

Review

Effects of androgen suppression and radiation on prostate cancer suggest a role for angiogenesis blockade

WA Woodward¹, P Wachsberger², R Burd² & AP Dicker^{2*}

¹Department of Radiation Oncology, UTMD Anderson Cancer Center, Houston, TX, USA; and ²Department of Radiation Oncology, Jefferson Medical College of Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, Pennsylvania, USA

Antiandrogen therapy is an important modality in the treatment of prostate cancer. Recent research into the role of angiogenesis in tumour growth and metastasis has uncovered links between antiandrogen therapy, radiation therapy and angiogenesis, which have exciting implications for the treatment of prostate cancer. Angiogenic cytokines such as vascular endothelial growth factor (VEGF) have been identified in prostate cancer cells and tumours, and androgens appear to stimulate VEGF. This article assesses the antiangiogenic effects of hormonal therapy and assesses the role that angiogenesis may play in the observed cooperation between hormonal and radiation therapies for prostate cancer.
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Introduction

Prostate cancer is the most common malignancy affecting men in the United States, and is the second leading cause of cancer death, with approximately 20% of diagnosed men expected to die of the disease.^{1,2} Prostate cancer is frequently hormone dependent, and therefore antiandrogen therapy has been an important aspect of treatment for patients with prostate cancer. While initial management of prostate cancer with antiandrogen therapy has been primarily limited to patients with metastatic disease, androgen ablation therapy has been used increasingly over the past decade as neoadjuvant therapy in conjunction with surgery, radiation therapy and prostate brachytherapy.

Multiple studies have examined the role of angiogenesis in tumour growth and metastasis and have recently

discovered links between antiandrogen therapy, radiation therapy and angiogenesis, which have exciting implications for the treatment of prostate cancer. The aim of this article is to examine the effects of hormonal therapy on angiogenesis and focus on this process as a mechanism that may account, at least partially, for the additive effect seen when hormonal and radiation therapies are used in combination.

Methods

Data for this review were identified by searches of MEDLINE, Current Contents, PubMed and references from relevant articles using the search terms 'hormonal therapy', 'radiation therapy', 'antiangiogenesis', 'VEGF' and 'prostate cancer'. Only papers published in English between 1980 and 2004 were included.

Current treatment modalities in prostate cancer

Currently, the appropriate treatment of adenocarcinoma confined to the prostate at the time of diagnosis (stages

*Correspondence: AP Dicker, Department of Radiation Oncology, Jefferson Medical College of Thomas Jefferson University, Kimmel Cancer Center, 111 South 11th St, Philadelphia, PA 19107-5097, USA.
E-mail: adam.dicker@mail.tju.edu
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T1–T2) includes either radical prostatectomy or external-beam radiation therapy (EBRT)/intensity-modulated radiation therapy (IMRT), with prostate brachytherapy used as monotherapy for selected patients (Gleason score <7, prostate-specific antigen level <10 ng/ml) or in combination with EBRT/IMRT for intermediate-risk patients. EBRT also plays a major role in the treatment of locally advanced (T3–T4) prostate cancer.³ Androgen deprivation therapy is being used with increasing frequency as primary monotherapy in appropriately selected patients with localised disease.⁴ The efficacy of adjuvant hormonal therapy with radiation therapy has been demonstrated in several studies.⁵

