

# Novel Targeting of Apoptosis Pathways for Prostate Cancer Therapy

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**Abstract:** Selection of treatment options for clinically localized prostate cancer is based on a host of factors including the patient's age, overall health status, potential complications, clinical tumor stage and Gleason score. It is widely acknowledged that androgen independent disease remains the main obstacle to improving the survival and quality of life in patients with advanced prostate cancer. Apoptosis as a genetically regulated process has a critical endpoint that coincides with the therapeutic goal of successful treatment of androgen-dependent and androgen-independent prostate cancer. Opportunities to alter the apoptotic threshold of prostate cancer cells using antisense technology and gene therapy certainly exist, but the scope and extent of their applicability and action depends upon research delineating the many subtleties within the apoptotic pathway. Most epithelial and endothelial cells undergo apoptosis when they loose contact with the extracellular matrix (ECM), via the phenomenon of anoikis. Signaling interaction between growth factor apoptosis-signaling pathways and cellular effectors of anoikis potential and tumor vascularity provides a new molecular basis for optimizing combination approaches for the effective treatment of advanced prostate cancer. Agents that induce epithelial or endothelial cell apoptosis by antagonizing integrin binding are considered for cancer therapy via their ability to inhibit tumor vascularization. This review summarizes the current knowledge of the therapeutic benefit of apoptosis induction within the context of tumor neovascularization inhibition, and provides an insight into the consequences of anoikis induction (by different agents) in targeting angiogenesis in prostate cancer cells.

## INTRODUCTION

Prostate cancer is major contributor to cancer mortality in American males causing the death of 30,200 in the year 2002 [1]. Therapeutic modalities such as radical prostatectomy and radiotherapy are considered curative for localized disease, however, no treatments are available for metastatic prostate cancer which shows a significant increase in survival [2]. Androgen ablation therapy either through medical or surgical castration has been utilized in the treatment of advanced prostate cancer since the idea's original inception by Charles Huggins. Understanding the conversion from an androgen-dependent to androgen-independent phenotype of prostate epithelial cancer cells is essential for the development of effective therapeutic modalities for the treatment of advanced disease. Clinical and experimental evidence implicates two components as contributors towards the emergence of the androgen-independent phenotype: activation of apoptosis suppression (such as overexpression of the anti-apoptotic bcl-2 protein gene) and dysfunctional growth factor signaling (such as TGF- $\beta$ ).

The onset of prostate cancer is initially dependent on androgens for development, due to the ability of androgens to block apoptosis of androgen-dependent prostate cancer cells [3]. Although AR mutations have been reported to be rare and probably without a significant causal function in the

initial phase of prostatic carcinogenesis, the presence of a significant number of mutations in the metastatic disease indicates that receptor mutations may play a role in the most advanced phases of prostate cancer promoting acquisition of the metastatic phenotype [4].

The homeostatic equilibrium of the normal prostate gland is regulated by the level of trophic hormones like 5 $\alpha$ -dihydrotestosterone (DHT), and following depletion of this androgenic steroid, the prostate gland undergoes dramatic involution due to the apoptotic death of the luminal epithelial cells [5]. Sensitivity to the apoptotic pathway has been shown in prostate cancers involving mutations/deletions in tumor suppressor genes and the overexpression of certain oncogenes [6]. The current understanding of the molecular pathways operating in prostate cancer progression has led to intensified efforts towards the development of chemotherapeutic strategies for targeting key apoptosis regulators in androgen-dependent and androgen-independent prostate cancer. Androgens are believed to regulate tumor neovascularization of hormone responsive prostate cancer and a positive correlation of pathological stage and vascularization has been established [7]. Thus therapeutic targeting of apoptotic players is of vital significance since resistance to apoptosis is not only critical in conferring therapeutic failure to standard treatment strategies (including androgen ablation), but in addition, anoikis (apoptosis upon loss of attachment to ECM) is of major significance in the process of angiogenesis and metastasis of malignant cells [6,8].

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## CONVERSION TO ANDROGEN-INDEPENDENCE: LIMITATIONS OF HORMONAL THERAPY FOR PROSTATE CANCER

The classic studies by Charles Huggins more than sixty years ago established the androgen-dependence of prostate tumors [9]. Today, in the midst of advanced molecular technology and "exotic" therapeutics, androgen ablation monotherapy is still the primary treatment for metastatic prostate cancer. Other hormonal-based therapies include surgical castration or medical castration with LHRH analogues, estrogen agonists, anti-androgen regimes, and maximal androgen blockade (MAB). The gold standard in reducing circulating androgens is still by orchiectomy [10]. The use of luteinizing hormone releasing hormone (LHRH) analogues has also become a common therapeutic approach to androgen ablation with efficacy comparable to orchiectomy, and is important physical and psychological because there is no surgery involved. LHRH agonists suppress endogenous testicular gonadotrophin synthesis that causes a hypogonadal condition with chronic administration, along with suppression of LH and follicle-stimulating hormone (FSH) levels. Acute effects may include voiding symptoms and patients with late-stage effects may have a worsening of bone pain, urinary obstruction, and cord compression, all of which are attributable to rapid cancer growth where LHRH agonist therapy would be ceased [11].

