3 and 4 days and subjected to RT-PCR using bFGF and VEGF-specific primers.

FIGURE 4. Expression of bFGF and VEGF mRNA in an SV-40-transformed decidual cell line at 33 °C (permissive) and 39 °C (non-permissive) temperatures. SV-40-transformed decidual cells were cultured at 33 °C (permissive) until 75% confluent and total RNA was isolated from these cells. RNA was subjected to RT-PCR using (A) bFGF and (B) VEGF-specific primer. A group of cells that were confluent at 33 °C were cultured at 39 °C (non-permissive) for 4 days. Total RNA was prepared from these cells and subjected to RT-PCR for detecting bFGF and VEGF mRNAs. L19 was used as an internal control. A single transcript of size 354 bp was discernible for bFGF mRNA, whereas three transcripts of sizes 631, 564 and 434 bp were detected for VEGF mRNA.

L19 was used as internal control. bFGF mRNA was detected in these cells cultured at both 33 °C and 39 °C, without any appreciable change (Fig. 4A). RT-PCR amplification of the total RNA obtained from the decidual cell line cultured at 33 °C and 39 °C for several days using VEGF-specific primers revealed three PCR products corresponding to 635, 564 and 434 bp respectively (Fig. 4B). The VEGF gene is alternatively spliced and gives rise to three transcripts encoding for protein of 188, 164 and 120 amino acids respectively that in turn form the active dimeric factors. The mRNAs for the VEGF₁₆₄ and VEGF₁₂₀ subunits are the dominant forms, whereas mRNA for the VEGF₁₈₈ is inactive (Cullinan-Bove & Koos 1993).

To determine the progesterone regulation of expression of bFGF and VEGF mRNA, we cultured the SV-40-transformed decidual cells at 33 °C until they were 75% confluent and then at 39 °C for 3 days. We have previously shown that these cells, when cultured at 39 °C, express progesterone receptor mRNA (Srivastava *et al.* 1995). Cells were then treated with progesterone (0, 1, 10, and 100 ng/ml) for 48 h. Total RNA prepared from these cells was subjected to RT-PCR to detect the mRNA expression of both bFGF and VEGF. However, progesterone at these doses was ineffective in altering the mRNA expression of either bFGF or VEGF (Fig. 5A and B, respectively).

To evaluate the regulation of bFGF and VEGF mRNA expression by PRL, we cultured the

FIGURE 5. Effect of different concentrations of progesterone (P₄) on the expression of bFGF and VEGF mRNA in SV-40-transformed decidual cells. Decidual cells were cultured at 33 °C for several days until they were 75% confluent and then at 39 °C for 72 h. Cells were treated with progesterone (0, 1, 10 and 100 ng/ml) for 48 h. Total RNA was isolated from these cells and subjected to RT-PCR using (A) bFGF- and (B) VEGF-specific primers (B). L19 was used as an internal control for both reactions. Lower panel represents the quantification of the data (*n*=3). Gels were exposed to films for 24 h.

decidual cell line at 33 °C until they were 75% confluent and then at 39 °C for 3 days. These cells were incubated with ovine PRL (0, 0.01, 0.1, 1 and 10 µg/ml) for 48 h. PRL treatment caused a rapid increase in the bFGF mRNA levels in the decidual cells (Fig. 6A); however, VEGF mRNA expression remained unchanged by PRL treatment (Fig. 6B).

DISCUSSION

The results of this investigation clearly demonstrate that the rat decidua expresses the genes for both bFGF and VEGF. Developmental studies revealed that VEGF mRNA remained expressed at similar levels throughout decidual development. In sharp contrast, bFGF mRNA expression was found to be

FIGURE 6. Effect of ovine prolactin (PRL) on the expression of bFGF and VEGF mRNA in SV-40-transformed rat decidual cells. SV-40-transformed decidual cells were cultured at 33 °C until they were 75% confluent and then at 39 °C for 72 h. Cells were treated with different concentrations of PRL (0, 0.01, 0.1, 1 and 10 µg/ml) for 48 h. Total RNA isolated from these cells was subjected to RT-PCR using (A) bFGF- and (B) VEGF-specific primers. L19 was used as an internal control. Right panel represents the quantification of the data ($n=3$). Gels were exposed to films for 24 h.

