American Journal of Pathology, Vol. 146, No. 1, January 1995

Expression of Vascular Permeability Factor/ Vascular Endothelial Growth Factor by Human Granulosa and Theca Lutein Cells

Role in Corpus Luteum Development

Brinda R. Kamat, Lawrence F. Brown, Eleanor J. Manseau, Donald R. Senger, and Harold F. Dvorak

From the Departments of Pathology, Mt. Auburn and Beth Israel Hospitals and Harvard Medical School, Boston, Massachusetts

Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) is a cytokine that is overexpressed in many tumors, in healing wounds, and in rheumatoid arthritis. VPF/VEGF is thought to induce angiogenesis and accompanying connective tissue stroma in two ways: 1), by increasing microvascular permeability, thereby modifying the extracellular matrix and 2), as an endothelial cell mitogen. VPF/VEGF has been reported in animal corpora lutea and we investigated the possibility that it might be present in human ovaries and have a role in corpus luteum formation. We here report that VPF/VEGF mRNA and protein are expressed by human ovarian granulosa and theca cells late in follicle development and, subsequent to ovulation, by granulosa and theca lutein cells. Therefore, VPF/VEGF is ideally positioned to provoke the increased permeability of thecal blood vessels that occurs shortly before ovulation. VPF/VEGF likely also contributes to the angiogenesis and connective tissue stroma generation that accompany corpus luteum/corpus albicans formation. Finally, VPF/VEGF was overexpressed in the hyperthecotic ovarian stroma of Stein-Leventhal syndrome in which it may also have a pathophysiological role. (Am J Pathol 1995, 146:157–165)

Vascular permeability factor (VPF), also known as vascular endothelial growth factor (VEGF), is a dimeric glycoprotein of M_r 34 to 42 kd that possesses potent vascular permeability-enhancing activity and that also serves as a selective mitogen for cultured endothelial cells.[1–9] Synthesis and secretion of VPF/VEGF have been demonstrated in many tumors and tumor cell lines of both rodent and human origin.[3,10–16] In tumors VPF/VEGF is thought to induce stroma both as an endothelial cell mitogen and by provoking vascular hyperpermeability to plasma proteins. As a consequence of increased microvascular permeability, plasma fibrinogen extravasates and clots to form an extravascular provisional matrix that favors and supports the ingrowth of new blood vessels and fibroblasts, which, in turn, organize the avascular provisional fibrin matrix into mature, vascularized connective tissue stroma.[17–21] The VPF/VEGF gene is also expressed at low levels by many normal adult tissues and at higher levels in kidney, heart, lung and adrenal glands and also by activated macrophages.[22–24] In addition, VPF/VEGF is widely expressed in the developing embryo[25] and has been implicated in the pathogenesis of certain nonneoplastic processes (eg, wound healing[26] and rheumatoid arthritis[27,28]) that, like growing tumors, are characterized by angiogenesis and new stroma formation.

Recent studies from two different laboratories have demonstrated that VPF/VEGF is expressed in the corpus luteum (CL) of rats and primates[29,30] and in cultures of human granulosa cells.[31] These findings suggested that VPF/VEGF might also have a role in the pathogenesis of CL generation and its subsequent regression, in the absence of pregnancy, to a corpus albicans. CL/corpus albicans generation share a

Supported by USPHS NIH Grants CA-50453 (H.F.D.) and CA-43967 (D.R.S.) from the National Cancer Institute, by the Beth Israel Hospital Pathology Foundation, and under terms of a contract from the National Foundation for Cancer Research.

Accepted for publication August 31, 1994.

Address reprint requests to Dr. Brinda R. Kamat, Department of Pathology, 330 Mt. Auburn Street, Cambridge, MA 02238.

Table 1. *Summary of Patients Studied with Clinical Indications for Surgery and Routine Pathological Findings*

Case	Age	Clinical diagnosis, surgical indications	Pathology findings, ovary
1	29	Ectopic pregnancy with ovarian cyst	CL of pregnancy
2	42	Leiomyomata	Organizing CL
3	33	Ovarian cyst	CL of pregnancy, corpus albicans
4	30	Wedge resection, cyst	CL, developing follicle
5	42	Hysterectomy for leiomyomata	CL
6	42	Adenomyosis	Corpus albicans, resting follicles
7	38	Leiomyomata, adenomyosis	Organizing CL, corpus albicans
8	30	Ovarian cyst	CL of pregnancy
9	42	Adenomyosis	CL
10	36	Ovarian cyst	CL of pregnancy
11	23	Ovarian biopsy, Stein-Leventhal syndrome	Cystic follicle
12	39	Ovarian cyst, pelvic adhesions	Organizing CL
13	48	Leiomyomata	CL
14	21	Ovarian cyst, Stein-Leventhal syndrome	Cystic follicle
15	20	Ovarian biopsy, Stein-Leventhal syndrome	Cystic follicle

number of features with tumors, healing wounds, and rheumatoid arthritis, including leaky blood vessels, plasma protein extravasation, fibrin deposition, angiogenesis, and deposition of vascularized connective tissue. The goal of the present investigation, therefore, was to determine whether VPF/VEGF was also expressed in the normally cycling human ovary and to relate any such expression to the histogenesis of CL/corpus albicans formation. We also investigated VPF/VEGF expression in three patients with polycystic ovary/Stein-Leventhal syndrome.[32]

