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Targeting angiogenic pathways involving tumor–stromal interaction to treat advanced human prostate cancer

Mitchell H. Sokoloff[1] and Leland W.K. Chung[1,2]
[1]*Molecular Urology and Therapeutics Program, Department of Urology and* [2]*Department of Cell Biology, University of Virginia, Charlottesville, Virginia, USA*

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Abstract

Interfering with and preventing tumor angiogenesis is an attractive therapeutic approach for treating cancer metastases. This commentary presents treatment strategies that may enhance the effectiveness of anti-angiogenic therapy by selectively targeting newly sprouting and immature vessels, inhibiting the production of angiogenic factors, and disrupting extracellular matrices. We propose several clinical paradigms, including hormonal ablation, intermittent androgen suppression, chemotherapy, and radiation therapy, that 'injure' nascent vasculature and interrupt the cancer cell–stromal relationship, thereby potentiating the efficacy of experimental anti-angiogenic agents. These stromal–epithelial interactions play an important role in the development, proliferation and dissemination of prostate cancer, as well as guiding the processes of tumor neovascularization. Successful utilization and targeting of tumor angiogenesis requires an increased understanding of tumor cell–stromal cell–endothelial cell relationships, most notably the intricate intracellular signalling cascades mediated by growth factors and the extracellular matrix.

Introduction

Despite advances in prevention and early detection, refinements in surgical technique, and improvements in adjuvant radio- and chemotherapy, the ability to cure many men with prostate cancer remains elusive. This is especially apropos to the successful management of metastatic and recurrent hormone-refractory disease. Clinical protocols using either androgen deprivation therapy or chemotherapeutic agents have shown some promise in treating advanced prostate cancer [1,2]. Unfortunately, the proportion and durability of complete remissions have been limited and new therapeutic approaches are desperately needed.

Stromal–epithelial interactions are paramount to the development, proliferation, and spread of prostate cancer. Studies in our laboratory have established that a bi-directional relationship between tumor cells and their surrounding stroma contributes to the growth and dissemination of prostate cancer [3–5]. These mesenchymal–epithelial interactions are responsible for maintaining the functional integrity of the normal adult prostate gland. Irregularities in the constituents of the stromal–epithelial milieu or aberrations in their interactions can induce genomic instability, enhance tumor cell proliferation, and drive both metastatic spread and progression to a hormone-refractory state. Consequently, novel therapeutic protocols are being developed that target not only prostate tumor epithelial components, but surrounding stromal and extracellular matrix (ECM) elements as well.

For a prostate cancer to grow and metastasize, endothelial cells from this surrounding stroma must be recruited to form an endogenous microcirculation to support the developing neoplastic mass [6,7]. Similar angiogenic processes are necessary at sites of metastasis if disseminated tumor cells are to become securely entrenched and, subsequently, propagate. Although prostate cancer cells produce inherent pro-angiogenic signals, integration of downstream signaling involving soluble factors and stromal and ECM components are critical to promoting and maintaining neovascularization [8].

