New Paradigms in Microtubule-Mediated Endocrine Signaling in Prostate Cancer

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Abstract

Metastatic prostate cancer has limited therapeutic options and has remained a major clinical challenge. Historically, prostate cancer has been widely recognized as a chemotherapy-resistant disease. However, clinical studies with anti-microtubule agents over the past decade have shown important efficacy in improving survival in patients with advanced disease. The favorable outcomes with microtubule-targeted agents have thus rekindled interest in such therapies for the clinical management of prostate cancer. Microtubules are dynamic polymers of tubulin molecules that play diverse roles within the cell. The dynamic property of microtubules is responsible for forming the bipolar mitotic apparatus, the mitotic spindle, that functions to precisely segregate the chromosomes during cell division. Thus, owing to the pivotal role that they play in the orchestration of mitotic events, microtubules provide excellent targets for anti-cancer therapy. Recent evidence also suggests that microtubules play a crucial role in the regulation of endocrine signaling pathways. Interestingly, microtubule-targeted agents such as taxanes not only inhibit cell division but also impair endocrine receptor signaling in prostate cancer. Herein, we provide an overview of the current status of microtubule-targeted therapies that are used in the treatment of prostate cancer and discuss novel mechanisms by which such therapies modulate endocrine signaling in prostate cancer. We also address the emerging roles of microtubule regulatory proteins in prostate carcinogenesis that could serve as attractive targets for prostate cancer therapy and might also serve as predictive biomarkers to identify patients who may benefit from endocrine and/or chemotherapy. This may have important implications in designing mechanism-based and targeted-therapeutic strategies for prostate cancer. Mol Cancer Ther; 12(5); 555–66. ©2013 AACR.

Introduction

Prostate cancer is the most frequently diagnosed malignancy and the second leading cause of cancer mortality in men in Western countries. As prostate cancer growth is driven by circulating androgens (mainly testosterone), the mainstay of first-line treatments for metastatic prostate cancer is suppression of gonadal androgens. Androgen deprivation therapy, either by medical or surgical interventions, is initially effective at blocking tumor growth. However, these therapies eventually fail, leading to a drug-resistant androgen-independent stage, which is invariably fatal. Unfortunately, androgen deprivation at best can only achieve a "hormone-reduced" rather than a "hormone-free" state. It is believed that residual levels of intratumoral androgens following androgen deprivation therapy are sufficient to activate the androgen receptor (AR) and drive prostate tumor growth. This trend of prostate cancer progression has led to the recent renaming of this stage of the disease as "castration-resistant" instead of "hormone-refractory" disease. Although androgen deprivation by castration produces dramatic and rapid declines in prostate-specific antigen (PSA), bone pain, and urinary tract obstruction, limited options are available for patients whose disease progresses despite a castrate testosterone level. Bone metastasis occurs in a majority of patients with advanced disease and such patients with bone lesions cannot be cured. Thus, the clinical management of patients with castration-resistant prostate cancer (CRPC) has remained a major challenge.

Microtubules, which are one of the integral components of the cytoskeleton, have proven to be an effective therapeutic target for CRPCs. They are intrinsically dynamic protein polymers made up of alternating subunits of α- and β-tubulin. The formation of microtubules involves a highly dynamic process of polymerization and depolymerization of α- and β–tubulin heterodimers. This dynamic property is crucial for the assembly of the mitotic spindle and precise segregation of the chromosomes during cell division. Disruption of microtubule dynamics, either by inhibiting polymerization or by preventing depolymerization of tubulin, interferes with the regulation of the mitotic spindle that results in cell-
cycle arrest and eventually cell death (5, 6). Thus, owing to their indispensability in mitosis and cell division, microtubules provide excellent targets for anti-cancer therapy. In addition to their role in spindle regulation, microtubules also play an important role in endocrine signaling pathways. In this review, we describe the current status of microtubule-targeted therapies that are used in the treatment of prostate cancer and discuss novel mechanisms by which such therapies modulate endocrine signaling in prostate cancer. We also address the emerging roles of microtubule regulatory proteins in prostate carcinogenesis that might serve as attractive targets for prostate cancer therapy and might also serve as prognostic and predictive tools to identify patients who would benefit from endocrine and/or chemotherapy. This may have important implications in designing mechanism-based and targeted-therapeutic strategies for prostate cancer.

