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Review Article

Impact of α-adrenoceptor antagonists on prostate cancer development, progression and prevention

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Abstract: Two decades following the discovery that α1-adrenoceptor antagonists suppress prostate tumor growth at the molecular and cellular level, the impact of α-blockade as re-purposed treatment strategy in the medical management of prostate cancer is gradually being recognized. Prostate cancer is the second most common cause of cancer deaths among males in the United States, yet the disease maintains inconsistent recommendations for prevention and screening. The functional relationship between α-adrenergic signaling and smooth muscle cells in the stroma of the prostate gland and the bladder neck empowered the use of α-adrenoceptor antagonists for the relief of urethral obstruction and clinical symptoms associated with benign prostatic hyperplasia (BPH). Adrenoceptors are G-protein-coupled receptors (GCPRs) that are functionally bound by catecholamines: epinephrine (ER) and norepinephrine (NE). The α1A adrenoceptor subtype is primarily responsible for smooth muscle contraction in the bladder neck and prostate gland. α1-adrenoceptor antagonists are clinically indicated as first-line therapies for the relief of BPH, hypertension, and post-traumatic stress disorder (PTSD). Compelling evidence from cellular and preclinical models have identified additional effects of α1-adrenoceptor antagonists regarding their ability to induce apoptosis-mediated suppression of prostate tumor growth and metastasis. Additionally, early epidemiologic data suggest that they may serve as a safe treatment to reduce the risk of prostate cancer. Optimization of quinazoline based compounds (doxazosin) to exploit pharmacologic targeting of tumor growth and vascularization revealed high efficacy of the lead novel compound DZ-50 against prostate tumors. This review discusses the experimental and pre-clinical evidence on the impact of α-blockade on prostate cancer.

Keywords: Alpha blockers, prostate cancer risk, sympathetic blockade

Introduction

The clinical burden of prostate cancer and benign prostatic hyperplasia (BPH)

Prostate cancer is the most frequently diagnosed cancer in males and the second leading cause of cancer deaths in males with an estimated 29,430 deaths in the United States for 2018, following only respiratory malignancies for mortality [1]. There is an estimated incidence of 164,690 new cases of prostate cancer in the United States for 2018 [1]. These cases account for approximately 19.2% of all estimated new cases of cancer in males in the United States. The five-year survival for patients with non-metastatic prostate cancer is 98.9% (measured between 2005 and 2011) but patients with metastatic prostate cancer on initial diagnosis (4% of prostate cancer patients) had only a 28.2% five-year survival [1]. The morbidity and mortality associated with advanced prostate cancer calls for preventive tools that reduce the likelihood of developing prostate cancer and impairing growth, metastases, and progression to therapeutic resistance. Despite the high morbidity of prostate cancer in the United States there are currently no drugs indicated for the prevention of prostate cancer in at-risk individuals. Recurrent prostate cancer progresses to resistance to androgen deprivation therapy (ADT) and/or taxane-based chemotherapeutic drugs [2]. The stromal microenvironment plays a major role in the progression of prostate cancer to ADT resistance by conferring a process called epithelial-to-mesenchymal transition (EMT) [2].

BPH is a benign proliferative pathology of the prostate glandular epithelium, connective tissue, and smooth muscle that affects males older than 30 years of age [3]. Histopathological evaluations demonstrate that 68% of men over the age of 50 show cellular changes associated with BPH [4]. Evidence derived from a population-based study has shown that 75% of men over the age of 70 describe at least one lower urinary tract symptom associated with BPH [5]. A contributor to the etiology of BPH is the reversion of the normal cellular environment to an embryologic growth phase where new epithelial gland formation and stromal cell inductive potential is aberrantly restarted from stem cell progenitors [6, 7], and deregulated cell survival signaling [8]. The relationship between adrenergic signaling and smooth muscle cells of the stroma introduced the use of adrenoceptor antagonists for the relief of urethral obstruction in BPH [9]. The clinical utility of α1-adenoceptor antagonists has long been established as first-line therapy to relieve lower urinary tract symptoms (LUTS) secondary to BPH (BPH-LUTS) in aging men [10].

The economic impact of treatment of BPH, including direct costs (drugs, procedures, imaging, office visits), indirect costs (lost earnings), and intangible costs (pain and suffering) is approximately $4 billion in the United States alone [11]. Evaluation of claims data demonstrate BPH associated costs begin to rise as early as men in the 4th decade of life, with 4.7% of men between the ages of 45-54 seeking treatment, and 14.3% of men between 55-64 seeking care for this condition [12]. The relatively rapid onset of the BPH effects in aging males is indicated by the prostate growth doubling time of only 4.5 years between the ages of 31-50 and 10 years between ages 50-70 [3]. The estimated prevalence of BPH for males over the age of 30 in the United States for 2015 was 38,000,000 [4], with 12 million seeking active treatment. Of those actively managed, 54.8% choose drug management, primarily with α1-blockade [13]. The medical management of patients with BPH demonstrates an estimated 23% of all visits to urologic practices in the US, second only to urinary tract infections (UTIs) [13]. The impact of this prostatic condition at both the clinical and economical level is expected to grow as the population of the United States ages. By 2030, 20% of the male population in the United States is expected to be 65 years of age or older, with the 85 and older subset being the fastest growing portion of the population [14].

In this review we discuss the pre-clinical and epidemiological evidence (spanning 20 years) on the impact of α-blockade (in clinical use for the treatment of BPH) on the cellular landscape and clinical outcomes in prostate cancer progression to advanced disease.

