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INVITED EDITORIAL

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The role of angiogenesis in prostate development and the pathogenesis of prostate cancer

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Abstract New vessel formation, a highly-regulated, active process commencing in the embryo and evident notably during the pubertal growth spurt, is essential for normal prostate development. Reactivation of this process in response to physiological stimuli, particularly hypoxia in mature tissues, occurs with new vessels forming principally from stromal components. Although angiogenesis is complex, putatively involving a multitude of angiogenic factors and inhibitors, there is powerful evidence of the importance of the VEGF system in the development of both the normal prostate and prostate cancer. Recent advances include an understanding of how castration acts through the VEGF system to inhibit angiogenesis. Stromal-endothelial and epithelial-endothelial interactions are just beginning to be investigated. A better understanding of how physiological angiogenesis is controlled should help to provide further insights into the mechanism of disregulated angiogenesis in tumours. Ultimately, new antiangiogenic agents are likely to find a role in the management of patients with prostate cancer.

Keywords Angiogenesis · Prostate · Prostate cancer · VEGF

Introduction

Under normal circumstances such as embryo development, reproduction and wound healing, angiogenesis is a highly regulated process characterised by short periods of activity followed by periods of complete inhibition. In contrast, the growth of primary tumours and metastases is characterised by persistent unregulated angiogenesis. Indeed, angiogenesis is a prerequisite for the expansion of solid tumours beyond 1–3 mm^3 [19].

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Normal development of the prostate

Prostatic ducts develop from solid epithelial cords of the endodermal urogenital (UG) sinus that grow into the surrounding UG sinus mesenchyme. This process of branching morphogenesis, whereby solid epithelial cords elongate, branch and then canalise to form ducts surrounded by mesenchyme-derived smooth muscle cells and fibroblasts, is dependent on the production of androgens by the fetal testes [7]. In a series of elegant experiments, Cunha showed that mesenchymal-epithelial interactions modulated by growth factors play a critical role in this process. Androgen receptors are undetectable in fetal prostatic epithelium and UG sinus epithelium is unable to undergo normal prostatic development by itself. However, in the presence of UG sinus mesenchyme and androgens UG sinus epithelium develops into normal prostatic epithelium. Fibroblast growth factors (FGF) such as FGF-7, FGF-10 and members of the transforming growth factor-β (TGF-β) family are thought to be involved in this epithelial-mesenchymal interaction. It is known that branching morphogenesis occurs in a similar fashion in other organs such as the lung, kidney etc. but, in these structures this process is not androgen-dependent.

Vasculogenesis and angiogenesis

The early embryonic vasculature is formed before the heart starts beating by a process termed vasculogenesis. Vasculogenesis is the process whereby the primary vascular plexus is formed by differentiation of angioblasts

from paraxial and lateral plate mesoderm. Once the primary vascular plexus has been formed, further blood vessels are generated by a process termed angiogenesis. These new vessels form either by sprouting from a pre-existing vessel (sprouting angiogenesis) or by the formation of transcapillary pillars that split pre-existing vessels (non-sprouting angiogenesis). In each case, further pruning and remodelling eventually results in a mature vascular system [56]. The exact mechanism of formation of the prostatic vasculature remains unknown but extrapolating from what is known in other organs, it is likely that the intraprostatic vasculature is of angiogenic origin.

Control of angiogenesis

Endothelial cell turnover is low in the normal microvasculature but endothelial cells can undergo rapid proliferation when active angiogenesis is required such as wound healing. Specific molecules both initiate angiogenesis and arrest the process. In certain microenvironments, nonvascular cells such as mast cells, macrophages and fibroblasts can modulate angiogenesis. The major positive angiogenesis molecules are VEGF, bFGF and IL-8 with synergism present with VEGF and bFGF.

Vascular endothelial growth factor (VEGF) is a prominent, if not the most prominent, cytokine responsible for endothelial cell differentiation, migration, proliferation, tube formation and vessel assembly [21,58]. In addition to VEGF, basic fibroblast growth factor (bFGF) also plays an important role. A number of other angiogenic polypeptides have been identified in normal human prostate epithelial cells in vitro including transforming growth factors α and β1 (TGFα, β1), tumour necrosis factor α (TGFα), G-CSF and GM-CSF [6]. The angiogenic molecules are listed in Table 1.

