REVIEW

Angiogenesis of prostate cancer and antiangiogenic therapy

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Abstract : Tumor-associated angiogenesis refers to the growth of new vessels toward and within the tumor. Several studies have revealed that increasing intratumoral microvessel density, a major of tumor-associated angiogenesis, correlates with greater aggressiveness of prostate cancer. Angiogenesis consists of multiple, sequential, and interdependent steps dependent on the local balance of proangiogenic and antiangiogenic molecules. Many proangiogenic and antiangiogenic molecules have been demonstrated to regulate growth and metastasis of prostate cancer.

As tumor-associated angiogenesis is a crucial step in the process of prostate cancer development, inhibition of tumor neovascularization, and/or destruction of tumor vasculature (antiangiogenic therapy) may maintain the tumors in a dormant state or, perhaps in combination with cytotoxic therapies, potentiate shrinkage of tumors. Recently, therapeutic agents targeting the receptors of proangiogenic molecules and their signal transduction cascade have been developed.

In this article, the role of angiogenic molecules in prostate cancer biology, and the application of angiogenesis inhibition to therapeutics for prostate cancer are reviewed.

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INTRODUCTION

Prostate cancer is the most common cancer and the second leading cause of death in men in North America (1). In Japan, age-standardized mortality has increased by 150% over the 25 years up to 1997 (2). The major cause of death from this disease is metastasis of hormone-refractory cancer cells. The metastases are commonly found in lymph nodes or bones (1, 3), and the specific organ microenvironment can influence the biological behavior of metastatic cells, including their response to systemic therapy (4). Stephen Paget proposed that some tissues may provide a better environment than other tissues for the growth of certain tumor cells (the seed) are compatible with a particular organ tissue (the soil). Metastasis only resulted when the seed and soil were compatible (5). A prime example of this principle is the contribution of angiogenesis to the growth and metastasis of cancer. The growth and spread of prostate cancer are dependent on the formation of adequate vasculature, *i.e.*, angiogenesis regulated by angiogenic factors (6-8).

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TUMOR-ASSOCIATED ANGIOGENESIS

Angiogenesis consists of multiple, sequential, and interdependent steps dependent on the local balance of positive and negative regulatory factors. The major pro-angiogenic factors include vascular endothelial growth factor/vascular permeability factor (VEGF/VPF), inter-leukin-8 (IL-8), fibroblast growth factor 2 (FGF 2), epider-mal growth factor (EGF) and platelet-derived growth factor (PDGF), and the major antiangiogenic factors include interferon (IFN), endostatin, angiostatin, and thrombospondin (8-14).

Tumor-associated angiogenesis refers to the growth of new vessels toward and within the tumor. Tumors

can not grow no longer than 2-4 mm in diameter until they are vascularized (9, 15). Compelling data implicate tumor-associated angiogenesis as a central pathologic step in the process of tumor growth, invasion, and metastasis. Several studies have revealed that increasing intratumoral microvessel density, a major of tumor-associated angiogenesis, correlates with greater tumor aggressiveness, such as a higher frequency of metastases and/or decreased survival in prostate cancer and other solid tumors (15-17).

PROANGIOGENIC MOLECULES

1) Vascular endothelial growth factor (VEGF)

VEGF is one of the most potent facilitators of angiogenesis with affect on endothelial cell proliferation, motility, and vascular permeability. VEGF binds with high-affinity to the tyrosine kinase receptors *Flt-1* (VEGFR-1) and *Flk-1/KDR* (VEGFR-2) expressed by endothelial cells (18-22). VEGF expression has been demonstrated in prostate cancer specimens (23) and in LNCaP, PC3, and DU145 prostate cancer cell lines (24-26). Kuniyasu *et al*. evaluated the expression level of VEGF/VPF in archival prostatectomy specimens from prostate cancer patients using a rapid colorimetric in situ hybridization technique. The relationship between advancing pathological stage and expression of VEGF/VPF gene was highly significant. Increased expression of VEGF/VPF was associated with the Gleason score of the tumors (27). Monoclonal antibodies that neutralize VEGF inhibit both the growth and metastatic spread of DU145 prostate cancer xenografts in severe combined immune-deficient mice and decrease the growth of LNCaP tumors in nude mice, suggesting that VEGF is a critical factor for the progression of prostate cancer (28, 29).