Materials and Methods

Ovarian tissue was obtained from 15 patients, ages 18 to 44, to include examples of the various stages of ovarian cycling (Table 1). Immunohistochemistry (IH) was performed on routinely formalin-fixed and paraffin-embedded tissue with an avidin-biotin peroxidase conjugate technique as previously described[14] except that sections were subjected to wet heat autoclaving for optimal antigen retrieval (121 C for 15 minutes).[33] The primary antibody used to identify VPF/VEGF in tissues was an affinity-purified rabbit antibody raised against a peptide corresponding to the N-terminal 26 amino acids of human VPF/VEGF.[10,11,14,34] This anti-peptide antibody specifically binds VPF/VEGF in enzyme-linked immunosorbent assays and on immunoblots, blocks both VPF and VEGF activities, and, when linked to agarose, selectively binds VPF activity from solution[34]; because of its specificity and the low background associated with its use, we have found it to be the antibody of choice for demonstrating VPF/VEGF by IH.[10,11,14]

Normal rabbit IgG diluted to an equivalent protein concentration served as a control in place of the primary antibody. Other sections were similarly stained (but without wet autoclaving) with commercial antibodies to clotting factor VIII antigen (von Willebrand factor) to identify blood vessels and with a monoclonal anti-peptide antibody (the kind gift of Dr. Gary Matsueda) that recognizes fibrin but not fibrinogen.[35]

For *in situ* hybridization, freshly resected ovarian tissue was fixed for 2 hours in 4% paraformaldehyde at 4 C, cryopreserved by transfer to 30% sucrose in phosphate-buffered saline, pH 7.4, at 4 C overnight, and frozen on dry ice in OCT compound (Miles Inc., Elkhart, IN).[10,11] Frozen sections were attached to sialylated glass slides and hybridized with a 204-bp antisense riboprobe specific for VPF/VEGF as previously described.[22,26] This probe has been designed to recognize all four of the alternatively spliced forms

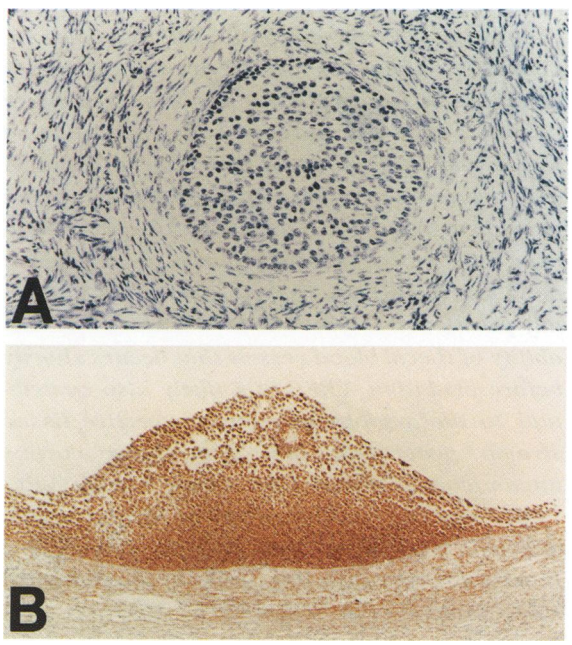


Figure 1. *IH staining for VPF/VEGF in a primary ovarian follicle* (**A**) *and in a maturing secondary follicle* (**B**). *Cells of the resting follicle* (**A**) *did not stain whereas granulosa cells, and to a lesser extent theca cells, exhibited intense cytoplasmic staining with antibodies to VPF/VEGF shortly before ovulation* (**B**). *Paraffin sections, counterstained with hematoxylin. Magnification,* ×155 (**A**) *and* ×57 (**B**).