Angiogenesis

It is known that multiple angiogenic cytokines, including basic fibroblastic growth factor (bFGF), transforming growth factor- α , transforming growth factor- β , tumour necrosis factor- α and vascular endothelial growth factor (VEGF), are expressed in normal prostate cells.⁶ Among these, perhaps the most significant is VEGF, the most potent and specific growth factor for endothelial cells.^{7,8} Angiogenic factors such as VEGF and bFGF are expressed in many human prostate cancer cell lines^{9,10} and VEGF expression is induced in many cancer cells as a result of multiple genetic alterations, including *P53* and *PTEN* loss of function, *RAS* and *SRC* gain of function and *Bcl-2* gain of function.¹¹ In addition, androgens can stimulate autocrine tyrosine kinase signalling pathways involving epidermal growth factor receptors (eg *HER2 neu*), which may lead indirectly to VEGF upregulation.¹² VEGF expression may also be upregulated by Cox-generated *PGE₂*.¹³ Moreover, human tumours can induce stromal cells to produce significant amounts of VEGF.¹⁴ In growing tumours, the distance from the supporting capillaries increases as cells multiply and tumour mass increases. When cells in the tumour mass grow too far from their blood supply, diffusion becomes insufficient for nutrient requirements and cells begin to die. This results in an equilibrium between the production of new cells in close proximity to the capillaries and the death of existing cells more than 1–2 mm from a blood supply.¹⁵ This equilibrium can apply to a stable carcinoma, which may exist for months or even years before changes leading to more invasive growth occur. One of the switches from a stable carcinoma to a more invasive phenotype occurs when a population of tumour cells develops the necessary cytokine-signalling mechanisms to induce neovascularisation, and the hypoxic or hypoglycaemic environment frequently experienced by tumours can result in upregulation of VEGF.¹⁶ Newly formed tumour blood vessels differ substantially from normal capillaries in several ways that make them accessible targets for antiangiogenic therapy.^{17–20} Some tumour vessels have a defective cellular lining composed of disorganised, loosely connected, branched, overlapping or sprouting endothelial cells.²¹ Tumour-induced neovasculature exhibits increased expression of receptors for angiogenic factors, and angiogenic endothelial cells proliferate up to 50 times more than normal endothelial cells.^{17,22}

Increases in VEGF, as well as in other angiogenic factors, have been shown to correlate with a worse

prognosis in multiple solid tumours and direct measurement of angiogenesis via microvessel density (MVD) assays has also correlated negatively with prognosis in multiple cancers, including prostate.^{23–27} VEGF expression and intratumoural MVD both correlate with decreased survival in men with prostate cancer, and MVD correlates with disease progression after radical prostatectomy.^{26–28} There is evidence that plasma levels of VEGF are increased in patients with metastatic prostate cancer^{29,30} and that anti-VEGF antibodies can prevent the growth of *in vivo* prostatic tumour models beyond the initial prevascular growth phase.^{31,32}

The effects of hormones and hypoxia on angiogenesis

Data addressing the stimulatory effect of androgens on angiogenesis suggest a possible role for androgens themselves as angiogenic survival factors in prostate cancer. In the prostate, testosterone stimulates angiogenesis in the ventral prostate and vascular regrowth in castrated adult rats,³³ with 1–2% of the endothelial cells in the intact ventral prostate proliferating, a process in which VEGF is likely implicated; castration downregulates VEGF in normal tissues as well as in malignant prostatic tissue, an effect that is reversed by testosterone.^{10,34,35} In both tumour xenografts and primary human tumours, the neovasculature contains a sizeable fraction of immature blood vessels, characterised by a lack of formed periendothelial cells, which undergo selective apoptosis in the absence of VEGF.³⁶ In hormone-responsive prostate cancer, VEGF expression is regulated by androgens: androgen deprivation leads to decreased VEGF mRNA and protein expression in LnCaP cells, and castration of mice bearing LnCaP tumours results in a rapid decrease in mRNA expression and markedly reduced tumour neovascularisation.^{9,10} In an androgen-dependent prostate cancer model (Shionogi carcinomas in castrated, severe combined immunodeficient (SCID) mice), androgen withdrawal has been shown to stimulate apoptosis in conjunction with tumour regression and decreased VEGF: on androgen withdrawal, tumour cell apoptosis is preceded by apoptosis of endothelial cells, resulting in tumour vessels adopting more normal vascular characteristics (ie, reduced diameter, tortuosity, vascular permeability and leukocyte adhesion).³⁷ In this system, 2 weeks after castration, extensive angiogenesis and tumour growth occurred, followed by apoptotic effects with a concomitant increase in VEGF expression, suggesting a mechanism for relapse following hormone ablation.³⁷ Most recently, it was shown that anti-VEGF treatment inhibits testosterone-stimulated prostate growth in castrated mice.³⁸

