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Angiogenesis and Prostate Cancer Tumor Growth

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Abstract The initiation of new blood vessels through angiogenesis is critical to tumor growth. Tumor cells release soluble angiogenic factors that induce neovascularization, without which nutrients and oxygen would not be available to allow tumors to grow more than 2–3 mm in diameter. This ''angiogenic switch'' or angiogenic phenotype requires an imbalance between proangiogenic and antiangiogenic factors since the formation of new blood vessels is highly regulated. This review discusses angiogenesis mediators, and the potential for manipulation of angiogenic factors as a practical cancer therapy, particularly in prostate cancer. J. Cell. Biochem. 91: 125–150, 2004. © 2003 Wiley-Liss, Inc.

Key words: prostate cancer; angiogenesis; VEGF

Angiogenesis became a recognized field of study after Folkman's observation that tumors are richly supplied with blood [Linde, 2001]. Subsequent research revealed that tumors are able to recruit their own blood supply [Gimbrone et al., 1972]. Folkman et al. were working on developing a freeze-dried blood substitute for the United States Navy and incidentally discovered that tumor cells which had become quiescent in vitro resumed growth when reintroduced into the donor animal and developed vascularization [Ezzell, 1998]. In 1973, Folkman also demonstrated tumor angiogenesis in vivo using a rabbit model [Gimbrone et al., 1973].

Diffusion is adequate for oxygen and nutrient uptake in small avascular tumors, but tumor growth requires an increased blood supply via new blood vessels [Folkman, 1992]. Tumor cells release soluble angiogenic factors inducing neovascularization [Folkman and Klagsbrun, 1987], a process referred to as the "angiogenic switch" is characteristic of an angiogenic phenotype [Hanahan and Folkman, 1996]. Tumor growth in vascular tissues may also co-opt existing blood supply [Holash et al., 1999] and some evidence suggests that developing tumors can promote angiogenesis by homeostatic mechanisms [Fidler, 1995]. The process involves degradation of the basement membrane surrounding capillaries, endothelial migration into the extracellular matrix (ECM) towards angiogenic stimuli, proliferation and reorganization of endothelial cells, and capillary differentiation and fusion into a tubular network of new blood vessels [Folkman et al., 1989]. Tumor cells can overexpress angiogenic factors or alter the regulation of endogenous angiogenic factors to establish an imbalance between proangiogenic and antiangiogenic factors (Table I) [Liotta et al., 1991].

Phenotypically, newly formed vessels often differ from mature vessels while tumor vasculature can differ from vessels in normal tissues. Vascular endothelial growth factor (VEGF) receptors are up-regulated in new blood vessels [Feng et al., 2000] and integrin $\alpha V\beta 3$ and $\alpha V\beta 5$ antibody staining is elevated in tumor vessels compared to normal vessels [Brooks et al., 1994]. Tumor endothelium also exhibits heterogeneity in binding specific peptide sequences [Pasqualini and Ruoslahti, 1996]. Interestingly, prostate-specific membrane antigen (PSMA) has been found in tumor-associated neovasculature, including tumors of non-prostatic origin, but not in benign tissue vasculature [Chang et al., 1999]. Endothelia from different tissues are also phenotypically distinct, vary in response to different angiogenesis regulators [Pasqualini and Ruoslahti, 1996], and are

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TABLE I. Angiogenic Factors in Prostate Cancer

	Proangiogenic	Antiangiogenic
Endogenous factors	Matrix metalloproteinases (MMPs)	Tissue inhibitor of metalloproteinase 1 (TIMP-1)
	VEGF (VEGF)	
	Basic fibroblast growth factor 2 (bFGF-2)	Interleukin-10 (IL-10)
	Fibroblast growth factor 4 (FGF-4)	
	Transforming growth factor β_1 (TGF-β_1)	Angiostatin
	Interleukin 8 (IL-8)	Endostatin
	Interleukin 6 (IL-6)	
	Interleukin 1β (IL-1β)	Prostate-specific antigen (PSA)
	Cyclooxygenase 2 (COX-2)	
	Nitric oxide (NO)	Interferon (IFN)
	Tumor necrosis factor (TNF) Insulin growth factor 1 (IGF-I)	
Pharmaceutical agents		Neutralizing antibodies
		MMP inhibitors
		Fumagillin analogue
		Linomide
		Carboxyamido-triazole

regulated by tissue-specific expression of cytokines and growth factors in the microenvironment [Fidler, 2001]. Interactions between tumors, adjacent tissues, and the surrounding ECM, as they relate to angiogenesis, have been demonstrated in mouse models: where human xenografts exhibited enhanced tumorigenesis, angiogenesis, and metastatic potential grown in an orthotopic location, as opposed to heterotopically implanted tumor cells [Naito et al., 1986; Fidler et al., 1990]. The implications for cancer progression to distant sites are significant since tumor cell migration into the circulatory system is directly related to the surface area of vessels within the tumor [Liotta et al., 1976].

Both endogenous factors and administered agents can regulate angiogenesis, either directly or indirectly. For example, therapeutic inhibitors of angiogenesis may exercise a direct cytostatic or cytotoxic effect or act indirectly, by recruiting secondary factors to regulate the angiogenic process. Combination therapies with antiangiogenic and cytotoxic agents have now shown some effectiveness in pre-clinical trials.

ANGIOGENIC MECHANISMS IN PROSTATE CANCER

VEGF

VEGF was first described in 1983 [Senger et al., 1983]. The gene encoding VEGF resides on chromosome 6p21.3 with a coding region spanning approximately 14,000 bases. The human VEGF gene contains eight exons. At least six isoforms of the protein are found secondary to alternative splicing of the messenger RNA (mRNA). All six spliced mRNAs are homologous in exons 1–5 and in exon 8, but vary in exons 6 and 7. The resulting isoforms are named VEGF plus the amino acid content of the protein: $VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$, and $VEGF_{206}$ [Robinson and Stringer, 2001]. VEGF isoforms are secreted as homodimers of the cysteine-knot superfamily and show similarity to the platelet-derived growth factor (PDGF) family [Muller et al., 1997]. VEGF binds three tyrosine kinase receptors; exon 3 codes for three acidic residues that binds the fms-like tyrosine kinase-3 receptor (Flt-1 or VEGFR-1), while three basic amino acid residues encoded in exon 4 bind the kinase insert domain-containing receptor/fetal liver kinase 1 (KDR/Flk-1or VEGFR-2) [Mustonen and Alitalo, 1995; Keyt et al., 1996]. Flt-4 (VEGFR-3) is related to VEGFR-1 and VEGFR-2 but is only found in embryonic lymphatic endothelium [Kaipainen et al., 1995].

Three isoforms of VEGF ($VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$) are preferentially expressed in VEGF-producing cells [Nicosia, 1998]. $VEGF_{165}$ has a moderate affinity for heparin via a heparin-binding domain, while $VEGF_{121}$ lacks the heparin-binding region [Fairbrother et al., 1998]. $VEGF_{121}$ is freely secreted, but $VEGF_{165}$ remains mostly associated with cells and the ECM, likely due to interactions with heparan sulfate proteoglycans [Houck et al., 1992]. $VEGF_{189}$ and $VEGF_{206}$ have additional heparin-binding domains and are completely sequestered to the ECM and cell surface [Park et al., 1993]. Recombinant $VEGF_{189}$ and

$VEGF_{206}$ were unable to stimulate endothelial cell proliferation, unlike recombinant $VEGF_{121}$ and $VDGF_{165}$ which did induce endothelial proliferation in their assay [Houck et al., 1991]. More recently, $VEGF_{189}$ has been shown to exert its biological effects by stimulating the fibroblast growth factor (FGF) pathway [Jonca et al., 1997]. Additional growth factors belonging to the VEGF family, sharing common receptors with VEGF, have been discovered, including placenta growth factor (PlGF) [Maglione et al., 1991] and VEGF B-E [Joukov et al., 1996; Paavonen et al., 1996; Yamada et al., 1997; Ogawa et al., 1998].

During angiogenesis, endothelial cells switch from a resting state to rapid growth induced by diffusible factors secreted by tumor cells [Folkman et al., 1989]. VEGF was the first selective angiogenic growth factor to be purified [Senger et al., 1986]. Many human tumor biopsies exhibit enhanced expression of VEGF mRNAs by malignant cells and VEGF receptor mRNAs in adjacent endothelial cells. Abrogation of VEGF function with monoclonal anti-VEGF antibodies completely suppresses prostate cancer-induced angiogenesis and halts tumor growth at the pre-vascular growth phase [Borgstrom et al., 1998]. Immunohistochemical (IHC) studies [Ferrer et al., 1997] demonstrate that in human prostate cancer tissues, cancer cells stained positively for VEGF, correlating with microvessel density (MVD). Conversely, benign prostatic hyperplasia (BPH) and normal prostate cells displayed little VEGF staining and vascularity [Ferrer et al., 1998]. IHC examination of VEGF and flk-1 staining found that in all benign glands, VEGF and flk-1 expression were confined almost exclusively to the basal cell layer. Whereas, in high-grade prostatic intraepithelial neoplasia (PIN), staining was seen in all neoplastic secretory cells as well as the basal cell layer. Furthermore, all carcinomas stained positive for both markers and a trend for increasing staining intensity with increasing cellular dedifferentiation was noted [Kollermann and Helpap, 2001]. Increased VEGF expression [Harper et al., 1996] has also been related to neuroendocrine differentiation in prostate cancer, a poor prognostic factor for survival [Noordzij et al., 1995]. These data suggest that the prostate tumor growth advantage conferred by VEGF expression is a consequence of angiogenic stimulation.