cell-specific and developmentally regulated. The mesometrial tissue where trophoblast invades and where extensive angiogenesis occurs (Christofferson & Nilsson 1988, Welsh & Enders 1991, Christofferson *et al.* 1994) showed a marked increase in bFGF mRNA expression between days 10 and 12, at a time when angiogenesis from pre-existing maternal venules and capillaries takes place in this tissue and when the newly formed vessels become continuous with the blood spaces within the trophoblast (Christofferson & Nilsson 1988, Welsh & Enders 1991, Christofferson *et al.* 1994). bFGF protein was also more abundantly expressed in mesometrial decidual cell as shown by immunocytochemistry. Because no change in VEGF mRNA could be detected in connection with angiogenesis in the decidua and because bFGF is released apparently only after cell damage, it is highly possible that mesometrial cells release bFGF after destruction of their cellular integrity during trophoblast invasion. The released bFGF may influence systemic blood vessels present in the decidua to proliferate into the cytotrophoblasts.

FGFs comprise a family of related proteins from at least nine distinct genes (Basillico & Moscatelli 1992, Miyamoto *et al.* 1993). Members of this growth factor family have been shown to have direct effects on endothelial cell growth, migration and protease synthesis. They are able to induce the formation of new blood vessels and, therefore, they have a direct role in angiogenesis (Gospodarowicz *et al.* 1987). bFGFs are not secretory proteins; they are, however, stored in the extracellular matrix and can be mobilized in a biologically active form by heparin or heparin sulfate (Vlodavsky *et al.* 1987, Folkman *et al.* 1988). The binding of FGF to heparin sulfate is a prerequisite for the binding of FGF to its high-affinity receptors on the cell surface (Rapraeger *et al.* 1991, Yayon *et al.* 1991). Cell-associated heparin sulfate proteoglycans appear to provide a sustained release reservoir that can mediate long-term responses to short exposures of bFGF (Moscatelli *et al.* 1991). bFGF is both chemotactic and mitogenic for endothelial cells (Friesel & Maciag 1995) and it induces a pathway leading to the phosphorylation and activation of mitogen-activated protein kinase (Johnson & Williams 1993). The expression of mRNA of this highly potent angiogenic factor in the decidua demonstrates that bFGF is instrumental in regulating the highly directional and stereospecific growth patterns of proliferating vessels during the decidual response. bFGF mRNA is expressed in decidua throughout the course of pseudopregnancy, and a striking feature of it is that it is expressed in abundance in the mesometrial decidua, where blood vessels gain access.

VEGF is a mitogen for endothelial cells *in vitro* and a potent angiogenic factor *in vivo* (Ferrara & Henzel 1989, Gospodarowicz *et al.* 1989, Ferrara *et al.* 1992). The presence of VEGF mRNA in the decidua suggests a role for it in angiogenesis. Such angiogenic activity of VEGF is probably due, not only to its mitogenic activity, but also to its ability to increase vascular permeability, which is believed to produce serum factors essential for new blood vessel growth (Connolly 1991). The mitogenic activity of VEGF is mediated by specific VEGF receptors, which are found on the cell surface of various endothelial cell types (Plouet & Moukadir 1990, Vaisman *et al.* 1990) and binding of VEGF to its cell surface receptor is dependent upon heparin-like molecules (Gitay-Goren *et al.* 1992). Recently, it has been shown that VEGF at approximately 2.2 nM concentration is capable of inducing the cells to invade the underlying matrix and form capillary-like tubules (Pepper *et al.* 1991), whereas bFGF is capable of inducing this response in concentrations about half those required with VEGF. Interestingly, these two growth factors have a potent

synergistic effect on the proliferation of endothelial cells and the induction of angiogenesis (Pepper *et al.* 1991, Goto *et al.* 1993). Therefore, it is plausible that this synergism between bFGF and VEGF might be playing an important part in the regulation of angiogenesis in the rat decidua.