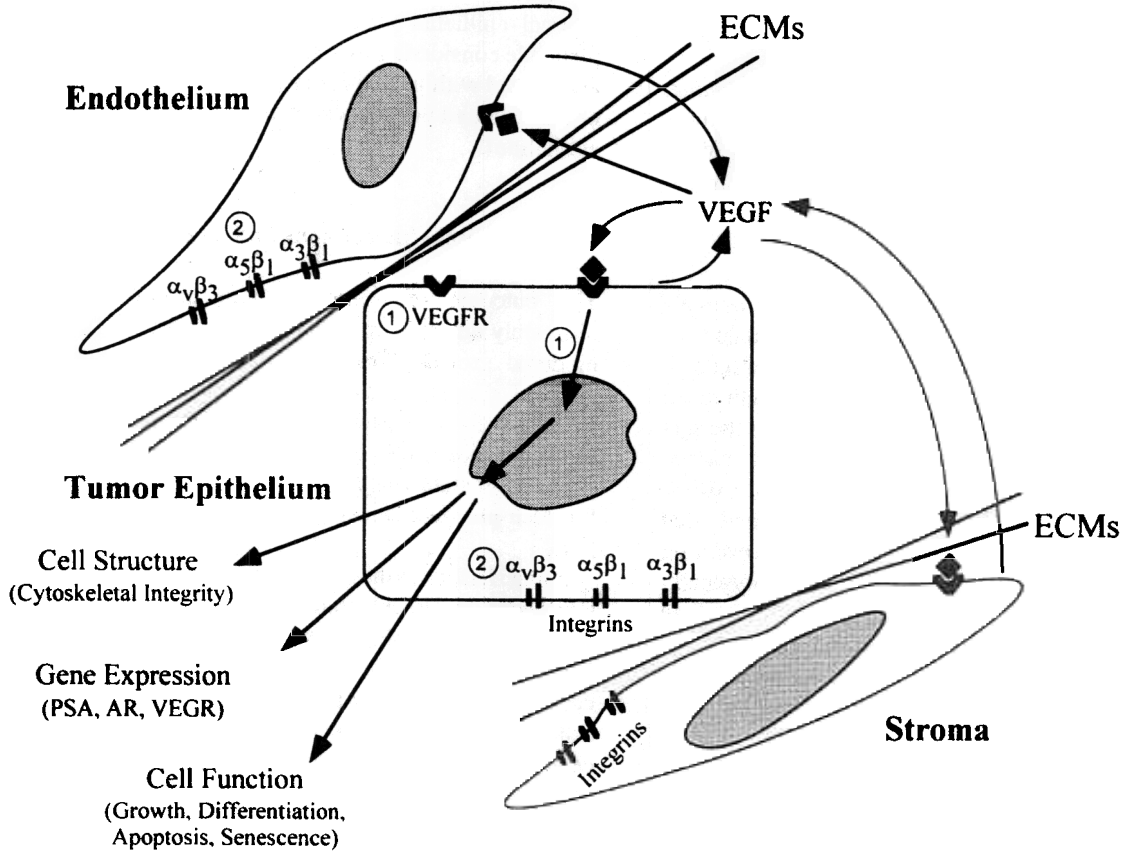

Figure 1. Prostate tumor cells express both vascular endothelial growth factor (VEGF) and its receptors (simplified here as a single entity, VEGFR). Intracellular communication between tumor epithelium, endothelium, and stroma is known to occur with signals mediated by such cell surface receptors as the integrins (e.g.: $\alpha_v\beta_3$), which confer cell–matrix interactions and the VEGF receptors (e.g.: Kdr/flk-1 and flt-1), which confer VEGF-induced intracellular signal activation. Resultant VEGF-VEGFR signaling is growth stimulatory for endothelial cells. In studies in our laboratory [11], we have demonstrated that VEGF has either no effect on or, surprisingly, can inhibit the growth of prostate tumor epithelial cells. Depicted in this illustration are two potential sites of interaction for an anti-prostate cancer anti-angiogenic agent: (1) VEGF – VEGFR interaction; and (2) extracellular matrix (ECM) – integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ interaction. Interruption of these cell-signaling pathways could be potentiated in the absence of the host androgen milieu or subsequent to any other therapy that injures the stromal–epithelial milieu and, hence, will adversely affect tumor cell survival in the stromal microenvironment. (Illustration courtesy of Song-Chu Ko, M.D., Ph.D.) [PSA, prostate-specific antigen; AR, androgen receptor].