VEGF expression is mediated by such external factors as hypoxia, growth factors, and cytokines. Regulation of VEGF can occur at transcriptional [Mazure et al., 1996], post transcriptional [Levy et al., 1996], and translational levels [Akiri et al., 1998]. Cytokines, growth factors, and gonadotropins that do not stimulate angiogenesis directly can modulate angiogenesis by altering VEGF expression in specific cell types and exerting indirect angiogenic or anti-angiogenic effects [Fidler, 2001]. Factors that can potentiate VEGF production include fibroblast growth factor 2 (FGF-2) [Seghezzi et al., 1998], fibroblast growth factor 4 (FGF-4) [Deroanne et al., 1997], platelet-derived growth factor (PDGF) [Finkenzeller et al., 1992], tumor necrosis factor (TNF) [Giraudo et al., 1998], transforming growth factor β (TGF-β) [Pertovaara et al., 1994], insulin growth factor 1 (IGF-I) [Warren et al., 1996], interleukin 1β (IL-1β) [Li et al., 1995], and IL-6 [Cohen et al., 1996].

In reciprocal regulation between VEGF and the small molecule nitric oxide (NO), nitric oxide up-regulates VEGF and the production of VEGF in turn up-regulates NO, in a positive feedback loop between these two factors [Dembinska-Kiec et al., 1997]. NO contributes to the blood vessel permeability effect of VEGF and VEGF-stimulated vasodilatation. NO synthetase-2 was recently reported to have an elevated expression by immunohistochemistry in both prostate cancer and PIN, compared to normal prostate tissue [Uotila et al., 2001]. VEGF may also directly stimulate prostate tumor cells via VEGFR-2-dependent autocrine and/or paracrine mechanisms [Jackson et al., 2002] and VEGF autocrine stimulation may coincide with progression to a malignant phenotype [Soker et al., 2001]. Additionally, androgen deprivation of LNCaP prostate cancer cells in vitro led to decreased vascular endothelial growth factor (VEGF) expression both at the mRNA and protein levels, at least partly in due to a fivefold destabilization of mRNA transcripts. In LNCaP tumor bearing mice, castration resulted in a rapid decrease in mRNA expression and markedly reduced tumor neovascularization in this study, and androgen withdrawal inhibited the hypoxic induction of VEGF mRNA [Stewart et al., 2001].

Soluble factors and other stimuli mentioned above are known to induce VEGF transcription, but the exact signaling intermediates used in

this process are less well defined. Recent articles have shed light on this issue, implicating *Ras*, *Raf*, and *Src* gene products as VEGF signaling intermediates [Rak et al., 2000]. Overexpression of activated forms of these genes is accompanied by marked elevation of both VEGF mRNA and secreted functional protein levels [Grugel et al., 1995]. VEGF expression is critical for ras-mediated tumorigenesis; while loss of expression causes dramatic decreases in vascular density and vascular permeability as well as an increase in tumor cell apoptosis [Grunstein et al., 1999]. Ras, Raf, and Src activating mutations are unusual in human prostate cancer, but may be major signaling molecules by which circuits can be corrupted [Konishi et al., 1997]. Any factor that stimulates Ras-, Raf-, and Src-mediated signaling pathways may contribute to solid tumor growth both by a direct effect on tumor cell proliferation and indirectly by facilitating tumor angiogenesis via VEGF induction.

VEGF transcription can also be regulated by cell surface contacts mediated by either cell–cell or cell–ECM interactions. A novel regulatory pathway mediating this effect was shown to involve focal adhesion kinase (FAK), Src, phosphatidylinositol 3 kinase (PI3K), Raf, and MAPK kinase (MEK) in a Ras-independent manner. This third major avenue of VEGF regulation may serve to explain a number of fundamental issues in prostate cancer biology such as the organ tropism of prostate cancer metastasis [Sheta et al., 2000].

Wartenberg et al. [2001] used a novel "confrontation culture" system with small clusters of embryonic stem cells and the human prostate cancer line DU-145 to demonstrate "vascularization" of cancer cell spheroids mediated by stem cells. In the confrontation culture, protein levels of VEGF and HIF-1 rose until vascularization became evident. When vascularization became clearly visible, levels of both proteins fell to approximately 20–30% of their day 3 peak levels. VEGF and the platelet endothelial cell adhesion molecule (PECAM) staining were most pronounced where the embryoid body contacted the DU-145 tumor spheroid. The authors found that the partial pressure of oxygen in the vascularized spheroid was lower than in the avascular spheroid, but evidence of central necrosis disappeared after vascularization. The authors suggested that central necrosis in the avascular spheroid may not be exclusively due to hypoxic conditions at the tumor center [Wartenberg et al., 2001].

Insertion of the SV40 T antigen gene under a prostate-specific promoter into the germ line of mice produced the TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP mouse) model. Heterozygous animals develop well-differentiated prostate tumors between 10–16 weeks of life. Progression to poorly differentiated primary prostatic tumors and metastatic lesions occurs at 18–24 weeks. Temporal and spatial expression patterns of PECAM, hypoxia-induced factor 1 (HIF-1), VEGF, and the cognate receptors VEGFR-1 and VEGFR-2 were characterized in this model system. Immunohistochemical and in situ analyses of prostate tissue specimens identified a distinct early angiogenic switch corroborated by the expression of PECAM-1, HIF-1 alpha, and VEGFR1 and the recruitment of new vasculature to lesions of high-grade PIN. HIF-1 expression localized to the nucleus, correlating with or preceding VEGF expression. Expression of the VEGF-165 isoform was not seen in normal prostate, high-grade PIN, or moderately or well-differentiated prostate adenocarcinoma. It was found in poorly differentiated prostate cancers. A distinct late angiogenic switch was consistent with decreased expression of VEGFR1, increased expression of VEGFR2, and the transition from differentiated adenocarcinoma to a poorly differentiated state [Huss et al., 2001].

Metastatic human prostate cancer cells exhibited enhanced VEGF production and tumor vascularity compared with prostate cancer cells of lower metastatic potential [Balbay et al., 1999]. This study evaluated the expression of VEGF in LNCaP, which does not exhibit a metastatic phenotype, and two of its derivatives: LNCaP-Pro5 (slightly metastatic) and LNCaP-LN3 (highly metastatic) after orthotopic implantation into athymic nude mice. In vitro, VEGF production by LNCaP-LN3 was significantly higher than those of both LNCaP and LNCaP-Pro5 cells. In vivo, LNCaP-LN3 tumors exhibited higher levels of VEGF mRNA and protein as well as VEGFR-2 protein and had higher microvessel density than either LNCaP tumors or LNCaP-Pro5 tumors.

Two other prostate cancer cell lines were evaluated for VEGF expression by enzyme-linked immunosorbent assay (ELISA). Orthotopic implantation of PC-3M (highly metastatic)

and DU145 (poorly metastatic) was performed in nude mice. Angiogenesis was much more evident in PC-3M tumors as compared with DU145 tumors, but ELISA-determined VEGF levels were approximately threefold higher in DU145 cell tumors and in the conditioned media of DU145 cells. To characterize this apparent contradiction, the authors detected VEGF isoforms by Western blotting in both solid tumor extracts and culture medium conditioned by the same cell lines. Western blotting showed that PC-3M tumors, but not conditioned media, contained VEGF isoforms not identified by an ELISA antibody raised against the $VEGF_{165}$ isoform. Other isoforms of VEGF may therefore predominate in PC-3M cells [Connolly and Rose, 1998].

Another study causally related tumor overexpression of VEGF to organ-specific tumor growth in bone using a prostate cancer xenograft model. Transfection of the LNCaP derivative C4-2, which is modestly tumorigenic and metastasizes preferentially to bone, with a full-length cDNA encoding $VEGF_{165}$ did not seem to affect in vitro cell growth. Although tumorigenicity and in vivo tumor growth rates were affected by overexpression after subcutaneous inoculation; no such effect was observed when cells were implanted orthotopically or into intrafemoral sites. These results suggest that the biological impact of prostate tumor VEGF overexpression is organ specific, leading to speculation that it has a role in the organ tropism of metastatic spread [Krupski et al., 2001].

Basic Fibroblast Growth Factor (bFGF) (FGF-2)

The bFGF gene is located at 4q25-q27, while the protein consists of 155 amino acids. Dimerization occurs when bound to the tyrosine kinase FGF receptor 1 (flg) requiring bFGF binding to cell surface heparan sulfate proteoglycans (HSPGs). The rabbit corneal model has demonstrated dose-dependent angiogenesis induction in response to bFGF [Gaudric et al., 1992]. Additionally, VEGF and bFGF are synergistic for induction of angiogenesis. Microvascular endothelial cells grown on the surface of three-dimensional collagen gels were stimulated with VEGF, inducing the cells to invade the underlying matrix and form capillary-like tubules. Furthermore, bFGF was twice as potent as VEGF at equimolar concentrations for stimulating angiogenesis. VEGF and bFGF together induced an in vitro angiogenic response far greater than additive, which occurred with greater rapidity than the response to either cytokine alone [Pepper et al., 1992].

When expression of bFGF and its receptor (flg) were compared in prostate cancer cell lines, androgen-sensitive and non-metastatic LNCaP cells did not produce measurable amounts of bFGF, expressed small but measurable amounts of FGF receptor mRNA, and did respond to exogenous bFGF. Androgen-independent but moderately metastatic DU145 cells produced measurable amounts of biologically active bFGF, expressed large amounts of FGF receptor mRNA, and responded to exogenous bFGF. Androgen-independent and highly metastatic PC-3 cells also produced measurable amounts of bFGF, but did not demonstrate a growth response to exogenous bFGF even though large amounts of FGF receptor mRNA were expressed [Nakamoto et al., 1992]. Another study examined co-cultured LNCaP cells with the bFGF-dependent human adrenal carcinoma SW-13 cell line as target cells. LNCaP cells stimulated SW-13 cell growth, and this stimulation was magnified in androgen-treated LNCaP cells. Specific anti-bFGF antibodies inhibited the LNCaP-stimulated growth of SW-13 cells, and no proliferation of SW-13 cells occurred in the absence of LNCaP cells. This study suggests that androgen may regulate bFGF secretion by LNCaP cells in vitro [Zuck et al., 1992]. Orthotopic tumors from PC-3M and DU145 cells were evaluated by ELISA for bFGF levels. The more aggressive PC-3M cell line was more angiogenic and displayed greater staining than the less aggressive DU145 cell line [Connolly and Rose, 1998].

