Vascular endothelial growth factor and endometriotic angiogenesis

J. McLaren

Preterm Birth Research Group, Department of Obstetrics and Gynaecology, University of Leicester, Faculty of Medical and Biological Sciences, CSB, PO Box 65, LRI, Leicester LE2 7LX, UK

Peritoneal endometriosis is a significant debilitating gynaecological problem of widespread prevalence. It is now generally accepted that the pathogenesis of peritoneal endometriosis involves the implantation of exfoliated endometrium. Essential for its survival is the generation and maintenance of an extensive blood supply both within and surrounding the ectopic tissue. The vascular endothelial growth factor (VEGF) family of angiogenic molecules is involved in both physiological angiogenesis, and a number of pathological conditions that are characterized by excessive angiogenesis. Increasing evidence suggests that the VEGF family may also be involved with both the aetiology and maintenance of peritoneal endometriosis. Sources of this factor include the eutopic endometrium, ectopic endometriotic tissue and peritoneal fluid macrophages. Important to its aetiology is the correct peritoneal environment in which the exfoliated endometrium is seeded and implants. Established ectopic tissue is then dependent on the peritoneal environment for its survival, an environment that supports angiogenesis. Our increasing knowledge of the involvement of the VEGF family in endometriotic angiogenesis raises the possibility of novel approaches to its medical management, with particular focus on the anti-angiogenic control of the action of VEGF.

Key words: angiogenesis/endometriosis/VEGF

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Introduction

Endometriosis, defined as the presence of tissue histologically similar to endometrium at sites outside the uterine cavity, is one of the commonest benign gynaecological conditions to affect women of reproductive age. It is estimated to be present in 10–25% of women presenting to gynaecologists in the UK and USA (Strathy et al., 1982). This disease affects women during their reproductive years and represents a major clinical problem with deleterious social, sexual and reproductive consequences. Although this is a common disease we have a relatively poor understanding of its pathogenesis. The current and most widely accepted hypothesis for the development of peritoneal endometriosis is the transplantation of exfoliated endometrium (Thomas and Prentice, 1992). This disease is essentially oestrogen dependent (Bergqvist, 1992) with its standard treatment being the reduction of the steroid hormones; unfortunately this can have serious implications for fertility. Ideally, therefore, we would require a treatment which could specifically target either its occurrence or control its development without unduly affecting other processes such as fertility. Central to the survival and growth of this tissue is the establishment of an effective blood supply (Nisolle et al., 1993). This review will focus on the role of angiogenesis in active peritoneal endometriosis in particular the part played by the vascular endothelial growth factor (VEGF) family. In addition, possible therapeutic interventions to prevent its action will be discussed.

Endometriosis

Appearance

Peritoneal endometriosis can have multiple appearances, classified as early, advanced or healed. Early lesions, which are considered to be the most active, appear as pink-red lesions and...
are characterized by intensive vascularization or haemorrhage (Figure 1). They usually contain glands and stroma, with no fibrosis. The area surrounding the lesion also displays increased vascularization and these lesions are thought to be the most active. The most severe forms of endometriosis are seen with deeply infiltrating ectopic tissue with pelvic pain as a common symptom (Cornillie et al., 1990; Koninckx et al., 1991). Advanced lesions are characteristically blue–black in

Figure 1. Red endometriotic papules. (Taken from Shaw, 1993 and reproduced with the permission of Parthenon Publishing Press).

Figure 2. Powder burn lesions overlying uterovesicle fold. (Taken from Shaw, 1993 and reproduced with the permission of Parthenon Publishing Press).

Figure 3. Hypervascularization in the cul-de-sac. (Taken from Shaw, 1993 and reproduced with the permission of Parthenon Publishing Press).
appearance; these lesions have less glands and stroma and are less active than early lesions (Figure 2) and are often described as powder burns.

