Sex-steroid regulation of vascular endothelial growth factor in breast cancer

S M Hyder

Dalton Cardiovascular Research Center, Department of Biomedical Sciences, University of Missouri, 134 Research Park Drive, Columbia, Missouri 65211, USA

(Requests for offprints should be addressed to S M Hyder; Email: hyders@missouri.edu)

Abstract

Angiogenesis is a key event, which occurs in both normal and pathological expansion of tissues and provides the nourishment necessary for growth. The role of angiogenic growth factors in breast pathology is rapidly gaining recognition since scientists and clinicians realized that these factors could function as molecular targets event 550 209822 for controlling tumor expansion. Vascular endothelial growth factor (VEGF) is a key regulator of angiogenesis. Although significant advances have been made in understanding the sex-steroid-dependent regulation of this key factor, the role of VEGF in controlling breast tumors is not well understood. In this review, I discuss recent studies describing the role of the female sex steroids estrogens and progestins in the regulation of VEGF in breast cancer cells. Furthermore, I present a summary of recent studies from other biological systems (mainly focused on tumor biology) directed towards providing us with a better understanding of the regulation of classical VEGF and VEGF receptors. I propose that by extending such studies we will gain deeper insights into how we might combat the progression of breast cancer by controlling hormone-dependent angiogenesis within tumor tissue. I believe that information gained from such studies will permit us to target angiogenic growth factors and their initiated signal transduction pathways in a more precise and selective manner, and thereby to control the formation of new blood vessels that fuel the rapid growth of breast tumors. Finally, it is my hope that the concepts discussed here will help elucidate molecular targets in the hormone-dependent angiogenesis pathway that will ultimately allow us to overcome anti-hormone resistance and to provide insights into how we might pursue the concept of chemoprevention by considering 'angioprevention' as the end point.

Introduction

A key function of the vasculature is to provide a nutrient supply to the organ and, in most species, three major types of regulation occur: vasodilation, changes in capillary permeability, and growth and development of new vessels, also known as angiogenesis (Folkman 2003, Hoeben et al. 2004, Carmeliet 2005). At the beginning of the 20th century, it was recognized in females that vasculature changes occurring in the reproductive tract were under the control of ovarian hormones. Since then, most studies have concentrated on developing our understanding of the role played by sex steroids in angiogenesis in uterine and ovarian tissue since there is evidence that neovascularization is influenced by ovarian steroids in these tissues (Findlay 1986, Reynolds et al. 1992, Brenner & Slayden 1994, Hyder & Stancel 1999, Augustin 2005). In most other adult tissues angiogenesis is a quiescent process. Since it was recognized that tumor expansion, irrespective of tissue origin, depends on the process of angiogenesis (Goldberg & Rosen 1997), a greater interest has been taken in the role of angiogenic growth factors. Expansion of many endocrine-dependent cancers, including many breast tumors, depends on sex steroids; consequently, there has been a recent upsurge in efforts to understand the role of these steroids in controlling both angiogenic and anti-angiogenic factors in normal and neoplastic breast tissue and cells. In the United States alone, about 200 000 new cases of breast cancer are detected annually and approximately 40 000 women die each year of this deadly disease. Endocrine therapy is the choice of treatment for those tumors that possess estrogen or progesterone receptors (ER or PR). However, many tumors either do not respond to this form of treatment or, after initially responding to treatment, go...
on to develop a population of cells that become resistant and therefore unresponsive to anti-hormone treatment. Alternate methods of treatment include chemotherapy or the use of aromatase inhibitors (Kaklamani and Gradishar 2005, Navolanic & McCubrey 2005). It is only recently that strong scientific evidence has been acquired proving that breast cancer is an angiogenic-dependent disease, which is potentially treatable by anti-angiogenic therapy (Longo et al. 2002, Atqur-Rehman & Toi 2003, Morabito et al. 2004, Moses et al. 2004, Ferrara & Kerbel 2005, Schneider & Miller 2005). Since the presence of both ER and PR influences the progression of the disease, it has been suggested that these receptors must be controlling angiogenic factors via their respective ligands (Hyder & Stancel 1999, 2000). Thus, studying the control of growth factors by sex steroids provides an area with important potential, since we may well be able to identify possible targets for anti-angiogenic therapy and control the disease before endocrine resistance develops. Furthermore, anti-hormones might also be modifying or inducing angiogenic inhibitors and thereby controlling tumor growth. These molecular targets representing components of the angiogenic pathway in breast cancer could be used for chemoprevention or for controlling tumor expansion. In this review, I will concentrate on evidence that breast cancer is an angiogenic-dependent disease and discuss the role of sex steroids (estrogens and progestins) in controlling the production of angiogenic growth factors. While several such vascular growth factors have been identified in breast cancer cells, which could influence cell growth, my discussion will concentrate on one of the most potent, termed vascular endothelial growth factor (VEGF). VEGF and its isoforms, in concert with specific receptors, comprise one of the most potent angiogenesis-promoting loops yet identified that significantly promotes tumor expansion (Rak et al. 1996, Saaristo et al. 2000, Zhang et al. 2000a, 2002, Huh et al. 2004). Consequently, VEGF represents a molecular target with immense therapeutic potential and I will focus on the role of estrogens and progestins in controlling production of VEGF in breast cancer cells. Finally, I will discuss the potential targets and strategies aimed at controlling the process of angiogenesis and curtailing this deadly disease.

### Angiogenesis in breast cancer

Angiogenesis is the process by which new capillaries are generated from existing vessels. During embryonic development, the formation of a primary vascular plexus (vasculogenesis) occurs from angioblasts, primitive blood vessels are formed and extensive angiogenesis then occurs by sprouting and branching (Risau 1997, Carmeliet 2005). 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 & Rosen 1997, Zetter 1998) and in a number of other diseases (Folkman 1995). Ovarian hormones may influence angiogenesis in breast tissue, but the details of how such a process occurs under normal physiological situations remains obscure.

Whether it occurs during development, normal physiological processes, or in pathological conditions, angiogenesis is an extremely complex process. New capillaries originate from a pre-existing microvasculature which consists mainly of capillaries and venules, rather than from larger vessels with a smooth muscle layer. One of the initial events 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 in-growth of endothelial cells and other elements to generate new vascularized stroma. For additional information on mechanisms of angiogenesis, interested readers are referred to several recent reviews about the basic process of angiogenesis (Goldberg & Rosen 1997, Zetter 1998, Nicosia & Villaschi 1999, Carmeliet 2005).