Studies evaluating the usefulness of urinary and serum VEGF and bFGF measurements have shown no correlation with prognosis and are not as useful as serum prostatic specific antigen (PSA) measurements [Walsh et al., 1999; Bok et al., 2001]. Reverse transcription polymerase chain reaction (RT-PCR) with primer sets for FGF-3, FGF-4, and FGF-6 was performed on 26 prostate cancer RNA samples. As opposed to normal prostate RNA, in which these FGF factors are not amplified, 14 of 26 samples expressed FGF-6 while no amplification of either FGF-3 or FGF-4 was detected. Further analysis by ELISA with a specific

antibody against FGF-6 showed an absence of the factor in normal prostate. It was elevated in 4 of 9 PIN lesions and in 15 of 24 prostate cancers. Immunohistochemical analysis with anti-FGF-6 antibody revealed weak staining of prostatic basal cells in normal prostate, which was markedly elevated in PIN lesions. Most prostate cancer cases revealed FGF-6 expression in the prostate cancer cells. In two cases, expression was present in prostatic stromal cells. The authors of this study found that exogenous FGF-6 could stimulate proliferation of primary prostatic epithelial and stromal cells, immortalized prostatic epithelial cells, and prostate cancer cell lines in tissue culture. They concluded that FGF-6 is increased in PIN lesions and prostate cancer and can promote the proliferation of the transformed prostatic epithelial cells via paracrine and autocrine mechanisms [Ropiquet et al., 2000].

Transforming Growth Factor Beta 1 (TGF-β_1)

TGF-β_1, a cytostatic inhibitor of epithelial cell growth, may stimulate the growth of stromal cells such as fibroblasts. The growth of prostate cancer cells in vitro is inhibited by TGF-β_1 under restrictive conditions, but this inhibition can be overcome by growth factors or ECM components [Morton and Barrack, 1995]. Prostate cancer cells in vivo secrete TGF-β_1 but seem to acquire resistance to this inhibition as they progress to more aggressive phenotypes. Overproduction of TGF-β_1 and loss of TGF-β receptor type II expression have been shown to be associated with poor clinical outcome in prostate cancer. Additionally, TGF-β_1 expression was found to correlate with tumor vascularity, tumor grade, and metastasis [Wikstrom et al., 1998]. Resistance to TGF-β_1 growth inhibition as well as TGF-β_1 stimulated angiogenesis and metastasis may explain this apparent paradox. Immunohistochemical studies have localized TGF-β_1 to intracellular and extracellular locations in prostate cancer and BPH, but extracellular and epithelial cell staining was more extensive in prostate cancer than BPH samples. Conversely, staining was more extensive intracellularly and in stromal cells within BPH samples compared to prostate cancer [Truong et al., 1993]. Similar results were shown by another group comparing prostate tissue from local and metastatic prostate cancers. Increased intracellular staining was found in patients with lymph node involvement as compared to patients with localized disease [Eastham et al., 1995].

Further data suggests that TGF-β_1 may play a role in the interaction between stroma and cancer angiogenesis. One study using a reactive stroma xenograft model, in which growth of LNCaP human prostate tumors and angiogenesis are promoted, found efficacy when using anti-TGF-β_1 agents. In the presence of TGF-β_1 latency-associated peptide (LAP) neutralizing antibody, tumors exhibited a reduction in MVD by 3.5-fold as well as a decrease in average weight [Tuxhorn et al., 2002].

One proposed mechanism by which prostate cancer could escape inhibitory regulation by TGF-β_1 was suggested by immunohistochemical studies on 2 of the 3 receptors for this factor. TGF-β type I and type II receptors were localized to epithelial cells in 8 specimens of benign prostatic hypertrophy, but in 32 specimens of prostate cancer loss of staining in 4 samples for type II receptor and in 8 samples in type I receptors was found [Kim et al., 1996]. A corroborative study also noted decreasing expression of type II receptors significantly related to increasing histological tumor grade [Williams et al., 1996]. Evaluation of TGF-β receptors in primary tumors and lymph node metastases displayed weak immunohistochemical staining compared to normal prostate with lack of staining for both type I and type II receptors in 25 and 45% of samples, respectively. Further analysis of mRNA by RT-PCR and Northern blotting revealed decreased expression of both type I and type II receptors secondary to down-regulation of gene transcription [Guo et al., 1997]. The expression of TGF-β_1 in several rat prostate adenocarcinoma models was also evaluated by Northern blotting and mRNA levels were found to be unchanged 2 weeks after castration [Steiner et al., 1994] but TGF-β_1 mRNA levels were much higher in rat prostate adenocarcinomas (Dunning R3327 MATLyLu, AT2, G, HI, and H sublines) than normal prostate.

Androgen and Macrophage Regulation

Androgens are involved in blood flow regulation in vivo in both the normal rat ventral prostate and in the Dunning tumor rat model [Hartley-Asp et al., 1997; Lekas et al., 1997]. Castration, a widely used antiandrogen therapy in patients with advanced prostate cancer, in-

hibits VEGF expression and induces apoptosis of endothelial cells that precedes the apoptosis of tumor cells in vivo [Jain et al., 1998]. Chemical castration 7–28 days prior to radical prostatectomy results in tremendous recruitment of inflammatory cells and induction of apoptosis in resected histologic specimens [Mercader et al., 2001]. Tumor-associated macrophages (TAMs), one such inflammatory cell, play an important role in tumor angiogenesis [Lissbrant et al., 2000]. Reduced infiltration of TAMs has been associated with prostate cancer progression [Shimura et al., 2000]. TAMs can influence each phase of the angiogenic process, such as alterations of the local ECM, induction of endothelial cells to migrate or proliferate, and inhibition of vascular growth with formation of differentiated capillaries [Sunderkotter et al., 1994]. Other studies have demonstrated that castration inhibited prostate tumor VEGF production, but had no effect on other angiogenic factors [Joseph and Isaacs, 1997].

Cyclooxygenase-2 (COX-2)

Cyclooxygenase is involved in hypoxia-induced angiogenesis through interactions with VEGF. Some evidence suggests that inhibition of this factor may also induce apoptosis in prostate cancer cells, although the relationship to angiogenesis is not defined [Hsu et al., 2000]. Immunohistochemical staining of 30 samples of benign prostatic hypertrophy and 82 samples of prostate cancer revealed that for both benign prostatic hypertrophy and prostate cancer, COX-1 expression was primarily in the fibromuscular stroma, with variable weak cytoplasmic expression in glandular neoplastic epithelial cells. In contrast, COX-2 expression differed markedly between benign prostatic hypertrophy and cancer. In benign prostatic hypertrophy membranous expression of COX-2 in luminal glandular cells occurred without stromal expression. In cancer, the stromal expression of COX-2 was unaltered, but expression by tumor cells was significantly greater with a change in the staining pattern from membranous to cytoplasmic. Additionally, COX-2 expression was significantly higher in poorly differentiated tumors. Immunoblotting confirmed these results represent four-time greater expression of COX-2 in cancer than in benign prostatic hypertrophy [Madaan et al., 2000].

Stromal–Epithelial Interactions and Hypoxia-Induced Angiogenesis

Normal human prostate epithelial cells express a variety of cytokines with angiogenic and/or endothelial cell-activating properties [Campbell et al., 1999]. Stromal fibroblasts co-inoculated with the human prostate cancer cell line PC-3 are required for angiogenesis in three-dimensional culture [Janvier et al., 1997]. Prostate cancer cells also express angiogenic factors, most notably VEGF and interleukin-8 (IL-8) [Ferrer et al., 1998]. Endothelial cell cultures have been stimulated by the addition of conditioned medium or co-culture with prostate cancer cells [Hepburn et al., 1997].

Hypoxia induces expression of the hypoxia-inducible factor-1 (HIF-1) [Semenza and Wang, 1992], shown to increase growth rate and metastatic ability in prostate cancer cells independent of the oxygen tension in the cellular environment [Zhong et al., 1998]. An immunohistochemical study found that HIF-1 protein levels are higher, relative to normal tissue, in 13 of 19 tumor types, including prostate cancer [Zhong et al., 1999]. Furthermore, hypoxia can up-regulate expression of VEGF in prostate cancer in vitro [Cvetkovic et al., 2001]. Zhong et al. [2000] linked the signal transduction pathway from receptor tyrosine kinases to phosphatidylinositol 3-kinase (PI3K), AKT (protein kinase B) and its effector FKBP-rapamycin-associated protein (FRAP), via autocrine stimulation or inactivation of the tumor suppressor, and VEGF-induced angiogenesis in prostate cancer. Growth factor and mitogen-induced secretion of VEGF, the product of a known HIF-1 target gene, was inhibited by LY294002 and rapamycin, inhibitors of PI3K and FRAP, in an in vitro prostate cancer system [Zhong et al., 2000]. Cyclooxygenase-2 (COX-2), an inducible enzyme that catalyzes the formation of prostaglandins from arachidonic acid, was induced by hypoxia. Moreover, the up-regulation of VEGF in PC-3ML human prostate cancer cells is accompanied by a persistent induction of COX-2 mRNA and protein and is significantly suppressed following exposure to a selective COX-2 inhibitor, NS398 [Liu et al., 1999].