Aetiology

The precise aetiology of endometriosis is still not clearly defined. However, it has been suggested that inheritance, race, exposure to ovarian steroids, infertility and peritoneal soiling may contribute to an individuals propensity to develop this disease (McLaren and Prentice, 1996; Tsaltas et al., 1998). Little evidence exists that race, age, social class or personality types are associated with this disease. Factors which affect the individuals exposure to endogenous oestrogen (Cramer et al., 1986) and increased menstrual soiling of the pelvis can influence the development of endometriosis (Liu and Hitchcock, 1986).

Histogenesis

There are numerous theories proposed for the histogenesis of endometriosis. The current and most widely accepted hypothesis for the development of endometriosis is the transplantation of exfoliated endometrium

Transplantation theory

This theory was put forward by Sampson (1927) in which endometriosis is due to the implantation of the retrograde endometrium, seeded in the peritoneal cavity by means of retrograde menstruation. The advances in our understanding of the pathogenesis of endometriosis, have recently been concerned with the investigation of the properties of eutopic/ectopic endometrium (Smith, 1998, Rogers et al., 1998, Sillem et al., 1998) and the peritoneal environment (Koninckx et al., 1998) in which the endometriotic tissue finds itself. Recent progress has included a clearer understanding of the mechanisms responsible for implantation (Regidor et al., 1998), vascularization (Nisolle et al., 1993), and the survival of the ectopic tissue in what is essentially an immunologically aggressive environment (Hill, 1992).

It is now generally accepted that the pathogenesis of peritoneal endometriosis involves, in part, the implantation of exfoliated endometrium which is present through retrograde menstruation (Thomas and Prentice, 1992). The phenomenon of retrograde menstruation commonly occurs in ~90% of women undergoing laparoscopy (Halme et al., 1984), however, only ~10% of women develop symptomatic endometriosis. This suggests that other factor(s) determine the susceptibility of an individual to implantation and growth of this tissue. These could include genetic susceptibility (Lamb et al., 1986), alterations in menstrual effluent (Cramer et al., 1986), differences in the peritoneal environment (Ramey and Archer, 1993), or changes in immunological tolerance (Oosterlynck et al., 1991). Regardless of these factors, central to the establishment and maintenance of endometriosis are the successful adherence, implantation, survival and growth of the exfoliated tissue. This sequence of events will depend on the role of certain adhesion molecules, proteolytic enzymes and most critically the establishment of an effective blood supply. The latter process will involve the generation of new blood vessels (angiogenesis) which requires the complex control of such divergent processes as endothelial cell proliferation, migration and permeability, as well as the involvement of cell adhesion molecules and proteolytic enzymes (Folkman and Shing, 1992).

Cell adhesion molecules

Adhesion of exfoliated cells to the peritoneal wall could result in the development of endometriosis (Van den Linden et al., 1994, 1995). Cell adhesion molecules, most notably the integrins and cadherins, are the two main mediators of cell–cell and cell–matrix adhesion (Kim and Yamada, 1997), and their expression on cells and tissue may be important for the initial adhesion of the exfoliated tissue. Studies show that integrins and cadherins are found on the endometrium, menstrual effluent and endometriotic samples (Van den Linden et al., 1994, 1995; Bridges et al., 1995; Regidor et al., 1998). Therefore the ability of the ectopic tissue to express integrins after retrograde menstruation, would suggest their potential to establish cell–cell and cell–matrix interactions with the surrounding peritoneum.

Proteolytic enzyme activity

The proteolytic digestion of the extracellular matrix (ECM) following initial attachment could be important for the successful implantation of the ectopic tissue (Sillem et al., 1998; Salamonsen, 1999). Two families of proteolytic enzymes are implicated in this process (Rodgers et al., 1994): (i) serine proteases (plasminogen/plasmin activation system); and (ii) matrix metalloproteinases (MMP). Expression of these enzymes occurs within the eutopic endometrium, with increased expression of MMPs in endometrial stroma during menstruation (Osteen et al., 1994; Rawdanowicz et al., 1994; Rodgers et al., 1994). The presence of these enzymes in exfoliated endometrium may be important in the establishment of endometriotic tissue within the peritoneal environment and in the determination of its invasive potential. Evidence suggests that ECM remodelling is occurring in endometriosis since studies by Spuijbroek (1991) have detected increased concentrations of procollagen type II fragments in the peritoneal fluid of women with endometriosis, and active red lesions express MMP-1 throughout the cycle (Kokorine et al., 1997). In endometriosis plasminogen and plasminogen activator (uPA) have been detected in higher concentrations in ectopic than in eutopic tissue (Sillem et al., 1998). MMP-3 and the MMP inhibitor, tissue inhibitor of matrix metalloproteinase-1.
Angiogenesis