The origins of the prostate gland

The prostate gland is a walnut-sized glandular organ found inferior to the urinary bladder and surrounds the proximal urethra that is found in human males [15]. Histologically the structure of the prostate gland is organized by two different schemas: “zones” or “lobes”. For this review we will be using the zone classification schema to better illustrate the localization of cell populations and their impact on BPH and prostate cancer. The four major zones of the prostate include the peripheral zone which makes up most of the glandular organ, the central zone that surrounds the ejaculatory ducts, the transition zone that surrounds the proximal urethra, and the anterior fibromuscular zone [16]. The function of the healthy prostate is the emission of prostatic fluid into the seminal fluid for the survival and function of spermatozoa in male ejaculate [17]. Prostatic fluid contains many proteins, including various enzymes that prolong the survival of spermatozoa in the ejaculate, prostate specific antigen (PSA), and highly concentrated zinc [17]. Despite the small size of the prostate, the gland is the site of major contributors to morbidity and mortality from diseases such as BPH and prostate cancer [17].

Embryologically the prostate gland arises from the primitive endoderm or ‘gut tube’. The hindgut region of the primitive endoderm swells into a larger hollow structure called the cloaca. The cloaca is separated into the ventral and dorsal outlets by the urorectal septum which give rise to the urogenital and anorectal structures, respectively. The ventral outlet is termed the primitive urogenital sinus. The urogenital sinus gives rise to the urinary bladder at the cranial end and the urethra caudally [17]. During the

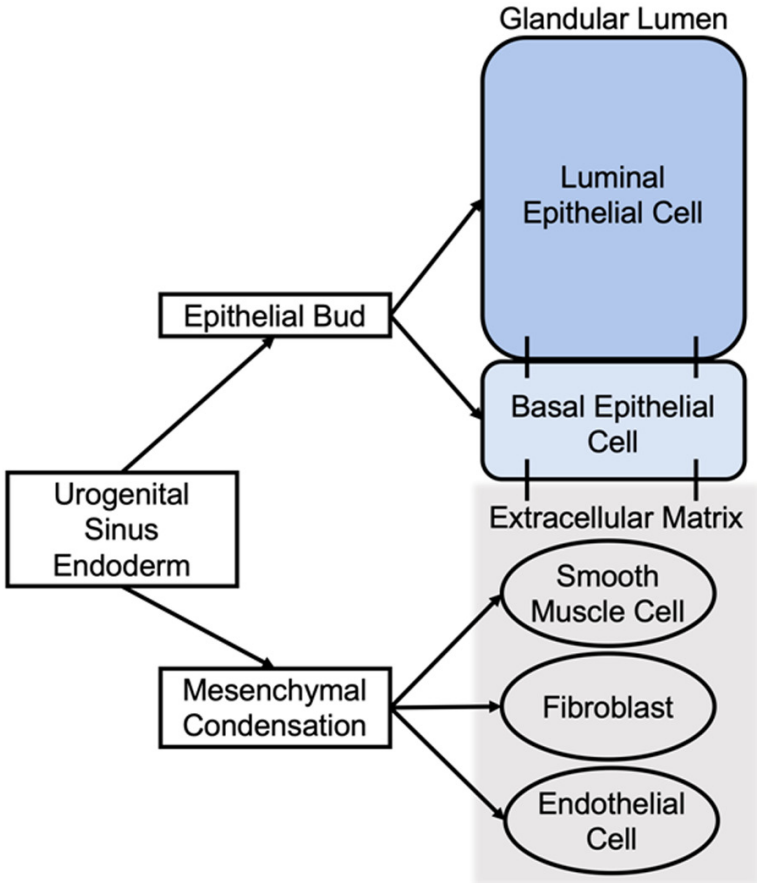


Figure 1. Embryologic order of development for prostate cells. Urogenital sinus gives rise to the epithelial and mesenchymal cells of the prostate. The epithelial bud differentiates into luminal, basal, and intermediate (not pictured) epithelium. The mesenchymal condensation gives rise to smooth muscle cells, fibroblasts, and endothelial cells that inhabit the extracellular matrix.

10th week of human gestation, the prostate forms caudal to the bladder neck by an interplay of early epithelial and mesenchymal cells which both arise from the urogenital sinus. In an androgen independent process (occurs in both male and female embryos), the urogenital sinus gives rise to loose connective tissue composed of mesenchymal cells into distinct regions that will determine the lobes of the prostate [18]. The release of mesenchymal cells into these distinct regions is called "mesenchymal condensation" [18]. The mesenchymal cells ultimately differentiate into the prostate stromal cells, the supporting cells of the fibromuscular tissue within the prostate microenvironment [18]. In male embryos, the urogenital sinus-derived epithelial buds are stimulated in an androgen dependent process by mesenchymal-secreted fibroblast growth factors (FGFs), transcription factors, and transforming growth factor-β (TGF-β) to invade the mesenchymal condensations and grow into the early prostate epithelium [18, 19]. The invading urogenital sinus epithelium differentiates into distinct epithelial cell populations of the prostate including the basal, intermediate, and luminal layers (**Figure 1**) [20]. The basal epithelial layer consists of non-secretory cells adjacent to the stroma, the luminal layer makes up the secretory columnar epithelium, and the intermediate layer is a population of epithelial cells with shared basal and luminal characteristics [20, 21]. These epithelial cells make up the functioning luminal structure (prostate glands). The mesenchyme gives rise to distinct cell populations that occupy the prostate gland microenvironment: smooth muscle cells, fibroblasts, and endothelial cells (**Figure 1**).

