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Invited Review

Regulation of VEGF in the reproductive tract by sex-steroid hormones

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Summary. Vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis. In adults, angiogenesis is an infrequent event in the normal tissue except in the female reproductive tract where angiogenesis occurs frequently during the cyclical repair and regeneration of the endometrium as well as in the ovary. Little is known about angiogenesis in the male reproductive tract. The role of VEGF in controlling reproductive tract physiology and the role of hormones in regulating this key regulator of angiogenesis is not well understood. Since reproductive tract physiology is largely under sex-steroid regulation, we have reviewed some recent studies describing the role of sex-steroid hormones in regulating VEGF. We have also included studies on the role of sex-steroids in regulating VEGF and angiogenesis in endometrial, breast and prostate pathologies. We have provided an extensive review of the classical VEGF and VEGF receptors with examples drawn from numerous studies in the literature using diverse biological systems to encourage similar studies in the area of reproductive tract physiology. It is speculated that such studies will provide insights into understanding the role of VEGF in reproductive tract development, causes of infertility, and cancer. Such knowledge would allow us to target VEGF for improving human reproductive tract abnormalities, for enhancing implantation and fertility, and for designing drugs for treatment of endocrine dependent cancers.

Key words: Vascular endothelial growth factor, Angiogenesis, Cancer, Estrogen, Progestin, Androgen, Steroid receptors

Introduction

Since time immemorial, women and men alike have undoubtedly recognized that pronounced changes periodically occur in the vasculature of the reproductive tract. However, it was not until the beginning of the 20th century that it became clear these changes were under endocrine control by ovarian hormones in females, and even more recently that angiogenesis plays a fundamental role in the normal physiology of the male reproductive tract. Within the last 2 decades specific genes that regulate vascular growth and function have been identified (Goldberg and Rosen, 1997), and we can now investigate their hormonal regulation. This is required to elucidate the physiological mechanisms that regulate changes in the endometrium throughout the menstrual cycle, the pronounced vascular changes that occur during implantation and placentation, and the pathophysiology of conditions such as dysfunctional uterine bleeding, uterine cancers, and endometriosis which are major women's health problems. Also it is becoming clear that both physiological and pathological mechanisms underlying angiogenesis in the male reproductive tract need further elucidation to understand the origin and expansion of male reproductive cancer and other abnormalities such as unexplained infertility. We will first present an overview of the effects of sex steroids on the vascular system of the uterus, and then more extensively discuss vascular endothelial growth factor (VEGF) since this gene is emerging as a key regulator of angiogenesis and vascular function. Next, we will discuss the role of VEGF in the male reproductive tract and we will then end with a section on role and regulation of VEGF in male and female reproductive tract cancer.

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Sex-steroids and the vasculature

A key function of the vasculature is to provide a nutrient supply to the female reproductive tract, and in most species 3 major types of regulation occur: vasodilation; changes in capillary permeability; and growth and development of new vessels. In virtually all species studied, estrogens produce dramatic increases in uterine blood flow due to vasodilation (Spaziani, 1975). For example, in the castrate ewe a single injection of 1 μg of estradiol can increase uterine blood flow 100-fold within 2 hours (Killam et al., 1973). In general, uterine hyperemia (i.e., an increase in blood in the tissue)

increases as the ratio of circulating estrogen/progesterone increases. The role of steroids on the male reproductive tissue is not well studied.

Since this hyperemic effect of estrogens is so rapid there has been considerable controversy as to whether this hormonal effect is mediated by the regulation of gene expression via the classical nuclear estrogen receptor-α. There have been periodic suggestions that other mechanisms, e.g., rapid activation of plasma membrane estrogen receptors (Pietras and Szego, 1977) might be involved, and several very recent reports have described effects of plasma membrane estrogen receptors (see Razandi et al., 1999) and references therein). Despite the fact that these dramatic changes in uterine hyperemia have been recognized for many years, the molecular mechanism(s) for this effect remains to be established and will not be further considered here.