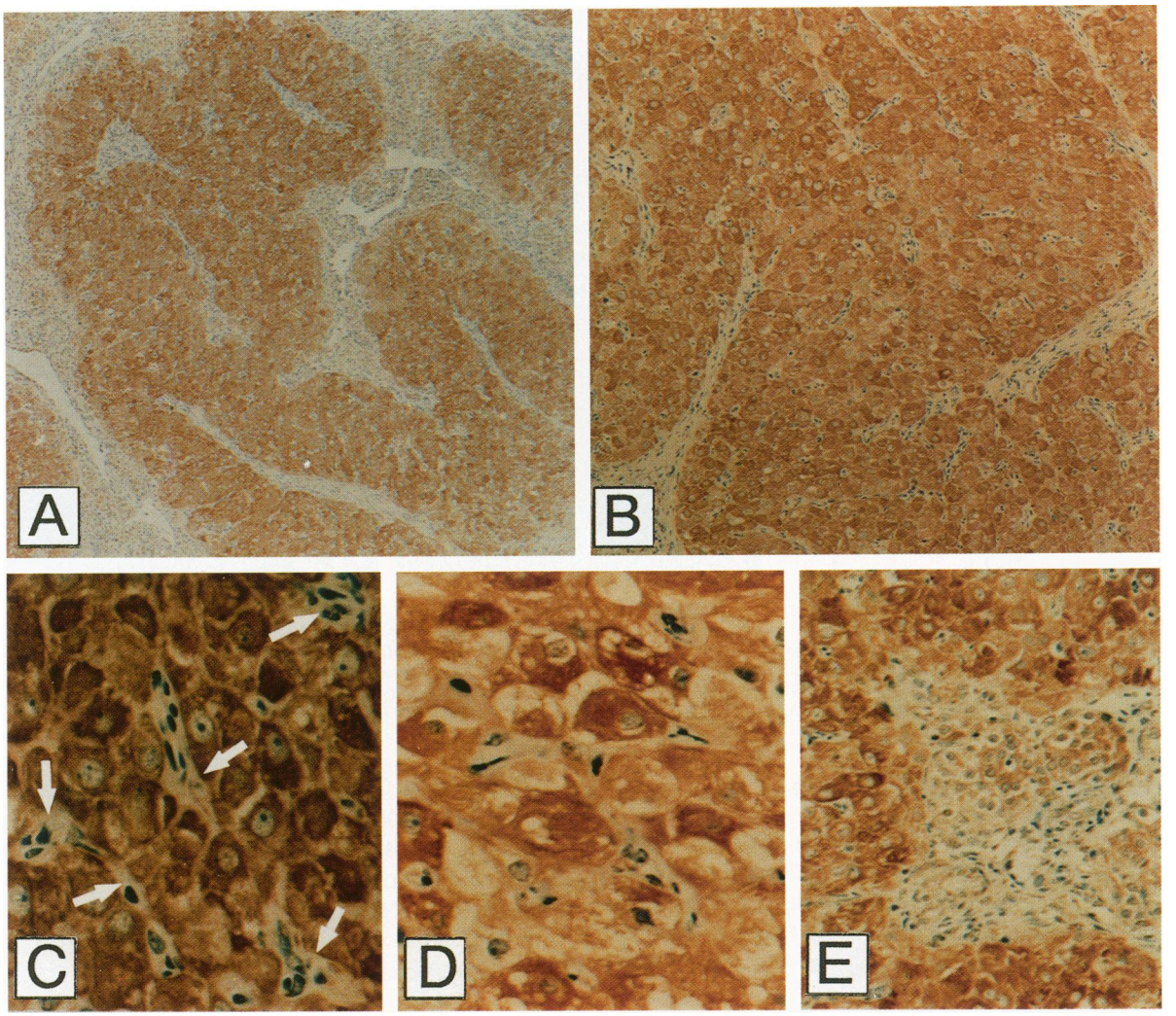


Figure 2. **A–E**. *IH staining for VPF/VEGF in CLs from four different patients with histologically normal ovaries. In early CLs* (**A–C**), *nearly all granulosa lutein cells stained strongly; however, with further CL maturation* (**D**), *granulosa lutein cell staining became more variable. Microvessels supplying the CL did not stain for VPF/VEGF* (*white arrows;* **C**). **E**: *Nests of theca lutein cells contained within bands of fibrous connective tissue stroma also stained for VPF/VEGF but less intensely than surrounding granulosa lutein cells. Paraffin sections, counterstained with hematoxylin. Magnification,* ×*51* (**A**), ×*81* (**B**), ×*410* (**C**), ×*365* (**D**), *and* ×*165* (**E**).

of VPF/VEGF mRNA.[36,37] It was transcribed to a specific activity of approximately 10^8 cpm/μg with [^{35}S]-UTP, purified on polyacrylamide gels, and used without reduction in length, all as described previously.[24] Probes of the same length, but in sense orientation, served as controls.

Results

Expression of VPF/VEGF was studied in the cycling ovaries of 12 patients who underwent surgery for the clinical indications listed in Table 1. In addition, we studied ovaries from 3 patients with polycystic ovaries/Stein-Leventhal syndrome (patients 11, 14, and 15; Table 1).

VPF/VEGF Expression in the Developing Ovarian Follicle

In primary follicles, the ovum is enveloped by layers of granulosa cells, theca cells, and fibrous ovarian stroma.[38] None of these cells stained for VPF/VEGF by IH in any of our patients with histologically normal ovaries (Figure 1A). As Graafian follicles formed and accumulated fluid, their granulosa cells began to stain, at first faintly and then more intensely, for VPF/VEGF (Figure 1B). Surrounding theca cells also became weakly positive (Figure 1B).