Neurologically, the prostate is innervated by both parasympathetic muscarinic and sympathetic adrenergic nerve plexuses from the pelvic nerve and hypogastric nerve, respectively. The glands of the prostate in the peripheral and central zones are innervated by muscarinic autonomic nerve plexuses, with high association between cholinergic signaling receptors and the epithelial lining, which drives secretions by the prostate [22]. While the stromal areas of the prostate gland also contain muscarinic autonomic neurons, the smooth muscle cells are predominantly autonomically innervated by adrenergic signaling [23]. Smooth muscle cells are primarily in the anterior fibromuscular portion of the prostate, and throughout the peripheral and central glandular zones of the organ [18]. Adrenergic innervation mediates the contraction of prostatic smooth muscle via norepinephrine [24].

Clinical recommendations for prostate cancer

Diagnosis

After the widespread implementation of PSA screening began in the 1990s, the rate of diagnosis of prostate cancer remains nearly twice the rate prior to the pre-PSA era, while the rate of mortality has slowly decreased since 1991, which suggests many of the cases diagnosed would otherwise be indolent and have no clinical significance [25]. While advanced stage prostate cancer carries significant mortality, there is growing evidence over the past decade that many low-grade diseases are best managed by observation [26].

Current recommendations by the American Urologic Association (AUA) advocate screening for prostate cancer by means of annual PSA and digital rectal exam in select groups of patients. This recommendation is not shared by all professional groups, as until recently the United States Preventive Services Task Force (USPSTF) provided a D rating (recommends against) for PSA-based screening for prostate cancer. This was recently updated in May 2018 to more closely mirror the AUA's current recommendation, and the USPTF now recommends shared discussion with men between the ages of 55-69 to discuss the risks and benefits of PSA screening for prostate cancer. The overall benefit of prostate cancer screening is also a debated topic. Two large population-based randomized trials have been conducted with conflicting results. The European Randomized Study of Screening for Prostate Cancer (ERPSC) and the United States Prostate, Lung, Colorectal, and Ovarian (PLCO) are the two most cited studies. The ERPSC demonstrated a 21 percent prostate cancer mortality improvement in men obtaining routine PSA screening vs the unscreened control, however, the PLCO trial showed no survival benefit [27, 28]. The controversy surrounding these trials is beyond the scope of this article. Prostate cancer diagnosis is confirmed with ultrasound-guided needle biopsy after an abnormal digital rectal exam and/or elevated PSA is noted [26]. Staging of prostate cancer is based on the American Joint Committee on Cancer Tumor/Node/Metastases (TNM) system that combines tumor size, lymph node involvement, metastases, PSA at time of diagnosis, T1-T4 stage, and Gleason score [29].

Prevention

Androgens are the major contributor to the development of malignancy in the prostate gland [30]. Several clinical trials have determined the relationship between 5α-reductase inhibitors and prostate cancer development, the most prominent being the PCPT and the REDUCE trials [31, 32]. Reduced overall incidence of low grade prostate tumors (Gleason 5 or 6) was found in both trials; however there was no significant change in the mortality, and the rates of high grade prostate cancer (≥ Gleason grade 7) were unaffected and possibly increased, resulting in a black-box warning for 5α-reductase inhibitors by the FDA [33]. A recent case-control study supported this association, citing that use of finasteride, a common 5α-reductase inhibitor used to treat BPH, was associated with increasing the risk of developing high-grade (Gleason scores ≥8) prostate cancer and lower risk of developing low-grade (Gleason scores <8) prostate cancer, fueling the controversy on the use of 5α-reductase inhibitors as therapeutic strategy in high risk patients for prostate cancer [34].

A number of other agents have been evaluated for prevention of prostate cancer with limited evidence including metformin, statins, Selenium, Vitamin C, D, and E, Retinoids, and dietary phytoestrogen. For men already diagnosed with low grade disease (Gleason scores 5-6), the REDEEM (reduction by dutasteride of clinical progression events in expectant management) trial demonstrated dutasteride reduced the progression of low risk prostate cancer (HR 0.62, P=0.009) [35]. To date, no professional medical organization currently recommends chemoprevention for prostate cancer and all therapies studied to date are ineffective or experimental. While not associated with overall increased risk of prostate cancer, obesity is associated with higher-grade prostate tumors at diagnosis [36, 37]. These effects are potentially mediated by recruitment of adipose stromal cells from areas of white fatty tissue to the tumor site where they induce the EMT phenotype [36]. This is a burgeoning area of prostate cancer research in the growing body of evidence that targeting EMT may prevent progression to therapeutic resistance.