Central to the establishment, survival and growth of endometriotic implants is the establishment of an effective blood supply, the development of which is termed angiogenesis (Folkman and Shing, 1992). Angiogenesis is a complex process involving a number of different but co-ordinated functions that must be undertaken before this process can be fulfilled. These include the proliferation, migration and extension of endothelial cells, adherence of these cells to ECM, remodelling of the ECM and ultimate formation of a new lumen (Folkman and Shing, 1992). This process is required in a number of different physiological processes notably, embryogenesis (Breier et al., 1992). In women, profound non-pathological angiogenesis is seen during corpus luteum formation (Ravindranath et al., 1991) and the menstrual cycle (Smith, 1998). This is unusual since normally vasculature is in a state of quiescence resulting from the balance between inhibitors and activators. Disruption of this balance in favour of excessive angiogenesis results in conditions such as rheumatoid arthritis, diabetic retinopathy, psoriasis and cancer (Hanahan and Folkman, 1996). It is now recognized that other mechanisms exist for the creation of new blood vessels, these include intussusception (Burri and Tarek, 1990) and vessel elongation (Ausprunk et al., 1974).

Angiogenesis and endometriosis

The peritoneal environment is highly angiogenic with increased angiogenic activity demonstrated in peritoneal fluid from women with endometriosis (Oosterlynck et al., 1993) and increased amounts of angiogenic factors (Koninckx et al., 1998). Laparoscopic examination of endometriotic tissue demonstrates this tissue deriving blood from the surrounding peritoneum (Shaw, 1993; Figure 3). In addition, extrapelvic endometriosis is found in well-vascularized sites, e.g. lung, skin and muscle. Angiogenesis is under the control of a number of inducers, including fibroblast growth factor (FGF), hepatocyte growth factor, transforming growth factor (TGF) α and β and inhibitors, such as angiostatin, endostatin and thrombospondin (Smith, 1998). Of particular importance is one family of glycoproteins, the VEGF family, which are being seen as increasingly significant in processes characterized by both physiological and pathological angiogenesis (Smith, 1998; Leenders, 1998).

Vascular endothelial growth factor

The VEGF family at present consists of five main members (for reviews see Ferrara and Davis-Smyth, 1997; Achen et al., 1998; Carmeliet and Collen, 1998; Eriksson and Alitalo, 1999): (i) VEGF-A (Leung et al., 1989); (ii) VEGF-B (Olson et al., 1994); (iii) VEGF-C (Joukov et al., 1996; Lee et al., 1996); (v) VEGF-D (Orlandini et al., 1996; Achen et al., 1998); and (v) placental growth factors (PLGF) (Maglione et al., 1991). A brief summary of the more important properties of each member of this family will be given below. Although PLGF is a member of this family it will not be considered here (for a review, see Persico et al., 1999).

VEGF-A

The original name for VEGF-A was vascular permeability factor (VPF), because of its ability to induce vascular permeability in guinea-pig skin (Dvorak et al., 1995). It is a 34–46 kDa glycoprotein which is a highly specific mitogen for vascular endothelial cells (Ferrara and Davis-Smyth, 1997). VEGF-A monomers are linked together by two disulphide bridges to form the homodimer.