Proteinases Acting on the ECM

Plasminogen activators (PAs) and matrix metalloproteinases (MMPs), two families of

proteinases, have been implicated in the degradation of the basement membrane [Rabbani, 1998]. Urokinase-type plasminogen activator (uPA), a PA family proteinase, and its receptor (uPAR) are expressed in aggressive human prostate cancer cell lines DU145 and PC-3 and absent in the less aggressive LNCaP cell line [Hoosein et al., 1991]. Blocking uPA receptor inhibits tumor growth and neovascularization in prostate cancer cells [Evans et al., 1997]. In vitro, the primary human prostate cancer cell line 1013L was found to express no uPA, while DU145, a cell line derived from a metastatic lesion, expressed high levels of uPA. Using a Xenograft mouse model, 1013L tumor homogenates had hardly detectable levels of uPA, 300-fold lower than found in the invasive prostate xenograft DU145 [Billstrom et al., 1995]. The human prostate carcinoma lines PC-3, DU145, and LNCaP also express enzymatic activity converting plasminogen to the endogenous inhibitor of angiogenesis angiostatin [Gately et al., 1996]. Basic fibroblast growth factor (bFGF), besides stimulating uPA production by vascular endothelial cells, also increases the production of receptors, modulating their capacity to localize this enzyme on the cell surface [Mignatti et al., 1991].

MMP-9 and MMP-2, type IV collagenases, are also involved in basement membrane degradation [Nagakawa et al., 2000]. Radical prostatectomy specimens from 40 patients were examined using rapid colorimetric in situ hybridization technique to evaluate the expression level of E-cadherin and MMPs types 2 and 9. While E-cadherin stained in a more central region of the tumor, both MMPs predominately stained at the leading edges of the tumor. Decreased expression of E-cadherin, as well as increased expression of MMP-2 and MMP-9 was significantly correlated with the Gleason score of the tumors. The authors also found that irrespective of serum prostate-specific antigen level or Gleason score, the ratio between expression of MMPs and E-cadherin at the invasive edge of tumors exhibited the strongest association with non-organ-confined prostate cancer.

E-cadherin and MMP expression levels may become useful to delineate organ-confined and non-organ-confined disease [Kuniyasu et al., 2000] since both MMP-9 and MMP-2 have been shown to be regulated by transforming growth factor beta 1 (TGF-β_1) in prostate cancer cell lines [Sehgal and Thompson, 1999]. MMP-2 and TGF-β_1 expression were both demonstrated to be directly related to the angiogenic and metastatic phenotype in the aggressive PC-3ML cell line. MMP-9 has been shown to be regulated both at the transcriptional and translational levels [Jiang and Muschel, 2002]. Inhibition of this phenotype was effected by IL-10, which has been shown to induce the tissue inhibitor of metalloproteinase-1 (TIMP-1) [Stearns et al., 1999b]. TGF-β_1 and IL-10 regulation were also linked in a study showing that TGF-β_1 transfected cells had greater metastatic growth and that these tumors stained poorly for IL-10 [Stearns et al., 1999a].

Tumor Suppressor Genes

Reports link up-regulation of VEGF to mutations in tumor suppressor gene *p53* in many different cancers, but the only report so far on prostate cancer concludes that VEGF expression was independent of p53 expression albeit p53 correlated with tumor grade, stage, and clinical outcome [Strohmeyer et al., 2000]. Inactivation of the tumor suppressor PTEN, detected in a minority of clinically localized prostate cancers, is common in metastatic disease and associated with increased angiogenesis [Giri and Ittmann, 1999]. Enhanced angiogenesis in this study, however, did not rely on reduction of thrombospondin 1 expression as previously reported in glioma cells in vitro [Wen et al., 2001].

Interleukin 8 (IL-8)

Interleukin 8 (IL-8) is a macrophage-derived mediator of angiogenesis, promoting angiogenesis in the rat cornea model. It is also a chemotactic and mitogenic factor for human endothelial cells from the umbilical vein [Koch et al., 1992]. IL-8 expression in normal human prostate epithelial cells was demonstrated by RT-PCR and in conditioned media by ELISA [Campbell et al., 1999]. Ferrer et al. [1997, 1998] compared VEGF expression with expression of IL-8 in human prostate cancer cells. Ex vivo immunohistochemical staining of human prostate cancer specimens, BPH, and normal prostate tissues showed that adenocarcinoma cells stained positively for VEGF (20 of 25 slides) and IL-8 (25 of 25 slides), while BPH and normal prostate cells displayed little staining for either angiogenesis factor. IL-8 was present throughout the cytoplasm of cancer cells and no

difference in staining pattern was detected between tumors of different Gleason grade. Additionally, DU145 cells grown in culture were stimulated with cytokines showed induction of both VEGF and IL-8. Interestingly, cytokine stimulation of DU145 cells resulted in differential stimulation, whereby tumor necrosis factor (TNF) predominantly induced VEGF while IL-1 was the predominant inducer of IL-8 [Ferrer et al., 1998]. An in vitro study on the highly metastatic human PC-3M-LN4 prostate cancer cell lines measured IL-8 mRNA by Northern bloting and colorimetric in situ hybridization techniques. Highly metastatic cell lines constitutively and uniformly expressed higher levels of IL-8 when compared to parenteral PC-3M cells or poorly metastatic cell lines. Prostate cancer cells implanted subcutaneously expressed less IL-8 mRNA than cells implanted orthotopically, indicating that expression of these genes depended on the organ environment [Greene et al., 1997].

Another group performed studies on the highly metastatic PC-3M-LN4 cell line, an IL-8 overexpressing derivative, and the poorly metastatic PC-3P cell line that expresses relatively low amounts of IL-8. In this study, investigators transfected PC-3P cells with full-length sense IL-8 cDNA and transfected PC-3M-LN4 cells with the full-sequence antisense IL-8 cDNA. In vitro, sense-transfected PC-3P cells overexpressed IL-8 mRNA and protein, resulting in up-regulation of MMP-9 mRNA and collagenase activity, as well as increased invasion through Matrigel. Conversely, antisense IL-8 cDNA transfection of the PC-3M-LN4 cells greatly reduced IL-8 and MMP-9 expression, collagenase activity, and invasion. After orthotopic implantation into athymic nude mice, the sense-transfected PC-3P cells were highly tumorigenic and metastatic, with significantly increased neovascularity and IL-8 expression compared to PC-3P cells or controls [Inoue et al., 2000]. Another study implicated expression of IL-8 by human prostate cancer cells is shown to correlate with induction of angiogenesis, tumorigenicity, and metastasis. Low and high IL-8-producing PC-3 clones were injected into the prostate of nude mice. PC-3 cells expressing high levels of IL-8 were highly tumorigenic, producing rapidly growing, highly vascularized prostate tumors with, and a 100% incidence of lymph node metastasis. Whereas low IL-8-expressing PC-3 cells were less tumorigenic, producing slower growing and less vascularized primary tumors and a significantly lower incidence of metastasis. In situ hybridization (ISH) analysis of the tumors showed that the expression level of IL-8, MMPs, VEGF, and E-cadherin corresponded with microvascular density and biological behavior [Kim et al., 2001].

MICROVESSEL DENSITY (MVD) IN THE PROSTATE AS AN INDEX OF ANGIOGENESIS

Microvessel density analysis (MVD) involves staining tissue with specific anti-endothelial antibodies and scoring the vessels under the microscope. The identification of the endothelial cell–cell adhesion molecule PECAM-1 [Albelda et al., 1991] in solid tumor cell lines, including prostate cancer lines DU145 and PPC-1, has made anti-PECAM-1 [Tang et al., 1993] antibodies useful for immunohistochemical quantitation of microvessels in tumor specimens. Other antibodies include anti-Factor VIII-related antigen, anti-von Willebrand Factor (vWF), and anti-CD34 [Brawer et al., 1992]. An early study of MVD in specimens from 74 invasive prostate cancers, 29 with metastatic disease, showed a significant correlation with disease progression. Patients with metastasis showed almost a twofold increase in MVD over patients with locally invasive cancers. MVD was also found to increase with Gleason score only in the poorly differentiated tumors [Weidner et al., 1993]. In addition to increases in MVD between clinically localized prostate cancers and locally invasive or metastatic tumors, PIN revealed increased MVD in acini and ductules compared to benign epithelium in 18 of 25 tumors [Brawer et al., 1994].

Microvessel density relies upon the observer visually identifying the area of the specimen with the greatest MVD, and then counting the vessels. The reproducibility of MVD quantitation by a single observer was recently studied by performing three repeated measurements on 60 specimens. MVD counts were similar, with a reliability coefficient of 0.82. The same group evaluated 100 randomly selected radical prostatectomy specimens to evaluate the usefulness of MVD as a prognostic marker, with a median follow-up of 36 months. Immunostaining with anti-PECAM-1 antibody was found not to be associated with Gleason score, tumor stage, surgical margin status, or seminal vesicle

invasion. Additionally, MVD counts did not associate with prostate specific antigen (PSA) failure in the 20 patients who had a biochemical relapse during follow-up [Rubin et al., 1999].

A long-term study evaluated radical prostatectomy specimens from 42 terminal patients who were not treated with adjuvant hormonal therapy. Pathologic specimens from these patients were stained for p53, retinoblastoma, chromogranin A, and MVD assay was performed. Multivariate analysis revealed that p53 and retinoblastoma had the greatest prognostic importance for disease-specific survival. Furthermore, Chromogranin A and MVD values were of no additional significance when p53 and retinoblastoma were assessed [Krupski et al., 2000]. However, many groups are now finding that increased MVD does have some prognostic value in prostate cancer. Immunostaining against von Willebrand factor and analysis by both a univariate and a multivariate method in 64 consecutive radical prostatectomy specimens demonstrated that the maximal MVD, in contrast to the mean MVD, was significantly associated with survival in prostate cancer patients [Offersen et al., 1998]. In an assessment of MVD using anti-factor VIII-related antigen in 221 prostate needle biopsies from the patients managed by watchful waiting (median follow-up of 15 years), MVD was statistically correlated with clinical stage ($P < 0.0001$) and histopathological grade ($P < 0.0001$). A multivariate analysis showed that MVD was a significant predictor of disease-specific survival in the entire cancer population ($P = 0.0004$) and the clinically localized cancer population ($P < 0.0001$) [Borre et al., 1998].