In addition to hyperemia, estrogens increase the permeability of uterine capillaries to water, small molecules, and proteins (Spaziani, 1975). While slightly slower in onset, changes in permeability are also quite rapid and are typically seen within several hours after hormone administration to experimental animals. These changes are thought to occur as the result of fenestrations that develop in the endothelial surface of capillaries following treatment with hormones (Martin et al., 1973; Spaziani, 1975; and references therein). Changes in blood flow and vascular permeability provide increased oxygen, nutrients, and plasma-born growth factors, i.e., a "perfusion" effect, and also cause stromal edema. This edema and increased blood flow could facilitate uterine growth by "diluting" or removing inhibitory factors. While admittedly speculative this possibility has not been well explored and we believe it merits consideration.

In addition to hyperemia and changes in permeability, marked changes also occur in the growth of vessels. In primates there is an increase in the length, branching, and coiling of the spiral arteries that supply the functionalis layer of the endometrium during each menstrual cycle as the endometrium is regenerated. The sole ovarian hormones required to produce these changes are estrogens and progesterone (Brenner and Slayden, 1994). In non-primates this cyclical regeneration does not occur in the estrus cycle, but the growth of new vessels takes place if implantation and placentation occur. In either case, the production of new vessels adds to the "perfusion" effect as noted above, but it is now recognized that endothelial cells secrete a large number of cytokines and growth factors (Rak et al., 1996). Increasing the number of endothelial cells and/or altering their production and release of growth factors thus represents a potential "paracrine" effect which could contribute to the growth and proliferation of uterine cells, the infiltration of migratory cells, and/or "autocrine" effects on the endothelial cells themselves. This is another possibility that has not yet been widely studied in the female reproductive tract, but which we believe also merits consideration.

Changes in blood vessel permeability and the growth of blood vessels may also be essential components of male reproductive tract development. For instance (Folkman, 1998) has recently suggested that prostate mass increases only if angiogenesis occurs in this tissue. Similarly other aspects of angiogenesis and of VEGF in particular are just now beginning to be investigated. We will discuss some of these aspects in detail later in this review.

Angiogenesis and angiogenic factors

Angiogenesis is the process of generating new capillaries from existing vessels. During embryonic development the formation of a primary vascular plexus (vasculogenesis) occurs from angioblasts; primative blood vessels are formed; and extensive angiogenesis then occurs by sprouting and branching (Risau, 1997). In the adult organism angiogenesis is limited to relatively few tissues including the ovary and uterus, but the formation of new vessels commonly occurs during tumor growth (Goldberg and Rosen, 1997; Zetter, 1998) and in several other diseases (Folkman, 1995) including endometriosis (Smith, 1997).

Whether it occurs during development, normal physiological processes, or in pathological conditions, angiogenesis is a complex process. New capillaries originate from a pre-existing microvasculature, mainly capillaries and venules, rather than from larger vessels with a smooth muscle layer. One of the first things that occurs in response to an angiogenic stimulus is an increase in capillary permeability (Dvorak et al., 1995). This allows fibrinogen and other serum proteins to exit the capillary space and form an extravascular fibrin gel. This matrix then supports the ingrowth of endothelial cells and other elements to generate new vascularized stroma. The generation of new capillaries involves: (1) local degradation of the basement membrane by matrix metalloproteinases; (2) movement of endothelial cells through the basement membrane and their ingrowth into the provisional perivascular matrix noted above; (3) proliferation of the endothelial cells; and (4) organization of the cells to form new vessels and the subsequent production of basement membrane along their surface. For additional information interested readers are referred to several recent reviews about the basic process of angiogenesis (Goldberg and Rosen, 1997; Zetter, 1998; Nicosia and Villaschi, 1999) and reviews of angiogenesis in the female reproductive tract (Reynolds et al., 1992; Smith, 1998).

Given the complexity of angiogenesis it is not surprising that a large number of factors have been identified that can stimulate or inhibit this process (Goldberg and Rosen, 1997; Zetter, 1998; Zheng et al., 1998; Ferrara, 1999; Nicosia and Villaschi, 1999). Despite this complexity, two factors emerging as key regulators of angiogenesis in most systems are basic fibroblast growth factor or bFGF (Lawson, 1990; Sherwood et al., 1992; Ware, 1993; Cronauer et al.,

1997; Ropiquet et al., 1997; Yazidi et al., 1998) and vascular endothelial growth factor or VEGF (Ferrara and Davis-Smyth, 1997; Ferrara, 1999). We will focus only on VEGF in this article since this growth factor is the most selective regulator of angiogenesis and it is a good potential candidate for antiangiogenic therapy of both male and female reproductive tract abnormalities.