Although angiogenic signalling molecules differ in their regulation by androgens (eg, castration was found to inhibit VEGF, but not bFGF, in the androgen-responsive PC-82 and A-2 human prostatic cancers when grown in SCID mice⁹), when taken together, these reports suggest that androgen withdrawal leads to vascular regression. This regression occurs by downregulation of VEGF expression in normal and malignant cells, firstly causing apoptosis of endothelial cells of immature vessels and then by contributing to the formation of a

more normal vascular phenotype in which vessels are supported by periendothelial cells. Immunohistochemical studies in untreated and androgen-ablated patients with prostate cancer have shown that significant levels of VEGF are present, and that VEGF expression is down-regulated by hormonal manipulation.³⁹

Hypoxic conditions create a microenvironment in which tumour cells become less dependent on angiogenesis, but more resistant to apoptosis, more capable of existing under hypoxic conditions and more malignant because of the development of genomic instability and mutant genotypes impacting on apoptosis/survival signalling pathways.⁴⁰ Hypoxia is present in localised prostate cancer: by measuring the partial pressure of oxygen using Eppendorf pO₂ microelectrodes, regions of prostate cancer have been shown to be hypoxic when compared with areas of normal prostate or adjacent muscle.^{41,42} Oxygen measurements from the pathologically involved portion of the prostate were significantly lower than those from normal muscle. Similarly, higher pO₂ readings were obtained from pathologically normal prostates (in patients with bladder cancer) compared with the prostates of patients with prostate cancer. Increasing levels of hypoxia were observed with increasing clinical stage. Movsas *et al*⁴¹ reported significant predictors of oxygenation including the type of tissue (pathologically involved prostate *vs* normal muscle or normal prostate), clinical stage and type of anaesthesia. This group found clinical evidence that increasing levels of hypoxia in prostate cancer are associated with increased expression of VEGF. A blinded comparison of pO₂ levels and VEGF staining intensity in radical prostatectomy specimens from 13 men demonstrated a significant correlation between increasing hypoxia and the percentage of cells staining positive for VEGF ($r = -0.721$, $P = 0.005$).⁴² This correlation was also significant when pO₂ levels were compared with the overall immunoreactive score, which takes into account staining intensity ($r = -0.642$, $P = 0.018$). This was the first study demonstrating a significant association between increasing levels of hypoxia and increased expression of VEGF in human prostate carcinoma.

Hypoxic tumour cells are characterised by the upregulation of the hypoxia-inducible factor-1 alpha (HIF-1 α), which, together with the constitutively expressed HIF-1 β , forms the heterodimeric transcription factor HIF-1.⁴³⁻⁴⁵ The expression of HIF-1 α is regulated by cellular oxygen tension and diverse signal transduction pathways.⁴⁶ Genes regulated by HIF-1 may be active in adapting tumours to the highly hypoxic and acidic microenvironments in which they are often found, and fall into four broad categories: angiogenesis-related genes; cell viability-related genes; tumour metabolism-related genes and genes coding for degradative enzymes involved in tumour invasion and metastasis.⁴⁷ Hypoxia, via HIF-1 α signalling, is the most potent stimulus for induction of VEGF. HIF-1 α regulates both hypoxia- and growth factor-induced VEGF expression in tumour cells, and genetic manipulation in human tumour xenograft models has established causal relationships between the level of HIF-1 activity, tumour growth and angiogenesis,⁴⁸ although non-HIF-1 α -mediated signalling pathways may also be involved.⁴⁹ It has recently been shown that upregulation of HIF-1 α is an early event in prostate carcinogenesis, which suggests that it may serve