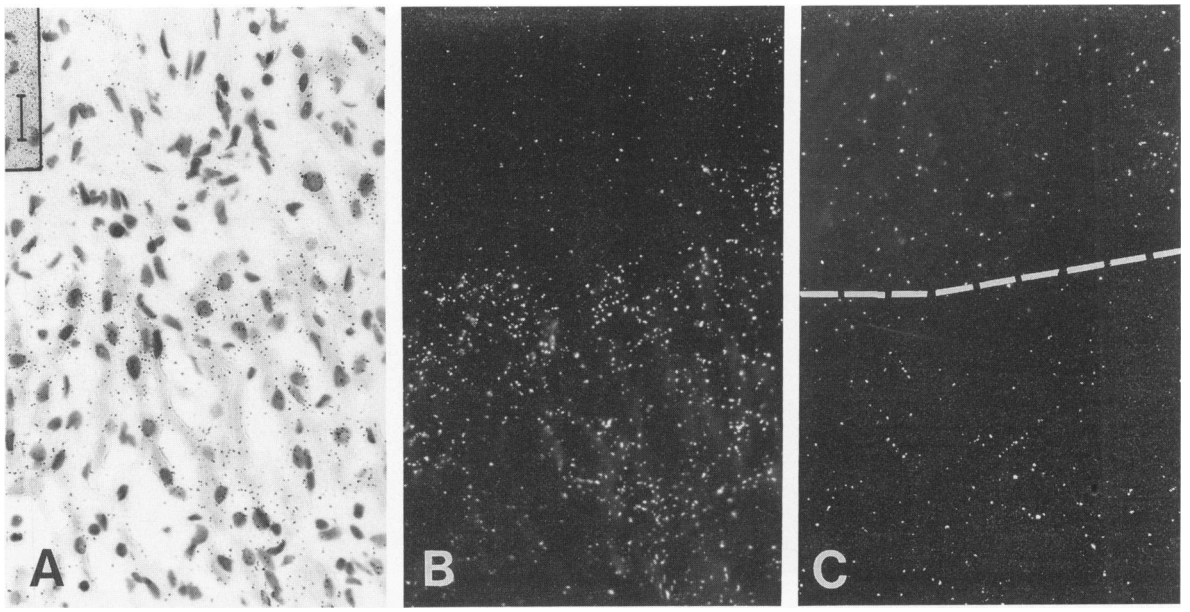


Figure 3. *In situ hybridization of a developing CL.* **A:** *Bright field, H&E-stained section hybridized with antisense riboprobe to VPF/VEGF, illustrating a CL (below) and ovarian stroma (above). Autoradiographic grains appear as faintly visible black dots and are better visualized in* **B**, *the same field as in* **A** *photographed with dark field illumination. Granulosa lutein cells (below) are heavily labeled whereas surrounding stroma (above) is negative.* **C:** *A section of CL similar to that in* **B** *but hybridized with VPF/VEGF control sense probe. Few grains are present and they do not conform to any recognizable anatomic pattern. The interface between CL (below) and ovarian stroma (above) is indicated with a broken white line. Paraffin sections, counterstained with hematoxylin. Magnification of* **A–C** *is indicated by a bar in* **A** *that equals 30 μ.*

VPF/VEGF Expression in the CL

After ovulation, follicular granulosa cells differentiate into granulosa lutein cells.[38] In early CLs, granulosa lutein cells exhibited strong cytoplasmic staining for VPF/VEGF protein (Figure 2 A–C). However, with further CL maturation, lutein cell staining for VPF/VEGF became more variable (Figure 2D). This variable staining pattern exhibited by individual, adjacent granulosa lutein cells was also observed in a mature CL of pregnancy (not shown).

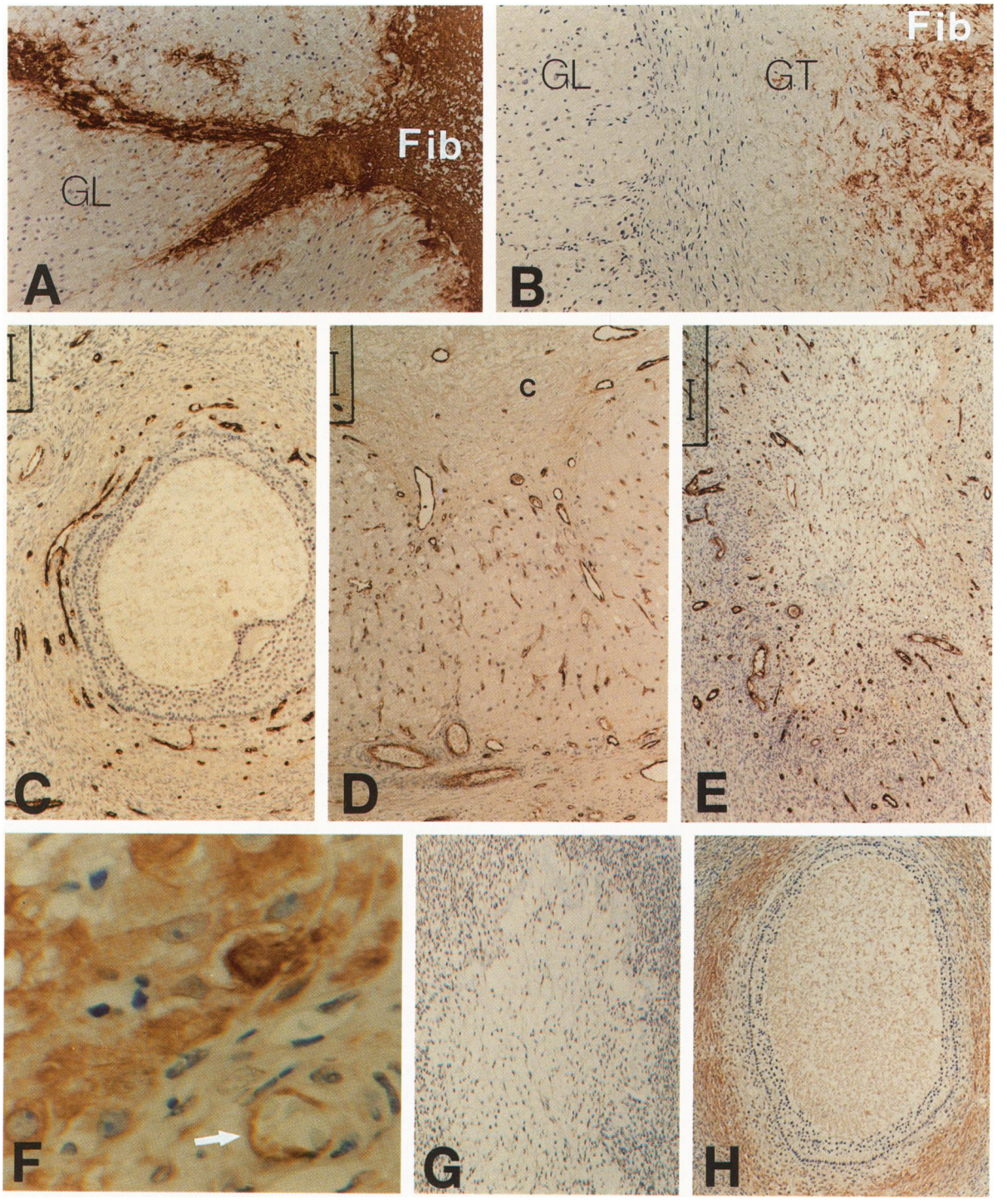
Follicular theca interna cells also become luteinized in developing corpora lutea[38] and these theca lutein cells were found to exhibit cytoplasmic staining for VPF/VEGF (Figure 2E); however, theca lutein cell staining was less intense than that exhibited by most granulosa lutein cells.