Vascular Endothelial Growth Factor (VEGF)/Vascular Permeability Factor (VPF)

VEGF is a multi functional cytokine that was originally identified as a protein produced by tumor cells that increased the permeability of capillaries to proteins (see Dvorak et al., 1995). It was subsequently discovered to be selectively mitogenic for endothelial cells and to stimulate angiogenesis (Ferrara and Davis-Smyth, 1997; Ferrara, 1999). This factor has been referred to as VPF, VEGF, or vasculotropin in the literature. VEGF is expressed in both epithelial and mesenchymal cells in a wide variety of tissues, and it is highly expressed in many tumors (Ferrer et al., 1997; Goldberg and Rosen, 1997; Ferrara, 1999; Neufeld et al., 1999; and references therein).

When the gene for VEGF was cloned, sequence alignment revealed some homology (18%) with the B chain of the platelet-derived growth factor. Both the human and murine VEGF genes contain 8 exons and 7 introns, and differential exon splicing generates 4 predominant forms containing 121, 165, 189, and 206 amino acids in the human, and corresponding forms with 1 amino acid less in the mouse (Ferrara, 1999). In most systems VEGF-121 and -165 are the major species expressed. VEGF 206 is not predicted for rodents due to an inframe stop codon resulting in the loss of one amino acid. A reproductive tract specific VEGF 145 has also been reported in the literature but this variant is not consistently observed. VEGF 165 lacks exon 6, VEGF 121 lacks exons 6 and 7, VEGF 189 has an insertion of 24 amino acids in the VEGF 165 sequence and VEGF 206 contains an additional insertion of 17 amino acids. A major difference between the different forms of VEGF is their heparin binding capability. VEGF 121 is freely diffusible, but the other major forms contain heparin binding regions that can mediate binding to cell surfaces and the extracellular matrix, and thus provide a potential reservoir for locally controlled release by heparinases or plasmin see (Ferrara and Davis-Smyth, 1997). VEGF 165, the major isoform, is a heparin binding basic protein which functions as a homodimeric protein of 45,000Da. VEGF 121 lacks the heparin binding ability and is weakly acidic, whereas the VEGF 189 and 206 are strong binders of heparin and are more basic than VEGF 165. VEGF 121 is the freely diffusible form but other isoforms remain attached to the extracellular matrix with VEGF 165 bound less tightly than the other two basic proteins. However, the bound forms can be released by heparin, heparinase or by plasmin. It is interesting that loss of heparin binding ability leads to a loss of mitogenic activity indicating that the proteolytically released fractions may have other unknown functions (Ferrara and Davis-Smyth, 1997).

The promoter for the human VEGF has been extensively analyzed (Ferrara, 1999 and references therein). It contains one major start site near a cluster of SP1 binding sites. In the vicinity of the promoter there are also a number of putative AP1 and AP2 elements. In contrast to the human gene, the 3'-untranslated region of the rat gene has been extensively explored. There are four potential polyadenylation sites and the one most frequently used is 1.9 kb downstream of the translation termination codon. Interestingly, the sequence in the 3'-untranslated region contains a number of regions which are known to be involved in regulating the stability of mRNA. From the point of view of this review, there are no hormone response elements that have been reported as yet in VEGF DNA regulatory sequences.

Quite recently a number of VEGF related genes referred to as VEGF B, C, D, E, and placental growth factor have been identified (Eriksson and Alitalo, 1999; Meyer et al., 1999). Some of these forms may have selectivity for certain functions, e.g., VEGF-C may be the factor that regulates capillary growth in the lymphatic system (Pajusola et al., 1996; Jeltsch et al., 1997). In addition since VEGF binds to its receptor as a dimer (see below), the various VEGFs can form heterodimers, either with variants of the same family or with members of another family, that may have different activities.

VEGF receptors

The role and regulation of VEGF receptors in the reproductive tract is not well studied, and the following discussion is thus based on studies of VEGF receptors in other biological systems. We hope to stimulate research in the field of reproductive tract biology by providing examples of VEGF receptor stimulated signal transduction pathways that have been observed in other biological systems. Readers are referred to several excellent reviews for detailed information on VEGF receptors (Eriksson and Alitalo, 1999; Neufeld et al., 1999; Ortega et al., 1999).