Additionally, expression of VEGFR-1 and R-2 in prostate cancer, prostatic intraepithelial neoplasia, and the basal cells of normal glands, has been reported (23, 30). In comparison with normal glands, the expression of VEGFR-1 and R-2 increases in prostatic intraepithelial neoplasia and well to moderately differentiated prostate cancer. These observations suggest that VEGF plays a role on tumor cell activation (autocrine), in addition to paracrine actions whereby it regulates endothelial cell functions and subsequent neovascular development (23).

Recently, vascular endothelial growth factor C (VEGF-C) which belongs to the platelet-derived growth factor (PDGF)/VEGF family of growth factors was identified as a ligand for the endothelial-specific receptor tyrosine kinases VEGFR-3 and VEGFR-2. The expression of VEGFR-3 was found to be highly restricted to the lymphatic endothelial cells (31). VEGF-C expression in prostate cancer cells is implicated in the lymph node metastasis (32).

2) Interleukin-8(IL-8)

IL-8, which belongs to the superfamily of CXC chemokines, has a wide range of proinflammmatory effects and is produced by various cells, including lymphocytes, monocytes, endothelial cells, fibroblasts, hepatocyte, keratinocyte, and various tumor cells including prostate cancer cells (33, 34). It has been shown that IL-8 enhances production and secretion of collagenase type IV by tumor cells, suggesting that it can modulate invasiveness, and/or extracellular matrix remodeling in the tumor environment. As cell proliferation, angiogenesis, migration, and invasion are important component of the metastatic process, IL-8 expression by tumor cells can influence their metastatic capabilities (35). Indeed, the expression of IL-8 has been shown to correlate with angiogenesis and the metastatic potential of human prostate cancer cells (36-38). When low and high IL-8-producing clones isolated from the heterogeneous PC3 human prostate cancer cell line were injected into the prostate of nude

Table 1. Tumorigenic potential of PC-3 cells with low or high expression of IL-8.

Number of Cells Injected[1]	PC-3 IL-8 low		PC-3 IL-8 high	
	Incidence[2]	Tumor Weight Median (range)	Incidence	Tumor Weight Median (range)
1.25×10^4	0/5	0	0/5	0
2.5×10^4	0/5	0	0/5	0
5×10^4	1/5	120	4/4	170(110-180)
1×10^5	1/5	100	5/5	220(100-240)
2×10^5	4/4	180(140-200)	5/5	670(420-780)
5×10^5	5/5	230(190-270)	5/5	860(560-1040)

[1]Nude mice (n=5) were given prostate injections of the incidental number of PC-3 IL-8 low or high cells. The mice were killed 5 weeks later at which point the prostate and tumors were removed and weighed (tumor weight is in milligrams).
[2]Number of positive mice/number of mice injected.

mice, PC3 cells expressing high levels of IL-8 were highly tumorigenic, producing rapidly growing prostate tumors. On the other hand, low IL-8-expressing PC-3 cells were less tumorigenic, producing slower growing tumors (Table 1). Additionally, prostate tumors produced by high IL-8-expressing PC-3 cells showed higher vascularity with significantly higher incidence of metastasis than the tumors produced by low IL-8-expressing PC-3 cells (38).

The pleiotropic transcription factor NF-κB regulates the expression of multiple genes including IL-8 and matrix metalloproteinase (MMP)-9 in several types of cells (39-42), and is constitutively activated in prostate cancer cells(43). Blockade of NF-κB activity in human prostate cancer cells inhibits *in vitro* and *in vivo* expression of VEGF, IL-8 and MMP-9, and hence decreases neoplastic angiogenesis (44).

3) Fibroblast growth factor 2(FGF 2)

FGF2 (*i.e.* bFGF) is synthesized mainly by stromal fibroblasts in prostate. When prostate cancer converts to an invasive phenotype, the cancer cells respond to FGF 2 through high-affinity FGFR 2IIIc receptor. Then, the cancer cells synthesize their own FGF 2 to propagate their own growth. In addition, secreted FGF 2 acts on the endothelial cells to promote tumor angiogenesis (45). Several studies have shown that the metastatic potential of prostate cancer cells directly correlated with the gene expression level of FGF 2 (46, 47). Metastatic variant of human prostate cancer cell line expresses higher FGF 2 mRNA than parental cell line (47).

4) Epidermal growth factor (EGF)

The interaction of EGF with its receptor EGF-R has been shown to play an important role in neoplastic angiogenesis (48-50). The expression of VEGF, major proangiogenic factor, is strongly induced by EGF and transforming growth factor-α (51, 52). The expression of EGF and EGF-R is observed in both benign prostatic hyperplasia and prostate cancer (53, 54). Several studies have shown that the metastatic potential of prostate cancer cells directly correlated with the expression level of angiogenesis-and metastasis-related genes including EGFR (19, 55).