The VEGF system

VEGF, which is the most potent and specific of the angiogenesis-related cytokines, is known to be selectively mitogenic for endothelial cells. VEGF stimulates angiogenesis and has multiple other functions [15]. It is a cytokine that is produced by a wide variety of cell types.

Table 1 Angiogenic molecules

VEGF
bFGF
IL-8
Angiopoientins
Also
TGFα
TGFβ
TNFα
G-CSF
GM-CSF

Five different isoforms of VEGF (VEGF-A, VEGF-B, VEGF-C, VEGF-D and VEGF-E) are generated by alternate splicing of a single gene. Most research has focussed on classical VEGF (VEGF-A) which is considered the most important form in tumour tissues, although other isoforms such as VEGF-B and VEGF-C have been found in tumours [57]. There are three VEGF tyrosine kinase receptors, VEGFR-1 (Flt-1), VEGFR-2 (flk) and VEGFR-3 (Flt4), the first two of which bind classical VEGF. VEGF-induced endothelial cell proliferation and differentiation is mediated by the VEGFR-2 receptor [52].

VEGF has been shown to play a role in normal vasculogenesis and angiogenesis in the embryo [58]. Inactivation of a single allele of VEGF in fetal mice results in impaired blood island formation and angiogenesis with death in utero [16].

BFGF System

Basic FGF is mainly synthesised by fibroblasts within the prostate. It is a multifunctional cytokine and is mitogenic for the growth and differentiation of a variety of cells and organ systems. It is also an angiogenic factor and induces migration, proliferation and differentiation of endothelial cells. In tumours, VEGF stimulates endothelial cells to produce bFGF [15].

Angiopoietins

Angiopoietin-1 and angiopoietin-2 are naturally occurring angiogenic factors that are antagonists for the Tie-2 tyrosine kinase receptor. Angiopoietin-1 is known to induce capillary-like tubule formation and stabilise the vascular network and is a survival factor for endothelial cells when acting in combination with VEGF. Immunohistochemical analysis of angiopoietin-1, angiopoietin-2, Tie-2 expression and localisation has demonstrated that there are significant differences between normal prostate and prostate cancer. Blood vessels in the normal prostate do not express angiopoietin-1 and Tie-2 whereas blood vessels associated with prostate cancer do. This suggests autocrine action of the angiopoietin-1/Tie-2 system in prostate cancer. Angiopoietin-2 is expressed in glandular prostate cancer but not intraductal adenocarcinoma [69].

Regulation of angiogenesis in the prostate by androgens

The acceleration in prostatic growth during adolescence is androgen induced through an enhanced cellular proliferation rate in concert with a low rate of apoptosis. Indeed, the prostate was one of the first structures in which apoptosis was observed following castration [41]. Apoptosis of prostate cells following androgen

deprivation has long been thought to be a direct response by prostate cells expressing the androgen receptor. However, recent studies in the rat ventral prostate showing that prostatic vasculature may be regulated by androgens, have provided an alternative explanation of the mechanism of castration-induced prostate cell apoptosis. For the effects of castration see Fig. 1.

It was shown as early as 1985 that castration reduces endothelial cell numbers and endothelial cell proliferation in the ventral rat prostate with reversal by testosterone therapy [13]. Blood flow in the ventral rat prostate is reduced by 92% 7 days after castration and, in concert with endothelial cell response, this reduction in blood flow can be reversed by testosterone replacement [43]. A series of experiments by Franck-Lissbrant and colleagues provide evidence that the normal prostate is under the control of the vascular endothelium. Analysis of cellular changes 7 days following castration showed reductions in the weights of blood vessel luminal walls and endothelial cells of around 50% so that castration reduces both prostatic blood flow and the volume of blood vessel-related structures. When testosterone was given to castrated rats at day 7, the total weights normalised after only 1–2 days whereas the weights of epithelial and stromal cells took several days to recover. These results strongly suggest that endothelial cell recovery precedes organ regeneration [23]. Larger blood vessels in the rat ventral prostate respond to androgen deprivation by constriction and this has been proposed to be integrally associated with the reduction of nitric oxide synthase and cyclic GMP [33].