Bostwick et al. [1996] reported a multi-institutional study of the predictive power of MVD analysis combined with Gleason score and serum PSA to predict extraprostatic extension. Randomly selected prostate needle biopsy specimens from 186 patients and matched samples from radical prostatectomy specimens were compared using an automated digital image analysis system to measure microvessel morphology and calculate the optimized microvessel density (OMVD) in the biopsy samples. When OMVD analysis was added to Gleason score and pre-operative serum prostatic specific antigen concentration, prediction of extraprostatic extension was increased significantly. Prediction by OMVD did not extend to outcome in patients with margin-free organ-confined prostate cancer with Gleason sums of 6–9 [Bostwick et al., 1996].

In another study, specimens from 147 radical prostatectomies were stained with an antibody against factor VIII-related antigen using the OMVD method. In this study, OMVD did not associate with DNA ploidy, Gleason grade, unilateral or bilateral disease, or pre-operative PSA; nor was OMVD a significant univariate or multivariate predictor of clinical or biochemical recurrence. Mean follow-up was 6 years and 58 patients demonstrated clinical or biochemical relapse. Twelve patients died during this period, 1 from prostate cancer and 11 from other causes [Gettman et al., 1998]. However, a study comparing anti-PECAM-1 and anti-CD34 MVD assays and their relationship to PSA biochemical failure in 102 patients with radical prostatectomy but not adjuvant hormonal therapy found that the disparate results of the use of MVD as a prognostic indicator may be related to the different antibodies used. The average MVD determined by CD31 staining was significantly lower than that obtained by CD34 staining. Using Kaplan–Meier analysis, anti-CD34 and anti-CD31 MVDs were associated strongly with PSA recurrence on a univariate level, but only anti-CD34 MVD was an independent predictor of PSA failure [de la Taille et al., 2000].

ANTIANGIOGENIC THERAPY FOR PROSTATE CANCER

Because prostate cancer tumors require angiogenesis for growth and metastasis, and angiogenesis is a complex multifactorial process, antiangiogenic therapy could offer a variety of avenues to arrest tumor growth, induce tumor regression, or block the ability of tumors to metastasize. Antiangiogenesis therapy has generally been seen as antiproliferative, but a study using angiostatin, an antiangiogenic molecule which inhibits endothelial cell response to angiogenic therapy, caused regression in three human and three murine primary carcinomas in mice without apparent toxicity. The human carcinomas regressed to microscopic dormant foci in which tumor cell proliferation was balanced by apoptosis when angiogenesis was blocked [O'Reilly et al., 1996]. Tumor regression may be an achievable goal with prolonged antiangiogenic therapy.

Some ongoing trials using antiangiogenic strategies in prostate cancer are listed in the National Cancer Institute database (Table I) found at http://www.cancer.gov/search/clinical_trials/. This page is regularly updated as an overview of current trials of anti-angiogenesis agents. Therapeutic strategies include inhibiting the release of proangiogenic molecules by tumor cells or cells surrounding tumors, inhibiting the angiogenesis-stimulating action of these molecules, and inhibiting endothelial cell response to proangiogenic molecules. Another approach is the delivery or induction of endogenous antiangiogenic molecules. Therapies may also target either the tumor or supporting cells directly, or target the endothelial cells. Modulating the endothelial cells has the advantage of easy intravenous drug delivery and decreased likelihood of developing endothelial cell resistance to the therapy. Table II lists some of the proangiogenic and antiangiogenic factors relevant to prostate cancer.

VEGF Inhibitors

A neutralizing anti-VEGF antibody (A4.6.1) was evaluated for effects on the growth and angiogenic activity of spheroids of the human prostatic cell line DU145 implanted subcutaneously in nude mice. Tumor cells were prelabeled with a fluorescent vital dye (CMTMR), allowing measurement of implanted tumor spheroids throughout a 2-week observation period. FITC-dextran was then used for plasma enhancement to visualize angiogenic activity. Tumors of control animals induced angiogenesis with high vascular density, but in animals treated with the anti-VEGF antibody angiogenesis was completely inhibited in the micro-tumors, resulting in complete inhibition of tumor growth after the initial prevascular angiogenesis-independent growth phase [Borgstrom et al., 1998]. Another study examined the effect VEGF inhibition on primary tumor growth and metastasis in vivo. Using luciferase as a reporter, DU145 cells, which were found to secrete VEGF, and DU145-luciferase were injected subcutaneously and consistently formed tumors in severe combined immunodeficient mice. After 6 weeks, luciferase assays were performed in whole lung lysates, showing significant activity consistent with micrometastasis. Twice weekly treatment with antibody A4.6.1 suppressed primary tumor growth and inhibited metastatic dissemination to the lungs. When treatment was delayed until the primary tumors were well established, further growth and metastatic progression were still inhibited [Melnyk et al., 1999]. A study using glioma cells showed that overexpression of transfected anti-sense-VEGF cDNA led to decreased expression of VEGF in vitro and greatly reduced tumor growth in vivo [Saleh et al., 1996]. The anti-VEGF receptor (flk-1) antibody DC101 showed some efficacy in orthotopic prostate cancer xenografts and prostate cancer bone metastasis. Orthotopically implanted PC-3M-MM2 and LNCaP-LN3 lines along with a PC-3-MM2 bone metastasis model were treated with paclitaxel, DC101, or a combination of both in early and late tumors. Treatment with DC101 alone or in combination with paclitaxel reduced tumor-induced neovascularity as measured by MVD, reduced tumor cell proliferation, and enhanced apoptosis. Additionally, significant inhibition of tumor growth was seen in the bone metastasis model. The authors note that the efficacy of DC101 was much greater in the treatment of early tumors [Sweeney et al., 2002]. Another study showed a cytostatic effect of an anti-VEGF antibody in an androgen-independent prostate cancer xenograft model, and an increased efficacy with the addition of paclitaxel [Fox et al., 2002]. These studies confirm the principle of tumor growth inhibition by targeting angiogenesis within tumors and support the use of anti-VEGF receptor agents in strategies for treating prostate cancer.

A gene therapy study using an antiangiogenic target was utilized in two murine prostate cancer models. Recombinant adenovirus encoding the ligand-binding ectodomain of the VEGF receptor 2 (flk-1) fused to an Fc domain was administered to immunocompromised mice bearing orthotopic LNCaP tumors and TRAMP. Treatment reduced tumor growth by 66% for orthotopic LNCaP tumors and by 42% for spontaneous tumors in TRAMP mice with a corresponding MVD reduction. Survival time was extended in the Ad flk-1–Fc-treated TRAMP mice relative to the control-treated animals [Becker et al., 2002].

Anti-IL-6 Antibodies

Anti-IL-6 monoclonal antibodies, with or without concurrent etoposide, caused tumor regression and apoptosis in a recent study [Smith and Keller, 2001] in which xenografts

TABLE II. Current Clinical Trials of Angiogenic Prostate Cancer Therapy Search Criteria* (http://www.cancer.gov/search/clinical_trials/)

Title of clinical trial	Protocol ID number(s)
Trials closed to accrual	
Phase I study of carboxyamidotriazole for refractory cancers	NCI-92-C-0054P NCI-MB-281 NCI-T91-0170N
Phase I study of SU006668 in patients with advanced solid tumors	UCLA-0004061 NCI-G01-2010 SUGEN-U6668.004
Phase I study of SU5416 with standard androgen ablation and radiotherapy in patients with intermediate or advanced-stage prostate cancer	UCCRC-NCI-4390 NCI-4390
Phase II randomized study of BMS-275291 in patients with hormone-refractory prostate cancer	UCD-CHNMC-PHII-32 CHNMC-PHII-32 NCI-5615
Phase II randomized study of CT-2584 in patients with hormone refractory, metastatic adenocarcinoma of the prostate	CTI-1038 CPMC-IRB-8781
Phase II randomized study of dexamethasone with or without SU5416 in patients with hormone refractory prostate cancer	UCCRC-10428 NCI-49 UCCRC-NCI-49
Phase II randomized study of docetaxel with or without thalidomide in patients with androgen-independent metastatic prostate cancer	NCI-00-C-0033 NCI-17
Phase II randomized study of oral thalidomide in patients with hormone refractory adenocarcinoma of the prostate	NCI-95-C-0178L NCI-CPB-372 NCI-T95-0038N
Phase II study of bevacizumab, estramustine, and docetaxel in patients with hormone-refractory metastatic prostate cancer	CLB-90006
Phase II study of immunization with prostate-specific membrane antigen-pulsed autologous peripheral blood mononuclear cells and IL-12 in patients with metastatic hormone-refractory prostate cancer	UCCRC-9845 NCI-1192
Phase II study of oral carboxyamidotriazole in patients with androgren-independent prostate cancer	NCI-97-C-0059C NCI-T96-0053
Phase II study of the safety and efficacy of shark cartilage (cartilade) in patients with advanced or metastatic cancer	MRMC-CTCA-9501 NCI-V96-1021
Phase III randomized study of low molecular weight heparin (Dalteparin) plus standard therapy versus standard therapy alone in patients with advanced cancer	NCCTG-979251 NCI-P98-0139
Phase III randomized study of matrix metalloprotease inhibitor AG3340 in combination with mitoxantrone and prednisone in patients with hormone refractory prostate cancer	AG-3340-009
Trials open to accrual	
Phase I randomized study of neoadjuvant celecoxib followed by prostatectomy in patients with localized prostate cancer	JHOC-J0007 JHOC-00030801 NCI-N01-95129 NCI-P01-0186
Phase II randomized Study of genistein in patients with localized prostate cancer treated with radical prostatectomy	NU-00U7
Phase II randomized study of zoledronate with or without BMS-275291 in patients with hormone-refractory prostate cancer	MAYO-MC0151 NCI-5361
Phase II study of APC8015 (Provenge) and bevacizumab in patients with progressive prostate cancer	UCSF-0155-01 NCI-2617 UCSF-01554
Phase II study of docetaxel, estramustine, and thalidomide in patients with hormone-refractory prostate cancer	NH-0139 NCI-V01-1681
Phase III randomized study of oral thalidomide versus placebo in patients with androgen-dependent nonmetastatic prostate cancer after limited hormonal ablation	NCI-00-C-0080 NCI-T99-0053

