

Featured Review Article

Cytoskeleton targeting value in prostate cancer treatment

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Abstract: Prostate cancer is a disease that affects hundreds of thousands of men in the United States each year. In the early stages of advanced prostate cancer, the disease can be suppressed by androgen deprivation therapy (ADT). Eventually, however, most patients experience resistance to androgen deprivation, and their treatment transitions to alternative targeting of the androgen axis with abiraterone and enzalutamide, as well as taxane-based chemotherapy. Development of advanced castration-resistant prostate cancer (CRPC) is a consequence of lack of an apoptotic response by the tumor cells to treatment. Understanding the mechanisms contributing to prostate tumor therapeutic resistance and progression to metastasis requires dissection of the signaling mechanisms navigating tumor invasion and metastasis as mediated by cell-matrix interactions engaging components of the extracellular matrix (ECM), to form adhesion complexes. For a tumor cell to metastasize from the primary tumor, it requires disruption of cell-cell interactions from the surrounding cells, as well as detachment from the ECM and resistance to anoikis (apoptosis upon cell detachment from ECM). Attachment, movement and invasion of cancer cells are functionally facilitated by the actin cytoskeleton and tubulin as the structural component of microtubules. Transforming growth factor (TGF)- β has tumor-inhibitory activity in the early stages of tumorigenesis, but it promotes tumor invasive characteristics in metastatic disease. Recent evidence implicates active (dephosphorylated) cofilin, an F-actin severing protein required for cytoskeleton reorganization, as an important contributor to switching TGF- β characteristics from a growth suppressor to a promoter of prostate cancer invasion and metastasis. Cancer cells eventually lose the ability to adhere to adjacent neighboring cells as well as ECM proteins, and via epithelial-mesenchymal transition (EMT), acquire invasive and metastatic characteristics. Microtubule-targeting chemotherapeutic agents, taxanes, are used in combination with antiandrogen strategies to increase the survival rate in patients with CRPC. This review addresses the development of therapeutic platform for targeting the integrity of actin cytoskeleton to impair prostate cancer progression.

Keywords: Prostate cancer, taxanes, metastasis, tubulin, cofilin, talin, actin, tumor microenvironment, androgen receptor

Introduction

There were 241,740 new cases of prostate cancer diagnosed and approximately, 28,170 men died from the disease in 2013. Prostate cancer was ranked first in the number of new cancer cases diagnosed and number two in the leading cause of cancer related deaths behind lung cancer [1]. Death from prostate cancer occurs due to metastasis to pelvic and retroperitoneal lymph nodes, as well as bone [2]. The cytoskeleton plays a major role in the process which governs the cellular mechanism by which cells dissociate with their microenvironment and become metastatic. This process is known as

epithelial to mesenchymal transition (EMT). During this transition, cells take on a more fibroblastic appearance and can become more invasive and motile. Decreased focal adhesions due to downregulation of E-cadherin have been linked to the ability of the androgen receptor (AR) to influence the way the cancer cell behaves by upregulating N-cadherin and β -catenin, and downregulating E-cadherin [2]. E-cadherin is necessary for adhesion at the apical surface of the cell. In tumor cells, loss of E-cadherin results in the loss of epidermal adherent junctions that allow a cell to adhere to neighboring cells. The intracellular domain of this membrane protein provides a structural link to actin.

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In 1941, Charles Huggins, made the seminal discovery that prostate tumors respond to androgen deprivation establishing this endocrine-targeting approach as the lead therapy for prostate cancer [3]. Unfortunately, in a majority of cases, patients relapse because the tumor will become androgen independent and reactivate AR signaling pathways consequential to androgen ablation. Overcoming this therapeutic resistance and targeting these prostate tumors is a major clinical challenge and involves the use of cytotoxic agents targeting the microtubules as a first line chemotherapy for advanced disease [4]. These chemotherapeutic agents target the extracellular environment and cytoskeleton remodeling, impacting cell migration, invasion and metastasis. Cell motility is an important aspect of the development of the embryo and fetus *in utero*, as well as immune response to pathogen and wound healing [5]. Most migration controlling genes are turned off after development but through the course of tumorigenesis these genes can be turned back on, resulting in EMT [5]. Anokis inducing agents can induce stabilization of the cytoskeleton to restrict movement as well as halting intracellular processes that are necessary for tumor survival, and have been investigated as potential therapeutics for prostate cancer [6].

The microenvironment and prostate cancer

The ECM plays a vital role as a critical contributor to tumorigenic development, growth and metastasis of prostate cancer. Access to new blood supply and nutrients are particularly important in metastatic disease, in accordance with the Seed and Soil hypothesis [7]. Prostate cancer metastasizes to bone in approximately 68% of cases of metastatic cancer [8]. Bones secrete cytokines that allow for trafficking prostate cancer cells to bone, including but not limited to osteopontin, osteonectin, and stromal-derived factor 1 (SDF1) [9]. SDF1 facilitates the invasion and migration of prostate cancer cells toward bone secondary tumor metastatic sites from their primary tumor [9]. Once the cells penetrate the bone, they adhere to bone marrow derived endothelial cells and begin secondary tumor growth at that site. Attachment to the bone endothelial cells is mediated by interactions with antigens on their surface and E-selectin molecules on the endothelial cells [8].

The contribution of the tumor microenvironment to metastatic progression is dictated by diverse cell types. Pathological review of biopsies often find inflammation associated with prostate cancer [10]. Monocytes have been found in these biopsies and they are known to secrete tumor necrosis factor (TNF). Secretion of TNF into the microenvironment correlates with prostate cancer progression associated loss of AR responsiveness and eventually castration resistant prostate cancer (CRPC) [11]. Tumor associated macrophages (TAMs) are also known to play roles in promoting angiogenesis in hypoxic environments deep within a tumor. TAMs release several cytokines and growth factors that stimulate angiogenesis and migration including VEGF and many types of matrix metalloproteases. Secretion of these cytokines empower the blood vessels surrounding the tumor to reorganize themselves towards penetrating into the center of the tumor, increasing the blood supply and nourishing tissues [12]. The dynamic signaling interaction between the tumor and its microenvironment has long been recognized as a bonafide cellular target in the treatment of prostate cancer. Thus, prostate fibroblasts in the context of the reactive stroma signal effectors that navigate the ECM cell attachment. They secrete proteins like collagen and other protein based fibers such as fibronectin, laminin, and others found in tissues that serve as basement membranes. Cancer associated fibroblasts (CAFs) play an intricate role in initiating invasion into surrounding tissue by responding to TGF- β in the extracellular environment [13]. Recent work from this laboratory established the functional contribution of CAFs and TGF- β signaling in modulating the structural integrity of the cytoskeleton towards enhanced migration, invasion and metastasis [14]. Extracellular TGF- β from the microenvironment in concert with CAFs is capable of inducing intracellular responses correlating with enhanced actin polymerization and invasion [14].