It therefore seems likely that post castration apoptosis in the prostate gland might be mediated primarily by the reduction in blood flow that follows androgen deprivation. The absence of androgen receptors on endothelial cells [54] indicates that the effect of androgens

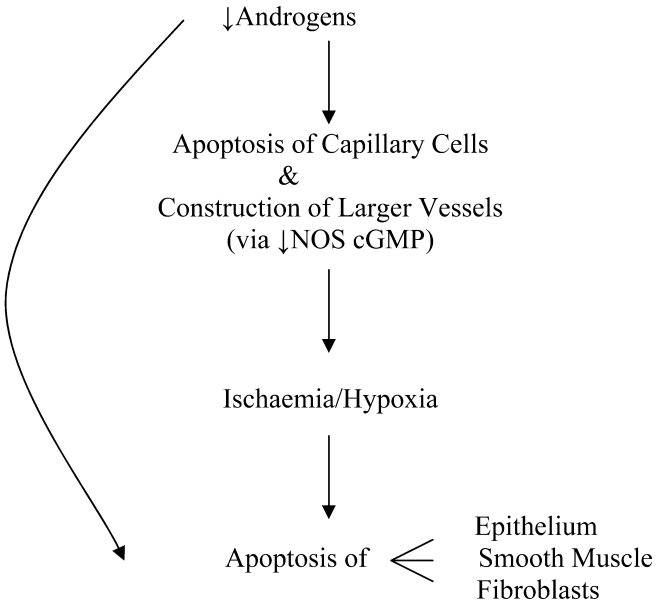

Fig. 1 Effects of castration on the prostate

on the vasculature is most probably mediated through stromal or epithelial cells. It is unknown whether this involves the induction of synthesis of angiogenic factors, reduction of production of angiogenic inhibitors or upregulation of expression of receptors to angiogenic factors present on endothelial cells.

There are a number of potential growth factors known to be present in the prostate and also under androgen regulation. VEGF is expressed by prostatic glandular epithelial cells and is an obvious candidate which has been studied in some detail. Its synthesis in rat prostate epithelial cells has been shown to be downregulated following castration and upregulated by testosterone [29]. In the same model, Burchardt found that VEGF-A is not downregulated until the second day following castration and concluded that it is an unlikely mediator of the immediate vascular response [5]. However, in human prostate fetal fibroblasts VEGF mRNA levels increase within an hour of androgen addition. This effect suggests a possible direct effect of androgens on the VEGF promoter but as yet a functional androgen response element has not been identified. VEGF was also found to be strongly expressed in the stroma of adult benign prostatic hyperplasia and cancer [44].

Cunha established the importance of stromal-epithelial interaction in the prostate, whereby stroma was required to mediate androgenic effects on epithelium. The homeostatic relationship of epithelial and stromal cells is disregulated following malignant transformation of adult epithelial cells, resulting in the loss of control of proliferation and cell death. When initiated prostatic epithelial cells are co-cultured with human prostatic carcinoma associated fibroblasts in vitro and in vivo in a tissue recombination system, the result is an increase in the growth of the epithelial cells with an alteration morphologically. Normal prostatic fibroblasts do not have this effect. Therefore, the carcinoma associated fibroblasts appear to be able to induce progression in the initiated epithelial cells [51].

It seems possible that, as an extension of Cunha's findings, a similar mechanism of androgenic stromal-endothelial interaction may exist.

The prostate is rich in other potent endothelial cell mitogens such as bFGF but this cytokine is actually upregulated by castration and downregulated by testosterone. Growth factors such as TGFβ1 and hepatocyte growth factor are similarly affected by androgens. In contrast, the expression of EGF is downregulated by castration and upregulated by testosterone. Mast cells are involved in angiogenesis in certain environments and are found close to blood vessels in the stroma of the rat prostate.

Angiogenesis and biological aggressiveness

Judah Folkman hypothesised that solid tumours have a prevascular and a vascular phase [20] and this is supported by early studies showing very low blood capillary

ratios on the majority of latent prostate cancers compared with tumours presenting with clinical symptoms [25,66].

Studies measuring microvessel density in a number of tumour types suggest that angiogenesis correlates with biological aggressiveness with many of these new vessels having weak or deficient basement membrane structures. The heterogeneity of prostate cancer and thus the heterogeneity of microvessel density has lead researchers to develop the concept of the "hotspot", the area of greatest density of endothelial cells [68]. The site of greatest microvessel density (MVD) usually occurs at the centre of the tumour. Reproducibility has been demonstrated but it is unclear whether hotspots are representative of the metastatic potential of the tumour. Nevertheless, there seem to be consistent differences in MVD when patients with metastases are compared with those without metastases [66].