*Date search: September 2003; location: all states and all countries; type of cancer: prostate; NIH Clinical Center only: no; type of trial: all types; type of treatment or intervention: antiangiogenesis therapy; stage/subtype of cancer: all; phase of trial: all phases; status of trial: open and closed; sponsor of trial: all.

of human prostate cancer cell line PC-3, which produces IL-6, were established in nude mice. Tumor volume and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end-labeling (TUNEL) assay were performed. Anti-IL-6 antibody, with or without etoposide, induced 60% tumor regression compared to initial tumor size in addition to apoptosis. Etoposide alone did not induce tumor regression or apoptosis in this animal model, and no synergy was demonstrated between anti-IL-6 antibody and etoposide. Although MVD and angiogenesis were not evaluated in this report, given the association of IL-6 induction of VEGF and angiogenesis [Cohen et al., 1996], reduction of angiogenesis may be one mechanism underlying the observed tumor effects. Dexamethasone therapy, which demonstrates some efficacy in patients with prostate cancer, may work through suppression of serum IL-6.

This suppression is likely through inhibition of androgen-independent activation of androgen receptor [Akakura et al., 2003].

Matrix Metalloproteinase (MMP) Inhibitors

The tissue inhibitors of MMPs are secreted by epithelial cells and are in part stimulated by IL-6 and -10 [Stearns et al., 1995]. Levels of MMPs and TIMPs secreted by epithelial cultures of normal, benign, and malignant prostate were compared in an early study. Analysis of conditioned media showed both normal and prostate cancer tissues grown in culture secreted latent and active forms of both MMP-2 and MMP-9. Normal juvenile and adult prostates secreted significant amounts of free TIMPs, but they were either markedly reduced or not detectable in conditioned media from neoplastic tissues [Lokeshwar et al., 1993]. Immunohistochemical staining of fetal and normal prostate tissues, BPH and prostate cancer showed that TIMP-1 and TIMP-2 were expressed at elevated levels in the stroma of Gleason 5 tissues, while TIMP-1 and TIMP-2 were not expressed in higher Gleason score tissues (8–10). TIMP-1 and TIMP-2 expression was high in organ-confined specimens, somewhat lower in specimens with capsular penetration, and low or negative in samples with positive surgical margins or seminal vesicle involvement and lymph node metastases [Wood et al., 1997]. Co-cultures of prostate cancer cells derived from primary and metastatic tumors with primary or immortalized stromal cells showed enhanced levels of pro-MMP-9 and reduced levels of TIMP-1 and TIMP-2. Enhanced expression of pro-MMP-9 occurred in prostate cancer cells. TIMPs were down-regulated in stromal cells. Induction of pro-MMP-9 and reduction of TIMP expression did not require cell–cell contact and was mediated by a soluble factor(s) present in the conditioned medium of the effector cell [Dong et al., 2001].

IL-10 treatment of PC-3ML cell tumors in a severe combined immunodeficiency (SCID) mouse model effectively inhibited spinal metastasis and increased tumor-free survival rates. IL-10 treatment of the PC-3 ML cells reduced the number of spinal metastases from 70% seen in the natural progression of the model to 5%. Following discontinuation of IL-10 treatment at 30 days, the mice remained tumor-free and mouse survival rates increased from less than 30% in untreated mice to about 85% in IL-10-treated mice. To explore the mechanism behind this result, the authors measured expression of MMPs and TIMPs by ELISA assay in IL-10 treated PC-3ML cells. IL-10 treatment of the PC-3 ML cells down-regulated expression of MMP-2 and MMP-9 and up-regulated expression of TIMP-1, but not TIMP-2. IL-10-treated mice exhibited similar changes in MMP-2, MMP-9, and TIMP-1 expression. IL-10 receptor antibodies blocked the effects of IL-10 on PC-3ML cells [Stearns et al., 1997]. Alendronate, a potent bisphosphonate compound, inhibited TGF-β_1-induced MMP-2 secretion in PC-3ML cells, while TIMP-2 secretion was unaffected. The relative imbalance between the molar stoichiometry of TIMP-2 to MMP-2 resulted in decreased collagen solubilization [Stearns, 1998].

Several well-tolerated, orally active MMP inhibitors (MMPIs) demonstrate efficacy in mouse cancer models. Marimastat (BB-2516) was the first MMP inhibitor to enter clinical trials and has completed phase I [Nemunaitis et al., 1998] and phase II trials in prostate and colon cancer patients. Marimastat was generally well tolerated in Phase I trials. Phase II trials used serum PSA as a marker in patients with prostate cancer. The authors reported a 58% response rate (no increase in serum prostatic specific antigen over the course of the study plus partial response defined as 0–25% increase in serum prostatic specific antigen per 4 weeks) using doses of greater than 50 mg twice daily [Steward, 1999].

The MMP inhibitor batimastat (BB-94) inhibited invasion of DU145 cells in Matrigel and in a murine diaphragm invasion assay [Knox et al., 1998]. Another study looked at the effect of batimastat on MatLyLu cancer cells in vitro and described its in vivo effect on tumor growth in the orthotopic cancer R3327 Dunning tumor rat model. Significant inhibition of tumor cell proliferation in vitro occurred. After orthotopic cell inoculation, tumors grew to mean weights of almost one-half the weight of the control group [Lein et al., 2000]. A novel inhibitor, Ro 28–2653, with high selectivity for MMP2, MMP9 and membrane type 1-MMP was evaluated in an orthotopic prostate cancer rat model. Ro 28–2653 reduced tumor weights by up to 90% in a dose-dependent manner, and had an inhibitory effect on established tumors. A significantly prolonged survival of Ro 28–2653-treated rats was also demonstrated [Lein et al., 2002]. Other

MMPIs are in various stages of pre-clinical and clinical trials, including Bay 12-9566 and prinomastat (Ag3340).

Over-expression of urokinase type plasminogen activator (uPA) by the rat prostate-cancer cell line Dunning R3227, Mat-LyLu, results in increased tumor metastasis to several sites. Histological examination of skeletal lesions showed them to be primarily osteoblastic. A selective inhibitor of uPA enzymatic activity, 4-iodo benzo(b)thiophene-2-carboxamidine (B-428), in this model resulted in a marked decrease in primary tumor volume, weight, and development of tumor metastases compared with controls [Rabbani et al., 1995]. A mutant recombinant murine uPA that retains receptor binding but not proteolytic activity was generated by polymerase chain reaction mutagenesis and transfected into the highly metastatic rat Dunning MAT-LyLu prostate cancer cell line. A clone stably expressing uPA was injected into Copenhagen rats. Tumors found in these animals were significantly smaller with fewer metastases than in control animals. Mean microvessel density in transfected tumors was fourfold lower than in animals with tumors derived from the control tumor cell line [Evans et al., 1997]. These studies demonstrate that uPA-specific inhibitors can decrease primary tumor volume and invasiveness as well as metastasis in prostate cancer models.

To determine the effect of bone cells on prostate cancer cell expression of basement membrane degrading proteins, serum-free conditioned medium harvested from osteoblast cultures was used to stimulate the in vitro chemotaxis of prostate cancer cells and invasion of a reconstituted basement membrane (Matrigel) This enhanced invasive activity was due to osteoblast cell-conditioned media stimulated secretion of uPA and matrix MMP-9. Inhibition of these matrix-degrading proteases by neutralizing antibodies or by inhibitors of their catalytic activity reduced Matrigel invasion, demonstrating that factors produced during osteogenesis by bone cells stimulate prostate cancer cell chemotaxis and matrix protease expression. This exchange presents a potential target for alternative therapies deterring prostate cancer metastasis to bone [Festuccia et al., 1999].

Stearns et al. [1999b] demonstrated that antibodies to MMP-2 and -9 inhibit induction of microvessel formation in vitro. MMP-9 expression is partly inhibited by anti-alpha-2-integrin antibody, a major collagen I receptor [Dong et al., 2001]. MMPs are primarily involved in the early stages of angiogenesis. Therefore, therapies against these molecules would probably best be used early in cancer development.