There is now considerable evidence that breast cancer is an angiogenic-dependent disease and that angiogenesis plays an essential role in breast cancer development, invasion, and metastasis (Lichtenbeld et al. 1998, Dabrosin et al. 2003b, Schneider & Miller 2005). Even though it has been recognized for decades that breast cancer is an endocrine-dependent disease, little attention has been paid to the role of sex steroids in the process of angiogenesis in breast cancer cells. Since breast tissue is a target for sex steroids and many breast tumors are hormone responsive, it is very likely that sex steroids control the production of angiogenic growth factors or help in the removal of factors, e.g. anti-angiogenic proteins that impede the process. Strong evidence that breast cancer is an angiogenesis-dependent disease comes from hyperplastic murine breast papillomas (Brem et al. 1977) and histologically normal lobules adjacent to cancerous breast tissue that possess higher levels of blood vessels (Jensen et al. 1982), suggesting that angiogenesis precedes transformation of mammary hyperplasia to malignancy (Gimbrone & Gullino 1976, Maiorana & Gullino 1978). In addition, transfection of breast cancer cells with angiogenic stimulatory peptides
increases tumor growth, invasiveness, and metastasis (Zhang et al. 1995, 2000a). Conversely, transfection of tumor cells with inhibitors of angiogenesis decreases growth and metastasis (Sledge & Miller 2003). It has been suggested that tumor progression occurs once the balance is attained that favors angiogenic activators over inhibitors of angiogenesis and a number of factors have been shown to alter from a pre-invasive to an invasive status within breast tumors (Folkman & Hanahan 1991, Engels et al. 1997a,b). This could involve either the induction of angiogenic growth factors (e.g. VEGF, fibroblast growth factor (FGF), matrix metalloproteinases, etc.) or the loss of inhibitors of angiogenesis such as thrombospondin-1 (TSP-1), soluble (s)VEGF receptors and tissue inhibitor of metalloproteinases, etc. Several factors such as hypoxia or loss of tumor suppressors and angiogenesis inhibitors can disrupt the angiogenic balance in a tissue leaning towards tumor progression. This is commonly referred to as an angiogenic switch and describes the sudden growth of tissues that otherwise remain quiescent for a long time (Folkman & Hanahan 1991, Bos et al. 2001, Bergers & Benjamin 2003, Naumov et al. 2006), as occurs in many human breast tumors. The effect of sex steroids on the angiogenic switch remains to be determined; however, the interest to find a connection has gained momentum over the past few years since modulation of this switch could prove crucial in preventing the progression of breast cancer.

There are several clinicopathologic conditions that also confirm the central role of angiogenesis in breast cancer progression. It has been shown, for example, that fibrocystic lesions with the highest vascular density are associated with a greater risk of breast cancer (Guinebretiere et al. 1994). Similarly, microvessel density (MVD) was shown to be highest with histopathologically aggressive ductal carcinoma in situ lesions (Guidi et al. 1994) and was associated with increased VEGF expression (Guidi et al. 1997). High MVD in premalignant lesions has been associated with high risk of future breast cancer (Guinebretiere et al. 1994). Also, high MVD in invasive disease correlates with a greater likelihood of metastatic disease (Weidner et al. 1991), shorter relapse-free intervals and reduced overall survival in patients with node-negative breast cancer (Weidner et al. 1992). A number of angiogenic factors are commonly expressed by invasive human breast cancers (Engels et al. 1997a,b, de Jong et al. 1998), VEGF 165 and VEGF 121–amino acid isoforms being the most predominant (Relf et al. 1997). VEGF expression has also been found to correlate with risk and outcomes in breast cancer (Linderholm et al. 2000a, 2001). Carriers of the C936T allele in the VEGF gene were more frequent among controls than among a cohort of breast cancer patients, implying a protective effect for carriers of the variant polymorphism (Krippel et al. 2003) and even improved metastasis-free survival time in patients with low-grade breast cancer (Krippel et al. 2004). Several studies found an inverse correlation between VEGF expression and overall survival in both node-positive and node-negative breast cancer (Gasparini 1997, 2000, Gasparini et al. 1999, 2005, Linderholm et al. 2000b). Increased VEGF and VEGF receptor expression is also associated with impaired response to tamoxifen or chemotherapy in patients with advanced breast cancer (Foekens et al. 2001, Ryden et al. 2005). Recently, VEGF and VEGF receptor expression has been successfully quantified via immunohistochemistry in breast cancer tumor specimens (Ragaz et al. 2004), the expression and intensity of expression correlating with a significantly inferior clinical outcome (Meunier-Carpentier et al. 2005).

One of the key features of VEGF is that it can promote permeability in tissues resulting in hyperemia (Dvorak et al. 1995). There is evidence that hormone replacement therapy increases breast tissue density and it is possible that some of these effects arise due to the hyperemic effects of estrogens via the production of VEGF, which may become more pronounced with the inclusion of progestins (Noh et al. 2005). Indeed, recent evidence suggests that progesterone may have effects on breast vaculature (Thomas et al. 2003). Thus, it is likely that both estrogens and progestins have direct effects on both normal and neoplastic cells as well as the resident endothelial cells, via their respective receptors. In addition, there is a possibility that a paracrine mechanism exists that could also arise from the production of angiogenic growth factors in breast tissue following hormonal treatment. It has been proposed that such paracrine mechanisms might even allow proliferation of tumor cells that lack the capacity to produce their own angiogenic growth factors in response to steroid hormones but which respond to VEGF produced from other cells, since they may retain the necessary cell-surface receptors (Liang & Hyder 2005). Further research is required to establish if such regulatory loops occur in vivo, although recent reports indicate the possibility that this process may operate in real clinical conditions. Thus, targeting both the angiogenic ligand and the receptors would seem to be the most comprehensive strategy for better controlling tumor proliferation.
Angiogenic growth factors in breast cancer

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 & Rosen 1997, Zetter 1998, Ferrara 1999, Nicosia & Villaschi 1999). However, we still do not know the overall extent of various angiogenic growth factors produced by malignant breast cells and whether there is a master regulator that should be targeted for therapy. Recent research has shown that indeed breast cancer cells and breast cancer biopsies do exhibit several angiogenic growth factors (Engels et al. 1997a,b, de Jong et al. 1998). One should be cautious when investigating ‘angiogenesis’ and angiogenic growth factors, since it is entirely possible that angiogenesis may proceed with no change per se in the levels of pro-angiogenic growth factors. However, the production of angiogenesis inhibitors may be blocked, or receptors for angiogenic growth or inhibitory factors may be reduced, as recently described in estrogen-dependent stimulation of angiogenesis in breast cancer (Elkin et al. 2004). In this case, sVEGFR-1, which binds and neutralizes the actions of VEGF, is decreased, thus creating a more favorable angiogenic environment for tumor progression. Despite this complexity, two factors emerging as key regulators of angiogenesis in most systems are VEGF (Ferrara 2005, Hicklin & Ellis 2005) and basic fibroblast growth factor (bFGF) (Cronauer et al. 1997, Yazidi et al. 1998). The detailed effects of sex steroids on bFGF in breast cancer cells remain to be elucidated; consequently, this review will concentrate on VEGF and its receptors, which, to date, have received most attention from endocrinologists, especially since recent clinical trials have shown it to be a good candidate for anti-angiogenic therapy (Ferrara et al. 2005, Jain et al. 2006). However, readers should be aware that other angiogenic proteins exist which play a role in breast cancer, although their modulation by steroid hormones is poorly understood. These include, but are not limited to, insulin-like growth factor-I and its receptor (Kucab & Dunn 2003) and interleukin-8, which is regulated by estrogen in breast cancer cells (De Larco et al. 2001, Lin et al. 2004).