Anti-angiogenic therapy has been demonstrated to be effective in several preclinical solid tumor animal models [9,10]. Moreover, since stromal factors can affect the multistep processes of both carcinogenesis and neovascularization, many of the factors involved in tumor angiogenesis are also associated with prostate cancer proliferation, progression, and dissemination. Hence, these stromal factors and interactions present an attractive therapeutic target for the treatment of hormone-refractory prostate cancer (see Figure 1). This commentary will review the importance of prostate tumor cell–stromal cell interactions in tumor angiogenesis. In addition, we will conjecture how conventional therapies directed at interfering with the prostate tumor–stromal cell relationship may influence the extracellular milieu in such a manner that the anti-neoplastic effects of putative anti-angiogenic agents are enhanced. Finally, we will postulate how combining these two therapeutic approaches may improve our capacity to successfully manage men with advanced and metastatic prostate cancer.

Stromal–epithelial cell injury may potentiate anti-angiogenic therapy

We have recently demonstrated that *squalamine*, an anti-angiogenic sterol isolated from shark liver, has potent anti-prostate cancer activity [11]. When squalamine was applied concomitantly with androgen withdrawal in a human prostate cancer xenograft model, an absolute and lasting eradication of both PSA and subcutaneous tumors was achieved. No such result was observed in intact tumor-bearing animals or in tumor-bearing animals treated with squalamine post-castration, yet subsequent to the appearance of androgen independent lesions. Immunohistochemical staining of responding and non-responding tumors indicated that combined squalamine and castration substantially diminished integrin $\alpha_v\beta_3$ expression. Additional histologic data established that squalamine's actions were most potent in preventing proliferation of the freshly sprouting, phenotypically immature blood vessels that, we believe, emerge in the tumor tissue as it acquires hormone independence. This finding corroborated prior studies which have determined that squalamine's efficacy is significantly enhanced when tumor vasculature and cancer cells are 'injured' immediately antecedent to its application [12,13].

Accordingly, we have developed two theories to explain the powerful anti-angiogenic as well as anti-prostate cancer effects of coincident androgen ablation and squalamine administration. First, androgen deprivation, may so effectively 'stun' the stromal–epithelial environment that normally prevalent and active pro-angiogenic factors are diminished, inactivated, or eradicated. At this juncture, the diminution of angiogenic forces may be sufficient enough that the otherwise ineffectual squalamine (if used in the absence of hormone-ablation) is consequently rendered potent. Alternatively, during the immediate post-orchiectomy rejuvenated proliferative phase (when stromal-mediated and autonomous epithelial tumor cell growth is actively acquiring hormone independence) the cellular and vascular architecture may become somewhat pliable and plastic, predisposing these precarious cells to either anti-angiogenic or cytotoxic effects of squalamine.

These interesting observations and speculations have led us to develop the following hypothesis: that the effectiveness of anti-angiogenic agents can be increased when applied coincident with other therapeutic interventions that injure components of the stromal–epithelial milieu. This premise is particularly plausible considering that most pro-angiogenic factors are located within the stromal milieu, either as soluble or ECM-bound factors, which affects survival of tumor epithelium.

Tumor–stromal interactions and angiogenesis

The outcome of a patient with prostate cancer ultimately depends upon the tumor's capacity for unhindered growth, local invasion, and the establishment of distant metastasis. Local factors, produced by mesenchyme, epithelial cells, or as a consequence of bi-directional mesenchymal–epithelial interactions between prostate tumor and stromal cells, are necessary for such proliferative, invasive, and migratory events [14].

Numerous cytokines and growth factors have been implicated in either enhancing or impairing a given prostate tumor's inherent tumorigenic and metastatic phenotype [15]. While some act directly upon the tumor cells, others influence prostate tumor cell proliferation by modulating their interactions with the extracellular matrix interactions through either soluble or matrix-associated signaling. This can significantly alter tumor cell heterogeneity with the propensity of selecting androgen-independent and metastatic variants.

Angiogenesis

Angiogenesis refers to the formation of new blood vessels from pre-existing, nascent vasculature. It is a multistep sequential process involving the recruitment and proliferation of endothelial cells, their subsequent migration to the tumor mass, morphogenesis into a tubular structure, and maturation into a stable structure [16,17]. It is important to note that the structure of tumor vessels differ from those of normal tissues, especially with regard to cellular composition, tissue integrity, vascular permeability, and regulation of cell proliferation and apoptosis [18]. It is presumed that these many differences may impart selective susceptibility of tumor vessels to the effects of anti-angiogenic agents.