The human VEGF-A gene, which is located to chromosome 6p21.3 (Vincenti et al., 1996) comprises eight exons separated by seven introns (Houck et al., 1991; Tischer et al., 1991), and alternative splicing of the VEGF-A gene results in the generation of at least five splice variants (isoforms), each containing 121, 145, 165, 189 and 206 amino acids respectively (Charnock-Jones et al., 1993; Ferrara, 1999). VEGF-A165 is the predominant variant produced by a variety of normal and transformed cells, with VEGF-A121, 189 being found in most cells that express the VEGF-A gene. VEGF-A145 and VEGF-A206 are more restricted in their expression, with the VEGF-A206 form only being identified in a human fetal liver cDNA library (Houck et al., 1991). VEGF-A165 and VEGF-A189 bind to heparin and heparin sulphate proteoglycans, whereas VEGF-A121 does not (Houck et al., 1991; Poltorak et al., 1997). VEGF-A121 is a freely soluble protein, whilst VEGF-A165 is also secreted but a certain proportion remains bound to the cell surface of ECM, whilst both VEGF-A189 and VEGF-A206 are almost completely bound to the ECM (Ferrara, 1999). VEGF-A expression is induced by hypoxia (Carmeliet and Collen, 1998).

VEGF-B

VEGF-B is a 44% identical amino acid sequence with VEGF-A, forms disulphide-linked homodimers and, like VEGF-A, exists as isoforms predominately those of 167 and 186 amino acids, arising from alternative RNA splicing (Gimmond et al., 1996). It is strongly expressed in the heart of the developing embryo and in adult cardiac and skeletal muscle (Gimmond et al., 1996; Olofsson et al., 1996a). VEGF-B167
binds to heparin, whilst VEGF-B<sub>186</sub> is freely secreted from cells and is mitogenic for endothelial cells (Olofsson et al., 1996b). The gene expression of VEGF-B overlaps that of VEGF in a number of tissues and can heterodimerize with VEGF-A (Olofsson et al., 1996a). However, unlike VEGF-A, VEGF-B is not induced by hypoxia (Enholm et al., 1997).

**VEGF-C**

VEGF-C exhibits ~30% identity to VEGF-A, and induces vascular permeability and endothelial mitogenesis in vitro (Joukov et al., 1996). It is induced by pro-inflammatory cytokines but not hypoxia (Enholm et al., 1997; Ristimaki et al., 1998) and high concentrations can induce an angiogenic response in the chick chorioallantoic membrane model. In general, VEGF-C induces a lymphangiogenic response and unlike VEGF-A and VEGF-B, is secreted as a mature protein, therefore previous proteolytic processing must have occurred, and will thus regulate the activity of VEGF-C since only fully processed VEGF-C activates VEGF receptors (Joukov et al., 1996).

**VEGF-D**

VEGF-D was first described as a 'c-fos-induced growth factor' (Orlandini et al., 1996; Eriksson and Alitalo, 1999) but was renamed VEGF-D due to its functional properties (Achen et al., 1998). VEGF-D shares similar receptor-binding specificities to VEGF-C (Achen et al., 1998), and similar proteolytic processing. VEGF-D is mitogenic for endothelial cells in vitro and its gene expression is induced by the transcription factor c-fos (Orlandini et al., 1996b).

**VEGF receptors**

Two principle VEGF receptor kinases have been identified (for reviews see Terman et al., 1992; Shibuya et al., 1999; Figure 4). The VEGFR-1 (fms-like tyrosine kinase) (Vries et al., 1992) and VEGFR-2 (kinase domain receptor; KDR) (Terman et al., 1992), both bind the VEGF family with high affinity. Murine version of KDR (fetal liver kinase, Flk-1) shares 85% sequence identity with human KDR. VEGFR-1 and 2 have seven immunoglobulin (Ig)-like domains in the extracellular domain, a single transmembrane region and a consensus tyrosine sequence which is interrupted by a kinase-insert domain (Shibuya et al., 1990; Terman et al., 1992). VEGFR-1 has a higher affinity for rhVEGF<sub>165</sub> (kDa 10–20 pM (Vries et al., 1992) than VEGFR-2 (kDa 75–125 pM) (Terman et al., 1992). The VEGF receptors 1 and 2 are expressed predominately on endothelial cells (Jakeman et al., 1992, but are also found on non-endothelial cells including peripheral blood monocytes (Shen et al., 1993), malignant melanoma cells lines (Gitay-Goren et al., 1993), ovarian carcinoma tumour cells (Bocock et al., 1995), trophoblast (Charnock-Jones et al., 1994) and peritoneal fluid macrophages (McLaren et al., 1996a).