VEGF and vascular permeability factor

VEGF is a multi-functional cytokine, originally identified as a protein produced by tumor cells that increased the permeability of capillaries to proteins (Dvorak et al. 1995, Ferrara et al. 2003a). It was subsequently discovered to be selectively mitogenic for endothelial cells and to stimulate angiogenesis (Ferrara & Davis-Smyth 1997, Ferrara 1999). This factor has been referred to in the literature as vascular permeability factor (VPF), VEGF, or vasculotropin. VEGF is expressed in both epithelial and mesenchymal cells in a wide variety of tissues, and it is highly expressed in many tumors, including breast cancers (Goldberg & Rosen 1997, Ferrara 1999, Neufeld et al. 1999, Ferrara et al. 2004a,b, Carmeliet 2005, Hicklin & Ellis 2005).

Both the human and murine VEGF genes contain eight exons and seven introns, and differential exon splicing generates up to six isoforms of VEGF (Neufeld et al. 1999). The best characterized are the four predominant human forms, containing 121, 165, 189 and 206 amino acids, and corresponding forms with one fewer amino acid in the mouse (Ferrara 1999). In most systems, VEGF 121 and VEGF 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. 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 heparinas or plasmin (see Ferrara & Davis-Smyth 1997). VEGF 165, the major isoform, is a basic homodimeric protein of 45 kDa which binds heparin. 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 & Davis-Smyth 1997).

The promoter for human VEGF has been extensively analyzed (Ferrara 1999, Pages & Pouyssegur 2005). 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 that are known to be involved in regulating the stability of mRNA. From the point of view of this review, both functional estrogen and progesterone responsive
regions have been located in the VEGF gene (Hyder et al. 2000a, Mueller et al. 2000, 2003, Buteau-Lozano et al. 2002, Stoner et al. 2004, Wu et al. 2005a). Interestingly, a recent study using the chromatin immunoprecipitation procedure reports additional ER sites on the rat VEGF promoter and suggests that these mediate the estrogen response (Kazi et al. 2005). However, in our studies, we have found that not all sites that bind steroid receptors (as demonstrated by chromatin immunoprecipitation studies or using gel shift assays) are necessarily functionally active (Hyder et al. 1995, J Wu & SM Hyder, unpublished data). Thus, receptors may have additional functions yet to be determined on the VEGF promoter. Further studies are needed to confirm the exact locations of functional ER- and PR-binding sites on the VEGF promoter, although irrespective of their location, both hormones have a profound effect on VEGF induction, especially in the uterus (Cullinan-Bove & Koos 1993, Hyder et al. 1996). Their effects in breast cells are controversial as will be discussed below.

A number of VEGF-related genes referred to as VEGF B, C, D, E, and placental growth factor have been identified (Eriksson & 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 (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 the members of another family that may have different activities. Interestingly, there are recent reports of a naturally occurring VEGF isoform, termed VEGF 165b, that is a negative regulator of cellular functions promoted by VEGF 165, including angiogenesis (Woolard et al. 2004). VEGF 165b has the same number of amino acids as VEGF 165 but six amino acids in the COOH terminal region usually coded for by exon 8 are different. The COOH terminal of VEGF 165 is necessary for determining mitogenic signaling, therefore changes in this region are believed to influence its functions. The new open reading frame is termed exon 9 (Woolard et al. 2004). Unlike the other VEGF isoforms, which stimulate angiogenesis, VEGF 165b is an endogenous inhibitory form of VEGF, which decreases VEGF-induced proliferation and migration of endothelial cells. Although it can bind to VEGFR-2 (Flk-1/KDR), VEGF 165b binding does not result in receptor phosphorylation or activation of downstream signaling pathways (Woolard et al. 2004). No information is available on the regulation of VEGF 165b by sex steroids, and the presence and role of VEGF 165b in breast cancer remains to be established. Since VEGF 165b may be an important player in both tumor angiogenesis as well as normal tissues, there is an urgent need to confirm the early reports by other investigators.

Regulation of VEGF is under both transcriptional and translational control (Akiri et al. 1998). While sex steroids have been shown to induce transcription of the VEGF gene (Hyder et al. 1996, 2001), the effect of hormones on post-transcriptional regulation of VEGF in breast cancer cells remains to be explored. Recent studies, however, suggest that a post-transcriptional event is indeed likely (Wu et al. 2004, 2005b). There may be several points of interaction between sex steroids and VEGF regulation and I will briefly review those factors that may be important for future consideration in the field of breast cancer. Hypoxia, one of the most potent inducers of VEGF mRNA synthesis, induces a powerful transcriptional activator, hypoxia-inducible factor (HIF)-1α, which binds to a hypoxia-responsive region on the VEGF promoter and induces gene transcription (Hicklin & Ellis 2005). HIF-1α is upregulated in breast cancer and serves as a prognostic factor (Bos et al. 2001, 2005). It has been suggested that estrogens may involve the HIF transcription factor for VEGF induction in uterine cells (Kazi et al. 2005). However, hypoxia has been shown to reduce ER and ER function in breast cancer cells (Kurebayashi et al. 2001), clouding the issue of the role of HIF in ER function in the uterus, although it is possible that HIF effects may be tissue specific. VEGF is also regulated by several growth factors and cytokines, as well as oncogenes and tumor suppressor genes (Rak et al. 1995, Hicklin & Ellis 2005), many of which are frequently observed in breast cancer. Much focus has centered on the role of tumor suppressor p53 in VEGF regulation since elevated levels of VEGF promote tumor progression and p53 is implicated in the molecular pathology of many solid malignancies. It has been shown that direct interaction of the p53 protein with the transcription factor Sp1 prevents transcriptional activation of the VEGF promoter in breast cancer cells (Pal et al. 1998, 2001, Milanini-Mongiat et al. 2002). Several studies have also shown that genetic alterations of tumor suppressor genes, such as p53, can lead to induction of additional factors such as HIF-1α (Blagosklonny et al. 1998), leading to increased VEGF production and angiogenesis. Similarly, we recently discovered that loss of wt p53 allows PR to induce VEGF in breast cancer cells (Liang et al. 2005). This novel observation is further strengthened by the fact that transfection of wt p53 into breast cancer cells expressing mtp53 can suppress progesterone-dependent VEGF release from tumor.
cancers (Speirs & Atkin 1999, Nakopoulou et al.) and epithelial and stromal cell types of breast carcinomas (Hicklin & Ellis 2005). Subsequently, they established how steroid receptors interact with p53 and the VEGF promoter. Elucidation of these mechanisms is essential if we are to better understand the role of p53 in the induction of angiogenically active VEGF.