There are three members of the VEGF receptor family: VEGFR-1 (Flt), VEGFR-2 (flk/KDR) and VEGFR-3 (Flt4) and all three are receptor-type tyrosine kinases. The first two bind the classical VEGF (VEGF A) and the latter interacts with VEGF C and D. These receptors are tyrosine protein kinases expressed primarily, but not exclusively, on endothelial cells. At present the mitogenic actions of VEGF are thought to be mediated primarily by VEGF-R2. Several other forms of VEGF receptors have been identified, but their physiological functions are not clearly established (see Neufeld et al., 1999; Ortega et al., 1999).

These receptors form a subfamily distinguished by the presence of seven immunoglobin-like domains in their intracellular domain and these receptors are crucial

for embryonic development. VEGFR1 binds the ligand with a greater affinity than VGEFR2. An alternatively spliced form of VEGFR1 has been identified in HUVEC cells, and this receptor lacks the seventh immunoglobin-like structure, the transmembrane domain and the cytoplasmic domain. Since this receptor binds VEGF with high affinity and prevents VEGF-induced mitogenesis it may represent a negative regulator of angiogenesis.

VEGFR1 and VEGFR2 are expressed predominantly in endothelial cells and autoradiographic studies have localized these receptors to both proliferating and quiescent endothelia. Gene knock-out studies indicate that the two receptors exhibit different functions in vivo and several biochemical studies have shown that flk but not flt is responsible for mediating the mitogenic signal in the endothelial cells. The biological roles of these receptors include their involvement in cell proliferation, migration, vascular permeability, induction of protease activity, and in vitro angiogenesis. Recently, neuropilin, the neuronal cell guidance receptor for the semaphorin ligands, has also been identified as a VEGF receptor. This receptor binds VEGF 165 specifically but because of its short intracellular domain it is unlikely to function as an independent receptor. This assumption is based on the fact that when expressed alone this receptor is unable to modulate responses to VEGF 165, but neuropilin can influence VEGF 165 binding to its other receptors. Neuropilin may thus represent a co-receptor for VEGF 165. It is interesting to note that several non-endothelial cell types express neuropilin and many breast and prostate cancer cell lines have abundant amounts of this receptor although the role of neuropilin in the latter tissue is not understood.

Both VEGFR-1 and VEGFR-2 are expressed in several different cell types. VEGFR-1 transcripts are made up of several species of mRNA (8.0, 3.0 and 2.2 kb). VEGFR-2 is generally encoded by a 5.5 kb transcript. The *VEGFR-1* gene consists of 30 exons spanned across 140 kb of chromosomal DNA. The genomic organization for *VEGFR-2* is not yet determined. The promoter region of the human *VEGFR-1* has been examined and reveals that it is a TATA box containing gene with a single start site, flanked by several transcription factor binding sites including ETS and CRE. The promoter region for both human and mouse *VEGFR-2* has also been analyzed. There is no consensus TATA box for this gene and the start site is located 303 bp upstream of the first methionine. This region is flanked by several SP1 binding sites. Several endothelium specific regulatory elements have been identified on the *VEGFR-2* promoter.

Limited information is available on the signal transduction mechanisms for the VEGF receptors and except for a few cases selected binding of downstream signaling molecules to one or the other receptor has not been demonstrated. As indicated previously cells transfected with VEGFR-1 fail to show a mitogenic response to VEGF in contrast to cells transfected with VEGFR-2. It is speculated that lack of MAP kinase activation in cells transfected with VEGFR-1 is responsible for lack of VEGFR-1 effect on proliferation. However, some recent reports indicate that both VEGFR-1 and -2 can activate MAP Kinases Erk1 and Erk2 making it difficult to understand how the two receptors mediate distinct effects in vivo. As with all tyrosine kinases, signal transduction by VEGF receptors depends on binding of Src Homology 2 Domain (SH2) containing molecules to bind to the tyrosine phosphorylation sites of the receptor.