Ovaries with CLs were available from three patients in a form suitable for performing *in situ* hybridization (patients 11, 14, and 15; Table 1). In all three, lutein cells hybridized strongly with an antisense probe to VPF/VEGF mRNA (Figure 3A, B); in contrast, no significant hybridization was detected with the corresponding sense probe (Figure 3C).

Fibrin Deposition, Angiogenesis, and Connective Tissue Stroma Generation, Integral Components of CL and Corpus Albicans Development

The developing follicle is characterized by a substantial increase in the number and permeability of thecal blood vessels (Figure 4C).[39–42] At ovulation, variable

Figure 4. **A** *and* **B:** *IH staining of an early (***A***) and an organizing (***B***) CL for fibrin. Prominent fibrin deposits (Fib) accumulated in the follicular cavity after ovulation and extended spoke-like projections peripherally that separated masses of differentiating granulosa lutein (GL) cells. Fibrin persisted until it was organized (replaced by granulation tissue: GT), a process that began at the periphery (left side of* **A** *and* **B**) *and proceeded centrally (right side of* **A** *and* **B**). **C–E:** *IH staining of a developing Graafian follicle (***C***), a CL (***D***), and a corpus albicans (***E***), with antibodies to factor VIII antigen (von Willebrand factor) to demonstrate blood vessels. In the developing follicle (***C***), blood vessels supply the theca and ovarian stroma but do not enter the follicle. With formation of a CL (***D***) new blood vessels have extensively infiltrated the granulosa lutein layer (midportion of micrograph) and a few vessels have penetrated into the fibrin-filled central cavity (c). Later, as a CL regresses into a corpus albicans (***E***), lutein cells and central fibrin have disappeared and central granulation tissue is largely replaced by relatively avascular fibrous connective tissue.* **F–H:** *IH staining for VPF/VEGF. F. Maturing CL with VPF/VEGF-positive granulosa lutein cells (upper left) and a band of fibrous connective tissue (lower right). The connective tissue band is of the type separating nests of granulosa lutein cells (Figure 2A, B); it includes a microvessel (white arrow) whose endothelial cells stain strongly with antibodies to VPF/VEGF. The VPF/VEGF staining of such vessels stands in contrast to the negative VPF/VEGF regularly observed in capillaries that infiltrate granulosa lutein cells (compare with Figure 2C).* **G:** *Corpus albicans is negative for VPF/VEGF staining.* **H:** *Cystic follicle from a case of polycystic ovary/Stein-Leventhal syndrome, illustrating strong VPF/VEGF staining of hyperthecotic stromal cells. All paraffin sections, counterstained with hematoxylin. Magnification, ×85 (***A***), ×90 (***B***), ×84 (***C***), ×61 (***D***), ×57 (***E***), ×690 (***F***), ×70 (***G***), and ×55 (***H***). Bars in* **C**, **D** *and* **E** *represent lengths of 83, 106, and 103 μ, respectively.*


GL
Fib
A
GL
GT
Fib
B
C
C
D
E
F
G
H

amounts of blood and plasma extravasate into the follicular cavity where they form a fibrin clot that extends spoke-like projections peripherally among groups of granulosa cells (Figure 4A).[38] Subsequently, as granulosa and theca cells become luteinized, an extensive network of new blood vessels grew inward from pre-existing thecal vessels, infiltrating both the lutein cells and the fibrin clot (Figure 4C, D). The vessels infiltrating the granulosa and theca lutein cells were almost all simple capillaries with little supporting connective tissue stroma; they were uniformly VPF/VEGF-negative (Figures 2C, D and 4D).

Coincident with these events, new blood vessels also infiltrated the fibrin deposits that filled the follicular cavity. Over time, these new vessels and fibroblasts organized the fibrin deposits into radiating bands of vascular connective tissue (Figures 2A, B and 4B). Organization proceeded from outside in such that more peripherally situated fibrin deposits were replaced first and more central deposits later (Figure 4B). In contrast to the capillaries that interdigitated among granulosa lutein cells, many of the blood vessels that invaded the fibrin matrix and that persisted after fibrin was organized into more mature connective tissue had the appearance of venules, which were often lined by endothelial cells that were VPF/VEGF-positive (Figure 4F).

When pregnancy does not ensue, the CL becomes a CL of menstruation and regresses into a corpus albicans.[38] In the course of this regression, luteinized cells gradually disappeared and eventually the entire structure, including the fibrin-rich central zone, became organized first into vascularized connective tissue and later into a relatively avascular, hyalinized scar, the corpus albicans (Figure 4D, E, G). At late stages, lutein cells could no longer be identified and neither VPF/VEGF nor fibrin staining persisted (Figure 4G).

Polycystic Ovaries/Stein Leventhal-Syndrome

Ovaries from patients with polycystic ovary/Stein-Leventhal syndrome exhibited multiple cystic follicles surrounded by luteinized theca and hyperplastic stromal cells.[32] In contrast to normal ovaries, those from the three cases of polycystic ovary/Stein-Leventhal syndrome we studied exhibited hyperthecotic stromal cells and variable numbers of follicular granulosa cells (not illustrated) that stained strongly for VPF/VEGF (Figure 4H).