The post-transcriptional regulation of VEGF is a well-studied area of research. A protein known as eukaryotic initiation factor 4E (eIF4E) plays a major role in the translation of VEGF mRNA (Kevil et al. 1996). eIF4E, a 25 kDa mRNA cap-binding phosphoprotein, is the rate-limiting factor responsible for delivering cellular mRNAs to the eIF4F complex that facilitates ribosome loading and mRNA translation (Mamane et al. 2004). eIF4E has been shown to be upregulated in many tumors with an associated increase in VEGF message (Nathan et al. 1997, De Fatta et al. 1999). The role of sex steroids in controlling the translational factors that control angiogenic growth factor mRNA is not well established (see Neufeld et al. 1999, Ortega et al. 1999, Hicklin & Ellis 2005).

VEGF receptors

Receptors for VEGF are members of the tyrosine kinase family and were initially identified on endothelial cells (Hicklin & Ellis 2005). Subsequently, they have also been observed on non-endothelial cells, including epithelial and stromal cell types of breast cancers (Speirs & Atkin 1999, Nakopoulou et al. 2002, Liang & Hyder 2005), although reports are limited regarding the role and regulation of VEGF receptors by sex steroids in breast cancer cells. It should be noted that VEGF receptors have been shown to function differentially (Hicklin & Ellis 2005), implying that if differential regulation by sex steroids occurs, then it is likely that different functions can be assigned to VEGF receptors during hormone-induced proliferation of breast cancers. Readers are referred to several excellent reviews for a detailed summary of our understanding of VEGF receptors (Eriksson & Alitalo 1999, Neufeld et al. 1999, Ortega et al. 1999, Hicklin & Ellis 2005). A discussion of some of the salient points follows.

There are three members of the VEGF receptor family: VEGFR-1 (Flt), VEGFR-2 (Flk/KDR), and VEGFR-3 (Flt4). All three are receptor-type tyrosine kinases, which are expressed primarily, though not exclusively, by endothelial cells. The first two bind the classical VEGF (VEGF A) and the latter interacts with VEGF C and D. At present, the mitogenic actions of VEGF are thought to be mediated primarily by VEGFR-2. Several other forms of VEGF receptors have been identified, but their physiological functions have not been well established (see Neufeld et al. 1999, Ortega et al. 1999, Hicklin & Ellis 2005).

VEGF receptors are crucial for embryonic development. VEGFR-1 binds the ligand with a greater affinity than VGEFR-2. An alternatively spliced form of VEGFR-1, lacking the seventh immunoglobulin-like structure and both the transmembrane and cytoplasmic domains, has been identified in HUVEC cells. Since this receptor binds VEGF with high affinity and prevents VEGF-induced mitogenesis, it may represent a negative regulator of angiogenesis. Autoradiographic studies have localized these receptors to both proliferating and quiescent endothelia. Gene knockout 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 within endothelial cells. VEGF receptors have diverse biological roles, including 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 (Hicklin & Ellis 2005). 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. The role of neuropilin and its regulation by sex steroids is poorly understood, both in prostate and breast cancer cells and requires further analysis.

Limited information is available on the signal transduction mechanisms for VEGF receptors and, apart from a few cases, selected binding of downstream signaling molecules to one or the other receptor has not been demonstrated. A number of proteins are phosphorylated following the interaction of VEGF with its receptors. Occupancy of VEGF receptors leads to autophosphorylation as well as phosphorylation of other effectors, including phospholipase C, the GTPase activating protein Ras-GAP and the adaptor proteins Nck and Shc. Also, both receptors possess the Grb2-binding site. Both Grb2 and Shc could potentially induce the Ras pathway, however, the involvement of Ras in the VEGFR signaling pathway has not been 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, vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression, and hexose transport (Pekala et al. 1990, Ferrara 1999). Special note should be made of VEGFs’ ability to induce fenestrations in capillaries in vivo (Roberts & Palade 1995), which is reminiscent of estrogenic effects in the female rodent reproductive tract (Martin et al. 1973). Whether such effects occur in the mammary gland remains to be determined, although they would provide a probable explanation for the increased breast density seen following hormone replacement therapy (HRT) (Colacurci et al. 2001, Erel et al. 2001). If such an effect can be shown in the breast, it may explain breast tenderness and increased mammographic density, which occur as a result of HRT.

Several important differences have recently been reported between the two VEGF receptors (reviewed in Hicklin & Ellis 2005). For example, when VEGFR-2 is expressed in cells and exposed to ligand, a strong ligand-dependent tyrosine phosphorylation of the receptor occurs. In contrast, VEGFR-1 does not show such an intense signal. It is therefore possible that the intense signal, which occurs with VEGFR-2, may be required for eliciting a full response spectrum for mitogenic events to occur. VEGFR-1 has been shown to interact with the p85 subunit of phosphatidylinositol 3-kinase implicating higher levels of phosphatidylinositol involvement in cell signaling pathways, although it is not known whether this is a receptor selective effect. VEGFR-2 has been shown to associate with the protein tyrosine phosphatases SHP1 and 2 suggesting that these two proteins may be involved in some form of signal modulation. However, it is not known if these proteins also interact with VEGFR-1. It has been shown that the immigration of monocytes in response to VEGF only occurs through VEGFR-1-induced signaling (Barleon et al. 1996). Most interestingly, VEGF-treated cells containing VEGFR-1 have been shown to contain the phosphorylated Src family members, lyn and yes. These proteins, however, are not phosphorylated in VEGFR-2-containing cells, indicating that subtle differences may exist in the signal transduction pathways of these two receptors. The role of VEGF receptors in breast cancer remains to be explored since it is likely that they affect processes such as angiogenesis, tumor cell motility, invasion, survival and metastasis. Emerging evidence supports such roles (Price et al. 2001, Nakopoulou et al. 2002, Ryden et al. 2003, Wedam et al. 2006).

Sex-steroid regulation of VEGF and VEGF receptors in breast cancer

As discussed earlier, 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 (Gasparini 1997, 2000, Gasparini et al. 2005). 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. 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, McLeskey et al. 1998, Ryden et al. 2005). The data with respect to the effects of estrogens on regulation of VEGF and its receptors in breast cancer are emerging in the literature, although a clear picture is still not available (Hyder & Stancel 2000, Elkin et al. 2004). Limited data are, however, available on the regulation of VEGF and its receptors by progestins in breast cancer. There is now considerable evidence that, compared with women treated with estrogens or placebo, those undergoing HRT containing a progestin component, develop tumors more readily (Ross et al. 2000, Writing Group for Women’s Health 2002, Chlebowski et al. 2003), leading to renewed interest in the role of progesterone in breast cancer (Moore 2004). It is possible that human tumors may expand under the influence of progestins, and in rodents progestins have been shown to be associated with tumor expansion (Pasqualini 2000, Lanari & Molinolo 2002, Schairer 2002, Connelly et al. 2003).