There are several proteins that are phosphorylated following VEGF interaction with its receptors. Occupancy of VEGF receptors leads to autophosphorylation as well as phosphorylation of other effectors including phospholipase C-γ; the GTPase activating protein Ras-GAP, and the adaptor proteins Nck and Shc. Also, both receptors have the binding site for Grb2. Both Grb2 and Shc could potentially induce the Ras pathway however the involvement of Ras in the VEGFR signaling pathway is not established.

Following receptor activation in endothelial cells a number of cellular responses occur that are likely to play a role in angiogenesis and tissue remodeling, e.g., induction of urokinase and urokinase receptors, tissue plasminogen activators (PA), PA-inhibitors, metalloproteinase activity, VCAM-1 and ICAM-1 expression, and hexose transport (Pekala et al., 1990; Ferrara, 1999). Special note should be made of VEGF's ability to induce fenestrations in capillaries in vivo (Roberts and Palade, 1995) which are reminiscent of estrogenic effects in the female rodent reproductive tract (Martin et al., 1973). In contrast to bFGF which affects multiple cell types including endothelial cells, smooth muscle cells, and fibroblasts, VEGF is much more selective and exerts its actions primarily on endothelial cells.

There are some distinct differences that have recently been noted for the two VEGF receptors. It has been demonstrated that when VEGFR-2 is expressed in cells and exposed to ligand, there is a strong ligand dependent tyrosine phosphorylation of VEGFR-2. VEGFR-1 in contrast does not show such an intense signal. It is therefore possible that such an intense signal may be required for eliciting a full spectrum of response for mitogenic events to occur. VEGFR-1 has been shown to interact with the p85 subunit of phosphatidyl inositol 3-kinase implicating higher levels of phosphatidyl inositol involvement in cell signaling pathways. It is not known if this is a receptor selective effect. VEGFR-2 has been shown to associate with protein tyrosine phosphatases SHP1 and 2 suggesting that these two proteins may be involved in some form of signal modulation. It is not known if these proteins also interact with the VEGFR-1. It has been found that the migration of monocytes in response to VEGF apparently only occurs through VEGFR-1 induced signaling. Most interestingly, VEGF treated cells containing VEGFR-1 have been shown to contain phosphorylated Src family members, fyn and yes but these proteins are not phosphorylated in VEGFR-2 containing cells, indicating

suttle differences may be inherent in the signal transduction pathways of these two receptors.

VEGF in the female reproductive tract

In 1993 VEGF expression was initially reported in the uterus of the mouse (Shweiki et al., 1993), human (Charnock-Jones et al., 1993), and rat (Cullinan-Bove and Koos, 1993), and has since been identified in the ewe (Reynolds et al., 1998), the rabbit (Das et al., 1997), and the monkey (Greb et al., 1997). These and other studies have found that expression of this growth factor and its mRNA occurs primarily in the epithelial and stromal layers of the uterus, but expression of VEGF (Charnock-Jones et al., 1993; Harrison-Woolrych et al., 1995) and its receptor (Brown et al., 1997) has also been observed in the myometrium. As in other tissues, multiple forms of VEGF are produced in the uterus including VEGF-189, -165, -145, and -121 in the human (Charnock-Jones et al., 1993), VEGF-188, -164, and -120 in the rat (Cullinan-Bove and Koos, 1993; Hyder et al., 1996), and VEGF-189, -165, and -121 in the monkey (Greb et al., 1997). In all these systems VEGF -121(120) and -165(164) appear to be the predominant forms produced, and immunological measurements have demonstrated that VEGF protein is also produced in monkeys (Greb et al., 1997), mice (Shweiki et al., 1993; Karuri et al., 1998), rabbits (Das et al., 1997), and humans (Shirfren et al., 1996; Zhang et al., 1998). VEGF expression has also been reported in human endometrial carcinoma cells (Charnock-Jones et al., 1993), leiomyoma (Harrison-Woolrych et al., 1995), endometriosis (Smith, 1997; Shirfren et al., 1996; McLaren et al., 1996), and various gynecological malignancies (Abulafia et al., 1999).