Mounting evidence suggests that activation of androgen signaling in hormone-independent disease still occurs. The androgen regulated gene, *PMEPA*, exhibits abundant expression in androgen-independent tumors [12]. Furthermore, in a recent micro-array study it was found that the AR gene is the most frequent target, and many times the first target, of amplification during prostate cancer progression; in contrast to AR, amplifications in *c-myc* oncogene and *CCND1* gene were also seen [13]. The evidence that androgen-independent prostate carcinomas with AR gene amplification show increased PSA protein expression further supports this concept [14].

Expression of estrogen receptor (ER), both  $\alpha$  and  $\beta$  forms, is severely decreased in androgen-independent tumors compared to the normal prostate gland, usually in the presence of enhanced AR levels. An increased AR expression in prostate cancer probably does not act alone in the transition to a hormone-independent state and ER may play a key role [15]. Two nuclear receptor coactivators, transcriptional intermediary factor-2 (TIF-2) and steroid receptor coactivator-1 (SRC-1), have been shown, along with the AR, to have an increased expression in cancerous prostate cells. This overexpression increases AR transactivation at physiological concentrations of adrenal androgen [16]. Other recently discovered AR coregulators (ARA54, ARA55, ARA70, ARA24, and ARA160) are also altered in prostate cancer [17]. Androgen-independent tumors possess the ability to convert adrenal androgens to DHT, which may provide a higher intracellular androgen level in contrast to the low levels found in serum [18]. Indeed, this evidence may provide a molecular mechanism underlying the function of AR to enhance sensitivity to androgens towards the convergence of a hormone-independent phenotype. Prostatic tumors that have acquired the ability to survive and grow in an androgen-independent manner may activate the

AR pathway by way of a broadened ligand specificity, which allows activation of growth factor signaling pathways. In the absence of androgens, IGF-I, keratinocyte growth factor (KGF), EGF, luteinizing hormone-releasing hormone (LHRH), neuropeptides, and interleukin-6 (IL-6) have been shown to enhance the transcriptional activation of AR reporter genes [19].

The cytokine IL-6 and its receptor have been closely evaluated for their role in the development and progression of androgen-independent prostate cancer. Prostate tumors contain and secrete significantly higher IL-6 than normal cells [20]. Similarly, in patients with hormone-independent prostate cancer, IL-6 levels were markedly increased when compared to healthy controls [21]. Since IL-6 inhibits apoptosis and increases the secretion of MMP-7 that is involved in tumor invasion [20,22], it is tempting to speculate on its potential involvement in anoikis induction in malignant prostate cells. Androgen-independent and metastatic prostate cancer cells also exhibit resistance to the antiproliferative and pro-apoptotic effects of another cytokine, TGF- $\beta$ 1 [23]. TGF- $\beta$ 1 can confer a potent metastatic advantage in prostate cancer cells by promoting angiogenesis via extracellular matrix production [24]. Furthermore, *bcl-2* overexpression, an apoptotic defect frequently detected in prostate tumors, may contribute to the development of androgen-independence by inhibiting the normal effects of TGF- $\beta$ 1 [25].

Estrogen therapy has long been known to be of benefit in prostate cancer by reducing androgen levels. Diethylstilbesterol (DES) and other synthetic estrogens work by inhibiting luteinizing hormone (LH) production, thus, decreasing testosterone. However, its use has been limited because of the potential cytotoxic effects. Estrogen stimulation results in cellular proliferation by causing local production of growth factors including TGF- $\alpha$ , IGF, and EGF and inhibiting the expression of growth inhibitory factors like TGF- $\beta$  [26]. An increase in estrogen levels leads to an enhanced sensitivity to androgens by increased AR expression, and in the presence of androgens, estradiol causes adenocarcinoma of the prostate [27,28]. Interestingly enough however estrogen may exert effects on prostate cancer cells in the absence of androgens [29]. Further dissection of the molecular pathways mediating the estrogen-regulatory effects on androgen-independent prostate cell growth may provide exciting new avenues for investigating the therapeutic targeting of the transcriptional partners of ER and or AR in advanced prostate cancer.

## ANOIKIS REGULATION

Advanced stages of prostate cancer and malignant transformation are not only characterized by androgen-independence, but also anchorage independence. *In vivo*, cells such as fibroblasts, endothelial cells, mammary epithelial cells, bronchial epithelial cells, kidney epithelial cells, neuronal cells, thyroid epithelial cells, pancreatic islet cells, and prostate epithelial cells all require attachment to the extra-cellular matrix (ECM) for cell survival-not only for structural support, but also for critical survival signals [30]. Loss of anchorage to the ECM results in a detachment-induced form of apoptosis defined as anoikis [8]. Anoikis is

involved in normal cellular processes like tissue regression of mammary epithelium and capillaries, and terminal differentiation of keratinocytes [31,32]. In displaced cells, anoikis induction prevents aberrant migration and growth, and reduced sensitivity to anoikis appears to be an important hallmark of oncogenic transformation.