Given the importance of estrogens and progestins in the regulation of breast cancer, and the clear evidence that angiogenesis plays an 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 context. This is clearly an important area for further study, especially since many reports show that these hormones regulate VEGF expression in normal estrogen and progestin target tissues (Cullinan-Bove & Koos 1993, Hyder et al. 1996, 2001, Hyder & Stancel 1999).
Estrogen regulation of VEGF and VEGF receptors in breast cancer cells

Recent studies have shown that VEGF acts as a growth stimulator and a proliferation factor for mammary cancer cells (e.g. Liang & Hyder 2005, Schoeffner et al. 2005). In addition, it has been shown that estrogens can regulate VEGF in mammary and uterine cells (Hyder et al. 1996, Nakamura et al. 1996, 1999) and that the proliferation rate of the breast epithelium peaks in the luteal phase of the menstrual cycle (Anderson et al. 1982, Soderqvist et al. 1997). Taken together, these findings suggest that estradiol in combination with progesterone likely stimulates the proliferation rate of breast cells and potentially might also promote angiogenesis in the normal and neoplastic breast tissue accompanying the steroid-dependent increase in tissue mass (as recently discussed in prostate) (Folkman 1998).

While it has been shown that many human breast cancers are under hormonal regulation, data are surprisingly limited regarding the regulation of VEGF and its receptors by sex-steroid hormones in breast cancer. Zhang et al. (1995) 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 into nude mice compared with non-transfected MCF-7 cells. Similarly, Brodie’s group (Nakamura et al. 1996) showed that estradiol regulates the expression of VEGF in estrogen-responsive 7,12-dimethyl-1,2-benz[a]anthracene (DMBA)-induced rat mammary tumors, and the levels of extracellular VEGF have been shown to increase in response to estradiol in murine mammary cancer cells (Dabrosin et al. 2003a). Garvin et al. (2005) showed that estradiol elevated VEGF levels in human breast cancer cells while simultaneously decreasing sVEGFR-1, thus increasing the angiogenic milieu. The latter effects were suppressed by tamoxifen. An estrogen-responsive element in both the rat and human VEGF gene has been identified (Hyder et al. 2000a, Mueller et al. 2000, Buteau-Lozano et al. 2002, Stoner et al. 2004) that interacts with both ERα and ERβ. While estrogens consistently induce VEGF at transcriptional levels in uterine tissue in a receptor-dependent manner (Hyder et al. 1996, 1997), and the gene contains an estrogen responsive region, the response is not so robust in human breast cancer cells (Hyder et al. 1998). In fact, a number of investigators have failed to demonstrate such an effect; others show only a modest response (Hyder et al. 1998, 2001, Ruhola et al. 1999, Bermont et al. 2001, Buteau-Lozano et al. 2002, Stevens et al. 2003, Mirkin et al. 2005), and even inhibition of VEGF mRNA by estradiol in breast cancer cells has been reported (Bogin & Degani 2002), although the latter results should be interpreted with caution since very high levels of estradiol were used (30 nM) and only a semi-quantitative method was utilized to reach this conclusion. It has also been reported using transient transfection assays that ERβ represses VEGF promoter in breast cancer cells, although whether such an effect occurs in a cell line that expresses high levels of ERβ remains to be determined (Buteau-Lozano et al. 2002). Sengupta et al. (2003) provide evidence that induction of VEGF by estradiol in MCF-7 breast cancer cells is a biphasic event, with increases in VEGF message at 2 and 24 h post-estrogen treatment, although not 6 h after estrogen administration. However, a biphasic effect is not observed in an in vitro DMBA-induced mammary tumor model, in which VEGF induction occurs by 2 h in response to estradiol and reaches a peak after 6–8 h, before declining by 24–48 h (Nakamura et al. 1996).

It is likely that wide variations in in vitro VEGF induction within breast cancer cells will arise as a consequence of different lengths of exposure to the hormone, variations in hormone concentration used and alterations in cellular characteristics (e.g. endogenous transcription factor levels) which might occur due to various growth conditions used for cells in culture (Hyder 2002). It has been proposed that HIF-α may influence estrogen-dependent increases in VEGF message in the rat uterus (Kazi et al. 2005). A similar mechanism may exist in breast cancer cells and fluctuation of such factors (or ER itself) may dictate estrogen-dependent VEGF induction (Coradini et al. 2004). Interestingly, studies suggest that post-transcriptional events might also influence estrogen-dependent release of VEGF from human breast cancer cells, an effect that is suppressed by tamoxifen (even though tamoxifen increases both mRNA and protein levels of VEGF in MCF-7 cells). This may explain why tamoxifen causes regression of ER-containing tumors (Garvin & Dabrosin 2003), while at the same time actually increasing VEGF levels in breast cancer cells (Ruhola et al. 1999, Wu et al. 2004). A recent study showed that estrogen acts as an angiogenic switch in breast cancer cells by downregulating soluble VEGFR-1 (s-flt) which is believed to sequester available VEGF, although whether this is a direct or an indirect effect of hormone remains to be determined (Elkin et al. 2004, Garvin et al. 2005). It has been suggested that loss of s-flt provides an increase in the net balance towards estrogen-dependent angiogenesis in breast cancer cells.
In contrast to what we know regarding the role of sex steroids in controlling VEGF and angiogenesis in cancer cells, little is known about how angiogenesis is regulated in normal human breast tissue. VEGF mRNA levels were found to be increased in the mammary gland of baboons in the luteal phase of the cycle, when estradiol and progesterone levels are elevated (Greb et al. 1997). Dabrosin (2003, 2005b) showed that in healthy women, bio-available VEGF levels fluctuate in normal human breast tissue during the same (luteal) phase of the menstrual cycle, when estradiol and progesterone levels are high; local extracellular levels of VEGF in the breast were doubled compared with the follicular phase, while plasma levels of VEGF did not show a cyclic variation. These results suggest that sex steroids affect VEGF production in normal breast tissue, although it is not yet clear whether this is an effect of estradiol alone or whether progesterone is also involved. Addition of progesterone to estradiol in tissue culture experiments with normal breast biopsies did not alter the estrogen-induced VEGF levels (Dabrosin 2005b). Importantly, it has not been proven whether VEGF in the normal breast can be associated with the initiation or progression of tumors. Other evidence that sex steroids may regulate VEGF in the breast comes from observations that premenstrual breast tenderness and edema is a common symptom in women and studies suggest that VEGF may be involved in mechanisms that cause premenstrual mastalgia (Ader et al. 2001).