Estrogens regulate VEGF mRNA expression in human endometrial adenocarcinoma cells (Charnock-Jones et al., 1993), primary cultures of human uterine stromal cells (Shirfren et al., 1996; Huang et al., 1998) and the rat uterus in vivo (Cullinan-Bove and Koos, 1993; Hyder et al., 1996). These are likely to be primary estrogen receptor mediated effects because induction is very rapid, is blocked by pure anti-estrogens (Hyder et al., 1996, 1997a), and is inhibited by actinomycin D but not puromycin or cycloheximide (Cullinan-Bove and Koos, 1993; Hyder et al., 1996), although an estrogen response element has not yet been identified in the *VEGF* gene. Partial estrogen agonists such as tamoxifen (Hyder et al., 1996) and related triphenylethylene antiestrogens (Hyder et al., 1997b) also induce VEGF expression in the uterus which is consistent with their agonist activity in that tissue. This regulation by estrogens is consistent with most studies on the expression of VEGF throughout the estrus cycle in rodents (Shweiki et al., 1993; Karuri et al., 1998) and the menstrual cycle in humans (Shirfren et al., 1996). In contrast, others have reported that VEGF expression is highest in *hypoestrogenic* monkeys (Greb et al., 1997). One possible explanation of this apparent discrepancy is that the endometrium of hypoestrogenic animals may be relatively hypoxic, since hypoxia is known to be a strong inducer of VEGF transcription (Ferrara, 1999; Ferrara and Davis-Smyth, 1997).

There are several reports that progestins can also induce VEGF expression in human stromal cells (Shirfren et al., 1996; Greb et al., 1997) and the rodent uterus (Cullinan-Bove and Koos, 1993; Hyder et al., 1998a). The effects of progestins on VEGF expression have not been as well studied as those of estrogens and little information is available about the possible molecular mechanism of progesterone regulation of VEGF. However, progesterone increases VEGF protein expression in a human breast cancer cell line, and this effect is blocked by RU-486 suggesting it is mediated by the progesterone receptor (Hyder et al., 1998b). The overall picture which emerges from these studies is that VEGF expression is likely to be under the control of both estrogens and progestins, although very little is known about the molecular mechanisms that regulate expression in the reproductive tract at present, and substantial differences may exist between cell types and in different species.

A number of studies have also investigated expression of VEGF and its receptor in the uterus and placenta during pregnancy in the rat (Srivastava et al., 1998), mouse (Chakraborty et al., 1995), rabbit (Das et al., 1997), and human (Cooper et al., 1995; Athanassiades et al., 1998). VEGF expression correlates spatially and temporally with changes in angiogenesis and vascular reactivity at implantation sites and in decidua and placental tissue in most cases, except in the rat where these changes correlate primarily with bFGF, but not VEGF, expression (Srivastava et al., 1998). On balance, however, these studies support an important role for VEGF in implantation and placentation.

VEGF in the male reproductive tract

Few studies have shown the presence of VEGF in the male reproductive tract. Brown et al. (1995b) first showed the presence of both VEGF mRNA and protein in the prostate and seminal vesicle epithelium. These authors also reported a high content of VEGF in the semen suggesting an important role of this factor in male fertility. However, Korpelainen et al. (1998) showed that overexpression of VEGF in the testis and epididymis of transgenic mice led to severe histopathological abnormalities and caused infertility. This study thus indicated that cells other than those of endothelial origin could also be the targets for VEGF. It could be questioned however whether overexpression of VEGF is creating an artificial system which the cells may not normally experience. Campbell et al. (1999) report that epithelial cells from prostatic origin can produce VEGF. They found that VEGF was the most predominant cytokine produced by prostatic epithelium. The authors conclude that the the ability to stimulate angiogenesis is a function of normal prostate epithelial cells, rather than

a function acquired during prostate oncogenesis.

It was suggested by Folkman (1998) that prostate growth is most likely dependent on vascular endothelial cells. This report suggested that angiogenic molecules must be synthesized to allow for the formation of new blood vessels and tissue growth.