Discussion

VPF/VEGF is overexpressed in a number of processes (tumors, wound healing, and rheumatoid arthritis) that are characterized by a common set of pathophysiological features that include the following: hyperpermeable blood vessels with extravasation of plasma proteins, clotting of extravasated plasma fibrinogen to fibrin, replacement (organization) of fibrin by well vascularized connective tissue stroma (granulation tissue), and remodeling of granulation tissue into hyalinized and relatively avascular connective tissue (referred to as desmoplasia, scar, and corpus albicans in tumors, healed wounds, and cycling ovaries, respectively). In all of these examples, fibrin provides a provisional stromal matrix that induces and supports the ingrowth of new blood vessels as well as the fibroblasts responsible for synthesizing and secreting the structural proteins and proteoglycans that typically comprise mature connective tissue. VPF/VEGF is thought to have an important role in initiating these events, serving as a selective endothelial cell mitogen and rendering blood vessels hyperpermeable to plasma proteins, thereby provoking fibrinogen extravasation and consequent deposition of a fibrin provisional matrix. The correlative data presented here suggest that VPF/VEGF may exert similar functions in the normal cycling human ovary with regard to follicle maturation and CL/corpus albicans generation. VPF/VEGF may also play a role in ovarian pathology as is suggested by the aberrant pattern of VPF/VEGF expression found in polycystic ovary/Stein-Leventhal syndrome.

Developing ovarian follicles accumulate proteinaceous fluid (liquor folliculi), at least a portion of which is derived from plasma. Moreover, fluid accumulation accelerates after the preovulatory gonadotropin surge at a time when capillaries of the thecal layer of the follicle become hyperpermeable in several species.[40,41,43–45] It is just at this time, shortly before ovulation, that both granulosa and theca cells of the human ovary stain positively for VPF/VEGF (Figure 1B). Thus, it is reasonable to postulate that local secretion of VPF/VEGF, likely under hormonal regulation, renders thecal microvessels hyperpermeable, leading to increased extravasation of plasma and accumulation of antral fluid in maturing Graafian follicles.

After ovulation, fibrin clot filled the follicular cavity and radiated peripherally among islands of developing granulosa lutein cells (Figure 4A). Such fibrin deposits have long been recognized and have generally been attributed to the tearing of small blood vessels at the time of follicular rupture. In support of this view,

red blood cells are often a prominent component of the fibrin clot that is deposited in the follicular cavity. However, VPF/VEGF may also have a role in the process of fibrin accumulation by rendering thecal blood vessels hyperpermeable to plasma fibrinogen. In favor of this hypothesis, human granulosa lutein cells, like those of rat and monkey,[29,30] have now been shown to express VPF/VEGF, both by antibody staining and by *in situ* hybridization (Figures 2–4). Therefore, as in tumors, healing wounds, and rheumatoid arthritis, VPF/VEGF synthesis and secretion correlate with fibrin deposition, angiogenesis, and new stroma formation.[17–19,26–28] In addition, although permeability measurements have not to our knowledge been performed on human ovaries, careful studies in other species (ewe and rat) indicate that microvessels associated with the developing CL, in contrast to those of ovarian stroma, are unusually permeable to plasma and plasma proteins[40]; in fact, the vessels that supply the CL are reported to be among the leakiest in the body.[43,44] The basis for the hyperpermeability of CL blood vessels has not been established, but our results, which correlate VPF/VEGF synthesis temporally and spatially with extravascular fibrin deposition, suggest that VPF/VEGF is a likely mediator.

Additional evidence supporting this thesis comes from the finding that many of the microvessels present in the bands of connective tissue that replaced fibrin provisional stroma (Figure 2A, B) stained strongly for VPF/VEGF protein (Figure 4F). This finding provides direct evidence for an interaction between VPF/VEGF and local blood vessel endothelium. Moreover, a similar pattern of VPF/VEGF staining has been observed in blood vessels supplying tumors[10–12,14,15] and joints affected with active rheumatoid arthritis[27,28]; VPF/VEGF is overexpressed in tumors and rheumatoid arthritis and in both is thought to have an important role in generating new blood vessels and stroma. Not explained by our data is the absence of VPF/VEGF staining of the capillary-like vessels most intimately associated with granulosa lutein cells (Figure 2C). Studies are in progress to determine whether these IH findings are related to the presence or absence of VPF/VEGF receptors on these vessels.[46,47]

The fibrin deposited in the residual follicular cavity after ovulation likely contributes to the induction of angiogenesis and stroma characteristic of CL/corpus albicans formation. Deposits of fibrin planted in the subcutaneous space of guinea pigs, whether free or included in plexiglass chambers, stimulate the ingrowth of new blood vessels and fibroblasts,[18,21,48] and such deposits are likely to exert a similar effect in tumors, wound healing, and rheumatoid arthritis.[1,26,27] In all of these circumstances, new blood vessels and fibroblasts grow into a fibrin provisional stroma, replacing it over time with granulation tissue and later with dense fibrous connective tissue. A very similar sequence of events occurs as an ovarian follicle matures, ruptures, and evolves into a CL and subsequently, in the absence of pregnancy, into a corpus albicans.

The signals governing the cyclic expression of VPF/VEGF synthesis in the developing CL have not yet been elucidated. Hypoxia stimulates VPF/VEGF synthesis in tumors and in some cultured cells, including ovarian granulosa cells.[10–12,15,16,31,49] However, other factors (eg, cytokines and hormones) may also have a role; thus, platelet-derived growth factor, phorbol esters, and transforming growth factor-α have been found to induce VPF/VEGF expression in cultured cells of different types.[50,51] It is of more than passing interest that, simultaneous with VPF/VEGF expression in the CL of pregnancy, the uterus also undergoes striking changes that include both vascular hyperpermeability and angiogenesis.[44,52] Also, a protein with significant homology to VPF/VEGF has been isolated from the placenta.[53] Therefore, members of the VPF/VEGF family of cytokines may exert heretofore unanticipated effects on many aspects of pregnancy.