Clinicians would like to be able to use MVD as a prognostic biomarker in patients presenting with clinically localised prostate cancer but as yet no compelling evidence has been provided that it can be used in this way. The original study by Brawer et al. used archival tissue from 32 radical prostatectomy specimens and five patients with metastatic disease who had transurethral resections. They found that MVD was an independent predictor of pathological stage in logistic regression analysis [4]. Multivariate analysis is important in assessing the predictive value of MVD because MVD correlates with tumour grade in other studies [32, 66, 68]. MVD proved to be a powerful independent predictor of progression after radical prostatectomy for clinically organ-confined disease [64] even though it was not predictive of pathological stage [59]. Furthermore, in a study of 125 patients with clinically localised prostate cancer managed expectantly and with a median follow up of 15 years, a low MVD was a predictor of disease-specific survival in multivariate analysis [3]. Overall, these studies suggest that there is a correlation between angiogenesis and the development of progressive disease and the metastatic phenotype.

The VEGF system in prostate cancer

VEGF expression

Experiments in rats have demonstrated that androgens regulate VEGF expression not only in the normal prostate but also in prostate cancer. The VEGF content of the ventral rat prostate was reduced to less than 20% within a week of castration and reversed with an eight-fold increase within a week if the animals were given androgens [40]. Similarly, castration resulted in reduced VEGF expression and a reduction in microvessel density in the rat model of prostate cancer [50]. Immunohistochemical expression of VEGF has been shown to be stronger in human prostate cancer than in the normal prostate. Mirroring the finding in rodents, androgen deprivation in humans results in the downregulation of VEGF expression [47]. Stewart et al. found that in the LNCaP human prostate cancer cell line, androgen deprivation resulted in a reduction of VEGF mRNA expression. In mice bearing LNCaP tumours, castration resulted in reduced VEGF expression within 24 h and decreased neovascularisation within 3 days. Tumour regression occurred at about day 8 [63].

VEGF and metastatic potential

There is evidence that VEGF expression is associated with metastatic capability. In Dunning tumours in rats, VEGF mRNA was highest in the metastatic sublines compared with the non-metastatic subline and VEGF protein levels in the serum showed a similar correlation [30]. It has also been shown that plasma VEGF levels are higher in patients with metastatic prostate cancer than in patients with localised disease or healthy controls [12]. By enhancing hyperpermeability of vessels, endothelial cell proliferation and endothelial cell migration, it is easy to envisage how VEGF may facilitate the entry of tumour cells into the circulation and thus be associated with the metastatic phenotype. Although the association warrants further assessment, as yet there is no evidence that plasma VEGF levels may be used as a marker of biological aggressiveness to predict stage or prognosis in a clinically useful way. An alternative potential measure of angiogenic activity might be angiogenic cytokines measured in urine samples. Patients with hormone refractory prostate cancer in a randomised trial of suramin had urine samples prospectively collected and the VEGF and bFGF levels measured. Low pre-treatment urine VEGF levels, but not bFGF levels, predicted improved survival [1].

The importance of VEGF in prostate cancer growth in animal models has further been demonstrated by the use of neutralising antibodies. Monoclonal anti-VEGF antibodies inhibited tumour growth and the development of metastases of DU-145 human prostate tumours in SCID mice [2, 49] (Fig. 2).

VEGF receptors

VEGF receptor mRNA for VEGFR-1 and VEGFR-2 is expressed by all of the sublines of the Dunning tumour

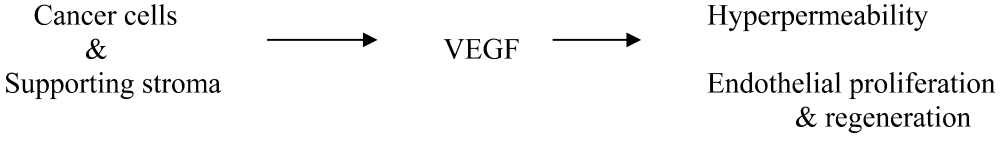

Fig. 2 VEGF actions

[30]. In human tissues, Hahn et al. demonstrated immunohistochemically the expression of the VEGFR-1 receptor in epithelial cells of benign prostatic hyperplasia with a loss of intensity in prostatic intraepithelial neoplasia (PIN) and further loss of intensity in cancer. The lack of supportive mRNA studies meant that it was uncertain whether VEGFR-1 protein staining represented the presence of a functional receptor [31]. However, a recent study has identified a functional VEGFR-1 receptor in a rat prostate cancer cell line and it has been postulated that VEGF may have an autocrine effect on tumour cell proliferation or tumour cell motility in addition to its known angiogenic role [61]. However, non-angiogenic effects of VEGF in prostate cancer remain poorly understood and the reason for the very strong expression of VEGFR-1 protein on BPH epithelial cells compared with prostate cancer is unknown.