VEGFR-1 is indispensable during embryonic development, since VEGFR-1 knock-out mice die due to disruption of blood vessel formations (Fong et al., 1999a). However, in adults VEGFR-1 is only weakly phosphorylated by the VEGF family and its biological significance is unclear. The VEGFR-2 receptor, on the other hand, if blocked inhibits tumour angiogenesis and growth and therefore it is this receptor which is thought to be responsible for mediating the effect of VEGF in the tumour angiogenic process (Millauer et al., 1994).

A soluble form of VEGFR-1 (sVEGFR-1) exists which binds VEGF-A with high affinity (kDa 1–20 pM), and has been located in a number of tissues, including human endometrium (Krussel et al., 1999) and placenta (Clarke et al., 1998). This soluble form may negatively regulator of the action of VEGF (Kendall et al., 1996) on two levels: firstly, it may bind and sequester VEGF, thereby reducing its bioavailability, and secondly, by occupying the VEGF receptors it will prevent VEGF occupancy and the resulting signal transduction.

VEGFR-3 (Pajusola et al., 1992; Taipale et al., 1999) is not a receptor for VEGF-A or B but rather binds VEGF-C, (Joukov et al., 1996; Lee et al., 1996). VEGF-D can also bind to and activate VEGFR-2 and 3 (Achen et al., 1998).

**VEGF-A function**

VEGF-A is pleiotropic in function (for reviews see Ferrara and Davis-Smyth, 1997; Achen et al., 1998) but is recognized predominately as a potent inducer of endothelial cell proliferation (Ferrara and Davis-Smyth, 1997) and angiogenesis (Kim et al., 1993). VEGF-A also increases the expression of fibrillar collagen degrading matrix metalloproteinases in human umbilical vein endothelial cells (HUVEC), and is capable of inducing vascular permeability (Dvorak et al., 1987; Donnez et al., 1998). Dvorak et al., 1995, 1987) proposes that vascular permeability is a crucial step in angiogenesis due to formation of extravascular fibrin gel, which is a substrate for endothelial and tumour cell growth. VEGF-A also increases the expression of vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) in endothelial cells (Melder et al., 1996) and the promotion of monocyte chemotaxis (Clauss et al., 1990). VEGF-A may also facilitate tumour growth by allowing the tumour to avoid the induction of an immune response (Gabrilovich et al., 1996).

**Pathological angiogenesis**

VEGF-A is involved in pathological conditions that are dependent on excessive angiogenesis. These include proliferative retinopathy (Adamin et al., 1994), rheumatoid arthritis (Houck et al., 1991) and psoriatic skin (Detmar et al., 1994). In a number of human tumours there is also a positive correlation
between VEGF-A expression, vascular density and degree of malignancy (Takahashi et al., 1995; Viglietto et al., 1996).