Finally, it is of interest that VPF/VEGF, a protein mediator, is expressed by granulosa and theca lutein cells best known for their capacity to synthesize and secrete steroid hormones. However, this finding is not without precedent in that normal adrenocortical cells also express abundant VPF/VEGF mRNA.[22]

References

1. Dvorak HF, Nagy JA, Berse B, Brown LF, Yeo K-T, Yeo T-K, Dvorak AM, Van De Water L, Sioussat TM, Senger DR: Vascular permeability factor, fibrin, and the pathogenesis of tumor stroma formation. Ann NY Acad Sci 1992, 667:101–111
2. Senger D, Van De Water L, Brown L, Nagy J, Yeo K-T, Yeo T-K, Berse B, Jackman R, Dvorak A, Dvorak H: Vascular permeability factor (VPF, VEGF) in tumor biology. Cancer Metastasis Rev 1993, 12:303–324
3. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF: Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 1983, 219:983–985
4. Senger DR, Connolly DT, Van De Water L, Feder J, Dvorak HF: Purification and NH_2-terminal amino acid sequence of guinea pig tumor-secreted vascular permeability factor. Cancer Res 1990, 50:1774–1778
5. Connolly DT, Olander JV, Heuvelman D, Nelson R, Monsell R, Siegel N, Haymore BL, Leimgruber R, Feder J: Human vascular permeability factor: isolation

from U937 cells. J Biol Chem 1989, 264:20017–20024
6. Ferrara N, Henzel WJ: Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 1989, 161:851–858
7. Gospodarowicz D, Abraham JA, Schilling J: Isolation and characterization of a vascular endothelial cell mitogen produced by pituitary-derived folliculo-stellate cells. Proc Natl Acad Sci USA 1989, 86:7311–7315
8. Keck PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, Connolly DT: Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science 1989, 246:1309–1312
9. Leung DW, Cachianes G, Kuang W-J, Goeddel DV, Ferrara N: Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 1989, 246:1306–1309
10. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Senger DR, Dvorak HF: Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. Cancer Res 1993, 53: 4727–4735
11. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Dvorak HF, Senger DR: Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas. Am J Pathol 1993, 143: 1255–1262
12. Brown L, Berse B, Jackman R, Tognazzi K, Guidi A, Dvorak H, Senger D, Connolly J, Schnitt S: Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in breast cancer. Hum Pathol 1995 (in press)
13. Senger DR, Perruzzi CA, Feder J, Dvorak HF: A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. Cancer Res 1986, 46:5629–5632
14. Dvorak HF, Sioussat TM, Brown LF, Berse B, Nagy JA, Sotrel A, Manseau EJ, Van De Water L, Senger DR: Distribution of vascular permeability factor (vascular endothelial growth factor) in tumors: concentration in tumor blood vessels. J Exp Med 1991, 174:1275–1278
15. Plate KH, Breier G, Weich HA, Risau W: Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas *in vivo*. Nature 1992, 359:845–848
16. Shweiki D, Itin A, Soffer D, Keshet E: Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 1992, 359:843–845
17. Dvorak HF, Orenstein NS, Carvalho AC, Churchill WH, Dvorak AM, Galli SJ, Feder J, Bitzer AM, Rypysc J, Giovinco P: Induction of a fibrin-gel investment: an early event in line 10 hepatocarcinoma growth mediated by tumor-secreted products. J Immunol 1979, 122:166–174
18. Dvorak HF, Dvorak AM, Manseau EJ, Wiberg L, Churchill WH: Fibrin-gel investment associated with line 1 and line 10 solid tumor growth, angiogenesis, and fibroplasia in guinea pigs: role of cellular immunity, myofibroblasts, microvascular damage, and infarction in line 1 tumor regression. J Natl Cancer Inst 1979, 62:1459–1472
19. Dvorak HF: Tumors: wounds that do not heal: similarities between tumor stroma generation and wound healing. N Engl J Med 1986, 315:1650–1659
20. Brown LF, Lanir N, McDonagh J, Czanecki K, Estrella P, Dvorak AM, Dvorak HF: Fibroblast migration in fibrin gel matrices. Am J Pathol 1993, 142:273–283
21. Dvorak HF, Harvey VS, Estrella P, Brown LF, McDonagh J, Dvorak AM: Fibrin containing gels induce angiogenesis: implications for tumor stroma generation and wound healing. Lab Invest 1987, 57:673–686
22. Berse B, Brown LF, Van De Water L, Dvorak HF, Senger DR: Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. Mol Biol Cell 1992, 3:211–220
23. Breier G, Albrecht U, Sterrer S, Risau W: Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation. Development 1992, 114:521–532
24. Brown LF, Berse B, Tognazzi K, Manseau EJ, Van De Water L, Senger DR, Dvorak HF, Rosen S: Vascular permeability factor mRNA and protein expression in human kidney. Kidney Int 1992, 42:1457–1461
25. Millauer B, Wizigmann-Voos S, Schnurch H, Martinez R, Moller N, Risau W, Ullrich A: High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell 1993, 72:835–846
26. Brown LF, Yeo K-T, Berse B, Yeo T-K, Senger DR, Dvorak HF, Van De Water L: Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. 1992, J Exp Med 1:1375–1379
27. Fava R, Olsen N, Spencer-Green G, Yeo K-T, Yeo T-K, Berse B, Jackman R, Senger D, Dvorak H, Brown L: Vascular permeability factor/endothelial growth factor (VPF/VEGF): accumulation and expression in human synovial fluids and rheumatoid synovial tissue. J Exp Med 1994, 180:341–346
28. Koch A, Harlow L, Haines G, Amento E, Unemori E, Wong W, Pope R, Ferrara N: Vascular endothelial growth factor: a cytokine modulating endothelial function in rheumatoid arthritis. J Immunol 1994, 152: 4149–4156
29. Phillips HS, Hains J, Leung DW, Ferrara N: Vascular endothelial growth factor is expressed in rat corpus luteum. Endocrinology 1990, 127:965–967
30. Ravindranath N, Little-Ihrig L, Phillips HS, Ferrara N, Zeleznik AJ: Vascular endothelial growth factor messenger ribonucleic acid expression in the primate ovary. Endocrinology 1992, 131:254–260
31. Koos RD, Olson CE: Hypoxia stimulates expression of