VEGF regulation

The genetic changes underlying increased VEGF expression in prostate cancer remain unknown. In renal cell carcinoma, it appears that mutations of the von Hippel-Lindau gene are responsible for disregulation of the VEGF system. There is no evidence that *VHL* mutations are involved in prostate cancer angiogenesis and other candidates such as *RAS* are uncommon in prostate cancer. Mutations of *p53* are a relatively late event and too infrequent to be the dominant factor in regulating VEGF expression [65].

PTEN

Mutations of the tumour suppressor gene PTEN occur in a 5–15% of primary prostate cancers and 30% of those with metastatic disease. PTEN inactivation is associated with tumour progression and angiogenesis [27]. Recently, evidence has been presented showing how PTEN inactivation may lead to increased angiogenesis [71]. VEGF is secreted in response to hypoxia-inducible factor 1α (HIF-1α), which is positively regulated by the Phosphatidylinositol 3-kinase/AKT/FRAP signal transduction pathway. This pathway is negatively regulated by the tumour suppressor PTEN. Demonstration that modulation of the PI3K/AKT/FRAP pathway alters the expression of HIF-1α protein, HIF-1α dependent transcriptional activity and VEGF protein in human prostate cancer cell lines, provides one possible explanation of how VEGF expression and angiogenesis is increased in non-hypoxic conditions in prostate cancer. HIF-1α expression and VEGF mRNA have been found in PIN in the TRAMP model and clinical prostate cancer specimens leading to the proposal that an initiation angiogenic switch is an early event [70]. The same group demonstrated a late progression angiogenic switch related to transition from differentiated to poorly differentiated carcinoma and associated with decreased expression of VEGFR-1 and increased VEGFR-2 [35].

EGF

The mechanism of upregulation of VEGF in hormone-refractory prostate cancer is uncertain. It has been shown that EGF can stimulate a dose-dependent increase in VEGF mRNA in the prostate cancer cell lines PC-3 and DU-145 [55]. Human prostate cancer cells are known to have upregulated autocrine expression of EGF but the significance of these results in vivo remains unclear.

bcl-2

Another potential pathway of angiogenesis control has recently been described. The *bcl-2* oncogene is commonly overexpressed in hormone-refractory prostate cancer and is known to inhibit apoptosis. It was demonstrated that human prostate cancer cell lines transfected with *bcl-2* expressed significantly higher levels of VEGF than control-transfected cells and that these cell lines grew more aggressively as xenografts with increased angiogenesis [14].

Interleukin-8

Interleukin-8, a mitogen for endothelial cells, may also play a role in regulating angiogenesis. Prostate cancer cells express IL-8 and IL-8 expression correlates with metastatic potential [28, 17]. In the PC-3 cell line, IL-8 appears to increase angiogenesis and metastasis through induction of matrix metalloproteinase 0 (MMP-9) expression [36].

Angiogenesis inhibitors

The major angiogenesis inhibitors include thrombospondin-1 (TSP-1), interferon α and interferon β, angiostatin and endostatin (Table 2).

Thrombospondin-1

TSP-1 is a cell matrix adhesion molecule that acts as an inhibitor of angiogenesis and is known to be upregulated by wild-type p53. In a study of 98 patients with localised prostate cancer undergoing radical prostatectomy or TURP, mutant p53 (mp53) was associated with low levels of TSP-1 and increased microvessel density.

Table 2 Major angiogenesis inhibitors

Thrombospondin-1
Interferon α
Interferon β
Angiostatin
Endostatin

Moreover, when mp53, TSP-1 and angiogenesis scores were integrated, the resulting angiogenesis index was an independent predictor of survival in multivariate analysis [48].