The establishment and maintenance of such a vascular supply is imperative to prostate carcinogenesis and involves the cooperation of a variety of molecules either constituting or inhabiting the ECM. A variety of

growth and survival factors present within the extracellular matrix have been associated with angiogenesis [19–21]. These include, but are not limited to, TGF-α and β, b-FGF, IGF-1, EGF, HGF/SF, PDGF, TNF-α, VEGF, and IL-6 and 8. As it is beyond the scope of this commentary to discuss all of these factors in much detail, we will briefly mention the best characterized and most studied soluble pro-angiogenic factors of the prostate cancer cell-ECM milieu: *vascular endothelial growth factor* (VEGF) [22–24].

VEGF is a potent stimulatory factor for angiogenesis and is a highly specific mitogen for vascular endothelial cells. It has been implicated in promoting prostate carcinogenesis and metastasis, as well as angiogenesis. It is commonly accepted that VEGF influences tumor growth indirectly, through angiogenic activity, although a direct effect has never been excluded and more recent data suggests a non-angiogenic mechanism (see below). VEGF has also been shown to stimulate cell migration, implicating it in tumor metastasis as well as angiogenesis. VEGF is expressed by prostatic cancer epithelium, and expression positively correlates with increasing grade and tumorigenicity [25–28]. In the LNCaP cell line, VEGF appears to be androgen regulated, suggesting that hormone ablation therapy may act, in part, through the inhibition of VEGF production [29,30]. These results, however, have been contradicted in other studies using different prostate cancer cell models (see below).

Integrin signaling

Although the ECM is an important determinant in tumor cell growth, survival, and dissemination, its potency depends upon successful tumor cell–ECM signaling. Cell–ECM interaction is signaled primarily through integrins, which are comprised of over twenty combinations of α and β heterodimers [14,31]. ECM-integrin signaling has been broadly implicated in regulating angiogenesis, as well as tumor cell motility, migration, and metastasis [14,20]. In the LNCaP/C4-2 progression model of human prostate cancer metastasis [32], we have documented a functional integrin switch from $\alpha_6\beta_4$ to $\alpha_3\beta_1$ and $\alpha_v\beta_3$ during prostate cancer progression. This switch appears early in the progression to androgen independence and has been confirmed biochemically by immunoprecpitation of biotinylated prostate tumor cells followed by subsequent western blotting of these products using avidin-conjugated peroxidase antibody. We have further demonstrated that integrin $\alpha_v\beta_3$ preferentially attaches to vitronectin and osteopontin, key components of bone matrix, and that osteopontin is overexpressed in pathologic specimens from men with hormone-refractory prostate disease. This is likewise associated with a coexistent overexpression of integrin $\alpha_v\beta_3$. Osteopontin, a potent autocrine and paracrine growth factor, is secreted by both prostate cancer epithelial and bone stromal cells, exerting a direct stimulatory effect on prostate epithelial cell proliferation *in vitro* [33].

Integrin $\alpha_v\beta_3$ has a prominent function in tumor angiogenesis [31,34]. Furthermore, integrin $\alpha_v\beta_3$ activity is modulated by several growth factors that reside in the ECM, such as VEGF. In chick chorioallantoic membrane (CAM) assays, angiogenesis can be disrupted by treatment with either a cyclic peptide or monoclonal antibody antagonist to $\alpha_v\beta_3$ [35]. *In vivo* studies have demonstrated that tumor growth can be inhibited when integrin $\alpha_v\beta_3$ expression is likewise constrained [36]. Since integrin $\alpha_v\beta_3$ influences angiogenesis, reorganization of cytoskeletal structures, basement membrane attachment, and cellular proliferation, a switch to integrin $\alpha_v\beta_3$ in prostate cancer may provide advantages to growth of androgen independent tumor cells at both primary and distant sites.