In response to TGF- β , prostate epithelial cells activate transcription factors such as Snail and Twist, functionally responsible for the down regulation of E-cadherin and upregulation of N-cadherin [15], towards promoting EMT [16]. E-cadherin, a glycoprotein associated with cell to cell adherence, is often lost during EMT while N-cadherin is up regulated [16]. Integrins associated with the ECM upon interaction with

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E-cadherin (adherens junctions) can initiate cell movement and/or attachment. These trans-membrane protein interactions between E-cadherin and integrins provide links by which actin can anchor, and mediate cell to cell adhesion. When E-cadherin is down regulated during EMT, resulting in loss of cell to cell contact and anoikis [17]. An intricate dynamic web is thus built that enables a vital platform for therapeutic targeting of advanced prostate cancer. A working knowledge of how the intracellular and extracellular proteins, as well as the genes that regulate the cytoskeleton can lead to novel pharmacological platforms for targeting castration resistant prostate cancer.

Actin

Actin is the most abundant protein within a cell and responsible for cellular movement within the microenvironment. Actin is found in all cells in the body including macrophages, myocytes, and epithelial cells. Cells rearrange the components of their microfilaments in order to drag themselves toward or away from stimuli [18]. Filopodia of moving cells contain parallel actin filaments that reach to an attachment site. The filaments grow in the direction of the stimuli by adding ATP-actin monomers, also known as G-actin, to the growing end. The growing, or (+) end, gains more ATP-actin than the (-) end. This facilitates the directional movement of the cell. The ATP is hydrolyzed, leaving ADP-actin monomers behind, which eventually disassociate from the (-) end resulting in a “treadmilling” action [19]. The actin subunits sequestered into the total helical filament that is formed is called F-actin (filamentous actin). The process is continuous until the stimulus from the chemo-attractant dissipates [20]. At times, cells must move more quickly than normal, causing activation of members of the ADF/ cofilin family of proteins, as well as other major actin binding proteins. Cofilin acts to sever the ADP-actin monomers from (-) end of the actin filament allowing the formation of a branch point and subsequent nucleation of a new (+) end filament, facilitating moving the filopodia toward the chemoattractant more rapidly [21, 22]. Regulation of the actin cytoskeleton is imperative to normal cell function. Cells that move within the body remodel the cytoskeleton to facilitate locomotion through tissues. For example, macrophages and other immune cells must be able to adhere and move within their microenvironment to engulf pathogens and cellular

debris. Actin filament dynamics are also regulated by phosphoinositides as well as a number of other actin binding proteins. The phosphoinositides, however, interact directly with the other binding proteins as well as providing an intracellular scaffold for proteins such as the Rho family small GTPases [23]. RhoC and RhoA play roles in tumor cell migration and EMT in tumor progression. F-actin is colocalized in the protrusions of migrating cells [24]. The Rho family of small GTPases acts in several protein cascades that ultimately activate actin polymerization and can be linked to metastatic cancer. Actin binding proteins like cofilin, WASP (Wiskott-Aldrich syndrome protein), and cortactin are involved in the signaling cascade that, when dysfunctional, leads to overactive filopodium protrusions and enhanced migration and invasion potential into surrounding tissue [25].

The WASP family of proteins is involved in actin polymerization and branching through the action of the Arp2/3 complex, which forms a new nucleation site stimulating (+) end microfilament polymerization [26]. Under normal conditions, WASP is auto-inhibited until activation by phosphoinositides and Cdc42, thereby stimulating actin polymerization [27]. There are several different mutations in this pathway that can lead to aberrant activation of WASP, including mutations in the protein itself, overexpression of the protein leading to increased activation of the Arp2/3 complex, mutations or over activation of effectors upstream of WASP such as G-proteins or PI3 kinase. Extracellular overstimulation of receptors associated with cell motility can also lead to an increased activation of the pathway and ultimately enhanced actin polymerization [28]. N-WASP is a ubiquitously expressed protein which binds phosphatidylinositol (4,5) biphosphate, Cdc42, and possesses an actin depolymerizing domain similar to cofilin [29]. Loss of N-WASP expression reduced the number of invadopodia structures seen *in vivo* in mammary glands. Loss of N-WASP impacts the metastatic ability of cancer cells navigated by the localization of invadopodia, further implicating the WASP proteins in metastatic disease [29]. Supporting evidence revealed that WASP-Arp2/3 complex mediated events lead to invadopodia formation in prostate cancer cells [30].

Cofilin

Cofilin acts as an actin filament severing protein creating a branch point between two fila-

ments of F-actin [31]. By creating a cut in the actin filament, it creates a branch point for more monomeric actin to bind, increasing the rate of actin filament polymerization to aid in cell locomotion, by generating new barbed ends on the (-) end of the F-actin filament independent of help from capping proteins [31]. This allows for the recycling of actin at the leading edge of the lamellipodia, such as is seen in prostate epithelial cells that have undergone EMT [32]. Cofilin protein is often complexed with Arp2/3 and associated with the leading edge of actin filaments of cell protrusions [33]. Under normal conditions, cofilin activity is inhibited by phosphorylation of the third serine in the protein by LIMK or by binding to phosphatidylinositol 4,5-bisphosphate, preventing it from branching actin filaments and enhancing migratory capacity [34]. Using site directed mutagenesis to perform a serine to alanine mutation at the third amino acid position (S3A) in prostate cancer cell line PC3, recent work by Collazo et. al has shown this S3A mutant cannot be phosphorylated (inactivated) leading to aberrant constitutive activation of cofilin and increased invasion and migration [14]. Cofilin and Arp2/3 functionally interact in an intracellular environment with excess monomeric actin to generate barbed ends. In cancer cells, cofilin initiates the assembly of actin filaments, localizing them to the lamellipodia [35]. Overexpression of catalytically active LIMK1 also results in highly metastatic potential in prostate cancer cells as seen in metastatic lesions from patients [36]. Overexpression of LIMK1 also results from a chromosomal translocation of the gene, resulting in increased levels LIMK1 gene transcripts [36].