Treatment

Treatment recommendations for localized prostate cancer are based on a risk stratification

scheme that integrates serum PSA, Gleason score (grade), and clinical stage [26]. The recommendation for very low risk disease is active surveillance including routine PSA surveillance and ultrasound or MRI-guided imaging [26]. Options for low-risk disease include active surveillance or interventions including radical prostatectomy or radiotherapy [26]. With progression from low-risk localized to intermediate- or high-risk localized disease radical prostatectomy or radiation with ADT are recommended therapy options [26]. ADT is considered first line therapy for metastatic and some stages of biochemically (PSA) recurrent prostate cancer. ADT includes bilateral orchiectomy or drugs to eliminate testosterone production by manipulation of the hypothalamic, pituitary, and gonadal axis in combination with androgen receptor (AR) antagonists [26]. Prostate cancer patients may ultimately progress to castration resistant prostate cancer (CRPC) disease, defined as rising PSA in the setting of castrate levels of testosterone [2]. With emergence of CRPC, the treatment recommendation is continued ADT until progression to radiographic or symptomatic metastasis [38]; there has been no treatment, however, to increase cancer specific survival in CRPC. The PROSPER trial demonstrated patients treated with enzalutamide had a 71% lower risk of progression to metastatic CRPC (mCRPC) or death, while the SPARTAN trial demonstrated patients treated with apalutamide had an increased metastatic free survival and time to symptomatic progression when compared to placebo. With mCRPC the treatment recommendation is second generation antiandrogen (abiraterone) with prednisone or taxane chemotherapy with immunotherapy (docetaxel with sipuleucel-T) [38, 39].

Adrenoceptor signaling and prostatic disease

Mechanism of α-adrenoceptor antagonism

The prostate gland is innervated by the autonomic nervous system, with the glandular epithelium largely muscarinic-innervated, and the stroma associated with adrenergic-innervation (responsive to adrenoceptor stimulation) [22, 23]. Adrenoceptors are a class of G-protein coupled receptors (GCPRs) distributed throughout the body and constructed of seven transmembrane domains that are physiologically responsible for mediating responses to endogenous catecholamines, namely epinephrine and norepinephrine [40-42]. The adrenoceptors are found throughout the body in neuronal and non-neuronal tissues, serving as regulators of many autonomic nervous functions [40]. Adrenoceptors are divided into α (alpha) and β (beta) groups, where α-adrenoceptors mediate predominantly excitatory functions like smooth muscle contraction and vasoconstriction and β-adrenoceptors mediate predominantly muscular inhibitory functions like vasodilation, smooth muscle relaxation, and bronchodilation, as well as excitatory cardiac function [40]. The α-adrenoceptors are divided into α1 and α2, where the α1 group was originally characterized as a separate group by binding affinity to the quinazoline-based α-antagonist prazosin, which had a minimal effect when administered to the α2 group [40]. The α1-adrenoceptors can be further sub-classified into α1A, α1B, and α1D based on binding and functional studies [40]. Of interest, the α1A subtype is localized to the prostate, vas deferens, and urethra in humans providing a localized drug target for patients suffering urinary symptoms [23, 40, 43, 44]. Compared to the normal adult prostate, α1-adrenoceptor mRNA and α1-adrenoceptors increase throughout in the aging gland and with BPH manifestation [45-48]. With aging, the topological distribution of the α1-adrenoceptors in the prostate surrounding the prostatic urethra, contributes to BPH-LUTS development [49]. Early pathophysiologic studies demonstrated prostatic tissue contraction when exposed to nonselective α-blocker inhibition [50], due to the abundance of α1A adrenoreceptors localized to the prostatic stroma [51]. Significantly, functional studies have demonstrated α1-adrenoceptor antagonists also relieve LUTS via mechanisms independent of prostatic muscle contraction [52].

α1-adrenoreceptor antagonists have long been used clinically as first-line therapy to relieve BPH-LUTS [10]. BPH is characterized by non-malignant proliferation of prostatic glandular epithelium and stroma (connective tissues, smooth muscle) resulting in obstruction of the prostatic urethra and bladder outlet. This obstruction causes LUTS that may be worsened by an increase in prostate smooth muscle tone [10, 53]. α1-adrenoreceptor antagonists act to relieve BPH-LUTS by antagonizing the smooth muscle contractility induced by the catechol-

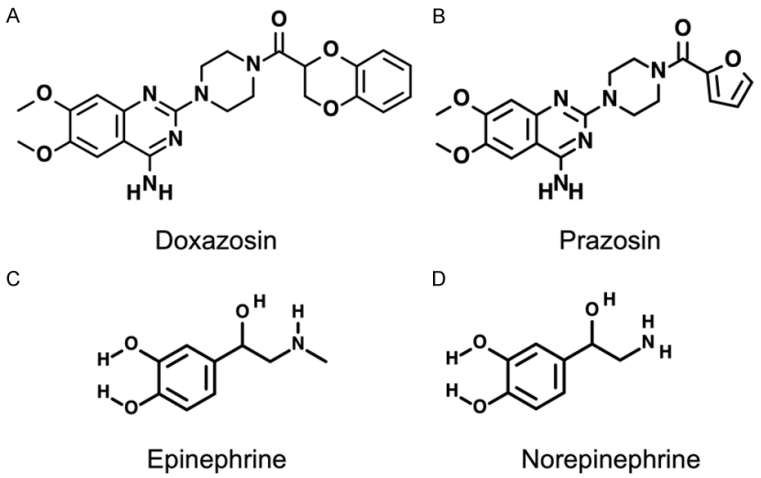


Figure 2. Structures of quinazoline-based α1-adrenoceptor antagonists doxazosin (A) and prazosin (B), and endogenous adrenergic agonists epinephrine (C) and norepinephrine (D).