as a surrogate marker for detecting premalignant lesions of the prostate.⁵⁰

The effects of ionising radiation

Several lines of evidence support a role for antiangiogenic effects in explaining the cooperation between androgen suppression and ionising radiation in prostate cancer treatment, and the rationale for combining inhibitors of angiogenesis with ionising radiation has recently been reviewed by Wachsberger *et al*.⁵¹ Zietman *et al*⁵² demonstrated that androgen deprivation enhances the ability of radiation to eradicate tumours in SCID mice: when radiation was combined with orchiectomy, tumours were significantly more likely to be controlled than when radiation was used alone. Androgen deprivation beginning 12 days prior to radiation produced a significantly greater decline in the dose of radiation needed to control tumours than androgen deprivation beginning 1 day or 12 days after radiation. In Lewis lung carcinomas (LLC) growing in syngeneic mice, the efficacy of experimental radiation therapy was found to be potentiated by brief exposure to angiostatin, and ionising radiation-induced VEGF in LLC both *in vivo* and *in vitro*.^{53,54} Ionising radiation-induced killing of human umbilical vein endothelial cells (HUVEC) was potentiated by anti-VEGF antibody and decreased by the addition of VEGF, leading investigators to propose a model whereby ionising radiation induction of VEGF contributes to tumour radioresistance.⁵⁴ Although ionising radiation may induce VEGF in tumors, over a protracted fraction schedule, the destruction of the microvasculature results in an antiangiogenic effect. Using cell culture systems, Abdollahi *et al*⁵⁵ reported a potent antiangiogenic effect for ionising radiation, inhibiting endothelial cell survival, but showed that VEGF and bFGF were able to reduce the radiosensitivity of endothelial cells. However, in PC3 tumour cells, radiation induced angiogenic factor production (that was abrogated by the addition of antiangiogenic agents), suggesting a radiation-inducible protective role for tumour cells in support of their associated vasculature. In a genetic investigation of the role of VEGF in the tumour response to ionising radiation in RAS-transformed murine fibrosarcoma cell lines, Gupta *et al*⁵⁶ showed that VEGF + / + xenografts were more resistant to the cytotoxic effects of ionising radiation than VEGF - / - xenografts. Antitumour strategies targeting VEGF and other endothelial cell survival mechanisms may enhance the cytotoxic effects of radiation therapy. Indeed, in combination with a small-molecule VEGF receptor tyrosine kinase inhibitor, ionising radiation abrogated VEGF-dependent proliferation in HUVEC in a dose-dependent way. Also, combined treatment exerted a substantial, more than additive tumour growth delay and decrease in MVD for radiation-resistant P53-dysfunctional tumour xenografts derived from SW480 colon adenocarcinoma cells, supporting a model of a cooperative antitumoral effect between angiogenesis inhibition and irradiation.⁵⁷

These data suggest that cooperation between androgen suppression and radiation in the treatment of prostate cancer may be the result of anti-VEGF activity by antiandrogen therapy, resulting in potentiation of

ionising radiation-induced lethality. Studies that have examined factors upstream of VEGF have suggested additional benefits of VEGF-targeting by hormonal therapy. One such factor, HIF-1, mediates a response to hypoxia partly through the transcriptional activation of several genes including VEGF.^{58–60} Increased expression of HIF-1 α in rat and human prostatic carcinoma cell lines is associated with increased cell growth rates and metastatic potential.^{61–63} In addition, HIF-1 α is also expressed in normoxic cancer cells, suggesting that HIF-1 α may be dysregulated in prostate cancer and thus drive the transcription of hypoxia-adaptive genes such as VEGF. Loss or inactivation of *P53*, *PTEN* or *pVHL* or oncogenic activation can all lead to increased HIF-1 activity, providing cancer cells with survival and proliferation advantages to promote the formation of vascular tumours.⁶⁴

Why, then, does antiangiogenic activity mediated by hormone ablation enhance the effects of radiation? It is known that hypoxic cells are radioresistant, requiring 2–3 times more radiation to kill them,⁶⁵ that hypoxic regions exist in human prostate carcinoma and that tumour hypoxia is an independent prognostic indicator of poor outcome in prostate and other tumours.^{41,66,67} Radioresistance may therefore arise because of the hypoxic tumour microenvironment.⁶⁸ There is evidence to suggest that, paradoxically, antiangiogenic therapy may actually increase delivery of oxygen to the tumour by reducing vascular permeability, which allows for increased oxygen delivery by more normal vasculature.^{69–73} Therefore, an additive effect between radiation therapy and androgen ablation in prostate cancer may

arise by a mechanism whereby decreased VEGF expression, resulting from androgen ablation, leads to endothelial cell death in immature tumour vessels, decreased tumour hypoxia and radiosensitisation of tumour cells. Such a model may account for the apparent additivity between hormonal therapy and EBRT for the treatment of prostate cancer.