The role of androgens in regulating VEGF has recently been studied in both normal cells and in tumor cells obtained from male genital tract. Cavett et al. (1997) reported that sustained delivery of testosterone led to increased angiogenesis in the rat ventral prostate. Also study by Ferrer et al. (1997) found overexpression of VEGF in prostate tumors and in prostate epithelial in culture, and Sordello et al. (1998) showed that testosterone can increase VEGF levels in both the prostate epithelial cells in culture as well as in the ventral prostate lobe in vivo following testosterone injection in adult rats. In addition, Levine et al. (1998) have shown that VEGF is under direct regulation by dihydroxytestosterone in the primary cultures of human prostate fetal fibroblasts suggesting that fibroblasts may have an indirect role in promoting angiogenesis in the prostate. Role for androgens in regulating VEGF and controlling tumor growth came from a recent elegant study by Jain et al. (1998). These authors caused regression of an androgen dependent tumor by castration. Interestingly, in this study the tumor vessels started to regress in preference to the vessels in normal sorrounding tissue and this effect preceded the regression of tumor volume. However, two weeks later vessels began to grow again indicating that tumor had acquired an angiogenic phenotype independent of testosterone levels. Another study by Haggstrom et al. (1998) also provided evidence for involvement of testosterone in the development of prostate tumors. These authors reported that castration decreased VEGF mRNA levels in the rat ventral prostate.

VEGF and endocrine-related cancer

There is voluminous literature on angiogenesis in endocrine related cancers of the reproductive tract and since space will not allow us to cover all the pertinent information in this review article, interested readers are referred to a recent review for a detailed discussion (Gasparini, 1997). We will briefly cover the more limited number of reports that have investigated the role of sex-steroid hormones in the regulation of VEGF and its possible relevance in uterine, breast and prostate cancers.

Sex-steroid regulation of VEGF in uterine cancer

Several recent studies have established the importance of angiogenesis (increase in microvessel density) in the development and progression of endometrial hyperplasia and carcinoma, survival in patients with gynecological malignancies, and in the recurrence of uterine tumors (Kirshner et al., 1996; Abulafia et al., 1999; Obermair et al., 1999). A recent study by Guidi et al. (1996) has shown strong expression of VEGF at both mRNA and protein levels in endometrial carcinoma compared with control endometrium. The same study also showed strong expression of VEGF receptors indicating that all the components of a potential autocrine/paracrine "VEGF loop" are overexpressed in endometrial tumors, and similar findings have been reported by Doldi et al. (1996). A number of studies noted in previous sections have established that estrogens regulate VEGF expression in normal uterine tissues and cells, but surprisingly, the study by Charnock-Jones et al. (1993) and Zhang et al. (1995b) are the only reports that estradiol regulates VEGF expression in normal endometrial cells and in human uterine carcinoma cell lines.

Another study of potential importance to uterine cancer is the report by Hyder et al. (1996) that uterine VEGF expression is increased by both estradiol and tamoxifen in the rodent uterus. A tamoxifen-mediated increase in endometrial VEGF expression could contribute to the uterine abnormalities that are now being reported in breast cancer patients receiving tamoxifen for adjuvant therapy. This effect of tamoxifen appears to be an estrogen receptor mediated response since it is abolished by the pure antiestrogen ICI 182,780 (Hyder et al., 1997a). Since ICI 182,780 is an effective agent for suppressing VEGF production in uterine cells and is devoid of partial agonistic properties associated with tamoxifen, it is suggested by the authors that this antiestrogen should be considered for adjuvant treatment of endometrial tumors. Similar conclusion have also been reached in an elegant study recently published by Jordans group (O'Regan et al., 1998).

Several studies have shown that progesterone enhances VEGF expression in the rodent uterus (Cullinan-Bove and Koos, 1993; Hyder et al., 1998a), but it is not clear if this hormone has a similar effect in uterine tumors. Jikihara et al. (1996) found that medroxyprogesterone acetate inhibited angiogenesis in endometrial tumor tissue that was implanted in rabbit cornea. However, in a study by Kim et al. (1996) medroxyprogesterone acetate was unable to regulate VEGF in an explanted human endometrial tumor in nude mice, and Hyder et al. (1998b) failed to observe regulation of VEGF expression by progestins in Ishikawa cells which are a human endometrial carcinoma line. The failure to observe regulation of VEGF expression by progestins or estrogens in these studies does not necessarily mean that VEGF is not important in uterine tumors, since many of the tumor cells used in these studies exhibit very high constitutive levels of VEGF expression. One possibility is that sex steroids are needed to regulate VEGF expression in normal uterine tissue, but that expression of this angiogenic factor becomes constitutive or is regulated by other factors in many uterine tumors.