Early in tumor development TGF- β , functions as a growth suppressor, but during the late stages of tumor progression it enhances cell invasion and metastasis. Different factors in the extracellular fluid as well as binding sites in the ECM allow prostate cancer cells to proliferate and thrive. TGF- β and EGF are both stimulators of aberrant cofilin expression or production [37]. TGF- β has been shown to play a major role in prostate tumor metastasis and invasion [37]. Once TGF- β ligand is bound, TGF β receptor complex I phosphorylates the intracellular Smad proteins. Activated Smad proteins enter the nucleus and act as DNA binding proteins that activate cell cycle inhibitory proteins, protecting the cell from progressing to a tumor

phenotype [38]. Thus “normal” TGF β inactivates CDK4 and CDK6 through activation of a p16^{INK4B} family tumor suppressor gene p15^{INK4B} [39]. TGF β can also act upon the Rho/ROCK pathway, limiting LIMK1 activity and therefore, cofilin activation [14]. Decreased LIMK1 activity inhibits filopodia formation and cytoskeleton remodeling, and thusly limits invasion to secondary sites of tumor formation [14]. *In vivo* inhibition of TGF- β signaling by expression of a dysfunctional type II TGF β receptor significantly inhibited tumor formation and invasiveness [40]. This evidence provided proof of principle that TGF- β signaling is required for initiation of the invasive properties towards metastatic events in tumor progression [40]. More recent studies by Collazo et. al established that cofilin a key signaling effector for TGF- β is capable of navigating in the context of actin cytoskeleton remodeling prostate cancer progression to metastasis [14]. Cofilin was previously identified as a non-canonical effector of TGF β signaling that has the capacity to facilitate TGF β induced invasive properties towards metastasis [37]. Prostate cancer cells exposed to TGF- β *in vitro*, exhibited an increased filopodia formation, as well as an increased intracellular localization of cofilin and actin reorganization [14]. Furthermore the prostate cancer cells PC-3 S3A cells (harboring the S3A mutation conferring a constitutively active cofilin), were more migratory and metastatic than wild type cofilin expressing cells [14].

Talin

Talin1 is an important focal adhesion regulator. It acts as an intermediate between integrins and actin. Interaction between these proteins facilitates migration of cells in addition to focal adhesion. Talin1 is a 270 kD protein dimer that has a structure similar to beads on a string [41]. Talin1 has two domains, one binds to the cytosolic portion of the transmembrane integrin protein and the other binds actin. When dimerized, the molecule possesses a dumbbell shape based on thrombin cleavage experiments, where each side of the connecting rod has a globular head, effectively anchors the actin cytoskeleton and plasma membrane together [42]. Talin1 binds to the β_1 cytoplasmic domain of the receptor, activating it, with the head domains only binding to β_1 domain with high affinity [42]. Talin1 is mechanistically upregulated and downregulated in invasion and

anoikis, respectively [43]. Talin1 was detected in higher levels in secondary metastatic sites compared to primary sites and benign tumors of the prostate. However, loss of talin1 expression impairs the cellular ability to adhere to neighboring cells, where EMT can potentially facilitate anoikis [43]. Talin1 is involved in the FAK/AKT signaling pathway and the MAPK pathway. Talin1 overexpression confers anoikis resistance in prostate cancer cells. Functional studies further showed that talin1 is associated with invasion because cells that received the knockdown treatment did not invade as readily [44]. Src kinase is an activating substrate for FAK to phosphorylate, allowing mediators of survival and anoikis to be turned on [45]. Activation of ERK1/2 via phosphorylation by Src kinase leads to EMT migration and resistance to anoikis. The increased activation of ERK1/2 during early stages of tumor development facilitate tumor growth and proliferation. ERK translocates to the nucleus and phosphorylates members of the ternary complex, leading to the transcription of genes for growth proteins. Later in tumor progression, activation of ERK1/2 results in increased survival and inactivation of cell death machinery [45]. Src kinase transfers a phosphate to PI3K/AKT, which activates NF- κ B pathways associated with anoikis resistance as well as blocking cell death machinery BAD, Bcl-xL and mitochondrial cytochrome C release and caspase 9 activation [45].

Tubulin

Tubulin is a dynamic part of the cytoskeleton that plays an essential role in cell movement, cellular cargo transport and chromosomal segregation during cell division [46]. It is composed of α and β tubulin protein monomers that form heterodimers; both components have a nucleotide binding site for GTP, called the N-site and E-site, for α and β tubulin, respectively. The GTP bound form of the heterodimer binds to the macrostructure of tubulin, causing the hydrolysis of GTP to GDP. At both the (+) and (-) ends of the structure, there is a GTP cap, that when hydrolyzed, causes the structure to destabilize and dissociate [46].

Tubulin plays an important role in facilitating translocation of cellular cargo. Motor proteins walk along the microtubule structure toward their cellular destination. Kinesin and dynein

are ATP driven motor protein families that carry the molecular cargo [47]. In prostate cancer, microtubules are involved in translocating the androgen receptor (AR) from the cytoplasm into the nucleus upon association with its cognate ligand, dihydrotestosterone (DHT). There are two proposed mechanisms of kinesin walking [48]. The first called the *hand over hand* mechanism states that kinesin is an ATP driven protein that moves along the microtubule in eight nanometer steps. It is composed of two head portions with neck linker domains connected to a coiled coil attached to the intracellular cargo. ATP binds to one of the neck-linker domains of the head region of the kinesin molecule and is hydrolyzed, causing a conformational change. Upon hydrolysis, the other head swings forward [48]. The second is the *inch worm* model. This mechanism describes the same ATP binding and then hydrolysis, but rather than swinging the head that has just changed forward, past the stationary one, it swings to a position equal to it on the microtubule [49]. The kinesin-13 family of proteins is ATP dependent microtubule catastrophe inducing proteins that also sequester tubulin subunits, ensuring that shrinking of the structure occurs [50]. MCAK, a member of the kinesin-13 family, binds to the ends of the microtubules, more specifically at the kinetochore and spindle poles of mitotic chromosomes, and cause their breakdown during mitosis [51]. In experiments investigating *kif2a*, loss of this microtubule disassembly protein results in morphological problems with the cell resulting from microtubule overextension [52]. Other microtubule disassembly proteins exist in a cell beyond the kinesin-13 family. The kinesin-8 family of proteins also participates in length dependent depolymerization of microtubules by binding to the growing end and accumulating there [50]. These particular microtubule binding proteins are imperative to alignment of chromosomes at the equator of the cell during mitosis. Knocking down Kif18A, the protein specifically responsible for this phenomenon, resulted in the failure of alignment of chromosomes in a cell ready to undergo division [53]. Dynein has a similar cellular function as kinesin, however, it is responsible for the transport of AR into the nucleus, an extremely integral event in prostate cancer. When a mutation in a subunit of dynein, dyactin, is overexpressed in cells, it results in a complete collapse of the dynein motor protein complex [54].