Integrins are the primary mediators of cell anchorage to the ECM and integrin-mediated signaling plays an important role in survival and neoplastic transformation. The process of anoikis involves dysfunctional cellular integrins and other components of the ECM including the two integrin-associated non-receptor kinases, focal adhesion-kinase (FAK) and integrin-linked kinase (ILK). Anoikis resistance arises from loss of apoptotic signaling via inhibition of caspase activity and bcl-2 overexpression or the activation of integrin signaling. Integrin signaling via FAK is an important regulator of growth, differentiation, adhesion, motility, and apoptosis [33,34]. During all stages of prostate tumorigenesis, this tyrosine kinase is overexpressed, appearing to be an early event in the development of prostate carcinoma, while it highly and uniformly expressed in most metastatic prostate cancers [35]. Alterations in the FAK/Src signal transduction pathway correlate with increased migratory capacity of prostate carcinoma cells [36]. In addition, FAK is believed to be a potential oncogene since it has been implicated in the progression of cancer to invasion and metastasis [37]. Integrin-linked kinase transmits integrin-mediated signals independently of FAK. While

FAK expression is relatively low in healthy prostate cells, prostatic tumors often show enhanced ILK activity, possibly as a result of a malfunctioning of upstream components in the integrin signaling pathways. Furthermore, ILK controls many downstream targets that are crucial to expression of the key cancer phenotypes apoptosis, angiogenesis, invasion/metastasis, and cell proliferation [38]. A recent report indicates that prevention of tumor cell invasion involves a novel mechanism of caspase-dependent inhibition of integrin and MMP expression and subsequent cleavage of FAK [39]. One could argue therefore that overexpression of FAK and ILK may block anoikis in prostate epithelial cells despite the loss of cell anchorage to the ECM (Fig. (1)).

Cell-to-cell adhesion is predominantly mediated by the cadherin family of proteins. Evidence suggests that E-cadherin can function as a tumor suppressor and decreased expression may be a marker of early neoplastic changes in prostate epithelial cells; therefore, diminished expression of E-cadherin in tumors correlates with an increased tumor cell invasion, migration, and metastasis [40]. This loss of E-cadherin function in prostate cancer may not be a result allelic loss or gene mutation alone; instead, decreased transcription due to methylation of its promoter or inactivation are also a likely agents [41]. An epigenetic mechanism of E-cadherin inactivation maybe due to cleavage via the  $Ca^{2+}$ -activated protease, calpain, which is involved in a variety of physiologic processes including signal transduction, cell-cycle regulation, and apoptosis. The