Prostate Specific Antigen (PSA)

PSA translation is regulated by androgen. There are two androgen response elements (AREs) in the 5′ untranslated region of PSA mRNA. The antiangiogenic property of PSA was delineated by administering PSA to bovine endothelial cells and human endothelial cell lines (HUVEC and HMVEC-d) with subsequent stimulation with FGF-2 or VEGF. PSA was antiproliferative in vitro, in a dose-dependent manner, in all three cell lines with an IC_{50} (concentration at which inhibition was 50%) from 0.6 to 4.0 μM after stimulation with FGF-2. However, PC-3 cells and murine melanoma cells (B16BL6) were not inhibited by PSA in vitro. HUVEC cells treated with PSA and stimulated with VEGF showed an IC_{50} of 4 μM versus an IC_{50} of 1.2 μM in FGF-2 stimulated cells in wound-migration assays. Similarly, Boyden chamber assays found PSA (5 μM) inhibited FGF-2 stimulated HUVEC invasion by 77%, while PSA (300 nM to 3 μM) inhibited tube formation of HUVEC in Matrigel by approximately 50%, in a dose-dependent manner. The authors state that on a molar basis, PSA inhibition on both endothelial cell proliferation and migration was 5–10-fold less potent than angiostatin and endostatin. PSA was then administered to mice at 9 μM for 11 consecutive days, after intravenous inoculation of B16BL6 melanoma cells, to assess its ability to inhibit the formation of lung colonies. PSA treatment resulted in a 40% reduction in the mean number of lung tumor nodules in this model [Fortier et al., 1999]. These authors then expressed a recombinant human PSA in the yeast *Pichia pastoris* and compared its activity with PSA purified from seminal plasma in a modified Boyden chamber migration assay. They found that this assay was more sensitive to the inhibitory effects of PSA and demonstrated that concentrations in the 100 nM range for both forms of PSA resulted in 50% inhibition of endothelial cell migration [Fortier et al., 2000].

Angiostatin

Angiostatin, a circulating inhibitor of angiogenesis, was first discovered in the presence of a murine Lewis lung tumor. A mouse corneal neovascularization model stimulated by a bFGF pellet detected circulating inhibitors of angiogenesis generated by PC-3 human prostate carcinoma grown in immunodeficient mice. These mice demonstrated significant inhibition of angiogenesis in the cornea, and significant inhibition of vessel length, clock-hours of neovascularization, and vessel density [Chen et al., 1995]. A mechanism behind angiostatin generation in human prostate carcinoma cell lines (PC-3, DU145, and LNCaP) was found in the expression of serine protease enzymatic activity, which can generate bioactive angiostatin from purified human plasminogen or plasmin [Gately et al., 1996]. Endogenous molecules sufficient for angiostatin generation were later identified as urokinase (uPA) and free sulfhydryl donors (FSDs) in PC-3 cells. Furthermore, in a defined cell-free system, angiostatin is generated from plasminogen by plasminogen activators such as uPA, tissue-type plasminogen activator (tPA), or streptokinases, in combination with one of a series of free sulfhydryl groups (*N*-acetyl-L-cysteine, D-penicillamine, captopril, L-cysteine, or reduced glutathione).

Cell-free derived angiostatin inhibited angiogenesis in vitro and in vivo and suppressed the growth of Lewis lung carcinoma metastases [Gately et al., 1997]. Serine protease PSA can convert plasminogen to biologically active angiostatin-like fragments. In an in vitro morphogenesis assay, purified angiostatin-like fragments inhibited proliferation and tubular formation of human umbilical vein endothelial cells with the same efficacy as angiostatin [Heidtmann et al., 1999]. Incubating plasminogen with conditioned media from prostate cancer cells resulted in purification of procathepsin D, a lysosomal proenzyme, which when converted to pseudocathepsin D generated two angiostatic peptides shown to inhibit angiogenesis both in vitro and in vivo [Morikawa et al., 2000]. Adenovirus-delivered angiostatin (AdK3) in association with docetaxel have been evaluated in a prostate cancer model and significant antitumoral effects were observed only with combined treatment [Galaup et al., 2003].

Endostatin

Endostatin, a 20 kDa C-terminal fragment of collagen XVIII, was discovered as an angiogenesis inhibitor produced by hemangioendothelioma. A potent inhibitor of angiogenesis and tumor growth, endostatin specifically inhibits endothelial proliferation. Primary tumors treated with endostatin regressed to dormant microscopic lesions similar to the effect in angiostatin-treated tumors [O'Reilly et al., 1996]. Immunohistochemistry revealed high proliferation balanced by apoptosis in tumor cells, blocking angiogenesis without apparent toxicity [O'Reilly et al., 1997].

The effects of endostatin treatment on spontaneous prostate cancer tumorigenesis were evaluated in a transgenic mouse model developed by insertion of an SV40 early-region transforming sequence under the regulatory control of a rat prostatic steroid-binding promoter. The SV40 Tag functionally inactivates p53 and Rb through direct binding to these proteins and appears to interfere with cell cycle regulation. Adenomas develop in about one-third of animals between 6 and 8 months of age and approximately 40% of male mice develop invasive prostate adenocarcinomas by 9 months. Mouse endostatin expressed in yeast was administered to mice 7 weeks before the expected visibility of tumors. No decrease in tumor burden was seen but the authors did demonstrate prolonged their survival time (an additional 74 days) [Yokoyama et al., 2000]. In 13 men heterozygous for the polymorphism D104N and 13 men homozygous for the allele, diagnosed with prostate cancer, a single nucleotide polymorphism (D104N) may have impaired the function of endostatin. Serum ELISA analysis demonstrated similar endostatin levels in both carriers and non-carriers of this mutation. The results of statistical analysis predict that individuals heterozygous for N104 have a 2.5 times greater chance of developing prostate cancer compared to men containing two wild-type endostatin alleles. Based on sequence comparison and structural modeling, this polymorphism in endostatin may inhibit the ability to interact with other molecules [Iughetti et al., 2001].

Interferons (IFNs)

Interferons have been used to modulate the immune regulation in many cancers but may

also affect angiogenesis in carcinomas. The antiviral activity of IFNs led to their discovery, but later data revealed that they also control cell growth and differentiation, inhibit expression of oncogenes, and activate T lymphocytes, natural killer cells, and macrophages [Krown, 1988]. IFNs have been extensively studied in clinical trials and have been shown to be effective against many vascular tumors. Some reports suggest that this effect is due to inhibition of angiogenesis [Dinney et al., 1998].

An in vitro study evaluated the effect of purified human fibroblast IFN-β and recombinant IFN-β on cell proliferation in PC-3 and DU145 cells. Both cell lines responded to the antiproliferative action of interferon, IFN-β being more effective than IFN-α. PC-3 cells were more sensitive than the DU145 cell line, showing 95% inhibition of cell proliferation at the highest concentration of IFN-β [Sica et al., 1989]. A human renal carcinoma cell metastatic line (SN12PM6) was established in culture from a lung metastasis and SN12PM6-resistant cells were selected in vitro for resistance to the antiproliferative effects of IFN-α or IFN- β. IFN-α and IFN-β, but not IFN-γ, down-regulated the expression of bFGF at the mRNA and protein levels by a mechanism independent of their antiproliferative effects. The withdrawal of IFN-α or IFN-β from the medium permitted SN12PM6-resistant cells to resume production of bFGF. Additionally, the incubation of human prostate carcinoma cells with non-cytostatic concentrations of IFN-α or IFN-β also produced down-regulation of bFGF production [Singh et al., 1995]. Thus, the inhibitory action of IFN-α and IFN-β on angiogenesis may act indirectly through down-regulation of bFGF.

Orthotopic and subcutaneous implantation of PC-3M human prostate cancer cells, engineered to constitutively produce murine IFN-β, as well as PC-3M-P and PC-3M-Neo cells in vivo in nude mice, demonstrated the antiproliferative effects of IFN-β. PC-3M-P and PC-3M-Neo cells produced rapid-growing tumors and regional lymph node metastases, whereas PC-3M-IFN-β cells did not. PC-3M-IFN-β also suppressed the tumorigenicity of bystander non-transduced prostate cancer cells. Immunohistochemical staining revealed that tumors were homogeneously infiltrated by macrophages. MVD assays showed that control tumors contained more blood vessels than PC-3M-IFN-β tumors. The authors suggest that suppression of tumorigenicity and metastasis of PC-3M-IFN-β cells is due to inhibition of angiogenesis and activation of host effector cells [Dong et al., 1999]. Small clinical trials have shown limited IFN-β effectiveness in patients with advanced hormone refractory prostate cancer [Bulbul et al., 1986]. IFN-β gene transfer was tested on PC3MM2 human prostate cancer cells in nude mice. In this study, intralesional delivery adenoviral vector encoding murine IFN-β (AdIFN-β) suppressed PC3MM2 tumors in a dose-dependent manner. At the highest dose (2×10^9 plaque-forming units, PFU), a single injection of AdIFN-beta (but not controls) suppressed orthotopic PC3MM2 tumors and development of metastasis by 80%, and eradicated the tumors in 20% of mice [Cao et al., 2001]. Inhibition of growth and metastasis of orthotopic human prostate cancer in mice by combination therapy with interferon-alpha-2b and docetaxel has also been shown [Huang et al., 2002].

A phase I trial based on prior studies demonstrating the effect of 13-*cis*-retinoic acid and interferon alpha (CRA/IFN) in decreasing the expression of the anti-apoptotic protein bcl-2 was reported. Investigators performed a study of weekly paclitaxel (TAX) in combination with CRA/IFN in patients with prostate cancer and other advanced malignancies. A total of 13 patients with prostate cancer or other advanced malignancies were treated with 1 mg/kg CRA on days 1 and 2, 6 MU/m^2 IFN subcutaneously on days 1 and 2, and TAX at increasing doses on day 2 each week for 6 weeks out of an 8-week cycle. The effect of CRA/IFN on bcl-2 expression was assessed in PBMCs by immunoblotting. The combination of CRA/IFN and TAX was well tolerated. Of 13 patients assessable by tumor markers or scans, 5 had stable disease and 2 had a biochemical partial response including a patient with a decrease in PSA of >50% while on study [Thalasila et al., 2003].