When a mutant form of the dynein protein is introduced, there is a significant decrease in nuclear AR translocation [53].

Microtubule associated binding proteins (MAPs) regulate the assembly of tubulin subunits in several ways. MAP2, MAP4, tau protein, and DCX are all microtubule binding proteins that act to suppress events called catastrophes, which is a rapid switch from growing to shortening [55, 56]. By binding to the microtubule macrostructure, Tau and MAP-2 increase the rate of polymerization by affecting the dissociating rate for the α -tubulin/ β -tubulin from the major filament [55, 56]. MAP4 is found in other tissues and perform the same roles as MAP2 and tau, as they are mostly found in neurons. MAPs play a role in microtubule dynamics during metaphase [57]. Microtubule disassembly proteins act to cause catastrophe events in a cell by inhibiting polymerization and causing disassembly of the structure. Moreover, centrosomes are made up of microtubules attached to kinetochores of chromosomes and subsequently pulling the chromosomes to opposite sides of the cell during anaphase. In prostate tumors with Gleason grades ranging from two to five, structural anomalies were detected in centrosomes [58]. In metastatic tumor cells, abnormalities in the centrosomes are severe and associated with chromosomal instability, as well as metastasis in high Gleason grade cancers [58]. Loss or gain of chromosomes results in severe deregulation of gene expression, contributing to tumor cell survival and disease progression [58]. Diverse post-translational modifications of the tubulin subunits are associated with prostate cancer, resulting in prostate tumor progression from androgen dependent to androgen independent disease [59]. For example, α -tubulin that has been deetyrosinated or polyglutamylated is increased in prostate cancer [59]. Both post-translational modifications play important roles in proliferation and interaction with MAPs and in centriole stability [59]. Once ligand-bound, AR uses the microtubule network in prostate cancer cells to translocate into the nucleus [60, 61]. Acetylation of the α -tubulin subunits normally plays a role in intracellular trafficking of proteins by increasing dynein binding affinity for it, enabling AR nuclear translocation [59]. During prostate tumorigenesis tubulin acetylation increases cancer cell motility and invasion [62].

The AR and its interaction with the cytoskeleton

The sensitivity of prostate tumors to castration-induced androgen deprivation was established by the seminal findings by Huggins and Hodges, who established the role of male steroid hormones in prostate cancer cell proliferation and that their withdrawal diminished prostate tumor growth [3]. In the absence of androgen, AR is sequestered in the cytoplasm associated with the Heat Shock Protein 90 (Hsp90) super complex and Filamin A awaiting its cognate ligand, DHT [63, 64]. Prior to binding DHT, the bipartite nuclear localization signal (NLS) of the AR is held in a structural conformation that does not allow its nuclear translocation [64]. Upon binding to DHT, AR proteins dimerize, undergo activating phosphorylation by Protein Kinase A (PKA) and translocate to the nucleus via dynein motor proteins that walk via ATP dependent mechanism along microtubule component of the cytoskeleton. The binding of DHT and dimerization of AR facilitate a conformational change that ensures cooperation between different domains of the protein by revealing the NLS and allowing nuclear translocation and subsequent binding of the activate AR dimers to androgen responsive elements (ARE) of the DNA, towards eliciting cellular responses such as proliferation, apoptosis resistance, and EMT [64]. Androgen deprivation therapy (ADT) is a cornerstone of the clinical treatment paradigm in the management of patients with metastatic prostate cancer that can be achieved by a variety of surgical and/or pharmacological methods. ADT ultimately fails however to cure prostate cancer patients due to recurrence and progression to castration resistant disease. Understanding the pathways via which prostate cancer cells become resistant by bypassing or inappropriately activating the AR signaling is an ongoing pursuit. In the hypersensitivity pathway, AR upregulation in prostate epithelial cells, sensitizes them to very low levels of circulating androgen [63]. Amplified sensitivity leads to prostate tumor aberrant growth due to augmented stability of the androgen/AR complex being able to bind to AREs on DNA [65]. Androgen responsive prostate cancer xenografts in castrated mice resulted in a switch to an androgen independent, CRPC phenotype [66]. This results in activation of AR independently of DHT bound to it, and induced gene expression leading to tumor development [66].

AR mutations become increasingly common after androgen ablation therapy, as samples collected before and after show different levels of mutation in the receptor. The first mutations were identified in LNCaP cancer cells, which express a mutant AR [67]. Mutations in this cell line included a missense mutation that lead to increase non-specific binding to the receptor and therefore random activation of growth associated genes [63, 67]. This promiscuous activation of the AR is not unique to only one mutation. The bypass pathway involves activating genes associated with growth and proliferation for example, the BCL-2 proteins and associated pathway has been implicated in apoptosis evasion in prostate cancer [63, 68]. BCL-2 over activation or overexpression results in blocking the p53 activated pathway that causes the release of cytochrome C from the mitochondria, resulting cell survival [68]. The lurker cell hypothesis implicates a small population of cells pre-existing in the initial tumor in an androgen independent state, until androgen deprivation therapy (ADT) kills the androgen-dependent cells [69]. Furthermore, over twenty splice variants of the AR, some lacking ligand binding domain, either in total or functionally, and therefore constitutively active have been identified [70-74].

The AR is involved in several pathways associated with EMT and metastasis, including cadherin switching, Wnt signaling, TGF β signaling, and Notch signaling events [64]. During EMT, E-cadherin expression is lost resulting in the subsequent loss of interaction with neighboring cells by downregulation of proteins involved in tight junctions [2]. Loss of E-cadherin also causing an increase of N-cadherin expression, further promoting a metastatic phenotype [2, 64, 75]. Expression of E-cadherin is repressed by Snail (SNAIL1) binding to the E-box of the E-cadherin promoter as well as activating expression of proteins involved in invasiveness [64]. Notch1 activates Snail1, leading to the repression of E-cadherin. Notch signaling is another classical component of EMT that normally functions in cell to cell communications. Notch1 signaling is functionally linked to prostate cancer recurrence and tumor progression [64, 76].