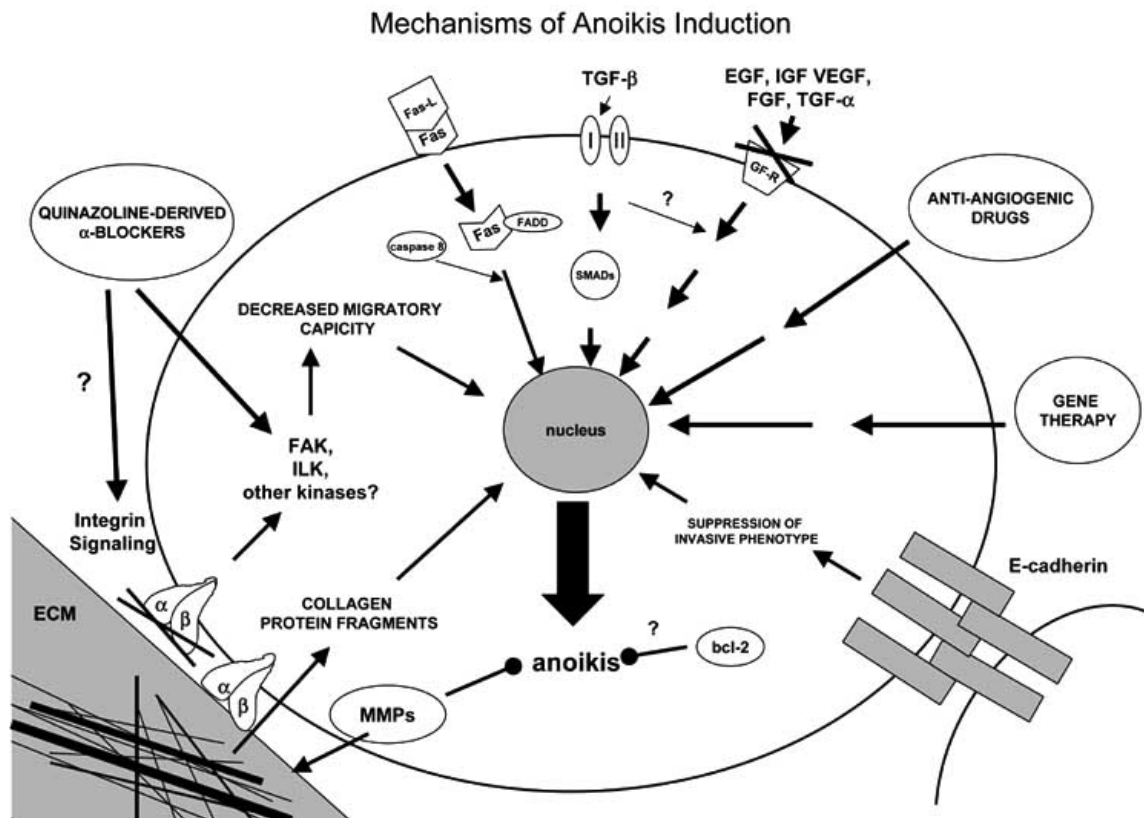


Fig. (1). Mechanistic model of targeting anoikis in prostate cancer cells.

recently demonstrated upregulation of calpain in localized and metastatic prostate tumors supports the concept that loss of E-cadherin function may result in the reduction of interepithelial adhesion thus promoting the invasive phenotype of prostate epithelial cells [42,43].

As illustrated on Figure 1 multiple and potentially overlapping signaling pathways may be functionally involved in dictating cell anchorage to the ECM and cell-to-cell contacts. These include FAK, ILK, caveolin, phosphoinositide-3 kinase (PI-3K), protein kinase B (PKB/Akt), ras-extracellular signal-regulated kinase (ERK), mitogen activated protein kinase (MAPK), glycogen synthase kinase-3 (GSK-3), adenomatous coli gene product (APC)/ $\beta$ -catenin, caspase, growth-factor receptors (GFR), Flip, Fas-associated death domain protein (FADD), cytochrome c, and Fas-L.

The bcl-2 family of proteins has been heavily implicated in prostate tumorigenesis since it has been established that bcl-2 protein levels are increased in androgen-independent tumors, and an overexpression directly correlates to the development and progression of androgen-independent prostate tumors [44,45]. Bcl-2 can protect prostate cancer cells from apoptotic stimuli *in vitro*, an apoptosis protection mechanism that directly correlates with the ability of bcl-2 to functionally contribute to the emergence of the hormone-independent prostate tumors *in vivo* as well as development of prostate tumor resistance to chemotherapy and radiotherapy [45-47]. Clinical studies revealed that prostate cancer patients who failed radiation therapy have elevated bcl-2 expression, evidence implicating bcl-2 as a marker of radioresistance of prostatic tumors [47]. Considering this evidence it seems plausible that bcl-2 could functionally contribute to anoikis-resistance in metastatic prostate cancer cells. However, one has to also consider other reports indicating that bcl-2-independent pathways in prostate carcinoma cells may regulate anoikis since cells surviving anoikis *in vitro* show lower levels of bcl-2 than apoptotic tumor cells [48].

The Fas-pathway has recently been shown to play a role in anoikis by the demonstration that the bulk of anoikis is preceded by activation of the pathway by its ligand, Fas-L, Fas/Fas-L interaction, Fas-FADD complex formation, and caspase-8 activation in endothelial cells, and inhibition of any of these events blocks anoikis [49]. Mechanistically caspases are the primary mediators of anoikis in target cells. Within minutes of epithelial cell detachment from the ECM the initiator caspases-2 and -9 are activated, followed by subsequent activation of caspases-3, -6 and -7, as well the release of cytochrome c following caspase activation [50-51]. It has yet to be established as to whether direct inhibition of caspase activation and/or cytochrome c release would confer anoikis-resistance in prostate cancer cells.

## ANGIOGENIC GROWTH FACTOR SIGNALING IN PROSTATE CANCER

In the prostate, androgen stimulation results in the production of many growth factors, including epidermal growth factor (EGF), transforming growth factor- $\alpha$  (TGF- $\alpha$ ), insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and TGF- $\beta$ .

This array of growth factors mediates cellular proliferation, differentiation, angiogenesis, and apoptosis upon binding to their membrane receptors in prostatic epithelial cells. Growth factors produced by the adjacent stromal cells can impact on epithelial cells in a paracrine manner to promote prostate growth [52].

Growth factors of the EGF family, including TGF- $\alpha$ , could contribute through autocrine and paracrine mechanisms to promote prostate cancer cell growth, proliferation, and angiogenesis by signaling through the same EGF receptor (EGFR) [53,54]. Evidence that EGF signaling is important in prostate cancer cell proliferation and invasion includes: human prostate cancer cell lines produce EGF and express the EGFR, adding EGF to prostate cancer cell cultures stimulates growth and this growth is blocked by an anti-EGFR antibody, EGFR levels are shown to be higher in prostatic tumors, and EGF promotes migration of human prostate cancer cells [52]. Interestingly, in androgen-dependent prostate cancer cells, DHT leads to an increased expression of TGF- $\alpha$  and EGFR, but not EGF synthesis [55,56].