In order to study the hormonal regulation of these growth factors in decidua, we used an SV-40-transformed decidual cell line (Srivastava *et al.* 1995). These cells express bFGF, VEGF and progesterone receptor mRNA and, as progesterone is essential for the process of decidualization, we studied the effect of progesterone *in vitro* on the mRNA expression of bFGF and VEGF, using this cell line. However, progesterone was not able to alter the mRNA expression of either bFGF or VEGF. Progesterone is essential for stromal cell proliferation (Galassi 1968, Sakamoto *et al.* 1983) and, when progesterone action is blocked using a progesterone receptor antagonist, RU 486, stromal cell bFGF expression is lost, these cells stop dividing, and implantation does not occur (Rider & Psychoyos 1994). Our failure to detect any alteration in the bFGF mRNA expression despite the fact that these cells do possess progesterone receptors is probably due to the fact that the cells are derived from highly differentiated decidual cells and not from the stromal cells.

Decidual tissue is the source of PRL-like hormones or decidual luteotropins and it also possesses the receptors for PRL (Jayatilak *et al.* 1985, Gu *et al.* 1996). Similarly, placental tissue also secretes proteins of the prolactin and growth factor family (Soares *et al.* 1991). In mouse placenta, two such proteins have been identified – proliferin and proliferin-related proteins – and a direct role for them in the process of angiogenesis has been implicated (Jackson *et al.* 1994). Interestingly, PRL administration up-regulated the expression of bFGF mRNA in the decidual cells, therefore it is quite probable that decidual PRL-like hormone secreted from the antimesometrial decidua has a role in regulating the process of angiogenesis in the decidua.

In conclusion, results of this investigation have revealed that, although VEGF mRNA is expressed almost equally in antimesometrial and mesometrial decidua, bFGF mRNA is highly expressed in the mesometrial decidua, where extensive angiogenesis occurs and where trophoblasts, by invading decidual cells, may promote the release of bFGF. It is also clear from this study that locally produced decidual PRL-like hormones might be playing an important part in regulating the process of angiogenesis, because of their capability to regulate the bFGF gene.

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REFERENCES

- Basillico C & Moscatelli D 1992 The FGF family of growth factors and oncogenes. *Advances in Cancer Research* **39** 115–165.
- Bell SC 1983 Decidualization: regional differentiation and associated function. *Oxford Review of Reproductive Biology* **5** 220–271.
- Chakraborty I, Das SK & Dey SK 1995 Differential expression of vascular endothelial growth factor and its receptor mRNAs in the mouse uterus around the time of implantation. *Journal of Endocrinology* **147** 339–352.
- Chirgwin JJ, Przbyla AE, MacDonalds RJ & Rutter WJ 1979 Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18** 5294–5299.
- Chomczynski P & Sacchi N 1987 Single-step method of RNA isolation by acid–guanidinium thiocyanate–phenol–chloroform extraction. *Analytical Biochemistry* **162** 156–159.
- Christofferson RH & Nilsson BO 1988 Morphology of the endometrial microvasculature during early placentation in the rat. *Cell and Tissue Research* **253** 209–220.
- Christofferson RH, Wassberg BE & Nilsson BO 1994 Angiogenesis in the rat uterus during pregnancy. In *Endocrinology of Embryo–Endometrium Interactions*, pp 77–93. Eds SR Glasser, J Mullohand & A Psychoyos. New York: Plenum Press.
- Connolly DT 1991 Vascular permeability factor: a unique regulator of blood vessel function. *Journal of Cell and Biochemistry* **47** 219–223.
- Cullinan-Bove K & Koos RD 1993 Vascular endothelial growth factor/vascular permeability factor expression in the rat uterus: rapid stimulation by estrogen correlates with estrogen-induced increase in uterine capillary permeability and growth. *Endocrinology* **133** 1829–1837.
- De Feo VJ 1967 Decidualization. In *Cellular Biology of the Uterus*, pp 191–290. Ed RM Wynn. New York: Appleton–Century–Crofts.
- Dennis PA, Saksela O, Harpel P & Rifkin DB 1989 α_2 -acroglobulin is a binding protein for basic fibroblast growth factor. *Journal of Biological Chemistry* **264** 7210–7216.
- Ferrara N & Henzel WJ 1989 Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochemistry and Biophysics Research Communication* **161** 851–858.
- Ferrara N, Houck K, Jakeman L & Leung DW 1992 Molecular and biological properties of the vascular endothelial growth factor family of proteins. *Endocrine Review* **13** 18–32.
- Folkman J & Klagsbrun M 1987 Angiogenic factors. *Science* **235** 442–447.
- Folkman J & Shing Y 1992 Angiogenesis. *Journal of Biological Chemistry* **267** 10931–10934.
- Folkman J, Klagsbrun M, Sasse J, Wadzinski M, Ingbar D & Vlodavsky I 1988 A heparin binding angiogenic protein basic fibroblast growth factor is stored within basement membrane. *American Journal of Pathology* **130** 393–400.