Clinically, the relationship between prostate cancer and angiogenic factors remains controversial [28,37–40]. In most studies, increases in tumor angiogenesis correlate with Gleason grade and with progression after prostatectomy. Angiogenic factors are also associated with a heightened metastatic phenotype and with poor prognosis. Furthermore, quantitation of tumor angiogenesis, based on microvessel density, appears to be a promising technique for estimating the extensiveness and aggressiveness of a given prostate tumor as well as predicting its response to various forms of treatment.

Combining conventional and anti-angiogenic approaches to treat men with advanced prostate cancer

Radical prostatectomy can cure patients with localized prostate cancer and its use in treating such tumors in younger and healthy patients is generally undisputed [41]. Nonetheless, almost 30 percent of patients with pathologically organ-confined cancer will experience an early relapse with recurrent disease despite successful treatment of the primary lesion [42]. Furthermore, current screening modalities fail to identify a significant subset of patients with locally-invasive tumors.

Recent studies report that up to 50 percent of patients who were thought to have organ-confined lesions were discovered to be understaged subsequent to surgery [43–45]. As a result, the majority of men with prostate cancer will eventually develop disseminated disease [46]. In addition to causing severe pain and morbidity, such metastatic disease is the primary cause of death in men with prostate cancer [47]. Androgen ablation therapy is the most widely accepted therapy for men with metastatic cancer. Because of its limited duration, however, certain chemotherapeutic agents have been incorporated in the treatment of advanced, hormone-refractory disease. Furthermore, radiation therapy (with or without hormonal therapy) is commonly used to treat locally-invasive lesions that are felt to be incurable by surgical means.

With regard to prostate cancer, androgen ablation therapy, chemotherapy, and radiation therapy share two common traits. First, by themselves, they customarily behave as temporizing agents resulting in disease remission, but are generally ineffective in curing advanced disease. Second, in addition to directly damaging prostate cancer cells, each of these treatment modalities induces injury to the surrounding stroma and extracellular matrix. In fact, studies demonstrating improved outcomes in men with prostate cancer after treatment with combined radiation and androgen ablation therapy attribute these findings, in part, to the resultant interference with the stromal–epithelial relationship [48]. Furthermore, p53, which can act as a radiosensitizer, may enhance the efficacy of radiation therapy via its anti-angiogenic properties, such as inducing expression of the anti-angiogenic ECM component, thrombospondin-1 [49,50]. Because of their individual and independent abilities to injure components of the extracellular milieu, androgen ablation therapy, chemotherapy, and radiation therapy are ideal therapeutic approaches to investigate for use in concert with anti-angiogenic agents to treat men with, or at risk for developing, advanced prostate cancer.

Taking all three of these treatment modalities into consideration, we can now reiterate our hypotheses as to how androgen ablation therapy, chemotherapy, and radiation therapy may induce sufficient injury to the stromal–epithelial environment that the effects of the subsequent utilization of anti-angiogenic agents would be potentiated. First, damage to the homeostatic cellular components of the stromal–epithelial milieu might decrease, or even completely suppress, the secretion of soluble pro-angiogenic factors and intracellular signaling of pro-angiogenic components, inhibiting many of the steps required for neovascularization. Such alterations in this constituency could greatly increase the sensitivity of the tumor to anti-angiogenic therapy. Second, injury to the vascular endothelial cells within the stroma could result in the immediate destruction of the tumor vasculature (resulting in the cessation of blood flow to the cancer cells) or weaken the vessels sufficiently that their susceptibility to attack by a second (anti-angiogenic) agent is increased. Furthermore, damage to established vessels might induce the formation of new vasculature to nourish and sustain both the tumor mass and the surrounding tissues, or alter the phenotype incipient vasculature so that it acquires the more immature characteristics of newer vessels. It is generally accepted that these younger, more immature vessels are most-susceptible to anti-angiogenic insult [51]. Third, immediately following intervention, some stromal and epithelial tumor cells are likely to overcome their injuries and begin to initiate repair pathways, in which they recapitulate a phenotypically younger and more unstable configuration. As the cellular architecture becomes increasingly precarious, these cells are more apt to be affected by the anti-angiogenic agents.