The IGF family is composed of IGF-I, IGF-II, and relaxin, all of which bind to IGF receptors type I and II and IGF-binding proteins (IGFBPs) [57]. Conflicting data shows that both prostatic epithelial and stromal cells may or may not secrete IGF-I and/or IGF-II; however, both molecules stimulate the growth of prostate epithelial cells and stimulate the EGFR pathway [52]. Prostatic IGF-I expression has been shown to contribute to the initiation and/or progression of prostate cancer, and independence from IGF-I receptor-mediated signaling correlates with metastasis and androgen independence [58]. Moreover, IGF-II up-regulation contributes to prostate cancer progression *in vivo* [58]. PSA has been shown to preferentially cleave IGFBP-3 and -5 that allows for further growth stimulation of prostate cells and overexpression of IGFBP-4 delays the onset of tumor formation [59-61].

Angiogenesis is the formation of new blood vessels by capillary sprouting from pre-existing vessels and plays a critical role in tumorigenesis and metastasis in prostate cancer [62]. In addition to tumor growth, angiogenesis required for normal physiological processes, such as reproduction, development, and wound healing. Tumor expansion requires the development of new capillaries from pre-existing blood vessels. The small non-growing tumor may remain in the dormant state for years, and the switch to aggressive malignancy is characterized by an angiogenic phenotype, involving a change in the local equilibrium between factors that induce the formation of blood vessels and those that halt or inhibit the process [63,64].

The mechanistic illustration on Figure 1 highlights that angiogenic capacity of cells in response to anoikis signals is a complex multistep process involving close orchestration of endothelial cells, extracellular matrix, and soluble growth factors and their membrane receptors. In prostate cancer, one of the most powerful stimulators of angiogenesis is VEGF, which induces endothelial cell-proliferation and increases vascular permeability [65]. Hypoxia stimulates angiogenesis through the binding of hypoxia-inducible factors to the hypoxia-response element on the vascular endothelial growth factor gene. Normal prostate expresses minimal levels of

VEGF due to the actions of the inhibitor, thrombospondin-1 (TSP-1). During the progression of prostate cancer TSP-1 expression is down regulated while VEGF expression is increased [66]. Moreover, overexpression of TSP-1 has been shown to decrease the growth of prostate tumors [67]. VEGF seems to be androgen responsive because VEGF expression by normal prostatic tissue is up regulated in response to exogenous androgen, and androgen withdrawal results in a decrease in VEGF levels, followed by prostate tumor regression [68,69]. VEGF can induce chemotactic migration of prostate cells, suggesting two roles for VEGF in tumor progression: a paracrine role as an angiogenic factor and an autocrine mediator of tumor cell motility [70].

Basic fibroblast growth factor, or fibroblast growth factor 2 (FGF2) is another major growth factor that plays a key role in prostate tumor progression via its ability to induce angiogenesis. Upon secretion by stromal fibroblast FGF2 directly acts on the prostate endothelial cells thus promoting angiogenesis during prostate cancer progression [71]. In addition, androgen-independent cells produce high levels of mRNA for both FGF2 as well as its receptor, while androgen-dependent cells do not synthesize significant amounts of FGF2 mRNA [72].

The transforming growth factor- $\beta$  family of proteins regulates the growth of normal and malignant prostate via autocrine and paracrine pathways. The actions of TGF- $\beta$  are mediated by its binding and complex formation of type I and type II TGF- $\beta$  receptors [73]. In the normal prostate, TGF- $\beta$ 1 is the predominate isoform and stimulates cell differentiation, inhibits epithelial cell proliferation, and induces apoptosis [74]. In some prostatic cancers there may be an overexpression of TGF- $\beta$ 1 where it appears to enhance prostate cancer growth and metastasis by stimulating angiogenesis, but other evidence suggests that the effects of TGF- $\beta$ 1 on these processes may be indirect via TGF- $\beta$ 1 stimulation of expression of angiogenic factors such as VEGF [75].

In the presence of physiological levels of androgens TGF- $\beta$ 1 can halt the proliferation of normal prostate epithelial cells by decreasing the expression of the proto-oncogene p53 and increasing retinoblastoma (Rb) tumor suppressor gene expression [76-78]. Increased levels of p15, p21 and p27 protein, accompanied by increased levels of hypo-phosphorylated Rb and decreased cdk2 kinase activity, are seen as a result of TGF- $\beta$ 1, which delays the cell cycle in G1 [79].

The inhibition of proliferation is temporally linked to apoptosis of prostate epithelial cells, and castration has been shown to cause apoptosis via an upregulation of TGF- $\beta$ 1 and both TGF- $\beta$ 1 receptors [80,81]. *In vivo* and *in vitro* studies established that development of resistance to the growth inhibitory effects of TGF- $\beta$ 1 contributes to prostate tumor progression [82]. Indeed while increased expression of TGF- $\beta$ 1 has been widely documented in human prostate tumors, a dramatic loss/decreased expression of TR $\beta$ I and TR $\beta$ II receptors abrogates the TGF- $\beta$  signaling pathway in prostate cancer [83,84]. Furthermore, activation of TGF- $\beta$ 1 signaling through the restoration of the TR $\beta$ II receptor in prostate cancer cells causes tumor suppression and caspase-1 mediated apoptosis [85].