Work from this laboratory provided support that modulating androgen exposure to androgen independent prostate cancer cells, leads to

EMT-MET cycling, actin cytoskeleton remodeling and microtubule rearrangement [61, 77]. Furthermore, an augmented EMT phenotype is detected in response to increasing concentrations of androgens [77]. These changes also correlated with the level of AR. Loss of E-cadherin and alterations in cytoskeleton structure may facilitate metastasis to secondary sites in the body as well as invasion [77]. The Wnt signaling pathway can also play a role in metastasis by preventing the degradation of β -catenin [64]. Ordinarily, β -catenin must be phosphorylated by GSK3 β to interact with phosphorylated Axin, a signaling scaffolding protein [64]. In tumor cells Axin is dephosphorylated and unable to facilitate the interaction of β -catenin with GSK3 β . This allows β -catenin to accumulate in the cytosol and eventually undergo nuclear translocation. Once in the nucleus, β -catenin can interact with T-cell factor (TCF), resulting in activation of oncogenes including c-MYC, c-Jun, fra-1, and the AR gene [64].

Targeting the cytoskeleton integrity in prostate cancer

Microtubule-targeting agents, the taxane family of chemotherapeutics cause stabilization of the components of the cytoskeleton, and have become the first line chemotherapy for metastatic CRPC. Because the main cytoskeletal components, actin and microtubules, play such vital roles in mitosis, cell motility, and movement of cargo into, out of, and around the cell, they are relevant targets for cancer chemotherapy [60, 61, 78-81]. Taxol, (paclitaxel), was the first taxane drug discovered in the 1970's as a molecule derived from the Pacific Yew tree [82]. Paclitaxel binds to microtubules with very high affinity [82], thus stabilizing the entire structure, and impeding the cells from undergoing mitosis, ultimately resulting in a mitotic block and apoptosis in rapidly dividing cells [78, 82]. Paclitaxel also induces cell cycle arrest at two different points in the cell cycle G₁ and M phase. During growth arrest, a p53 dependent pathway induces cell death, while if in prophase, a p53 independent pathway is activated [81]. Paclitaxel binds to the (+) end of the microtubule structure, specifically on the β subunit of tubulin when it heterodimerizes with the α -tubulin subunit [80]. This action explains the stabilization of the structure, since the β tubulin subunit must bind to a specific site on the

α -tubulin subunit towards structure elongation [80].

Recent paradigm-shifting evidence supports a new effect of microtubule-targeting chemotherapy on AR cellular localization and activity in prostate cancer cells [61], that provides novel mechanistic insights into cross-resistance between antiandrogens and taxanes in prostate cancer patients. Taxol inhibited PSA expression in prostate cancer cells and PSA protein expression in human clinical prostate specimens after docetaxel treatment [61]. Immunoprecipitation analysis of deletion mutants of AR revealed that the N terminal domain of the AR is associated with the α -tubulin subunit of the microtubule. Moreover, prostate tumors after docetaxel treatment showed significant translocation of AR from the nucleus to cytoplasmic fraction, where in untreated patients, AR was primarily localized to the nucleus [61]. By targeting the microtubule network of tumor cells, it is possible to prevent the translocation of AR into the nucleus and associated downstream transcriptional activation of AR target genes [60, 61]. Cabazitaxel is a second generation taxane, based on the rational design of the α -tubulin crystal structure [79]. It possesses a unique structural characteristic that confers a very low affinity for the P-gp (P-glycoprotein) efflux pump. Cabazitaxel is not recognized by this pump, thus enabling a rationale for its therapeutic use in taxane resistant cancer [79]. This rationally designed, new taxane drug, has extra methyl groups indirectly attached to ring structures, allowing the drug to pass the blood brain barrier, with major consequences for tumors metastasizing to the brain [79].

Epothilones are another class of antitumor drugs that targets the stabilization of microtubule structure in a similar way as taxanes [83]. They share the same binding sites on tubulin as paclitaxel, however, they also have some key differences that lead to their differential uses in clinical settings. Cancer cells exhibiting taxane resistance have over expression of P-glycoprotein efflux pumps. Epothilones and its derivatives are not affected by these pumps, similarly to cabazitaxel, therefore retaining their cytotoxicity [78, 83]. Mutations in β tubulin usually involve an alanine to threonine substitution that decreases the cytotoxicity of taxanes. However, expression of other isotypes of β tubulin can confer resistance to epothilones [83].

Pharmacological exploitation of the α 1-adrenoreceptor antagonist doxazosin® has led to the generation of novel quinazoline-based compounds, with the lead agent, DZ-50, having potent anoikis-inducing effects against prostate cancer cells [84, 85]. DZ-50 suppresses growth of human castration resistant prostate cancer xenografts and abrogates their metastatic potential *in vivo* by impairing angiogenesis, migration and invasion through targeting focal adhesions [86]. Recently conducted genome-wide analysis identified critical effectors of focal adhesion and tight junction interactions to be key molecular targets of the novel quinazoline agent. Significantly, DZ-50 elicits cancer cell anoikis by targeting talin and fibronectin in focal adhesion complexes [86]. In renal cancer cells functional loss of talin completely blocked focal adhesion complex formation by the lead compound [44]. DZ-50 also had downstream targeting effects, blocking AKT survival signaling pathway and enabling anoikis induction among tumor cells [86]. Ongoing studies focus on translating the antitumor action of DZ-50 to Phase I clinical trials for patients with metastatic CRPC.

Conclusions

The actin cytoskeleton provides a dynamic control of normal cell functions facilitating motility and intracellular trafficking. Dysregulation of protein effectors involved in actin polymerization and microtubule integrity plays a critical role in tumor progression, invasion and metastasis. Mutational activation of the TGF- β signaling network and its downstream effectors such as cofilin, during tumorigenesis induce EMT, facilitate aberrant actin polymerization and filopodia formation at the cell membrane, ultimately disrupting ECM interactions and promoting migration, invasion and metastatic spread. The focal adhesion effector, talin confers resistance to anoikis and activation of survival pathways towards metastasis. Microtubule-targeting chemotherapeutic agents, taxanes, have recently been shown to have additional action in mediating the nuclear translocation/cellular distribution of the AR in both androgen-sensitive and castration-resistant prostate tumors. Rapidly growing evidence implicates EMT as a cellular mechanism conferring resistance to docetaxel, development of metastases, and contributing to mortality. Since the androgen signaling has been closely

associated with EMT activation, one could argue that microtubule-targeting treatment (docetaxel) prior to androgen deprivation may result in improved therapeutic outcomes in patients with advanced prostate cancer.