Endometriosis and VEGF

**Eutopic endometrium**

Throughout the menstrual cycle extensive vascular remodeling occurs, principally involving steroid sensitive spiral arteries and sub-epithelial capillary plexus (for reviews see Healy et al., 1998; Smith, 1998). Ovarian steroids and in particular a number of angiogenesis promoting growth factors are known to regulate changes in the vascularization of the human endometrium. These include platelet-derived growth factor (PDGF), acidic and basic FGF, α and β TGF and tumour necrosis factor (TNF) (Smith, 1998). In addition, VEGF-A has been demonstrated in human endometrium (Charnock-Jones et al., 1993; Shifren et al., 1996; Donnez et al., 1998). Cyclic changes in the distribution of VEGF-A mRNA expression throughout the cycle are observed with an increased expression occurring during the secretory phase and during menstruation (Charnock-Jones et al., 1993). Within the eutopic tissue (Shifren et al., 1996, 1998) has shown that the ovarian steroids (oestrogen) can up-regulate the expression of mRNA encoding for VEGF-A in in-vitro cultures of eutopic endometrium. This data taken together with the observed cyclical changes indicates that VEGF-A is a steroid-sensitive angiogenic factor in endometrium. Using both immunohistochemical staining and in-situ hybridization experiments, VEGF-A has been localized in the luminal and glandular epithelium in the proliferative phase as well as the stroma (Charnock-Jones et al., 1993; Li et al., 1994; Shifren et al., 1996), with the expression remaining in the epithelial cells but not in the stroma during the secretory phase (Charnock-Jones et al., 1993; Torry and Torry, 1997). This data would suggest that retrograde menstruation expresses VEGF-A, indeed menstrual effluent does contain high values of VEGF-A expressing glandular cells.

The expression of VEGF-A in the menstrual effluent would suggest this tissue has the potential to undergo angiogenic blood vessel development and that abnormalities in this may contribute to the development of endometriosis. Donnez et al., 1998 has shown that VEGF-A content was significantly higher in the eutopic glandular epithelium of endometriotic patients during the late secretory phase. Wingfield et al., 1995 showed that elevated endothelial cell proliferation rates were related to endometrial-related disorders such as endometriosis. Studies have also shown that the ligation of integrin α5β3 is required for survival and maturation of newly-formed blood vessels (Brooks et al., 1994) and there is general support for this as an angiogenic marker (Sepp et al., 1994). It is suggested that the endometrium from women with endometriosis has enhanced proliferation and increased ability to implant and survive in ectopic locations (Healy et al., 1998). Therefore, even before it has entered the peritoneal cavity, differences in the retrograde endometrium, linked to angiogenesis and VEGF-A, may confer an increased susceptibility to implant, suggesting that the eutopic endometrium itself plays a critical role in the histogenesis of endometriosis.

**Ectopic endometrium**

Peritoneal ectopic tissue of differing appearances has been analysed for VEGF-A expression and these studies have confirmed the presence of VEGF-A in glandular epithelium, stromal cells and activated macrophages within the stroma (McLaren et al., 1996a; Shifren et al., 1996; Donnez et al., 1998). A difference in the expression was seen between different types of lesion with the early, highly vascularized lesions having a greater expression of VEGF-A than the later more inactive black powder burn lesions (McLaren and Prentice et al., 1996; Donnez et al., 1998). VEGF-A expression was also increased in lesions taken from women with severe compared to mild endometriosis. The authors show a similar VEGF-A expression in red lesions as that seen in eutopic endometrium and suggest that this is another argument in favour of the transplantation theory and that the red lesions should be considered as the first stage of endometriosis (Donnez et al., 1998). The expression of VEGF-A was also related to the expression of MMP-1 (Donnez et al., 1998). The presence of VEGF-A in ectopic tissue implies a role in the maintenance of the vascularization essential for it continued survival. The higher expression in the red lesions is probably a reflection of increased role in maintaining a higher degree of vascular development which is often more visible in the surround peritoneum (Figure 3).

**Peritoneal fluid**

The cellular (peritoneal fluid macrophages) and biochemical constituents (angiogenic growth factors) of the peritoneal fluid are increasingly being seen as important in endometriosis. It is in these areas that the majority of the most recent advances in our understanding of the pathogenesis of endometriosis have been made (for reviews see Ramey and Archer, 1993; Koninckx et al., 1998).