Androgen ablation therapy

Our data from the LNCaP-castrate xenograft model confirmed that squalamine's actions were most potent on the freshly sprouting, immature blood vessels that, we believe, developed during a rejuvenated proliferative phase of those prostate tumor cells acquiring hormone independence. The effect was independent of serum VEGF levels. There is ample evidence that androgen application can stimulate vasculogenesis whereas androgen deprivation can inhibit neovascularization, allegedly the result of increased or decreased VEGF production (respectively). Folkman has demonstrated that VEGF production by LNCaP cells is under tight regulation by androgen and that androgen withdrawal inhibited hypoxic induction of VEGF [52]. Isaacs and associates have demonstrated that the activity of Linomide, an oral anti-angiogenic agent which has demonstrated effectiveness in suppressing human prostate cancer in preclinical animal studies, was potentiated by concurrent androgen ablation, presumably due to down-regulation of VEGF [30].

It was recently demonstrated that prostate gland growth in a rat model was regulated by the vascular

endothelium, which, accordingly, was itself controlled by testosterone stimulation [53]. In this study, testosterone stimulation in castrated animals caused an escalation of endothelial proliferation and vessel development, antecedent of glandular tissue regrowth, allegedly the result of increased VEGF production. In the Dunning prostate adenocarcinoma model, however, castration had little effect on VEGF production, despite enhancement of VEGF flk-1 receptor expression and involution of tumor vasculature [54]. This critical observation suggests that castration can affect angiogenesis independently of VEGF production and receptor status and allows for the identification of multiple pathways of anti-tumor anti-angiogenic activity. The presence of many such pathways implies that in prostate cancer, a synergistic therapeutic approach could be developed by using androgen deprivation therapy along with other anti-angiogenic agents, thereby enhancing the effectiveness of each individual treatment modality [30].

Additional evidence to support using such a combined approach to managing human prostate cancer can be found in a recent article by Jain et al. [55]. Using the Shionogi tumor model, androgen ablation initially resulted in tumor involution, in which endothelial cells underwent apoptosis before neoplastic cells. Soon after castration, however, the regressing vessels began to exhibit changes in phenotype. Subsequently, they began to produce large quantities of VEGF and both neovascularization and tumor regrowth resulted. We propose that by working through one of the several putative anti-tumor/anti-angiogenic pathways postulated above, the application of an anti-angiogenic agent coincident with castration could inhibit this neovascularization and tumor regrowth, hence prolonging cancer remission.

Intermittent androgen suppression

The above data suggest that by injuring the prostate tumor cell–stromal cell–ECM environment, castration induces degeneration of vascular structures. This appears to precede the apoptotic effects seen in the tumor cells themselves and can be independent of VEGF production. As the effects of castration wane, there is a resurgence of pro-angiogenic influences with resulting vasculogenesis. These vessels are young, immature, pliable, and most prone to being affected by the administration of anti-angiogenic agents. In this commentary, we have proposed using orchiectomy in concert with anti-angiogenic therapy to maximize the potential of each to repress or, hopefully, eradicated prostate cancer. If castration by itself appears so promising as a neoadjuvant to anti-angiogenic therapy, intermittent androgen suppression (IAS) should, theoretically, have a more pronounced effect.