- Friesel RE & Maciag T 1995 Molecular mechanisms of angiogenesis: fibroblast growth factor signal transduction. *FASEB Journal* **9** 919–925.
- Galassi L 1968 Autoradiographic study of the decidual cell-reaction in the rat. *Developmental Biology* **17** 75–84.
- Gitay-Goren H, Soker S, Vlodavsky I & Neufeld G 1992 The binding of vascular endothelial growth factors to its receptors is dependent on cell surface-associated heparin-like molecules. *Journal of Biological Chemistry* **267** 6093–6098.
- Gospodarowicz D, Ferrara N, Schweigener L & Neufeld G 1987 Structural characterization and biological function of fibroblast growth factors. *Endocrine Review* **8** 95–114.
- Gospodarowicz D, Abraham JA & Schilling J 1989 Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculo-stellate cells. *Proceedings of the National Academy of Sciences of the USA* **86** 7311–7315.
- Goto F, Goto K, Weindel K & Folkman J 1993 Synergistic effects of vascular endothelial growth factor and basic fibroblast growth factor on the proliferation and cord formation of bovine capillary endothelial cells within collagen gels. *Laboratory Investigation* **69** 508–517.
- Gu Y & Gibori G 1995 Isolation, culture and characterization of the two cell subpopulations forming the rat decidua: differential gene expression for activin, follistatin and decidual prolactin-related protein. *Endocrinology* **136** 2451–2458.
- Gu Y, Jayatilak PG, Parmer TG, Gaudlie J, Fey GH & Gibori G 1992 α_2 -Macroglobulin expression in the mesometrial decidua and its regulation by decidual luteotropin and prolactin. *Endocrinology* **131** 1321–1328.
- Gu Y, Soares MJ, Srivastava RK & Gibori G 1994 Expression of decidual prolactin-related protein in the rat decidua. *Endocrinology* **135** 1422–1427.
- Gu Y, Srivastava RK, Ou JS, Krett NL, Mayo KE & Gibori G 1995 Cell-specific expression of activin and its two binding proteins in the rat decidua: role of α_2 macroglobulin and follistatin. *Endocrinology* **136** 3815–3822.
- Gu Y, Srivastava RK, Clarke DL, Linzer DIH & Gibori G 1996 The decidual prolactin receptor and its regulation by decidua-derived factors. *Endocrinology* **137** 4878–4885.
- Jackson D, Volpert OV, Bouck N & Linzer DIH 1994 Stimulation and inhibition of angiogenesis by placental proliferin and proliferin-related protein. *Science* **266** 1581–1584.
- Jayatilak PG, Glasser LA, Basuray R, Kelly P & Gibori G 1985 Identification and partial characterization of a prolactin-like hormone produced by rat decidual cells. *Proceedings of the National Academy of Sciences of the USA* **82** 217–221.
- Johnson DE & Williams LT 1993 Structural and functional diversity in the FGF receptor multigene family. *Advances in Cancer Research* **60** 1–41.
- Koos RD & Olson CE 1989 Expression of basic fibroblast growth factor in the rat ovary: detection of mRNA using reverse transcription-polymerase chain reaction. *Molecular Endocrinology* **3** 2041–2048.
- Miyamoto M, Naruo K-I, Seko C, Matsumoto S, Kondo T & Kurokawa T 1993 Molecular cloning of a novel cytokine cDNA encoding the ninth member of the fibroblast growth factor family which has a unique secretory property. *Molecular Cell Biology* **13** 4251–4259.
- Montesano R, Vassalli JD, Baird A, Guillemin R & Orci L 1986 Basic fibroblast growth factor induces angiogenesis *in vitro*. *Proceedings of the National Academy of Sciences of the USA* **83** 7297–7301.