## TARGETING ANGIOGENESIS FOR PROSTATE CANCER THERAPY

Cells undergoing angiogenesis are in a dynamic state and potentially lack firm attachment to the extracellular matrix, which makes them more vulnerable to anoikis. Thus, targeting prostatic endothelial cell survival by activating endogenous inhibitors of angiogenesis may provide a molecular basis for novel therapeutic strategies for metastatic prostate cancer. In addition, one of the most promising approaches is antivascular therapy with the use of pharmacological agents. Such as agents can be classified as antiangiogenic by acting to prevent neovascularization, inhibit cell proliferation, migration, and/or differentiation of endothelial cells, and antivascular by direct targeting of the existing tumor vasculature [86].

Degradation of the basement membrane during pathological processes can create protein fragments of collagens IV and XVIII that have been shown to act as angiogenesis inhibitors with promising therapeutic potential for prostate cancer (Fig. 1). Collagen type IV has been shown to be proteolytically cleaved into peptide fragments including tumstatin, canstatin, and arresten that act as angiogenesis inhibitors by inhibiting endothelial cell proliferation, inducing apoptosis, and suppressing tumor growth, respectively [87-89]. Collagen XVIII is cleaved into endostatin, which specifically inhibits endothelial proliferation and consequently angiogenesis [90].

Prostate-specific antigen (PSA) is regarded as a reliable surrogate marker for androgen-independent prostate cancer and has long been thought to adversely affect the outcome of prostate cancer, even in the absence of reports to indicate any direct effect of PSA on the proliferation or metastasis of prostate cancer cells. In contrast, emerging evidence suggest that PSA may act instead as an antiangiogenic agent by converting plasminogen to biologically active angiostatin, a documented endogenous inhibitor of angiogenesis [91]. Angiostatin has been shown not to induce stress-fiber formation when bound to integrins, suggesting that angiostatin may prevent angiogenesis by perturbing an integrin-mediated signal transduction pathway that may be necessary for angiogenesis [92]. Moreover, PSA inhibits proliferation, migration, and invasion of endothelial cells, but PSA does not appear to have direct stimulatory or inhibitory effects on the proliferation of cancerous cells [93].

### Interferon- $\alpha$ (IFN- $\alpha$ ) and IFN- $\beta$ are Cytokines that Possess Antiangiogenic Activity

Both the leukocyte-derived IFN- $\alpha$  and fibroblast-derived IFN- $\beta$  down-regulate the expression of bFGF at the mRNA and protein levels in prostate cancer cells [94]. Frequent systemic administrations of low-dose IFN- $\alpha$  or IFN- $\beta$  or the introduction of the IFN- $\beta$  gene to a tumor bed shows promising therapeutic potential in several animal models that may be relevant for human prostate cancer [95].

Matrix metalloproteinase's (MMPs) belong to a family of proteases that possess the ability to degrade extracellular matrix components and to destroy the basement membrane, and their activity is under regulation by tissue inhibitors of metalloproteinases (TIMPs). A clear association between increased MMP production and malignant aggressiveness has

been observed in prostate cancer. Moreover, both *in vitro* and *in vivo* work has demonstrated an important role for MMPs and TIMPs in tumor invasion and metastasis [96]. Low-grade prostate tumors have elevated TIMP-1 expression in contrast to MMP expression, and high-grade (Gleason 8–10) tumors exhibit increased MMP-2 and MMP-9 levels, while TIMPs were not expressed [97]. Elevated MMP-9, as well as increased ratios of MMP-2 and MMP-9 to TIMP-1 are found in malignant prostate cells, proving a potential proteolytic imbalance in prostate cancer [98]. MMP-9 has certainly been promoted to serve as a useful biomarker for prostate cancer progression [99].

Cyclooxygenase-2 (COX-2) is a key enzyme involved in the production of prostaglandins (PGs) from arachidonic acid recently implicated in the progression of prostate cancer and angiogenesis [100]. Mounting evidence derived from *in vitro* and *in vivo* studies demonstrated increased levels of PGs in prostate cancer. The influence of hormones and diet, particularly fat consumption, on prostate cancer can be partially mediated by their effect on PG synthesis in the prostate [101] and the prostaglandin, E2 (PGE2), up-regulates COX-2, while COX-2 overexpression has been shown to induce VEGF at the mRNA and protein level and COX inhibitors inhibit VEGF production in prostate cancer cells [102,103].

As discussed above, increased expression of TGF- $\beta$ 1 in primary human prostate tumor cells via transfection of the gene stimulates tumor growth, metastasis, and angiogenesis [75,82]. In contrast, transfection of interleukin IL-10 results in significantly reduced tumor growth and suppressed metastasis and angiogenesis. Further evidence indicates that TGF- $\beta$ 1 expression upregulates MMP-2, while IL-10 decreased MMP-2 expression while upregulating TIMP-1 in the transfected cells [104]. These studies suggest that IL-10 and TIMP-1 may suppress tumor growth by affecting angiogenesis.