the gene for vascular endothelial growth factor (VEGF), a putative angiogenic factor, by granulosa cells of the ovarian follicle, a site of angiogenesis. J Cell Biol 1991, 115:421a
32. Hughesdon P: Morphology and morphogenesis of the Stein-Leventhal ovary and of so-called "hyperthecosis". Obstet Gynecol Surg 1982, 37:59–77
33. Shin RW, Iwaki T, Kitamoto T, Tateishi J: Hydrated autoclave pretreatment enhances *tau* immunoreactivity in formalin-fixed normal and Alzheimer's disease brain tissues. Lab Invest 1991, 64:693–702
34. Sioussat TM, Dvorak HF, Brock TA, Senger DR: Inhibition of vascular permeability factor (vascular endothelial growth factor) with anti-peptide antibodies. Arch Biochem Biophys 1993, 301:15–20
35. Hui KY, Haber E, Matsueda GR: Monoclonal antibodies to a synthetic fibrin-like peptide bind to human fibrin but not fibrinogen. Science 1983, 222:1129–1132
36. Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW: The vascular endothelial growth factor family: identification of a fourth molecular species and characterization of alternative splicing of RNA. Mol Endocrinol 1991, 5:1806–1814
37. Tischer E, Mitchell R, Hartman T, Silva M, Gospodarowicz D, Fiddes JC, Abraham JA: The human gene for vascular endothelial growth factor: multiple protein forms are encoded through alternative exon splicing. J Biol Chem 1991, 266:11947–11954
38. Bloom W, Fawcett D: A Textbook of Histology. Philadelphia, W.B. Saunders, 1975, pp 858–880
39. Reynolds L: Angiogenesis in the female reproductive system. FASEB J 1992, 6:886–892
40. Morris B, Sass M: The formation of lymph in the ovary. Proc Soc Lond Biol 1966, 164:577–591
41. Moor R, Seamark R: Cell signaling, permeability, and microvasculatory changes during antral follicle development in mammals. J Dairy Sci 1986, 69:927–943
42. Zeleznik A, Schuler H, Reichert LJ: Gonadotropin-binding sites in the Rhesus monkey ovary: role of the vasculature in the selective distribution of human chorionic gonadotropin to the preovulatory follicle. Endocrinology 1981, 109:356–362
43. Okuda Y, Okamura H, Kanzaki H, Takenaka A, Morimoto K, Nishimura T: An ultrastructural study of capillary permeability of rabbit ovarian follicles during ovulation using carbon tracer. Acta Obstet Gynecol Jpn 1980, 32:859–867
44. Cullinan-Bove K, Koos R: Vascular endothelial growth factor/vascular permeability factor expression in the rat uterus: rapid stimulation by estrogen correlates with estrogen-induced increases in uterine capillary permeability and growth. Endocrinology 1993, 133: 829–837
45. Payer A: Permeability of ovarian follicles and capillaries in mice. Am J Ana 1975, 142:295–301
46. de Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT: The *fms*-like tyrosine kinase, a receptor for vascular endothelial growth factor. Science 1992, 255:989–991
47. Terman BI, Dougher-Vermazen M, Carrion ME, Dimitrov DC, Armellino D, Gospodarowicz D, Bohlen P: Identification of the KDR tyrosine kinase as a receptor for vascular endothelial cell growth factor. Biochem Biophys Res Commun 1992, 187:1579–1586
48. Dumonde D, Glynn L: The reaction of guinea pigs to autologous and heterologous fibrin implants. J Pathol Bacteriol 1965, 90:649–657
49. Goldberg M, Schneider T: Similarities between the oxygen sensing mechanisms regulating the expression of vascular endothelial growth factor and erythropoietin. J Biol Chem 1994, 269:4355–4359
50. Delmar M, Brown L, Claffey K, Yeo K-T, Kocher O, Jackman R, Berse B, Dvorak H: Overexpression of vascular permeability factor and its receptors in psoriasis. J Exp Med 1994, 180:1141–1146
51. Finkenzeller G, Marmé D, Weich HA, Hug H: Platelet-derived growth factor-induced transcription of the vascular endothelial growth factor gene is mediated by protein kinase C. Cancer Res 1992, 52:4821–4823
52. Christofferson RH: Angiogenesis as induced by trophoblast and cancer cells. Doctoral dissertation, Uppsala University, Uppsala, Sweden, 1988
53. Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG: Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor. Proc Natl Acad Sci USA 1991, 88:9267–9271