Angiostatin

The identification of the angiogenesis inhibitor angiostatin in the Lewis lung carcinoma model offered an example of how the production of an angiogenesis inhibitor by a primary tumour might actually inhibit the growth of metastases. It was found that prostate cancer cell lines were capable of generating angiostatin from plasminogen by expression of serine protease enzymatic activity [26]. Subsequently it was demonstrated that the serine protease, prostate specific antigen, can generate angiostatin-like fragments from Lys-plasminogen in vitro [34]. These findings offer a potential explanation for the relatively indolent course of many prostate cancers. It has been postulated that rising PSA levels in patients with prostate cancer may actually represent a host response to the tumour by production of an antiangiogenic protein. PSA has been found to inhibit the proliferation of cultured endothelial cells and block migration of FGF-2 stimulated endothelial cells. Human PSA was found to reduce the number of lung metastases in the B16BL6 mouse melanoma model [24]. Others have demonstrated that loss of PSA expression in prostate cancer specimens is associated with a higher MVD. However, this finding could be explained if PSA coexists with a non-angiogenic phenotype and does not imply that PSA suppresses angiogenesis [53]. It remains unclear whether PSA has significant in vivo activity in patients with prostate cancer.

The influence of interferon β on the angiogenic activity of human prostate cancer cells has been explored in PC-3 xenografts. PC-3 cells transfected with INF-β formed tumours exhibiting reduced angiogenesis, tumorigenicity and metastases than controls [11].

Antiangiogenesis therapy

Strategies for angiogenesis inhibition include blockers of VEGF and its receptors, matrix metalloproteinase (MMP) inhibitors and modulators of VEGF expression. The mechanism of action of some compounds is still unknown.

Monoclonal antibodies

Monoclonal antibodies against VEGF or VEGFR2 inhibit tumour growth and metastasis in animal models [2, 49, 60]. Phase I studies are underway using a synthetic VEGFR2 blocker [22].

Thalidomide

Thalidomide was originally marketed as a sedative but was found to be a potent teratogen that caused stunted limb growth. It is one of the first drugs found to inhibit angiogenesis in vitro [9] and has been shown to inhibit both VEGF- and bFGF-induced angiogenesis. In a phase II study in 63 patients with hormone refractory prostate cancer, 14% of patients showed a decline in the serum PSA of at least 50% [18]. One of the problems of using PSA as a surrogate marker of response is that thalidomide has been shown to increase PSA secretion in preclinical studies [10]. The authors contend that the clinical response may be better than the PSA response would suggest and that further studies in lower volume disease are justified. Thalidomide is being assessed in a neoadjuvant setting in patients undergoing radical prostatectomy and in combination with paclitaxel and estramustine chemotherapy in patients with metastatic hormone refractory prostate cancer [8]. Other angiogenesis inhibitors remain in an earlier stage of development [45, 67].

Linomide

Linomide inhibits angiogenesis induced by the VEGF, bFGF and TNFα systems and has shown potent activity in the rat model of prostate cancer [39]. The antiangiogenic effect of castration, resulting from a reduction in VEGF levels, is potentiated by linomide therapy which also acts through other systems [38]. It remains to be seen whether a combination therapy of castration and angiogenesis inhibitors will provide a more durable response than castration alone in patients with advanced prostate cancer.

COX-2 inhibitors

Cyclooxygenase-2 (COX-2) is an enzyme, induced in inflammatory cells and tumours by cytokines, growth factors and tumour promoters, that converts arachidonic acid to prostaglandins and other eicosanoids. COX-2 is upregulated in prostate cancer [42] and the prostaglandins generated have been shown to stimulate angiogenesis in a variety of tumours including prostate cancer [46]. COX-2 inhibitors have some activity in the prevention of colon cancer in patients with familial adenomatous polyposis [62] but as yet it is not known if they have a role in prostate cancer.

Gene Therapy

In vivo gene therapy using endogenous angiogenesis inhibitors may allow the balance of stimulators and inhibitors within the tumour to be favourably altered.

DU-145 xenografts transfected with thrombospondin-1 showed extensive areas of necrosis and reduced microvessel density [37]. Thus, if technical obstacles to successful gene therapy can be overcome, this represents a further method of interfering with tumour angiogenesis.

Conclusion

Although angiogenesis is a complex process probably involving a multitude of angiogenic factors and inhibitors, there is powerful evidence of the importance of the VEGF system in both normal prostate and prostate cancer. Recent advances include an understanding of how castration acts through the VEGF system to inhibit angiogenesis. Stromal-endothelial and epithelial-endothelial interactions are just beginning to be investigated. A better understanding of how physiological angiogenesis is controlled should help to provide further insights into the mechanism of disregulated angiogenesis in tumours. Ultimately, new antiangiogenic agents are likely to find a role in the management of patients with prostate cancer.

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