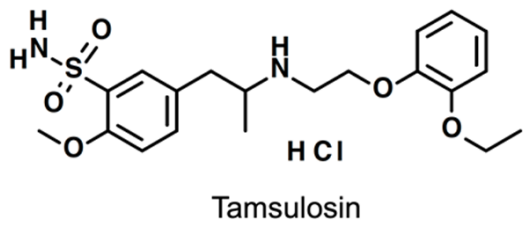
amine binding to α1A-adrenoreceptors in the prostate and bladder neck, providing relief of urinary obstruction by competitive inhibition [53]. Second-generation α-blockers (doxazosin, prazosin, and terazosin) are considered 'non-uroselective' where third-generation α-blockers (alfuzosin, tamsulosin, and silodosin) are considered 'uroselective' [54]. While both generations of α-adrenoceptor antagonists are indicated for first line treatment of BPH, second-generation α-blockers are highly associated with orthostatic hypotension, dizziness, tiredness, and ejaculatory problems due to less regionally selective α1-adrenoceptors antagonism [54, 55]. The function of α1-adrenoceptors as regulators of basal vascular tone, smooth muscle contraction, and arterial blood pressure provides the basis for the α1-antagonists use as hypertension treatment. Current guidelines from the American College of Cardiology and American Heart Association recommend the second generation α1-adrenoceptor antagonists as second-line agents for the treatment of hypertension, especially in the setting of male patients with concurrent LUTS [56, 57]. A review of the ALLHAT trial notes that patients with low risk for heart failure or young patients with hypertension with concomitant LUTS may benefit from monotherapy for both disease processes with a single agent [57].

Impact of α-adrenergic blockade on prostate cancer

The link between sympathetic nervous signaling and cancer neovascularization, metastasis, and survival has been well established in β-adrenergic signaling knockout models as well as epidemiologic cohort studies of β-blockade in patients [58]. The use of propranolol *in vitro* decreased viability and increased caspase activation in both hemangioblastoma and HeLa cell lines [59]. Treatment with propranolol decreased the hypoxia inducible factor (HIF) downstream transcription products, involved in angiogenesis, and extracellular matrix (ECM) degradation in HeLa cells, pointing to a mechanism underlying the anti-angiogenic effects of β-adrenergic blockade [59]. The *in vivo* silencing of β2 and β3 adrenoceptors in the prostate resulted in inhibition of angiogenic switch, mediated by pro-angiogenic factors, like vascular endothelial growth factor (VEGF) [60, 61].

Novel anti-tumor action by quinazoline-based α1-antagonists

Quinazoline-based α1-adrenoceptor antagonists, doxazosin, prazosin, terazosin, and alfuzosin, are structural competitive antagonists to epinephrine and norepinephrine, the predominant ligands of α-adrenoceptors (**Figure 2**). The structures of α1-adrenoceptor antagonists confer the ability to selectively antagonize adrenoceptors via post-synaptic blockade, inhibiting smooth muscle contraction, an effect that spares central action on blood pressure and neuronal adrenergic function, resulting in an effective drug class with few adverse or severe side-effects [41, 62, 63]. Subsequent work in the 1990s identified additional non-target quinazoline derivative mechanisms of action by impacting tumor vascularity and growth dynamics. Our group pioneered evidence on the apoptotic action of doxazosin mediated by TGF-β signaling disruption against benign prostate epithelial and stromal cells in pre-clinical models as well as in clinical specimens [64, 65]. Stimulation of α1-adrenoceptors with catecholamine ligands in prostate cancer epithelium promotes proliferation [66]. This response is mediated by induction of store-dependent Ca^{2+} entry resulting in activation of nuclear factor of activated T-cells (NFAT) [66]. Furthermore,



Tamsulosin

Figure 3. Structure of sulfonamide-based α1-adrenoceptor antagonist tamsulosin.

there is a correlation between α1-adrenoceptor activation and expression of VEGF and HIF-1α expression (inducers of angiogenesis and tumor invasion) [67]. Binding of the α1-adrenoceptors induces a second-messenger pathway via cAMP resulting in downstream PKA/PI3K/Akt/p70S6K pathway activation, driving HIF-1/VEGF-mediated angiogenesis in prostate cancer [67]. However, some pro-apoptotic mechanisms of action of quinazoline derivatives like doxazosin and terazosin are independent of the α1-adrenoceptor antagonism action [68]. Prostate cancer cells lacking α1-adrenoceptor undergo apoptosis in response to quinazolines, evidence supporting the α1-adrenoceptor-independent action of apoptosis induction [69]. Moreover, the sulfonamide-based third generation α1-adrenoceptor antagonist tamsulosin (**Figure 3**), had no effect on prostate cancer cell apoptosis [70]. Besides prostate cancer cells, breast and urothelial cancer cells, bladder smooth muscle cells, cardiac myocytes, pituitary adenoma cells, vascular endothelial cells, and HeLa cells undergo apoptosis in response to doxazosin [71-78]. The results of the ALLHAT trial that quinazoline-derived doxazosin doubled the risk of congestive heart failure resulted in investigation of the adrenoceptor blockade-independent mechanism of action for the pro-apoptotic activity in cardiac myocytes by these drugs [57, 73, 79]. Quinazoline-derived α1-adrenoceptor antagonist doxazosin induced apoptotic gene expression profiles in murine cardiac myocytes [73]. Specifically, doxazosin increased transcriptional activation of *gadd-153, C/epbβ,* and *DOC-1* genes, a profile associated with the ER stress apoptotic response. Downstream effects include the phosphorylation of p38 MAPK, GADD153 nuclear translocation, and phosphorylation of focal adhesion kinase (FAK) [73].