Progestin regulation of VEGF and VEGF receptors in breast cancer cells

As is the case with estrogen, the effects of progesterone on angiogenesis and VEGF expression in breast cancer are poorly understood. Consequently, we have scant information concerning the possible role of progesterone and its synthetic derivatives on angiogenesis and/or regulation of VEGF. Both animal studies and human clinical trials indicate a role for progestins in increasing the risk of breast cancer (Ross et al. 2000, Writing Group for Women’s Health 2002, Chlebowski et al. 2003, Chen et al. 2004). Recent epidemiological trials have rejuvenated the interest of the scientific community and there is an impetus to better understand the role of synthetic progestins in breast cancer, particularly in the area of progestin induction of angiogenic growth factors, which likely leads to tumor expansion (Hyder et al. 2001). Since many progestins have biological activities which differ from the natural hormone, studies are underway to better understand the role of various synthetic derivatives in regulating angiogenic growth factors in breast cancer cells. A connection between possible direct regulation of VEGF expression by progesterone was first described by Cullinan-Bove and Koos (1993) in the rat uterus and a role of progesterone in expansion of mammary tumors has been described (reviewed in Lanari & Molinolo 2002). We investigated the role of this class of hormone in angiogenic growth factor regulation in breast cancer. We showed that both natural and synthetic progestins used in HRT induced VEGF in breast cancer cells (Hyder et al. 1998, 2001, Liang et al. 2005), observations which have since been confirmed by other investigators (Mirkin et al. 2005). Progesterone response elements have been identified on the human VEGF promoter although it seems that the VEGF promoter is utilized in a cell-specific manner and different regions are involved in directing VEGF synthesis (Mueller et al. 2003, Wu et al. 2005a). In the case of breast cancer cells, the region that promotes VEGF induction involves the Sp-1 region since mutation in this region abolishes progestin induction of VEGF promoter (Wu et al. 2005a). This signifies that Sp-1 and PR may cooperate in producing this response (as was also found to be the case for estrogen-dependent induction of VEGF in ZR-75 breast cancer cells) (Stoner et al. 2004).

There is evidence that PR isoforms are important factors in VEGF induction in breast cancer cells; PR-B is a more potent inducer of VEGF than PR-A (Wu et al. 2004), suggesting that PR-B-rich tumors are likely to grow larger than tumors containing predominantly PR-A. Support for this hypothesis was provided in a recent study which demonstrated that larger tumors formed when PR-B-containing cells were grown in vivo (Sartorius et al. 2003). Furthermore, it appears that both anti-estrogens and anti-progestins induce VEGFs from breast cancer cells that are rich in PR-B, implying that anti-hormones are recognized differently based on the PR isoforms present (Wu et al. 2004, 2005b). Thus tumors with a high content of PR-B may be best treated by a combination of anti-hormones and anti-angiogenic agents since human tumors are heterogenous and may exhibit various combinations of steroid receptors and receptors for VEGF.

We showed that all synthetic progestins tested induced VEGF in breast cancer cells, via both phosphatidyl inositol 3 (PI3)-kinase and MAP-kinase pathways (Hyder et al. 2001, Liang et al. 2005). Interestingly, VEGF gene regulation occurred in a cell- and ligand-dependent manner (Wu et al. 2005a). Inhibitors of the PI3-kinase pathway suppressed both cellular VEGF expression and protein release. MAP-kinase inhibitors, however, were unable to inhibit gene
transcription in certain cells, while still suppressing protein secretion, suggesting that VEGF can be regulated at both transcriptional and post-transcriptional levels by progestins. Intriguingly, progestin-dependent induction of VEGF was blocked by the anti-estrogen ICI 182,780 at both the protein and transcriptional level, suggesting that there may be cross-talk mechanisms involved in such regulation between the two receptors (Hyder & Stancel 2002), a situation analogous to the previously described regulation of the c-src pathway (Ballare et al. 2003).

Using a small series of breast cancer cells, we recently showed that progestin-dependent induction of VEGF occurs only in PR-containing cells that either lack p53 or possess a mutation in the p53 gene (Liang et al. 2005). Progestin-dependent induction did not occur in cells that contained wild-type p53 protein or in cells that were transfected with the wild-type p53 expression plasmid (Liang et al. 2005). Furthermore, we demonstrated that progestin-induced VEGF was angiogenically active, inducing not only endothelial cell proliferation but also proliferation of breast cancer cells via autocrine and paracrine mechanisms as long as the recipient breast cancer cells exhibited VEGFR-2 (Liang & Hyder 2005). These observations lend further support to our hypothesis that increased risk of breast cancer due to progestin exposure may involve progestin-dependent proliferation of pre-neoplastic lesions together with cells possessing a mixture of PR-containing and PR-negative cells, as long as the latter contain VEGF receptors. Further support that progesterone can promote increased growth rates of breast tumors comes from studies in p53 knockout mice that were treated with DMBA (Medina et al. 2003), and we recently showed that progestins increase VEGF expression and accelerate tumors in a rat model of DMBA-induced tumorigenesis in a development stage-dependent manner (Benakanakere et al. 2005). These findings underscore the importance of the p53 tumor suppressor in keeping tumor growth and expansion under the influence of steroid hormones.

While we have information concerning the presence of VEGF receptors in breast cancer cells, our understanding of their regulation by progestins is lacking and requires further research. The picture which is emerging however, is that in breast cancer cells, regulation of the angiogenic pathway, which involves VEGF and VEGF receptors, is under the control of both estrogens and progestins. Currently, very little is known about the molecular mechanisms that regulate such expression, or the biological consequences which arise following induction or suppression of VEGF and VEGF receptors in breast tissue.

**VEGF and anti-hormone resistance**

As discussed above, both estrogens and progestins induce VEGF in breast cancer cells through their respective receptors and via characterized hormone response elements. Anti-estrogens and anti-progestins cause some hormone-dependent tumors to regress; however, some tumor cells invariably become resistant to anti-hormones and continue to grow (Gutierrez et al. 2005). In certain cases, anti-hormones can even stimulate tumor growth (Clarke et al. 2001). It is not known what specifically causes the resistant cells to continue to proliferate, though it has been suggested that growth factors may be involved (Manders et al. 2003, Nicholson et al. 2005). Interestingly, clinical studies have shown that tumors with high levels of VEGF fail to respond to hormone therapy or have an early recurrence (Foekens et al. 2001, Manders et al. 2003), suggesting that VEGF production may be responsible for anti-hormone resistance. These studies also re-affirm that VEGF may also be responsible for tumor cell proliferation as reported previously (Liang et al. 2005). In this respect, it is interesting to note that many reports indicate that hormone-dependent tumors secrete VEGF in response to either estrogens or progestins (Borgstrom et al. 1999), which might provide a partial explanation for the growth-promoting effects of steroid hormones in breast cancer as well as resistance to anti-hormones. Indeed, our recent data indicate that exposure of breast cancer cells to VEGF can override the effects of anti-hormone (Y Liang, R A Brekken & S M Hyder, unpublished observations), suggesting that a treatment regimen of both anti-hormones and anti-angiogenic agents may be better for tumor suppression than a single regimen alone. In certain cases, anti-hormones have themselves been shown to induce VEGF, suggesting that the nature of the ligand may be altered in a cell- and perhaps receptor isoform-dependent manner (Wu et al. 2004); both anti-progestins and anti-estrogens can induce VEGF in breast cancer cells that express high levels of PR-B (Wu et al. 2005b). For example, anti-estrogens can induce VEGF in MCF-7 cells (Ruhola et al. 1999, Wu et al. 2004, 2005b). Transcriptional and post-transcriptional effects may both be involved in elevating VEGF levels in breast cancer cells. Support for this notion was obtained recently with the identification of hormone response elements in VEGF as well as the analysis of VEGF production from tumor cells with altered levels of receptor isoforms (Wu et al. 2005b).
Potential sex-steroid targets for anti-angiogenic therapy of breast cancer