5) Platelet-derived growth factor (PDGF)

PDGF is a dimer that consist of AA, BB and AB proteins, and a ligand of PDGF receptor (PDGF-R), a member of a family of protein tyrosine kinases, encoded by two genes (PDGF-Rα and PDGF-R β)(56). PDGF and PDGF-R are co-expressed in many human cancers including prostate cancer (57). The binding of PDGF to PDGF-R can stimulate cell division (58-60), cell migration (61), and angiogenesis (62).

ANTIANGIOGENIC MOLECULES

1) Interferon (IFN)

IFNs are multifunctional regulatory cytokines involved in control of cell function and replication. IFN-α and IFN-β directly inhibit the proliferation of tumor cells of different histological origins (63-66). IFN-α and IFN-β can also down-regulate the expression of proangiogenic molecules, such as FGF2 (67-69) and IL-8(70, 71). The combined treatment with pegylated IFN-α and docetaxel inhibits neoplastic angiogenesis by inducing a decrease in the local production of proangiogenic molecules by human prostate cancer cells in nude mice, resulting in increased apoptosis of tumor-associated endothelial cells (72).

2) Endostatin

Endostatin is a 20 kDa C-terminal fragment of collagen XVIII. Endostatin specifically inhibits endothelial proliferation and potently inhibits angiogenesis (73). Endostatin treatment delays the onset of spontaneous mammary tumorigenesis in female transgenic mice, and prolonged survival time of male transgenic mice that develop prostate adenocarcinomas (74).

INHIBITION OF TUMOR-ASSOCIATED ANGIOGENESIS BY MOLECULAR TARGETING AGENTS

Therapeutic approaches targeting the receptors of proangiogenic molecules and their signal transduction cascade may result in small avascular tumors maintained in a dormant state or, perhaps in combination with cytotoxic therapies, they may potentiate shrinkage of tumors.

PDGF binding causes PDGF-R activation, which involves dimerization and autophosphorylation (i.e. activation) of specific tyrosines in the cytoplasmic domain of PDGF-R. Activation of PDGF-R has been shown to inhibit some apoptotic pathways in normal cells and in tumor cells (75, 76). We determined whether blockade of the PDGF-R signaling pathway by oral administration of STI571(Gleevec, Novartis Pharmaceuticals), PDGF-R tyrosine kinase inhibitor, inhibits the growth of PC-3 MM 2 human prostate cancer cells in the bone of nude mice. PC-3 MM2 induced lytic lesions in the bone and expanded into the surrounding muscle. Tumor cells adjacent to the bone expressed high levels of VEGF,