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Disclosure of conflict of interest

None.

Abbreviations

TGF- β , Transforming Growth Factor Beta; EGF, Epidermal Growth Factor; ECM, Extracellular Matrix; EMT, Epithelial to Mesenchymal Transition; VEGF, Vascular Endothelial Growth Factor; DHT, dihydrotestosterone; TNF, Tumor Necrosis Factor; CRPC, Castration Resistant Prostate Cancer; AR, Androgen Receptor; PSA, Prostate Specific Antigen; TAM, Tumor Associated Macrophages; FAK, Focal Adhesion Kinase.

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References

- [1] Siegel R, Naishadham D and Jemal A. Cancer Statistics, 2012. *CA Cancer J Clin* 2012; 62: 10-29.
- [2] Gravdal K, Halvorsen OJ, Haukaas SA and Akslen LA. A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent importance for the progress of prostate cancer. *Clin Cancer Res* 2007; 13: 7003-7011.
- [3] Denmeade SR and Isaacs JT. A history of prostate cancer treatment. *Nat Rev Cancer* 2002; 2: 389-396.
- [4] Sella A, Kilbourn R, Amato R, Bui C, Zukowski AA, Ellerhorst J and Logothetis CJ. Phase II study of ketoconazole combined with weekly doxorubicin in patients with androgen-independent prostate cancer. *J Clin Oncol* 1994; 12: 683-688.
- [5] McLennan R, Dyson L, Prather KW, Morrison JA, Baker RE, Maini PK and Kulesa PM. Multi-scale mechanisms of cell migration during development: theory and experiment. *Development* 2012; 139: 2935-2944.
- [6] Deschesnes RG, Patenaude A, Rousseau JL, Fortin JS, Ricard C, Cote MF and Petitclerc E. Microtubule-destabilizing agents induce focal adhesion structure disorganization and anoikis in cancer cells. *J Pharmacol Exp Ther* 2007; 320: 853-864.
- [7] Ribatti D, Mangialardi G and Vacca A. Stephen Paget and the 'seed and soil' theory of metastatic dissemination. *Clin Exp Med* 2006; 6: 145-149.
- [8] Langley RR and Fidler IJ. The seed and soil hypothesis revisited—The role of tumor-stroma interactions in metastasis to different organs. *Int J Cancer* 2011; 128: 2527-2535.
- [9] Taichman RS, Cooper C, Keller ET, Pienta KJ, Taichman NS and McCauley LK. Use of the stromal cell derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. *Cancer Res* 2002; 62: 1832-1837.
- [10] Platz EA and DeMarzo AM. Epidemiology of inflammation and prostate cancer. *J Urol* 2004; 171: S36-40.
- [11] Liotta LA and Kohn EC. The microenvironment of the tumour-host interface. *Nature* 2001; 411: 375-379.
- [12] Lewis CE and Polalrd JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 2006; 66: 605-612.
- [13] Varga J, Rosenbloom J and Jimenez SA. Transforming growth factor beta (TGF beta) causes a persistent increase in steady state amounts of type I and type III collagen and fibronectin mRNAs in normal human dermal fibroblasts. *Biochem J* 1987; 247: 597-604.
- [14] Collazo J, Zhu B, Larkin S, Horbinski C, Pu H, Martin SK, Koochekpour S and Kyprianou N. Cofilin drives cell invasive and metastatic responses to TGF-b in Prostate Cancer. *Cancer Res* 2014; [Epub ahead of print].
- [15] Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shiptsin M, Campbell LL, Polyak K, Briskin C, Yang J and Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008; 133: 704-715.
- [16] Takeichi M. Cadherins in cancer: implications for invasion and metastasis. *Curr Opin Cell Biol* 1993; 5: 806-811.
- [17] Yilmaz M and Christofori G. EMT, the cytoskeleton, and cancer cell invasion. *Cancer Metastasis Rev* 2009; 28: 15-33.
- [18] Doherty GJ and McMahon HT. Mediation, modulation, and consequences of membrane-cytoskeleton interactions. *Annu Rev Biophys* 2008; 37: 65-95.
- [19] Carlier MF, Pantaloni D and Korn ED. Evidence for an ATP cap at the ends of actin filaments