Conclusions

Advances in angiogenesis research have identified promising targets for therapeutic intervention in the treatment of cancer, including prostate cancer. Angiogenic cytokines such as VEGF have been identified in prostatic cells and tumours, and androgens appear to stimulate VEGF. The stimulatory impact of androgen on VEGF is significant, and there is compelling evidence that part of the antitumour effect of antiandrogen therapy is mediated by its downregulatory effect on VEGF. Both androgen suppression and anti-VEGF treatment target pathological neovasculature, which may result in increased oxygen delivery to hypoxic tumour areas (Figure 1), accounting for the additive effect observed between antiandrogens and radiation therapy. Addition of a VEGF inhibitor (or an inhibitor of other components of angiogenic signalling) to hormonal therapy could result in decreased tumour hypoxia and have the potential to further potentiate radiation therapy. Cytostatic antiangiogenic therapies may augment the cytotoxic impact of radiation therapy via complementary cell-killing mechanisms. Future laboratory and clinical

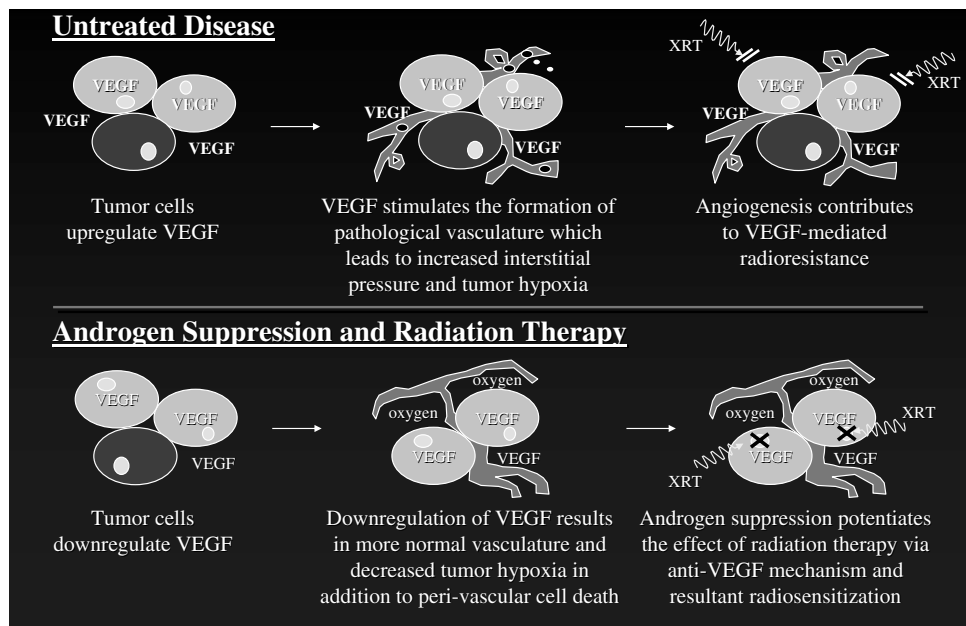


Figure 1 Possible mechanism for VEGF-mediated radioresistance in androgen-sensitive tumour cells. Androgen-exposed tumour cells upregulate VEGF leading to an increase in immature, leaky, tumour vasculature. This increase in leaky vasculature allows for increased interstitial pressure leading to increased hypoxia among tumour cells. Hypoxia decreases the sensitivity of this tumour cell population to cell killing by ionising radiation and consequently may allow for tumour progression in some patients. Androgen ablation can downregulate VEGF in androgen-sensitive tumour cells. Downregulation of VEGF, a critical survival factor for endothelial cells, initiates endothelial cell death and subsequent death of perivascular tumour cells. The death of immature vasculature cells results in a 'pruning' effect, which selects for mature, nonleaky vessels. This creates an environment with decreased interstitial pressure and decreased tumour hypoxia. The resulting increase in oxygenation in the tumour population increases the sensitivity of the cells to ionising radiation, ultimately resulting in potentiation of radiation therapy by anti-VEGF-mediated androgen ablation.

testing will determine if combinations of these modalities can provide new therapeutic options for possibly curable prostate carcinoma, as well as for hormone-resistant metastatic disease.

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