Angiogenesis is an extremely complex process, which involves many different cell types, all of which must coordinate their growth patterns to establish a well-defined vasculature for nourishment of either normal or tumor tissue. In the latter case, the vasculature can be quite haphazard (Ferrara & Kerbel 2005). Although this review concerns itself chiefly with our understanding of the role played by sex steroids in the control of VEGF in tumor epithelial cells, other cellular and molecular targets which might lead to an overall loss of angiogenic potential of tumor tissue should be considered as potential therapeutic or preventive strategies in breast disease. We need to better understand the role of sex steroids in the stromal compartment of tumor tissue and also in the endothelial cells in breast tumors. While endothelial cells in general do not show any genetic abnormality there are some recent reports that heterogeneity may exist in these cells between normal and tumor tissues (Ribatti et al. 2002). The role of sex steroids may therefore be different in such endothelial cells; there is evidence that endothelial cells do contain estrogen and PRs (Losordo & Isner 2001, Otsuki et al. 2001), although their role in tumor biology is not clear. Similarly, it is not known whether the stroma surrounding the tumor tissue may also be sensitive to anti-hormone. Thus, both endothelial cells and the stromal compartment in and around the tumor may also be targets for anti-hormones in addition to the classically considered epithelial cells.

A number of proteins with anti-angiogenic potential remain to be studied with regard to sex-steroid regulation of breast cancer cell metabolism. These include proteins such as soluble VEGFR-1 (sVEGFR-1), interferon inducible protein 10 (IP-10), endostatin, mammary serine protease inhibitor (maspin), TSP-1 and CD36, the receptor for TSP-1 (reviewed in Dabrosin 2005a). sVEGFR-1 is an alternative spliced form of VEGFR-1 and sequesters VEGF produced from tumor cells, thereby reducing the angiogenic environment. It has been reported that estrogen down regulates the expression of sVEGFR-1 in ER+ breast cancer cells and that this decreased expression is associated with a significant increase in angiogenesis (Elkin et al. 2004). However, a direct role for estrogens or ER in sVEGFR production has not been reported, although such a regulation could be important in designing anti-hormonal therapy of breast disease. Evidence that hormones can have an effect on sVEGFR-1 has come from the observation that during pregnancy, serum levels of sVEGFR-1 are elevated compared with postpartum levels (Koga et al. 2003). The biological significance of this is not known, but it is possible that sVEGFR-1 may be needed to antagonize excessive effects of VEGF on vasculature. Interferon-IP-10 seems to be a strong inhibitor of angiogenesis in vivo and is controlled by interferon (IFN)-γ (Angiolillo et al. 1995). Estradiol increases IFN-γ production and an estrogen-responsive element in the IFN-γ promoter has been found (Fox et al. 1991). In breast cancer, IP-10 was shown to be involved in continued progression of tumor growth and metastasis (Dias et al. 1998, Reome et al. 2004). Since IP-10 is upregulated by IFN-γ, there is a possibility that sex steroids could influence IP-10 production. It has been reported that exposure of murine mammary epithelial cells to estrogen may decrease the release of local chemokines and disrupt an IP-10-stimulated release of these chemokines from the cells (Aronica et al. 2004). However, in a study of normal breast tissue, in vivo IP-10 did not vary during the menstrual cycle, suggesting that sex steroids have minor effects on IP-10 secretion in the normal breast (Dabrosin 2003). Its role in breast cancer remains to be explored.

Recent studies in tumor biology indicate that TSP-1 and one of its receptors (CD36) possess anti-angiogenic capacities (Armstrong & Bornstein 2003, Simantov & Silverstein 2003). However, the role of TSP-1 is controversial and estrogens seem to induce this factor in breast cancer cells, indicating that it may have additional functions in the breast (S M Hyder, unpublished observation). Consequently, raising TSP-1 levels in breast cancer cells by exogenous means may first appear to be counterintuitive. The role of TSP-1 in proliferation of breast cancer cells has been well studied and it seems that the anti-angiogenic properties of this protein could be context-dependent, perhaps depending on the cleavage product formed as a result of other factors produced from cells, since inhibition of TSP-1 in mouse models prevents the proliferation of tumors (Wang et al. 1996). In addition, it has been shown that CD36, a receptor for TSP-1 that mediates the apoptotic effects of TSP-1 in endothelial cells, is downregulated by estrogens in breast tumor cells (Uray et al. 2004). This may explain why tumor cells proliferate in response to estrogens but do not undergo apoptosis as a result of the TSP-1 they simultaneously secrete, although the exact relationship between TSP-1 and CD36 remains to be explored in breast cancer cells. There are indications that TSP-1 may also be under sex-steroid control in other tissues besides breast cancer cells (Mirkin & Archer 2004). This raises the important question of whether or not it is a good idea to increase endogenous levels of proteins presumed to be anti-angiogenic, in the absence of crucial information.
as to whether such an action might have harmful as opposed to the desired anti-angiogenic effects.

As with TSP-1, the role of maspin also seems to be context dependent. Maspin was originally identified as a tumor suppressor protein that blocks primary tumor growth as well as invasion and metastasis (Shi et al. 2001). Overexpression of maspin in breast tumor cells has been shown to limit their growth and metastases in vivo and it has been demonstrated that maspin is an effective inhibitor of angiogenesis (Zhang et al. 2000b). It has been suggested that maspin is down-regulated during cancer progression, corresponding to loss of steroid receptors as tumors progress, although one study shows that increased expression of maspin correlates with an aggressive phenotype in breast cancer patients (Umekita et al. 2002). This seemingly contradictory result may be explained by a different biological activity of maspin, which is dependent on its subcellular distribution, since nuclear maspin staining appears to be a good prognostic factor, while cytoplasmic staining is associated with a poor prognostic marker (Mohsin et al. 2003). To date, few studies have investigated the effects of sex steroids on maspin expression, although much remains to be learned in this context. Tamoxifen increases secretion of maspin, whereas maspin secretion was reduced by estradiol in the myoepithelial cells surrounding the breast epithelium (Shao et al. 2000). It has also been demonstrated that tamoxifen induces maspin promoter activity, acting via ERα (Khalkhali-Ellis et al. 2004, Liu et al. 2004). However, correlation of maspin and sex-steroid receptor location in breast cancer needs to be further elucidated.