Chemotherapeutic approaches that have antiangiogenic activity must consider the difference between efficacy and toxicity because any cytotoxic agent dosed high enough will kill endothelial cells [105]. The taxanes are a group of compounds that are becoming increasingly popular as anticancer agents via their antiangiogenic activity [105]. Estramustine, a 17- $\beta$ -estradiol phosphate derivative, binds to microtubule associated proteins (MAPs) in the nuclear matrix and inhibits microtubule assembly/disassembly. Taxanes and estramustine are currently being investigated in phase II/III clinical trials [106], as monotherapy and in combination, for the treatment of androgen-independent prostate cancer.

TNP-470, a less toxic synthetic analogue of fumagillin, is a drug that directly inhibits the growth of endothelial cells [107]. In contrast, a phase I clinical trial reported that TNP-470 causes a moderate to substantial increase in PSA secretion in a concentration-dependent manner, and *in vivo* showed work this secretion is a result of increased PSA gene transcription [108]. Further studies demonstrated that TNP-470 inhibits endothelial cell growth by inducing p53 activation through a mechanism ultimately leading to p21<sup>CIP/WAF</sup> expression and subsequent growth arrest, in addition to targeting the cell-cycle regulator methionine aminopeptidase (MetAP-2) [109,110]. Significant changes in

tumor vascular characteristics have also been reported after treatment with TNP-470 can be detected using MRI [111]. Drugs that block angiogenic factors include SU5416, a selective inhibitor of VEGFR2, and SU6668, which blocks VEGF, FGF and PDGF-receptor signaling; both SU5416 and SU6668 are currently in clinical trials for the treatment of advanced malignancies [112,113].

Thalidomide is a potent endothelial cell growth inhibitor that causes embryotoxicity in humans. Recent *in vitro* data suggest that it inhibits angiogenesis; evidence emerging from clinical studies on phase II trial of thalidomide in patients with metastatic androgen-independent prostate cancer revealed a PSA decline of more than 50% in 14% of the patients, while approximately 28% of patients had more than 40% decline of PSA [114]. A subclass of thalidomide analogues has been shown to act as effective antitumor agents in prostate cells via induction of cell-cycle arrest associated with alterations in the balance of pro- and anti-apoptotic bcl-2 family proteins leading to caspase-dependent apoptosis [115].

Bevacizumab is a monoclonal anti-VEGF neutralizing antibody that blocks the binding of VEGF to its receptors. Inhibition of tumor-secreted VEGF by this neutralizing antibody is sufficient to significantly impair prostate tumor growth and its subsequent metastasis in an *in vivo* model of established advanced prostate cancer, which may prove an effective approach for inhibiting disease progression in patients [116]. Carboxyamido-triazole (CAI) is a calcium influx inhibitor that alters non-voltage-gated calcium-sensitive signal transduction pathways and suppresses the proliferative and metastatic potential of malignant prostate cells, and possesses antiangiogenic properties against prostate cancer cell lines *in vitro* and *in vivo* [117,118]. CAI has also been shown to decrease PSA secretion at concentrations having minimal cell killing effect in prostate cancer cells [118]. Recently, a phase II clinical trial of the effects of CAI on patients with androgen-independent prostate cancer and soft tissue metastasis, revealed no positive clinical outcome, yet following chronic treatment with CAI a significant decrease in serum concentration of VEGF was observed, but the strong toxicity of CAI that halted its further clinical testing [119].

## INDUCTION OF PROSTATE CANCER CELL ANOIKIS BY $\alpha$ 1-ADRENORECEPTOR ANTAGONISTS

The prostate has strong sympathetic innervations and contains a vast number of  $\alpha$ -adrenergic receptors. Catecholamines and  $\alpha$ -adrenoreceptor receptors may play an important role in the regulation of prostate cell growth and development of prostate cancer due to the discovery that catecholamines may act as growth factors in prostate cells *in vitro* [120]. Long-acting  $\alpha$ 1-adrenoreceptor antagonists such as the quinazoline-based doxazosin and terazosin, and the sulfonamide-derived tamsulosin, are clinically used for the relief of BPH symptoms by initiating changes in the periurethral tone of the prostate [121] via their ability to selectively antagonize the  $\alpha$ 1a-adrenoreceptors. The therapeutic benefits of have been widely demonstrated in clinical trials of BPH patients. Recent experimental and clinical evidence however indicates that induction of prostate

smooth muscle cell apoptosis by the quinazolines (doxazosin and terazosin) is one of the molecular mechanisms contributing to the overall long-term clinical efficacy of these medications in improving lower urinary tract symptoms in BPH patients [122]. The sulphonamide-based  $\alpha$ 1-adrenoceptor antagonist fails to induce the apoptotic response in prostate cells [123].