IAS has been applied in both pre-clinical models and in men with advanced prostate cancer in an attempt to prolong the anti-neoplastic effects of hormone ablation therapy [56–59]. The underlying biologic principle of IAS is straightforward. Androgen ablation inhibits proliferation of androgen-sensitive prostate tumor cells as well as inducing apoptosis. In the normal prostate, androgen-induced growth and castration-induced regression can be repeatedly cycled using androgen replacement and withdrawal, respectively, since normal prostatic epithelial cells do not acquire the ability to grow in an androgen-deficient environment. In prostate cancer, however, cells that escape the cytotoxic effects of hormone ablation emerge as a population of androgen resistant clones. This ultimately results in tumor growth and metastasis, with eventual death. Hormone-refractory tumor cells may result from the selection of pre-existing androgen independent cells resistant to apoptosis, or from upregulation of adaptive mechanisms to the androgen independent state. IAS is founded on the premise that with intermittent androgen exposure, tumor cells may delay their progression to an androgen-independent state. Conceivably, however, intermittent androgen exposure can also inhibit tumor cell growth through an androgen-repressed (or hypersensitivity to androgen) status [60]. Overall, IAS may restore apoptotic potential and delay the progression of prostate cancer cells to androgen-independence.

Few clinical trials with IAS have been completed to date. Some suggest slightly-improved survival rates, with substantial improvement in quality in life [57]. For our purposes, however, IAS offers an attractive opportunity to test our hypothesis that applying anti-angiogenic agents coincident with stromal injury and repair will enhance tumor kill. IAS involves cyclical androgen-induced growth and castration-induced regression. With each course, there is the same initial injury to the stroma and ECM seen with castration, however under the influence of hormone repletion, there is a resurgence of pro-angiogenic influences with resulting vasculogenesis. These are the young, immature, and pliable vessels most apt to being affected by the administration

of anti-angiogenic agents. By cyclically applying and withdrawing androgen, intermittent androgen blockade systematically injures tumor and vascular cells over a prolonged period of time. This repeated stress should prime them for the actions of an anti-angiogenic agent, resulting in the destruction of both tumor cells and the surrounding stroma.

Chemotherapy and radiation therapy

External beam radiotherapy, as well as brachytherapy, is frequently applied to the treatment of local and locally-advanced prostate cancer. Furthermore, radiation therapy is commonly used to treat symptomatic metastases. Radiation induces significant injury to both tumor and stromal cells [61,62]. There is often scarring and destruction of nascent vasculature, as well as damage to the surrounding stromal and ECM components. In both situations, much akin to androgen ablation therapy, the stromal–epithelial milieu initially experiences significant injury with damage to stromal cells as well as pro-angiogenic signals. With time, however, these cells attempt to repair the radiation-induced damage, and the stromal–epithelial compartments undergo neovascularization with the potential to support a tumor recurrence. Similarly, applying taxol and estramustine, two currently used agents in prostate disease and as a paradigm for other chemotherapeutic regimens, will similarly induce injury of both endothelial and epithelial tumor cells [63,64]. We propose that the application of an anti-angiogenic agent coincident with radio- or chemotherapy induced injury could inhibit stromal neovascularization and prevent tumor recurrence.

Concluding remarks

Anti-angiogenic agents have shown promise in several preclinical studies of prostate cancer. In our own experience squalamine, an aminosterol with anti-angiogenic properties, has demonstrated effectiveness when applied concomitant with castration. As many of the pro-angiogenic influences present in prostate carcinogenesis reside with the stromal–tumor cell–ECM environment, it is not surprising that castration, which induces widespread damage within the stroma (in addition to having direct cytotoxic effects), would potentiate the activity of an anti-angiogenic agent. In this commentary, we speculate that the potency of anti-angiogenic agents will be most pronounced when applied in conjunction with other therapeutic modalities that maximally injure the stromal and ECM. For now, this includes intermittent androgen suppression, radiation therapy, and chemotherapy, but may, with time, be applicable to gene therapy and novel molecular approaches being developed for the treatment of both localized and advanced human prostate cancer.

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Address for correspondence: Mitchell H. Sokoloff, Department of Urology, Box 422, Health Sciences Center, University of Virginia, Charlottesville, Virginia 22908, USA; *Tel:* 804 243 6512