The peritoneal fluid bathes the peritoneal cavity, in which is found the majority of endometriotic lesions, and can have a direct effect not only on the ability of the ectopic tissue to implant but also its survival, the degree of vascularization and extent of the disease (Ramey and Archer, 1993; Koninckx et al., 1998). In recent years, our understanding of the constituents of the peritoneal fluid has increased (McLaren et al., 1996b; Koninckx et al., 1998; Kupker et al., 1998). Peritoneal fluid contains a wide range of growth and angiogenic factors, some of which are elevated in endometriosis (Ramey and Archer, 1993). Increased angiogenic activity is seen in vivo in endo-
VEGF and endometriotic angiogenesis

Metriotic patients (Oosterlynck et al., 1993). These factors are important in the proliferation and vascularization of endometrial tissue as well as the recruitment and activation of the peritoneal macrophages. Elevated concentrations of the VEGF-A have been identified in peritoneal fluid of women with endometriosis (McLaren and Prentice et al., 1996), with the highest levels seen during the proliferate phases of the cycle, a time at which the peritoneum is exposed to the retrograde endometrium. A positive correlation exists between the severity of endometriosis and the concentration of VEGF-A in peritoneal fluid (Shifren et al., 1996). Work is continuing on further elucidating the constituents of the peritoneal fluid and any differences that may be evident in endometriosis.

Peritoneal fluid macrophages

There is increasing evidence to suggest that many of the growth/angiogenic factors found in the peritoneal fluid originate from peritoneal macrophages (Olive et al., 1991; Chao et al., 1995; McLaren et al., 1996a) and that activated macrophages and macrophage-derived factors play a critical role in the induction of neovascularization (Sunderkotter et al., 1994). Macrophages, as well as being an important cell in cellular immunity, can be viewed as a dispersal secretory organ capable of synthesising and secreting a large number of products (Auger and Ross, 1992). It is well known that in endometriosis there are increases in the number of peritoneal macrophages, possibly as a consequence of low grade inflammation or directly caused by endometriosis in women (Halme et al., 1983). Women with endometriosis have high chemotactic activity for macrophages in their peritoneal fluid (Weil et al., 1997) from factors, e.g. monocyte chemotactic protein-1 (MCP-1) (Akoum et al., 1995), and RANTES (regulated on activation and normally T cell expressed and presumably secreted) (Khorram et al., 1993; Hornung et al., 1997). It is also observed that these macrophages have an increased activity and secretory capacity. Activated peritoneal fluid macrophages have been demonstrated to be a potent source of VEGF-A in endometriosis and that this expression is directly regulated by ovarian steroids (McLaren et al., 1996a). These cells were also immunopositive for the VEGF receptors VEGFR-1 and 2 as well as the oestrogen and progesterone receptors (McLaren et al., 1996a). Co-expression of VEGF-A and its receptors raises the possibility of autocrine stimulation and of therapeutic strategies targeting this receptor-ligand interaction. VEGFR-1 expression was constant throughout the cycle in peritoneal fluid macrophages, but VEGFR-2 was up-regulated during the secretory phase, suggesting steroidal regulation of VEGF receptor. This suggests a direct link between VEGF-A, endometriosis and peritoneal fluid macrophage activity.

Anti-angiogenic treatments

The suggested link between endometriotic angiogenesis and VEGF-A opens the possibility of novel approaches to its medical management, in particular the use of anti-angiogenic treatments (for review see Leenders, 1998). Due to the complex nature of the angiogenic process there are a number of potential therapeutic sites of action. These will generally include the intervention at: (i) VEGF-A production; (ii) VEGF receptor binding; (iii) ECM degradation; (iv) endothelial cell proliferation; (v) endothelial cell migration; (vi) capillary tube formation; (vii) ECM synthesis; (viii) metastasis.

Prevention of VEGF-A production, bioavailability and receptor binding provides the most effective and specific routes for successful intervention.

Inhibition of VEGF production

Tumours transplanted into mice following treatment with either VEGF-A oligonucleotides or expression constructs showed greatly reduced vascularization and tumour growth, compared to controls (Saleh et al., 1996, Benjamin and Keshet, 1997). Recent work by Ke et al. (1998) has shown a down-regulation in VEGF-A mRNA expression and protein in glioblastoma multiforma following transfection with expression vectors containing hammerhead ribozymes. These experiments demonstrated the principle of targeted control of VEGF-A expression and its effect in vivo. However, the in-vivo manipulation of endogenous VEGF-A production needs to be achieved if this potential route can be seriously considered as a therapy. A number of workers have capitalized on the significant stimulatory effect of hypoxic conditions, controlled by the cSrc gene, on VEGF-A production, and by using specific antisense expression constructs to cSrc, in HT 20 colon tumour cells, have significantly reduced its expression (Mukhopadhyay et al., 1995).