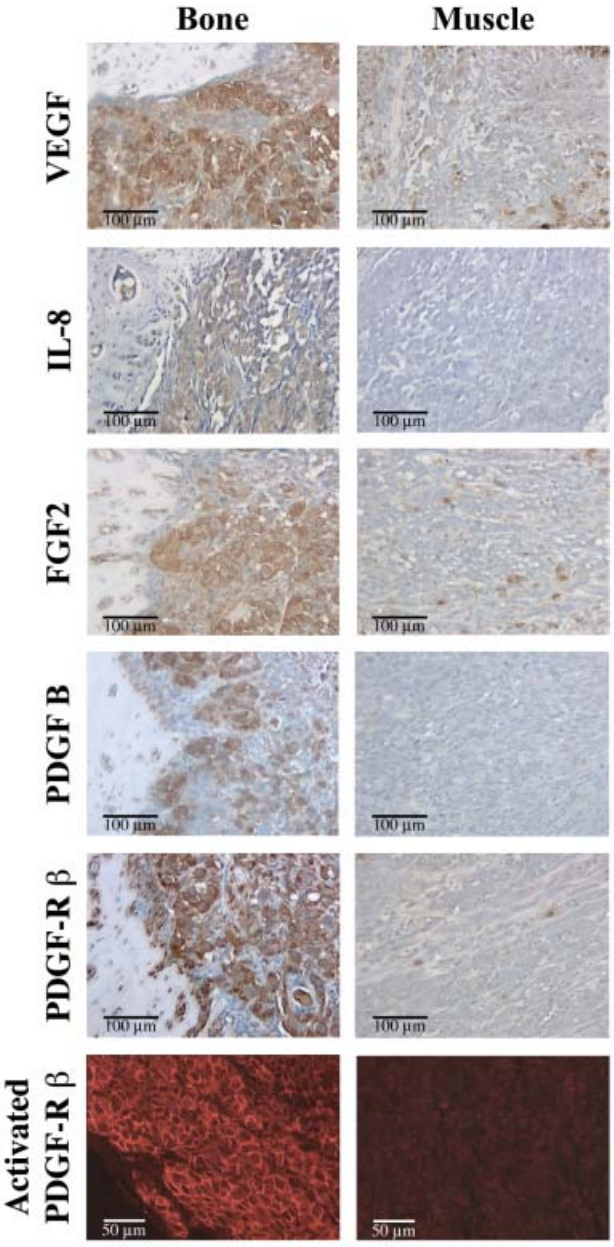

Fig.1. Immunohistochemical analysis of the different expression of vascular endothelial growth factor (VEGF), interleukin 8(IL-8), fibroblast growth factor 2 (FGF2), platelet-derived growth factor B (PDGF B), PDGF receptor β (PDGF-R β), and activated PDGF-R β by PC-3 MM 2 cells growing in the hind leg bones (**left column**) and surrounding muscles (**right column**) of control nude mice. The expression of VEGF, IL-8, FGF2, PDGF B, PDGF-R β (**brown**) and activated PDGF-R β (**red**), was higher in bone lesions than muscle lesions.

IL-8, FGF2, PDGF B, PDGF-R β, and activated PDGF-R β, compared with the tumor cells growing in the surrounding muscle (Fig.1). Treatment with STI571 or STI571 plus paclitaxel inhibited tumor growth and angiogenesis, and it was more effective to the tumor cells adjacent to the bone (77).

Agents targeting epidermal growth factor-receptor (EGF-R) and its signal transduction cascade include 1) monoclonal antibodies, directed against the extracellular binding domain of the receptor, or binding to the HER 2 receptor ; and 2) low-molecular-weight inhibitors of the EGF-R tyrosine kinase.

Cetuximab (C225, ImClone Systems, Inc.) is a chimeric monoclonal antibody with specificity for the external ligand-binding domain of EGF-R. Both dihydrotestosterone and EGF can stimulate proliferation of androgen-responsive prostate cancer cell lines, MDA PCa 2 a and MDA Pca 2 b, and this proliferation is associated with stimulation of cyclin-dependent kinase (CDK)-2 activity and downregulation of the CDK inhibitor gene $p27^{Kip1}$. Dual blockade of the EGF-R family with C225 and of androgen-receptor function resulted in significant growth inhibition (78). Treatment with C225 with or without paclitaxel inhibits growth, metastasis, and angiogenesis of PC-3M-LN4 prostate cancer cells implanted orthotopically in athymic nude mice(79).

ZD1839 (AstraZeneca), a low-molecular-weight anilino-quinazoline is a potent and specific inhibitor of EGFR tyrosine kinase activity. EGF-induced neovascularization of mice cornea is inhibited by ZD1839 treatment (80). Administration of ZD1839 and more so ZD1839 plus cytotoxic agents inhibit the growth of a wide range of human tumors grown as xenografts in nude mice, including prostate tumors (81). PKI 166 (Novartis Pharmaceuticals) is also a selective inhibitor of EGFR tyrosine kinase activity. Treatment with PKI 166 inhibits the growth and angiogenesis of PC-3 MM 2 human prostate cancer cells implanted in the bone of nude mice (82).

DC 101(ImClone Systems, Inc.) is a neutralizing monoclonal antibody that binds to the murine VEGFR-2/flk-1 receptor with high affinity and blocks ligand-induced receptor activation. Tumorigenicity, metastasis, and neovascularization in orthotopic prostate cancer xenografts in nude mice are reduced by a treatment with DC 101(83).

CONCLUDING REMARKS

Prostate cancer is the most common cancer in North America. The prostate cancer death rate is rapidly increasing in Japan. The major cause of death from prostate cancer is metastases that are resistant to therapy. In prostate cancer, same as other cancers, tumor-associated angiogenesis is a crucial step in the process of tumor growth, invasion, and metastasis, and depends on the local balance of proangiogenic and antiangiogenic factors. Therefore therapeutic agents and strategies are being devised either to interrupt or inhibit one or more of the pathogenic steps involved in the process of tumor neovascularization or to directly

target and destroy the tumor vasculature. Antiangiogenic therapy may provide an additional novel prostate cancer treatment suitable for combination with standard therapies.

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