A number of other proteins have been identified which might offer potential targets for triggering anti-angiogenic events in breast cancer, although so far they have received little attention. For example, the angiopoietins (Ang) belong to a family of growth factors, including Ang1, Ang2, Ang3 and Ang4, which can exert both agonist and antagonist action effects on the endothelial receptor tyrosine kinase Tie2 (Metheny-Barlow & Li 2003, Lee et al. 2004) and thereby function as pro-angiogenic or anti-angiogenic factors. For a further discussion on this topic, please refer to Dabrosin (2005a).

Finally, it is becoming increasingly clear that endothelial precursor cells (EPC) play a considerable role in tumor biology. These cells originate in the bone marrow, can be detected in the peripheral circulation and are incorporated into sites of ongoing angiogenesis where they differentiate into mature endothelial cells (Asahara et al. 1997, Lyden et al. 2001). VEGF has a potential role in such recruitment and there is evidence for EPC recruitment in breast cancer cells (Asahara et al. 1999, Shirakawa et al. 2002a, b, Spring et al. 2005). It has been reported that EPCs decline after treatment with anti-angiogenic therapy (Capillo et al. 2003) thus making these cells targets for such therapy (Rafi et al. 2002). However, to date, there is no information available on the role of estrogens or progesterins in causing EPCs to mobilize from bone marrow to the target site within the breast. Since EPCs could be targets for preventing tumor expansion and/or angioprevention, it is only a matter of time before studies are reported that address this issue in detail. Considerable effort will be required in this area to understand and establish the role of hormones and anti-hormones in influencing EPC mobilization and whether such mobilization is indeed involved in hormone-dependent incorporation of EPCs in new blood vessels.

Conclusions

Coordinated regulation of angiogenesis and vascular permeability is essential for the physiological functioning of any tissue such as the male and female reproductive tracts. Major health problems such as breast cancer, dysfunctional uterine bleeding, endometriosis, uterine cancer, prostate cancer and 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 regulation of VEGF in breast cancer cells and on the vasculature of the reproductive tract in general, although the overall picture on sex-steroid regulation of VEGF remains confusing. Moreover, far less is known about the molecular mechanisms that regulate these important actions. There is, therefore, an immense need for mechanistic studies in the area of angiogenesis in order to allow us to better understand the role of sex steroids in the production of angiogenic and naturally existing anti-angiogenic factors. This would likely lead to improved treatment and to a better understanding of how to develop prevention strategies geared to preventing tumors arising in the reproductive tracts of both men and women. The role of angiogenesis in breast cancer is now well recognized and with the recent success of clinically administered angiogenesis inhibitors (Morabito et al. 2004, Moses et al. 2004, Jain 2005, Ryden et al. 2005), the use of such compounds for “angioprevention” of breast cancer would seem to be on the horizon. It should be mentioned that a number of anti-angiogenic approaches are being taken in the field of breast cancer. It is an extremely exciting time to be working in this field of research and to explore how such treatment protocols might influence angiogenic
processes and arrest the growth of breast tumors. It will also be important to establish how hormones and anti-hormones influence the angiogenic balance in human tumors and to ascertain whether such alterations are dependent on polymorphisms in various genes related to angiogenesis. It remains to be established whether the area of angiogenesis, as it pertains to breast cancer, is ready for the application of a pharmacogenomic approach by which we might predict how individuals will respond to hormones and anti-hormones. Furthermore, it is imperative that we understand what controls the hormone-controlled angiogenic ‘switch’, since doing so will give us the potential to control or even eradicate breast tumors and gain a much-needed victory over this insidious and deadly disease.

Acknowledgements

I would like to apologize to those investigators whose work could not be cited due to the extent of this review. Our studies on the role of hormones on angiogenic growth factors in breast cancer cells were initially started in collaboration with Dr George Stancel at the University of Texas in Houston, whose contributions, support and advice were invaluable to the author. Thus, it would be appropriate to dedicate this review to his name. I am grateful to talented individuals in the laboratory, both past and present, who have been instrumental in obtaining the exciting data reported elsewhere and discussed herein. These include Drs Yayun Liang, Indira Benakanakere, Jianbo Wu, Sandra Brandt, Ms Jennifer Schnell, Ms Constance Chiappetta and Ms Lata Murthy. I am also grateful to various collaborators for their contribution; in particular to Drs George Stancel, Zafar Nawaz and Cynthia Besch-Williford. I would like to thank Dr Simon Slight for critically reading the manuscript and to Ms Schnell for compiling the references reported in the review.

Funding

The following sources are acknowledged for the support of studies on angiogenesis: NIH grants CA-86916 and HD-08615, the Susan G Komen Breast Cancer Foundation, American Heart Association, Fleming/Davenport Award from the Texas Medical Center in Houston, Department of Defense breast cancer concept award (W81XWH-05-1-0416), and COR grants from the College of Veterinary Medicine at the University of Missouri, Columbia. SMH is the Zalk Missouri Professor of Tumor Angiogenesis, and is grateful to Ms Thelma Zalk for her generous gift to the College of Veterinary Medicine, University of Missouri. Ms Zalk’s gift was instrumental in allowing us to make key discoveries related to the role of progestins and angiogenic growth factors in breast cancer. The author declares that there is no conflict of interest that would prejudice the impartiality of this scientific work.

References


Augustin HG 2005 Angiogenesis in the female reproductive system. EXS 94 35–52.


Chen WY, Hankinson SE, Schnitt SJ, Rosner BA, Holmes MD & Colditz GA 2004 Association of hormone replacement therapy to estrogen and progesterone receptor status in invasive breast carcinoma. Cancer 101 1490–1500.


Eskens FALM 2004 Angiogenesis inhibitors in clinical development; where are we now and where are we going? British Journal of Cancer 90 1–7.


Ferrara N 2005 The role of VEGF in the regulation of physiological and pathological angiogenesis. EXS 94 209–231.


Fox HS, Bond BL & Parslow TG 1991 Estrogen regulates the IFN gamma promoter. Journal of Immunology 146 4362–4367.


Huh JI, Calvo A, Stafford J, Cheung M, Kumar R, Philip D, Kleinman HK & Green JE 2004 Inhibition of VEGF receptors significantly impairs mammary cancer growth in C3(1)/Tag transgenic mice through antiangiogenic and non-antiangiogenic mechanisms. *Oncogene* 24 790–800.


Expression of growth factors, growth inhibiting factors, and their receptors in invasive breast cancer. I: An inventory in search of autocrine and paracrine loops. 
*Journal of Pathology** 184 44–52.


Nicolson RI, Hutcheson IR, Hiscox SE, Knowlden JM, Giles M, Barrow D & Gee JMW 2005 Growth factor signaling and resistance to selective oestrogen receptor modulators and pure anti-oestrogens: the use of anti-growth factor therapies to treat or delay endocrine resistance in breast cancer. Endocrine-Related Cancer 12 S29–S36.


Zhang HT, Scott PA, Morbidelli L, Peak S, Moore J, Turley H, Harris AL, Ziche M & Bicknell R 2000a The 121 amino acid isoform of vascular endothelial growth factor is more strongly tumorigenic than other splice variants in vivo. *British Journal of Cancer* 83 63–68.