- Moscattelli D, Flaumenhaft R & Saksela O 1991 Interaction of basic fibroblast growth factor with extracellular matrix and receptors. *Annals of the New York Academy of Sciences* **638** 177–181.
- O'Shea JD, Kleinfeld RG & Morrow HA 1983 Ultrastructure of decidualization in the pseudopregnant rat. *American Journal of Anatomy* **166** 271–298.
- Pepper MS, Ferrara N, Orci L & Montesano R 1991 Vascular endothelial growth factor (VEGF) induces plasminogen activators and plasminogen activator inhibitor-1 in microvascular endothelial cells. *Biochemistry and Biophysics Research Communication* **181** 902–906.
- Plouet J & Moukadir H 1990 Characterization of the receptor to vasculotropin on bovine adrenal cortex-derived capillary endothelial cells. *Journal of Biological Chemistry* **265** 22071–22074.
- Rapraeger AC, Krufka A & Olwin BB 1991 Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. *Science* **252** 1705–1708.
- Rider V & Psychoyos A 1994 Inhibition of progesterone receptor function results in loss of basic fibroblast growth factor expression and stromal cell proliferation during uterine remodeling in the pregnant rat. *Journal of Endocrinology* **140** 239–249.
- Sakamoto S, Abe A, Kudo H, Yamada N, Seki K & Okamoto R 1983 Effects of estrogen and progesterone on thymidine kinase activity in the immature rat uterus. *American Journal of Obstetrics and Gynecology* **145** 711–715.
- Shweiki D, Itin A, Neufeld G, Gitay-Goren H & Keshet E 1993 Patterns of expression of vascular endothelial growth factor (VEGF) and VEGF receptors in mice suggest a role in hormonally regulated angiogenesis. *Journal of Clinical Investigation* **91** 2235–2243.
- Soares MJ, Faria TN, Roby KF & Deb S 1991 Pregnancy and the prolactin family of hormones: coordination of anterior pituitary, uterine, and placental expression. *Endocrine Review* **12** 402–423.
- Soker S, Svahn CM & Neufeld G 1993 Vascular endothelial growth factor is inactivated by binding to α_2 -macroglobulin and the binding is inhibited by heparin. *Journal of Biological Chemistry* **268** 7685–7691.
- Srivastava RK, Gu Y, Zilberstein M, Ou JS, Mayo KE, Chou JY & Gibori G 1995 Development and characterization of a simian virus-40 transformed, temperature sensitive rat antimesometrial decidual cell-line. *Endocrinology* **136** 1913–1919.
- Thomas KA 1987 Fibroblast growth factors. *FASEB Journal* **1** 434–440.
- Vaisman N, Gospodarowicz D & Neufeld G 1990 Characterization of the receptors for vascular endothelial growth factors. *Journal of Biological Chemistry* **265** 19461–19466.
- Vlodavsky I, Folkman J, Sullivan R, Fridman R, Ishai-Michaeli R, Sasse J & Klagsbrun M 1987 Endothelial cell-derived basic fibroblast growth factor: synthesis and deposition into subendothelial extracellular matrix. *Proceedings of the National Academy of Sciences of the USA* **84** 2292–2296.
- Welsh AO & Enders AC 1991 Chorioallantoic placenta formation in the rat: II. Angiogenesis and maternal blood circulation in the mesometrial region of the implantation chamber prior to placenta formation. *American Journal of Anatomy* **192** 347–365.
- Yayon A, Klagsbrun M, Esko JD, Ledrer P & Ornitz DM 1991 Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell* **64** 841–848.

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