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- and its regulation of the F-actin steady state. *J Biol Chem* 1984; 259: 9983-9986.
- [20] Lambrechts A, Van Troys M and Ampe C. The actin cytoskeleton in normal and pathological cell motility. *Int J Biochem Cell Biol* 2004; 36: 1890-1909.
- [21] Saarikangas J, Zhao H and Lappalainen P. Regulation of the actin cytoskeleton-plasma membrane interplay by phosphoinositides. *Physiol Rev* 2010; 90: 259-289.
- [22] Chan AY, Bailly M, Zebda N, Segall JE and Condeelis JS. Role of cofilin in epidermal growth factor-stimulated actin polymerization and lamellipodia protrusion. *J Cell Biol* 2000; 148: 531-542.
- [23] Shen B, Delaney MK and Du X. Inside-out, outside-in, and inside-outside-in: G proteins signaling in integrin-mediated cell adhesion, spreading, and retraction. *Curr Opin Cell Biol* 2012; 24: 600-606.
- [24] Vega FM, Fruhwirth G, Ng T and Ridley AJ. RhoA and RhoC have distinct roles in migration and invasion by acting through different targets. *J Cell Biol* 2011; 193: 655-665.
- [25] O'Connor K and Chen M. Dynamic functions of RhoA in tumor cell migration and invasion. *Small GTPases* 2013; 4: 141-147.
- [26] Watanabe Y, Sasahara Y, Ramesh N, Massaad MJ, Yeng Looi C, Kumaki S, Kure S, Geha RS and Tsuchiya S. T-cell receptor ligation causes Wiskott-Aldrich syndrome protein degradation and F-actin assembly downregulation. *J Allergy Clin Immunol* 2013; 132: 648-655.
- [27] Vartiainen MK and Machesky LM. The WASP-Arp2/3 pathway: genetic insights. *Curr Opin Cell Biol* 2004; 16: 174-181.
- [28] Condeelis JS, Singer RH and Segall JE. The great escape: when cancer cells hijack the genes for chemotaxis and motility. *Annu Rev Cell Dev Biol* 2005; 21: 695-718.
- [29] Gligorijevic B, Wyckoff J, Yamaguchi H, Wang Y, Roussos ET and Condeelis JS. N-WASP mediated invadopodium formation is involved in intravasation and lung metastasis of mammary tumors. *J Cell Sci* 2012; 125: 724-734.
- [30] Loberg RD, Neeley CK, Adam-Day LL, Fridman Y, St John LN, Nixdorf S, Jackson P, Kalikin LM and Pienta KJ. Differential expression analysis of MIM (MTSS1) splice variants and a functional role of MIM in prostate cancer cell biology. *Int J Oncol* 2005; 26: 1699-1705.
- [31] Moriyama K, Iida K and Yahara I. Phosphorylation of Ser-3 of cofilin regulates its essential function on actin. *Genes Cells* 1996; 1: 73-86.
- [32] McGough A, Pope B, Chiu W and Weeds A. Cofilin changes the twist of F-actin; implications for actin filament dynamics and cellular function. *J Cell Biol* 1997; 138: 771-781.
- [33] Svitkina TM and Borisy GG. Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia. *J Cell Biol* 1999; 145: 1009-1026.
- [34] Yonezawa N, Nishida E, Iida K, Yahara I and Sakai H. Inhibition of the interactions of cofilin, destrin, and deoxyribonuclease I with actin by phosphoinositides. *J Biol Chem* 1990; 265: 8382-8386.
- [35] DesMarais V, Ghosh M, Eddy R and Condeelis JS. Cofilin takes the lead. *J Cell Sci* 2005; 118: 19-26.
- [36] Yoshioka K, Foletta V, Bernard O and Itoh K. A role of LIM kinase in cancer invasion. *PNAS* 2003; 100: 7247-7252.
- [37] Zhu B, Fukada K, Zhu H and Kyprianou N. Prohibitin and cofilin are intracellular effectors of transforming growth factor beta signaling in human prostate cancer cells. *Cancer Res* 2006; 66: 8640-8647.
- [38] Collazo J and Kyprianou N. TGF-Beta signaling dictates apoptotic and angiogenic response during prostate cancer progression. In: editors. *Angiogenesis and Therapeutic Targets in Cancer*. 2010. pp: 209-218.
- [39] Hannon GJ and Beach D. p15^{INK4B} is a potential effector of TGF-beta induced cell cycle arrest. *Nature* 1994; 371: 257-261.
- [40] Oft M, Heider KH and Beug H. TGF beta signaling is necessary for carcinoma cell invasiveness and metastasis. *Curr Biol* 1998; 8: 1243-1252.
- [41] Critchley DR. Cytoskeletal proteins talin and vinculin in integrin-mediated adhesion. *Biochem Soc Trans* 2004; 32: 831-836.
- [42] Isenberg G and Goldmann WH. Peptide-specific antibodies localize the major lipid binding sites of talin dimers to oppositely arranged N-terminal 47 kDa subdomains. *FEBS Lett* 1998; 426: 165-170.
- [43] Desinoitis A and Kyprianou N. Significance of talin in cancer progression and metastasis. *Int Rev Cell Mol Biol* 2011; 289: 117-149.
- [44] Sakamoto S, McCann RO, Dhir R and Kyprianou N. Talin1 promotes tumor invasion and anoikis resistance. *Cancer Res* 2010; 70: 1885-1895.
- [45] Mebratu Y and Tesfayiz Y. How ERK1/2 activation controls cell proliferation and cell death: Is subcellular localization the answer? *Cell Cycle* 2009; 8: 1168-1175.
- [46] Downing KH and Nogales E. Tubulin and microtubule structure. *Curr Opin Cell Biol* 1998; 10: 16-22.
- [47] Howard J and Hyman AA. Dynamics and mechanics of the microtubule plus end. *Nature* 2003; 422: 753-758.
- [48] Yildiz A, Tomishige M, Vale RD and Selvin PR. Kinesin walks hand over hand. *Science* 2004; 303: 676-678.