Figure 4. Diagram of vascular endothelial growth (VEGF) receptors binding different members of the VEGF family.
Bioavailability of VEGF

Another route to preventing the inappropriate action of VEGF-A is through controlling its bioavailability. This can be achieved principally by two methods: (i) sequestration of VEGF-A by soluble FLT-1 receptors (sflt-1), which have a high affinity for VEGF-A; and (ii) using specific neutralizing anti-VEGF antibodies. Studies by Goldman et al. (1998), showed that by transfecting HT-1080 human fibrosarcoma cells with a gene encoding for sflt-1 a significant inhibition in both implantation and growth was seen following transplantation into nude mice. Also Crystal et al. (1999) showed that the regional growth of tumours could be affected following transfection with adenovirus-vectors containing a gene encoding for sflt-1.

A considerable number of reports exist to show the effect of anti-VEGF-A antibodies on tumour growth (Kim et al., 1993; Asano et al., 1995; Brogstrom et al., 1996; Leenders, 1998). Recent work by Lichtenbeld et al. (1999) showed the advantage of local direct applications of an anti-VEGF-A antibody to tumour tissue growth in SCID mice over systemic application. Whilst Asano et al. (1999) undertook a comprehensive evaluation of the effects of the action of the anti-VEGF-A antibody MV833 on 25 tumour cell lines. Their results showed a strong suppression of tumour growth following transplantation into nude mice. This result not only illustrated the effectiveness of the anti-VEGF-A antibody but also demonstrated the global nature of the action of VEGF-A in these tumour cell lines.

VEGF receptor inhibition

Since VEGF receptors are confined to predominately endothelial cells and antagonism of Flt/KDR is sufficient to eliminate tumours in mice (Millauer et al., 1996) targeting VEGF receptors with VEGF receptor antagonists would seem an effective anti-angiogenic treatment. Evidence suggests that compounds such as FR118487 (Mu et al., 1996) and PD98059 (Liu et al., 1999) can inhibit VEGF mediated angiogenesis directly by blocking the receptor or the subsequent signal transduction. Indeed, work by Fong (1999b) have demonstrated the use of the compound SU5416 as a potent and selective inhibitor of the FLK/KDR receptor tyrosine kinase. As described in the previous section soluble FLT can alter the bioavailability of VEGF-A through its sequestration. However, another property of this truncated receptor is its ability to act in a ‘dominant-negative’ manner and interact with VEGF receptors preventing full receptor dimerization and the subsequent signal transduction (Lin et al., 1998). This mechanism would work in tandem with the sequestration of VEGF-A, providing an effective inhibition of the action of VEGF via two routes.

The increased expression of high affinity VEGF receptors in angiogenic blood vessels has been exploited as another possible anti-angiogenic treatment. This is through the specific targeting of these blood vessels by VEGF-toxin conjugates (Asano et al., 1995; Ramakrishnan et al., 1996). Elegant studies by Ramakrishnan et al. (1996) have successfully demonstrated the inhibition of endothelial cell proliferation, the neovascularization of chick chorioallantoic membrane and solid tumour using a VEGF–diphtheria conjugate (DT385).

This review has focused on the hypothesis that endometriosis is a result of excessive angiogenesis and that, based on current literature, VEGF-A is the member of the family which is predominantly involved. To date most of the potential anti-angiogenic therapies have focused on the treatment of the angiogenesis associated with tumour pathogenesis. Although these two conditions will have differences in their aetiology and pathophysiology they do share similarities in the characteristics of pathologic vascular development. In the future, therefore, therapeutic regimens developed and optimized for the treatment of cancer angiogenesis could equally prove useful for treatment of endometriosis.

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