The process of EMT has been implicated as a contributor to the emergence of therapeutic resistance in advanced prostate cancer; however the current understanding of the impact of exposure to α1 blockade on EMT phenotypic landscape is limited. Anoikis is an apoptotic phenomenon that occurs when cells lose sufficient cell-cell or cell-matrix interactions [80, 81]. Circumventing anoikis is a common loss of apoptotic control in therapeutically resistant prostate cancer that confers an aggressive metastatic phenotype, particularly among epithelial cancers that consequentially undergo EMT [2]. Quinazoline-based α1-adrenoceptor antagonists, doxazosin and terazosin, enhance prostate cancer cell and endothelial susceptibility to anoikis, resulting in decreased cell mobility, thus impairing neovascularization and metastasis [82, 83]. Doxazosin induces apoptosis of prostate tumor cells via activation of the canonical TGF-β signaling, and caspase-mediated cell cleavage *in vitro* [84, 85]. Prazosin was also found to exhibit a significant pro-apoptotic activity by induction of cell-cycle arrest [86]. Prazosin induces DNA strand breaks that result in cyclin-dependent kinase (CDK) phosphorylation, ultimately causing CDK1 inactivation leading to G2 checkpoint arrest [86]. Additionally, prazosin triggered caspase-mediated apoptosis in prostate cancer cell lines *in vitro* [86]. Oral administration of prazosin significantly reduced tumor mass in xenograft mice models [86]. Tamsulosin lacks the pro-apoptotic effect and demonstrates no induction of caspase-mediated cell death, unlike the quinazoline-based α1-adrenoceptor antagonists, suggesting the importance of structure in apoptotic induction [70, 84]. All α1-adrenoceptor antagonists have been shown on a large-scale meta-analysis to have similar efficiency in the reduction of urinary symptoms and improvement in flow rates, with differences relating to the specific side effect profile [87-89].

Optimization of quinazoline compounds into directed anti-tumor therapies

DZ-50 is a quinazoline-derived α1-adrenoceptor antagonist (**Figure 4A**) synthesized by replacing the 2,3-dihydro-benzo[1,4]dioxane-carbonyl moiety of doxazosin with a biphenyl aryl sulfonyl substituent, and the methoxy side chains replaced with isopropyl propxy functions [91]. These structural changes in the quinazo-

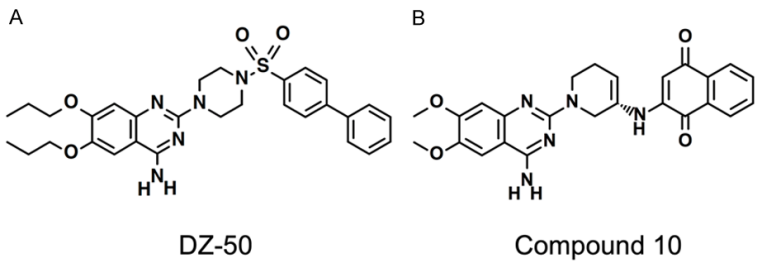


Figure 4. Structure of quinazoline-based modified prazosin derivative DZ-50 (A) and structure of quinazoline-based modified prazosin derivative Compound 10 (B).

line provide a cellular basis for targeting vascularity of pre-malignant or malignant prostate microenvironment with novel quinazoline derivatives [91]. DZ-50 significantly suppressed angiogenesis without increasing the apoptotic index *in vivo* [91], and exerted a metastasis impairing effect in prostate cancer pre-clinical models [91].

More recent studies from our lab have demonstrated the ability of DZ-50 to mediate the mesenchymal-to-epithelial transition (MET) phenotypic change that reverts de-differentiated EMT phenotype of prostate cancer cells into the differentiated epithelial phenotype [92]. The changes in cell-cell junction by DZ-50 are mediated by disruption of TGF-β1 and insulin-like growth factor (IGF) signaling axis, specifically IGF binding protein 3 (IGFBP3) [92]. The TGF-β1 and IGF signaling axes have been linked to cancer progression to metastatic, treatment resistant disease by altering the microenvironment via differentiation of fibroblasts into cancer-associated fibroblasts (CAFs), facilitating tumor survival, growth, and neovascularization, consequential to a dedifferentiated phenotype [93-96]. The IGF-I axis is responsible for upregulation of zinc finger E-box-binding protein 1 (ZEB1), a protein that transcriptionally represses E-cadherin [96]. Treatment of prostate cancer cells *in vitro* with DZ-50 results in drug sequestration of IGFBP3, the serum carrier of IGF ligand and promoter of IGF action; this leads to EMT reversal (MET) and ultimately anoikis [92, 96, 97]. The *in vitro* evidence so far provides a basis for advancing DZ-50 to a clinical trial for patients with metastatic CRPC [2].

Quinazoline structural optimization led to potent pro-apoptotic effects in prostate cancer cells, via piperazine ring substitution to the prazosin quinazoline nucleus [98, 99]. The most promising of the novel compounds from a recent study of quinazoline modification are compound 9 and compound 10 (**Figure 4B**), both demonstrated anti-proliferative effects in prostate cancer cells at a magnitude higher than doxazosin [99]. Compound 10, the S enantiomer of a prazosin quinazoline-quinone, was synthesized by substituting the piperazine side chain of prazosin with a 1,4-napthoquinone moiety [99]. The R enantiomer, compound 9, exhibits less binding affinity for the α1-adrenoceptor subtypes and lower apoptotic action, implicating the stereochemistry in the anti-cancer actions of the novel compounds [99].