Cytoskeleton targeting drugs for the treatment of prostate cancer

- [49] Hua W, Chung J and Gelles J. Distinguishing inchworm and hand over hand processive kinesin movement by neck rotation measurements. *Science* 2002; 295: 844-848.
- [50] Van Der Vaart B, Akhmanova A and Straube A. Regulation of microtubule dynamic instability. *Biochem Soc Trans* 2009; 37: 1007-1013.
- [51] Newton CN, Wagenbach M, Ovechkina Y, Wordeman L and Wilson L. MCAK, a Kin I kinesin, increases the catastrophe frequency of steady-state HeLa cell microtubules in an ATP-dependent manner in vitro. *FEBS Lett* 2004; 572: 80-84.
- [52] Homma N, Takei Y, Tanaka Y, Nakata T, Terada S, Kikkawa M, Noda Y and Hirokawa N. Kinesin superfamily protein 2A (KIF2A) functions in suppressions of collateral branch extension. *Cell* 2003; 114: 229-239.
- [53] Mayr MI, Hummer S, Bormann J, Gruner T, Adio S, Woehlke G and Mayer TU. The human kinesin Kif18A is a motile microtubule depolymerase essential for chromosome congression. *Curr Biol* 2007; 17: 488-498.
- [54] Burkhardt JK, Echeverri CJ, Nilsson T and Vallee RB. Overexpression of the dynamin (p50) subunit of the dynactin complex disrupts dynein-dependent maintenance of membrane organelle distribution. *J Cell Biol* 1997; 139: 469-484.
- [55] Drechsel DN, Hyman AA, Cobb MH and Kirschner MW. Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. *Mol Biol Cell* 1992; 3: 1141.
- [56] Kowalski RJ and Williams RC. Microtubule associated protein 2 alters the dynamic properties of microtubule assembly and disassembly. *J Biol Chem* 1993; 268: 9847-9855.
- [57] Dehmelt L and Halpain S. The MAP2/Tau family of microtubule-associated proteins. *Genome Biol* 2005; 6: 204.
- [58] Pihan GA, Purohit A, Wallace J, Malhotra R, Liotta LA and Doxsey SJ. Centrosome defects can account for cellular and genetic changes that characterize prostate cancer progression. *Cancer Res* 2001; 61: 2212-2219.
- [59] Hammond JW, Cai D and Verhey KJ. Tubulin modifications and their cellular functions. *Curr Opin Cell Biol* 2008; 20: 71-76.
- [60] Darshan MS, Loftus MS, Thadani-Mulero M, Levy BP, Escuin D, Zhou XK, Gyrezi A, Chanel-Vos C, Shen R, Tagawa ST, Bander NH, Nanus DM and Giannakakou P. Taxane-induced blockade to nuclear accumulation of the androgen receptor predicts clinical responses in metastatic prostate cancer. *Cancer Res* 2011; 71: 6019-6029.
- [61] Zhu ML, Horbinski C, Garzotto M, Qian DZ, Beer TM and Kyprianou N. Tubulin targeting chemotherapy impairs androgen receptor activity in prostate cancer. *Cancer Res* 2010; 70: 7992-8002.
- [62] Soucek K, Kamaid A, Phung AD, Kubala L, Bulinski JC, Harper RW and Eiserich JP. Normal and prostate cancer cells display distinct molecular profiles of alpha-tubulin posttranslational modifications. *Prostate* 2006; 66: 954-965.
- [63] Feldmann BJ and Feldmann D. The development of androgen-independent prostate cancer. *Nat Rev Cancer* 2001; 1: 34-45.
- [64] Martin SK, Fiandalo MV and Kyprianou N. Androgen receptor signaling interactions control epithelial-mesenchymal transition (EMT) in prostate cancer progression. In: editors. *Androgen-responsive genes in prostate cancer*. New York: Springer; 2013. pp: 227-255.
- [65] Koivisto P, Kononen J, Palmberg C, Tammela T, Hyytinen E, Isola J and Kallioniemi OP. Androgen receptor gene amplification: a possible mechanism for androgen deprivation therapy failure in prostate cancer. *Cancer Res* 1997; 57: 314-319.
- [66] Craft N, Shostak Y, Carey M and Sawyers CL. A mechanism for hormone-independent prostate cancer through modulation of androgen receptor signaling by the Her-2/neu tyrosine kinase. *Nat Med* 1999; 5: 280-285.
- [67] Veldscholte J, Berrevoets CA, Ris-Staplers C, Kuiper GGJM, Jenster G, Trapman J, Brinkmann AO and Mulder E. The androgen receptor in LNCaP cells contains a mutation in the ligand binding domain which affects steroid binding characteristics and response to anti-androgens. *J Steroid Biochem Mol Biol* 1992; 41: 665-669.
- [68] McDonnell TJ, Troncoso P, Brisbay SM, Logothetis CJ, Chung LW, Hsieh JT, Tu SM and Campbell ML. Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. *Cancer Res* 1992; 52: 6940-6944.
- [69] Isaacs JT. The biology of hormone refractory prostate cancer: why does it develop? *Urol Clin North Am* 1999; 26: 263-273.
- [70] Dehm SM, Schmidt LI, Heemers HV, Vessella RL and Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer cells. *Cancer Res* 2008; 68: 5469-5477.
- [71] Guo Z, Yang X, Sun F, Jiang R, Linn DE, Chen H, Chen H, Kong X, Melamed J, Tepper CG, Kung H-J, Brodie AMH, Edwards J and Qiu Y. A novel androgen receptor splice variant is upregulated during prostate cancer progression and promotes androgen depletion-resistant growth. *Cancer Res* 2009; 69: 2305-2313.
- [72] Hu R, Dunn TAW, Isharwal S, Veltri RW, Humphreys E, Han M, Partin AW, Vessella RL, Isa-

Cytoskeleton targeting drugs for the treatment of prostate cancer

- cs WB, Bova GS and Luo J. Ligand independent androgen receptor variants derived from splicing of cryptic exons signify hormone refractory prostate cancer. *Cancer Res* 2009; 69: 16-22.
- [73] Hu R, Isaacs WB and Luo J. A snapshot of the expression signature of androgen receptor splicing variants and their distinctive transcriptional activities. *Prostate* 2010; 71: 1656-1667.
- [74] Sun S, Sprenger CCT, Vessella RL, Haugk K, Soriano K, Mostaghel EA, Page ST, Coleman IM, Nguyen HM, Sun H, Nelson PS and Plymate SR. Castration resistance in human prostate cancer is conferred by a frequently occurring androgen receptor splice variant. *J Clin Invest* 2010; 120: 2715-2730.
- [75] Franco OE, Jiang M, Strand DW, Peacock J, Fernandez S, Jackson RS II, Revelo MP, Bhowmick NA, Hayward SW. "Altered TGF-Beta Signaling in a subpopulation of human stromal cells promotes prostatic carcinogenesis". *Cancer Res* 2011; 71: 1272-81.
- [76] Wang Z, Li Y, Kong D and Sarkar FH. The role of Notch signaling pathway in epithelial-mesenchymal transition (EMT) during development and tumor aggressiveness. *Curr Drug Targets* 2010; 11: 745.
- [77] Zhu ML and Kyprianou N. Role of androgens and the androgen receptor in the epithelial-mesenchymal transition and invasion of prostate cancer cells. *FASEB J* 2010; 24: 769-777.
- [78] Jordan MA and Wilson L. Microtubules as a target for anticancer drugs. *Nat Rev Cancer* 2004; 4: 253-265.
- [79] Paller CJ and Antonarakis ES. Cabazitaxel: a novel second line treatment for metastatic castration-resistant prostate cancer. *Drug design, Drug Des Devel Ther* 2011; 5: 117.
- [80] Pellegrini F and Budman DR. Review: Tubulin Function, Action of Antitubulin Drugs, and New Drug Development. *Cancer Invest* 2005; 23: 264-273.
- [81] Woods CM, Zhu J, McQueney PA, Bollag D and Lazarides E. Taxol induced mitotic block triggers rapid onset of a p53 independent apoptotic pathway. *Mol Med* 1995; 1: 506.
- [82] Schiff PB, Fant J and Horowitz SB. Promotion of microtubule assembly in vitro by taxol. *Nature* 1979; 277: 665-667.
- [83] Goodin S, Kane MP and Rubin EH. Epothilones mechanism of action and biologic activity. *J Clin Oncol* 2004; 22: 2015-2025.
- [84] Garrison JB, Shaw YJ, Chen CS and Kyprianou N. Novel quinazoline-based compounds impair prostate tumorigenesis by targeting tumor vascularity. *Cancer Res* 2007; 67: 11344-11352.
- [85] Garrison JB and Kyprianou N. Doxazosin induces apoptosis of benign and malignant prostate cells via death receptor mediated pathway. *Cancer Res* 2006; 66: 464-472.
- [86] Hensley PJ, Desinoitis A, Wang C, Stromberg A, Chen CS and Kyprianou N. Novel pharmacologic targeting of tight junctions and focal adhesions in prostate cancer cells. *PLoS One* 2014; 9: e86238.