Recently derived experimental data from this laboratory have shown that doxazosin and terazosin, but not tamsulosin, are effective at inducing anoikis and inhibiting growth in androgen-dependent and androgen-independent prostate cancer cells [124]. Interestingly, the apoptotic activity induced by these drugs occurs via a pathway not associated with the  $\alpha$ 1-adrenoreceptor blockade, but potentially involving a TGF- $\beta$  directed apoptotic signaling pathway [125,126]. Molecular dissection revealed that doxazosin-mediated apoptosis in human prostate cancer cells involves activation of latent apoptotic machinery via effector (Smad) activation of TGF- $\beta$ 1 signaling and  $\kappa$ B induction [127]. Additional signaling mechanisms involving disruption of cell attachment to the extracellular matrix and subsequent induction of anoikis are also believed to be involved [124] in a molecular cross-talk of the quinazolines' cell death actions (Fig. 1). Serious consideration should thus be given to such FDA-approved agents shown to induce prostate cancer cell anoikis for advanced prostate cancer therapy due to their ability to inhibit angiogenesis. Ongoing studies are focused on further characterization of these pathways and the functional significance of the overexpressed genes in specimens from doxazosin-treated patients. This will provide the molecular basis for assessing the potential therapeutic significance of quinazoline monotherapy in androgen-independent prostate cancer.

## GENE THERAPY

Gene therapy has recently become an attractive alternative therapeutic avenue for localized disease because many prostate cancers are highly resistant to the currently used local and systemic therapies for prostate cancer. The advantages to applying gene therapy to a clinical state are primarily two: a) the tumor is in a single fixed location allowing for easy introduction of a vector, and b) the efficiency of gene transfer can be determined following prostatectomy [128]. Recent *in vivo* studies using an adenoviral vector capable of efficient transduction and expression of the mRTVP-1 (mouse related to testes-specific, vespid, and pathogenesis proteins) (AdmRTVP-1) was used in an orthotopic, metastatic mouse model of prostate cancer [129]. The gene is a direct target of p53, which possesses proapoptotic activities in prostate cancer cells. A single intratumoral administration of AdmRTVP-1 gene therapy showed significant suppression in primary tumor compared with control, increased apoptosis and reduction in tumor vascularity [129]. An exciting insight into nonviral gene therapy against prostate cancer has recently emerged from studies using Epstein-Barr virus (EBV)-based plasmid vectors, to transfer the Fas ligand (FasL) gene in prostate cancer cells since most prostate cancer cells express the signal receptor molecule Fas (Apo-1/CD95). Intratumoral injections of the FasL vector into prostate cancer xenografts resulted in a significant

suppression of tumor growth due to enhanced apoptosis highlighting the significance of targeting specific apoptosis components for therapeutic strategies [130].

Gene therapy in prostate cancer has been transitioned from preclinical studies to clinical trials with the goal of developing effective treatment for advanced prostate cancer. Many proposed strategies apply the Herpes simplex virus-1 thymidine kinase gene (HSVtk), which converts nontoxic nucleoside analogues like ganciclovir (GCV) into phosphorylated compounds that act as chain terminators of DNA synthesis. In a recent phase I study, adenovirus-mediated suicide gene therapy and the creation of a cytosine deaminase/HSV-tk fusion gene (Ad5-CD/TKrep) were utilized for the locally recurrent prostate cancer. Intraprostatic administration of the Ad5-CD/TKrep adenoviral vector with concomitant 5-fluorocytosine (5-FC) and GCV prodrug therapy showed that 9 of 16 patients had at least a 25% decrease in serum PSA and two patients were negative for adenocarcinoma at 1 year follow-up [131].

Another approach to genetic alterations is with the use of corrective gene therapy to replace defective genes. The p53 gene replacement is attractive therapeutically because gene mutation is a late event in the progression of prostate cancer and is associated with metastasis, loss of differentiation, and the transition from androgen-dependent to androgen-independent growth and *in vitro* restoration of wild-type p53 in many tumor cell lines causes growth arrest and/or apoptosis [132,133].

Before the clinical consequences of any of these modalities can be fully realized, we must continue to determine the precise mechanisms underlying the apoptotic cross-talk signaling pathways so that effective implementation of targeted therapeutic approaches will lead to cure of prostate cancer promising survival of thousands of patients.

## ABBREVIATIONS

TGF $\alpha$	=	Transforming growth factor alpha
TGF $\beta$	=	Transforming growth factor $\beta$
EGF	=	Epidermal growth factor
KGF	=	Keratinocyte growth factor
FGF2	=	Fibroblast growth factor 2
VEGF	=	Vascular endothelial growth factor
PSA	=	Prostate specific antigen
DHT	=	5 $\alpha$ -dihydrotestosterone
ECM	=	Extra-cellular matrix
FAK	=	Focal Adhesion-Kinase
ILK	=	Integrin-linked Kinase
TSP-1	=	Thrombospondin-1
LHRH	=	Luteinizing hormone releasing hormone

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