Translational significance of cellular events

Based on the observed pro-apoptotic and anti-neovascularization actions of quinazoline-based α1-adrenoceptor antagonists summarized in **Figure 5**, investigators have completed retrospective risk analyses to elucidate the early translational evidence of these drugs as preventive tools. A 2018 meta-analysis by Cao *et al.* found non-significant association between the use of anti-adrenergic drugs and the risk of prostate cancer across 3 case-control studies (RR 1.22; 95% CI 0.76-1.96), and a significant decrease in risk across 2 cohort studies (RR 0.71; 95% CI 0.57-0.90) [100]. A case-control study by another team used the Finnish Cancer Registry and the national prescription database to observe the odds of developing prostate cancer for patients using α1-adrenoceptor antagonists for the treatment of LUTS (OR 1.79; 95% CI 1.67-1.91) [101]. However the exposed group of cases and controls were almost entirely users of sulfonamide-based α1-adrenoceptor antagonist tamsulosin (N=6,352) and only a small proportion represented quinazoline-based α1-antagonist alfuzosin (N=596) [101]. This is in accord with the pre-clinical evidence that only quinazoline-based antagonists confer the pro-apoptotic effect against prostate tumors, while the sulfonamide-derived α-adrenoceptor antagonist, tamsulosin, failed to exert such an effect [70].

More than a decade ago a retrospective study conducted by Harris *et al*. provided the first observational evidence that patients treated

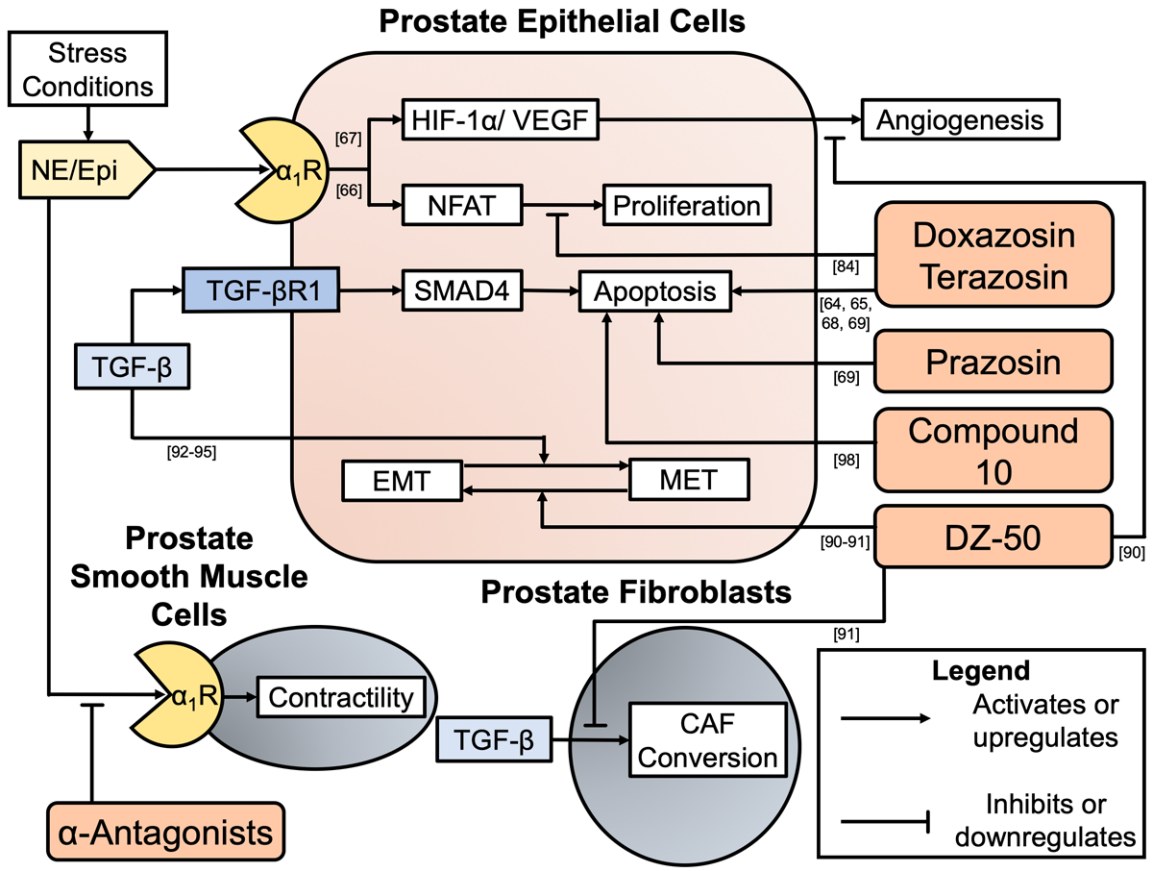


Figure 5. Summary of *in vitro* evidence demonstrating quinazoline-derived α1-adrenoceptor antagonist or modified quinazoline anti-cancer actions. Prostate epithelial-derived cancers and the microenvironment are influenced by α1-adrenoceptor antagonists. Blockade by α1-adrenoceptor antagonists at the smooth muscle cells in the prostate stroma mediate the anti-contractility benefits of the drugs when used for BPH-LUTS. Quinazoline derived α1-adrenoceptor antagonist doxazosin disrupts the proliferation axis induced by α1-adrenoceptor activation in a non-α1-adrenoceptor antagonism action ([84]). Quinazoline derived α1-adrenoceptor antagonists doxazosin, terazosin, and prazosin have demonstrated the ability to induce apoptosis in prostate cancer cells ([64, 65, 68, 69] and [69], respectively). Novel quinazoline-derived compound titled 'Compound 10' demonstrates anti-cancer activity with pro-oxidant DNA fragmentation in prostate cancer cell lines ([99]). Novel quinazoline-derived compound titled 'DZ-50' demonstrates multiple anti-cancer actions including induction of MET (reversal of EMT), inhibition of TGF-β-induced microenvironment fibroblast changes, and the inhibition of angiogenesis ([91-92], [92], and [91], respectively).