Fumagillin Analogue TNP-470

Despite many pharmacological studies, current knowledge of the molecular mode of action of TNP-470 is limited. TNP-470 exerts biphasic growth inhibition; reversible cytostatic activity toward endothelial cells at low doses (complete growth inhibition at 0.75 nM), but cytotoxic effects are observed at higher concentrations (75 μM) for all cell types tested [Kusaka et al., 1994]. Cytostatic inhibition is thought

to be responsible for its antiangiogenic effect, because the serum concentration of TNP-470 in rats after systemic administration was much lower than that required for cytotoxic inhibition. Incorporation of thymidine, but not uridine and leucine, in HUVEC is inhibited by TNP-470 treatment, suggesting specific inhibition of DNA synthesis. At the molecular level, TNP-470 does not inhibit early G_1 mitogenic events, such as cellular protein tyrosyl phosphorylation or the expression of immediate early genes [Abe et al., 1994]. TNP-470 potently inhibits the activation of CDK2 and CDC2 as well as retinoblastoma protein phosphorylation, although not through direct kinase inhibition [Abe et al., 1994]. In vitro, enhanced suppression of prostate tumor growth was obtained by combining C-CAM1 gene therapy with TNP-470 [Pu et al., 2002]. While in vivo, TNP-470 combined with nicardipine suppressed growth of PC-3 [Arisawa et al., 2002].

TNP-470 potently inhibits the tumor growth of hormone-independent prostate cancer PC-3 cell xenografts in vivo, with a maximum inhibition of 96%. Combination therapy with cisplatin and TNP-470 showed an additive antiproliferative effect against PC-3 cells. In vitro studies showed that PC-3 cells are considerably insensitive to TNP-470 in monolayer cultures (50% inhibitory concentration at 5 μg/ml), whereas TNP-470 did inhibit the anchorage-independent growth of PC-3 (50% inhibitory concentration at 50 pg/ml) [Yamaoka et al., 1993]. One group demonstrated that induction of TNP-470 therapy increased the secretion of PSA up to 1.5-fold irrespective of tumoricidal effects [Horti et al., 1999], which may erroneously suggest tumor progression unless clinicians are aware of this paradoxical effect.

A Phase I dose escalation trial of alternate-day intravenous TNP-470 therapy in 33 patients with metastatic and androgen-independent prostate cancer has been completed. The dose-limiting toxic effect was a characteristic neuropsychiatric symptom complex (anesthesia, gait disturbance, and agitation) that resolved upon cessation of therapy. No definite antitumor activity of TNP-470 was observed, but transient stimulation of the serum prostate-specific antigen concentration occurred in some of the patients treated [Logothetis et al., 2001].

Thalidomide

Thalidomide, once marketed in Europe as a sedative, was withdrawn 30 years ago because of potent teratogenic effects that cause stunted limb growth (dysmelia) in patients. However, in vitro data suggested that thalidomide has antiangiogenic activity induced by bFGF in a rabbit cornea assay [D'Amato et al., 1994]. In a recent report on a randomized Phase II study of thalidomide in patients with androgen-independent prostate cancer, with a total of 63 patients enrolled in the study, 50 patients on the low-dose arm received a dose of 200 mg/day and 13 patients on the high-dose arm received an initial dose of 200 mg/day that escalated to 1200 mg/day. A serum PSA decline of greater than or equal to 50% was noted in 18% of patients on the low-dose arm, but in none of the patients on the high-dose arm. A total of 27% of all patients had a PSA decline greater than or equal to 40%, often associated with an improvement of clinical symptoms. Only four patients were maintained for over 150 days. The most prevalent complications were constipation, fatigue, and neurological disorders. The authors note that the decline in PSA in these patients may be particularly important since pre-clinical studies showed thalidomide increasing PSA levels [Figg et al., 2001].

A study was undertaken using once daily thalidomide for up to 6 months in 20 men with androgen-independent prostate cancer. The mean time of study was 109 days (median 107, range 4–184 days). Three men (15%) showed a sustained decline in serum PSA of at least 50%, and 6 of 16 men (37.5%) treated for at least 2 months demonstrated a fall in absolute PSA by a median of 48%. Other findings included increasing levels of serum bFGF and VEGF associating with progressive disease; while five of six men who demonstrated a fall in PSA also showed a decline in bFGF and VEGF levels, three of four men with a rising PSA showed an increase in both growth factors. Peripheral neuropathy did occur from treatment in some of these patients [Drake et al., 2003].

Calcium Channel Blocker Carboxyamido-Triazole (CAI)

A Phase II clinical trial of the antiproliferative, antimetastatic, and antiangiogenic agent carboxyamido-triazole (CAI) was evaluated in fifteen patients with stage D2 androgen-

independent prostate cancer with soft tissue metastases. Because CAI was previously shown to decrease PSA secretion in vitro, this marker was not used. Fourteen of 15 patients were evaluated for response and all 14 patients demonstrated progressive disease at approximately 2 months. Twelve patients progressed by computed tomography or bone scan at 2 months, and two patients demonstrated clinical progression at 1.5 and 2 months. One patient was removed from the study at 6 weeks due to grade II peripheral neuropathy lasting over a month. No clinical responses were noted, but a 28% decrease in serum VEGF concentration was observed. CAI does not possess clinical activity in patients with androgen-independent prostate cancer and soft tissue metastases [Bauer et al., 1999].

Linomide

Linomide (*N*-phenylmethyl-1,2-dihydro-4-hydroxyl-1-methyl-2-oxo-quinoline-3-carboxamide) is a quinoline 3-carboxamide previously demonstrated to modulate immune response and produce antitumor effects when given in vivo. Five distinct Dunning R-3327 rat prostatic cancer subline models were treated daily with intraperitoneal injections of linomide, with an antitumor effect against all of the prostatic cancers tested, regardless of their growth rate, degree of morphologic differentiation, metastatic ability, or androgen responsiveness. This antitumor effect was observed only in vivo, not in vitro, and was cytotoxic to prostatic cancer cells. This cytotoxic response resulted in the retardation of the growth rate of both primary prostatic cancers and in metastatic lesions. Interestingly, the authors found that growth retardation due to linomide was reversible, and continuous daily treatment was required for maximal antitumor response. The antitumor effects of linomide were also demonstrated in prostatic cancer-bearing athymic nude rats. The antitumor effects of linomide against rat prostatic cancers may involve both immune and non-immune host mechanisms [Ichikawa et al., 1992].

This group recognized that linomide treatment has antiangiogenic activity, namely the observation that prostatic cancers from linomide treated rats have more focal necrosis than sized-matched tumors from untreated rats. They demonstrated that linomide has dose-dependent antiangiogenic activity in the rat using a Matrigel-based quantitative in vivo angiogenic assay [Vukanovic et al., 1993]. In another series of experiments, linomide was unable to inhibit either basal or hypoxia-induced secretion of VEGF in human prostate cancer cells [Joseph and Isaacs, 1997]. Linomide also has no effect on secreted bFGF levels. Castration inhibited tumor VEGF but had no effect on bFGF levels in both the androgen-responsive PC-82 and A-2 human prostatic cancers when grown in severe combined immunodeficient mice. When given in combination, castration potentiated the inhibition of tumor growth induced by linomide alone. This potentiation is not due to a further inhibition in tumor VEGF levels induced by castration. Although both castration and Linomide inhibit angiogenesis, the former accomplishes it by inhibiting VEGF secretion, whereas the latter has multiple effects at several steps in the angiogenic process other than VEGF secretion. Based on their different but complementary mechanisms of action, the combination of androgen ablation with linomide enhances therapeutic efficacy compared to either monotherapy alone. This promising modality appears to warrant testing in humans.

Immunoconjugate Therapy

Targeting endothelial cells with immunoconjugates that selectively occlude the vasculature of solid tumors [Ruoslahti, 2000] could offer advantages over challenging the tumors themselves. Tumor dependence on blood supply could lead to tremendous apoptotic response after interruption of the tumor vasculature. Secondly, the tumor vascular endothelium is in direct contact with the bloodstream for the delivery of endothelial cell toxic molecules. Thirdly, lack of tumor vascular endothelial cell transformation suggests that they are unlikely to acquire mutations that render them resistant to therapy. One study looked at the use of human tissue factor in tumor vascular endothelium in a mouse model. Tissue factor is the major initiating receptor for blood coagulation cascades. Assembly of cell surface tissue factor with factor VII/VIIa generates the functional tissue factor–factor VIIa complex which rapidly activates the serine protease zymogens factors IX and X by limited proteolysis, leading to the formation of thrombin and, ultimately, a blood clot. The investigators used a recombinant form of tissue factor containing only the

cell surface domain of the protein. This truncated tissue factor contains factor X-activating activity about five orders of magnitude less than native transmembrane tissue factor in an appropriate phospholipid membrane environment. Using an antibody to target this truncated form of tissue factor to tumor vascular endothelium; it was brought into proximity with a cell surface and partially recovered its native function, resulting in locally initiated thrombosis. Antibody-truncated tissue factor conjugate seems to have some potential to selectively thrombose tumor vasculature [Huang et al., 1997].

Integrin αVβ3 Antagonists

In a 1994 study, a single intravascular injection of a cyclic peptide or monoclonal antibody antagonist of integrin αVβ3 disrupted ongoing angiogenesis in the chick chorioallantoic membrane (CAM) assay, leading to the rapid regression of human tumors transplanted onto the CAM. The authors state that induction of angiogenesis by a tumor or cytokine promotes vascular cell entry into the cell cycle, resulting in expression of integrin αVβ3. After angiogenesis is initiated, antagonists of this integrin induce apoptosis of the proliferative angiogenic vascular cells, leaving pre-existing quiescent blood vessels unaffected [Brooks et al., 1994].

CONCLUSION

Angiogenesis is a critical requirement for local proliferation and metastasis in prostate cancer. Antiangiogenic therapy is a particularly attractive antitumor modality with many potential targets, considering that the regulation of tumor angiogenesis is profoundly multifactorial. As the above summaries show, a variety of avenues are being explored to delineate the molecular biologic mechanisms of tumor angiogenesis as well as potential antiangiogenic therapies.

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