Sex steroid regulation of VEGF in breast cancer

As with uterine cancer, it is now well established that breast cancer is an angiogenic dependent disease and that the level of angiogenesis in a tumor serves as an independent prognostic indicator for tumor growth, response to therapy, and clinical outcome. Importantly, both VEGF and its receptors are overexpressed in many breast tumors, and interested readers are referred to several recent articles and reviews which cover this topic in detail (Toi et al., 1994, 1995a,b; Brown et al., 1995a; Kolch et al., 1995; Anan et al., 1996; Yamamoto et al., 1996; Guidi et al., 1997; de Jong et al., 1998; Lee et al., 1998; Linderholm et al., 1998; Longcope, 1998; McLeskey et al., 1998). Surprisingly, however, limited data is available on the regulation of VEGF and its receptors by sex-steroid hormones in breast cancer. Zhang et al. (1995a) observed that MCF-7 cells transfected with a VEGF 121 expression vector grew much faster, formed tumors more rapidly, and were more angiogenic when implanted in the nude mice supplemented with estradiol compared to non-transfected MCF-7 cells. Similarly Brodie's group (Nakamura et al., 1996) has shown that estradiol regulates the expression of VEGF in estrogen responsive DMBA induced rat mammary tumors. Regulation of VEGF expression by estrogens may not occur in all breast cancer cells, however, since Hyder et al. (1998b) could not demonstrate estradiol regulation of VEGF in several different breast cancer cell lines. At present we are only aware of a single study of VEGF regulation by progestins in breast cancer cells (Hyder et al., 1998b) which showed that progestins regulate the levels of VEGF protein in the media of T47D human breast cancer cells. This effect was limited to T47D cells, however, and was not observed in several other breast or uterine tumor cell lines. It should be emphasized that in the study of Hyder et al. (1998b) only the secreted forms of VEGF were being measured in the culture media. It is not known if the other higher molecular weight forms of VEGF were under steroid regulation or not.

Given the importance of estrogens and progestins in the regulation of breast cancer, and the clear evidence that angiogenesis plays a important role in the onset, progression, and metastasis of this disease, it is surprising that so few studies have examined the regulation of VEGF by sex steroids in this disease. This is clearly an important area for further study, especially since many studies have demonstrated that these hormones regulate VEGF expression in normal estrogen and progestin target tissues.

Sex-steroid regulation of VEGF in prostate cancer

Several recent studies described a relationship between VEGF production and initiation, promotion and metastasis of prostate cancer (Ferrer et al., 1997; Jackson et al., 1997; Haggstrom et al., 1998; Balbay et al., 1999; Benjamin et al., 1999; Melnyk et al., 1999). Although several studies have shown overexpression of VEGF in both benign and malignant prostate tumors, very little data is available on the role of sex-steroids in regulating VEGF and its receptors in the prostate. Very recently, several reports have indicated that VEGF expression may be under androgen regulation in the normal prostate, prostate tumors, and in prostate tumor cell lines (Ware, 1993; Joseph et al., 1997; Levine et al., 1998; Sordello et al., 1998). In addition several unique VEGF isoforms have been identified in prostate tumors and in prostate tumors grown in nude mice, and this could account for the aggressive growth behavior of some prostate tumor cell lines (Jackson et al., 1997; Connolly and Rose, 1998). These isoforms are non-secretory forms of VEGF since they are not detected in the media of tumor cells by ELISA. Since the male reproductive tissues contain both isoforms of the estrogen receptor (Cooke et al., 1991; Hess et al., 1997; Prins et al., 1998) it will be interesting to investigate if estrogens have a role in regulating VEGF since such regulation of the potent angiogenic molecule may be amenable to antiestrogen mediated hormonal treatment.

Conclusions

Proper regulation of angiogenesis and vascular permeability is essential for the physiological functioning of both the male and female reproductive tract, and major health problems such as dysfunctional uterine bleeding, endometriosis, uterine cancer, prostate cancer and other male reproductive tract abnormalities almost certainly involve a vascular component. There is a large body of literature that describes the effects of sex steroids on the vasculature of the reproductive tract, but far less is known about the molecular mechanisms that regulate these important actions. It is therefore suggested that there is an immense need for mechanistic studies in these areas in order to improve treatment and prevention of these major women's and men's health problems related to the reproductive tract.

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