Table 1. Clinical evidence on impact of quinazoline α1-antagonists on prostate cancer

Study	Risk Ratio	Odds Ratio	Drug (s)	Drug Class	Source
Harris *et al.*	0.683 (95% CI 0.532-0.8776)	N/A	Doxazosin, prazosin, terazosin	Quinazolines	[102]
Van Rompay *et al.*	0.89 (95% CI 0.81-0.97)	N/A	Alfuzosin, doxazosin, prazosin, terazosin, or tamsulosin	Quinazolines (4), Sulfonamide (1)	[103]
Friedman *et al.*	N/A	1.17, 1.22 (95% CI 1.11-1.24, 1.16-1.29)	Prazosin, terazosin	Quinazolines	[104]

Epidemiological studies observing the relative odds or risk of developing prostate cancer amongst individuals treated with different α1-adrenoceptor antagonists.

for hypertension or BPH with quinazoline-derived α1-adrenoceptor antagonists doxazosin, prazosin, and terazosin [102], exhibited a reduced risk ratio of 0.683 for developing prostate cancer [102]. This study revealed no effect on the overall survival in men with prostate cancer who were exposed to quinazoline-based α1-adrenoceptor antagonists [102]. Potentially limited by the population age with group median exposure age of 68-yrs (**Table 1**). A more recent case-control study by Van Rompay *et al.* examined the overall risk of prostate cancer among patients taking the α1-antagonists alfuzosin, doxazosin, prazosin, terazosin, or tamsu-

losin, and found a protective risk ratio of 0.89 (**Table 1**) [103]. The average age at prescription of α1-antagonists in this study was 66.7, with a mean follow-up time of 6.3 years (exposure to α1-antagonists) [103].

A direct assessment of the link between norepinephrine signaling and cancer risk, by Friedman and colleagues, revealed both quinazoline-derived α1-adrenoceptor antagonists prazosin and terazosin increased the odds of developing prostate cancer (OR 1.17, 1.22; 95% CI 1.11-1.24, 1.16-1.29) [104]. These findings are challenged by evidence that terazosin showed statistically significantly reduction in the odds of developing colon and lung cancer with odds ratios of 0.86 and 0.89, respectively [104] (shown on **Table 1**). Users of naphthalene-based α1-adrenoceptor antagonist naftopidil had a risk ratio of 0.46 for developing prostate cancer when compared to users of sulfonamide-derived tamsulosin [105]. These findings drive the pursuit for further structural optimization of α1-adrenoceptor antagonists to mechanistically exploit the pathways causing prostate apoptosis towards the development of agents for prostate cancer prevention [2, 91, 92].

Conclusion

The epidemiologic data surrounding the use of quinazoline based α1-adrenoceptor antagonists as chemopreventive agents in prostate cancer is mixed and very limited. In order to further assess the role of these drugs as prostate cancer preventive agents requires a large-scale cohort of individuals exposed to quinazoline-derived antagonists for a more prolonged period than the existing studies. Given that the majority of studies investigating this relationship have a mean exposure age of more than 65 years old, the cohort should concern individuals treated at a younger age with α-antagonists for familial hypertension or BPH. As α-adrenoceptor antagonists are low risk drugs and widely available, a randomized controlled trial with a long-term cohort of young participants could provide the strongest evidence. The available *in vitro* and *in vivo* data provide an initial basis for quinazoline derivatives as cancer preventive agents. There is an immediate need for low risk and inexpensive chemopreventative agents for prostate cancer, a major contributor of morbidity and mortality. The existing data support the design of prospective studies using quinazoline derivatives, like doxazosin (in clinical use) and novel drugs like DZ-50, as prostate cancer chemoprevention drugs.

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Disclosure of conflict of interest

None.

Abbreviations

BPH, Benign Prostatic Hyperplasia; LUTS, Lower Urinary Tract Symptoms; EMT, Epithelial to mesenchymal transition; TGF-β, Transforming Growth Factor-β; ADT, Androgen Deprivation Therapy; ECM, extracellular matrix; AR, androgen receptor; ZEB1, E-box-binding protein 1; GCPRs, G-protein-coupled receptors; PCPT, Prostate Cancer Prevention Trial; REDUCE, Reduction of Prostate Cancer Events; PROSPER, Safety and Efficacy Study of Enzalutamide in Patients With Non-metastatic Castration-Resistant Prostate Cancer; SPARTAN, Selective Prostate Androgen Receptor Targeting with ARN-50; CRPC, Castration Resistant Prostate Cancer; NE, norepinephrine; PSA, Prostate Specific Antigen; MET, mesenchymal to epithelial transition; AUA, American Urological Association; MRI, Magnetic Resonance Imaging; FDA, Food and Drug Administration.

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