**Microtubule-targeted chemotherapeutics in prostate cancer**

Chemotherapy was once thought to play a clinically insignificant role in metastatic CRPCs. Although a variety of cytotoxic agents have been investigated for the treatment of CRPCs, most agents showed only marginal efficacy and did not extend survival. One of the reasons why prostate cancer cells may be so difficult to eradicate is that most chemotherapeutic agents are thought to be active in proliferating cells with a short doubling time. The classical view of the clinical success of chemotherapy is generally based on the notion that mitotic microtubules are highly dynamic and their rapid dynamics makes the cells more susceptible to cytotoxic agents leading to mitotic arrest in actively dividing cancer cells, eventually leading to apoptosis. In contrast, prostate cancer may have relatively long doubling time with only a small fraction of the tumor cells actively proliferating (7). Despite the slow growth kinetics of prostate cancer cells, microtubule-targeted agents (MTA) have shown considerable value in the treatment of CRPCs. Recent advances in our understanding of the biology of the microtubule machinery have led to a paradigm shift in the way we think how MTAs kill cancer cells. Although microtubules that make up the mitotic spindle have remained one of the key targets of MTAs in rapidly dividing cells, microtubules are as important in nondividing cells as they are in the mitotic spindle of dividing cells. Thus, it is not surprising that MTAs are effective against slower growing prostate tumors, where they might also interfere with interphase microtubule functions such as intracellular transport and signaling. This idea is supported by recent studies showing the involvement of the dynamic interphase microtubules in the regulation of transcription factors such as p53 and hypoxia-inducible factor (HIF)-1α (8, 9), implying a role for microtubules in transcription factor trafficking.

**Taxanes in prostate cancer**

Taxanes are a powerful class of chemotherapeutic agents that have shown significant clinical activity in a wide range of solid tumor malignancies including metastatic CRPCs. Taxanes function primarily by interfering with the dynamics of the mitotic spindle causing cell-cycle arrest and apoptosis. They are routinely used in many cancers in the neoadjuvant, adjuvant, and metastatic setting alone and in combination with drugs with different mechanism(s) of action and nonoverlapping toxicity profiles. In this section, we will discuss some of the taxane-based drugs that have helped to improve the outlook of patients with CRPCs.

**Docetaxel.** Docetaxel chemotherapy represented a key breakthrough in the treatment of CRPCs based on a series of clinical trials (10–12). Results of 2 pivotal phase III trials from independent teams formed the basis for docetaxel-based therapy as the first-line chemotherapy against CRPCs. Both trials randomized docetaxel versus mitoxantrone, an agent that was shown to improve quality of life but failed to show any survival benefit. First, in the TAX 327 trial, a total of 1,006 men were randomized to receive either docetaxel or mitoxantrone along with oral prednisone in both arms (12, 13). Treatment with docetaxel on a 21-day cycle was associated with an improvement in median overall survival (19.2 vs. 16.3 months, \( P = 0.004 \); refs. 12, 13). Second, Southwest Oncology Group (SWOG) 9916 trial randomized 674 patients to receive either docetaxel and estramustine or mitoxantrone and prednisone (11). As in TAX 327, the SWOG 9916 trial also showed a survival advantage associated with docetaxel therapy (17.5 vs. 15.6 months, \( P = 0.02 \); ref. 11). These studies led to the U.S. Food and Drug Administration (FDA) approval of docetaxel in 2004 and laid the foundation for additional studies of docetaxel for high-risk disease and novel docetaxel-based combinations for CRPCs. More recently, a phase I/IIa study was designed to examine the safety and efficacy of docetaxel in combination with radium-223 chloride for patients with CRPCs with bone metastases and this trial is ongoing (14). Table 1 summarizes some of the significant positive phase III taxane-based clinical trials in CRPCs that are already approved and those under investigation.

**Cabazitaxel.** Men with docetaxel resistance had, until recently, few options for treatment. However, in the past 2 years, there has been progress made in the treatment of this disease with the FDA approval of at least 3 drugs (sipuleucel-T, abiraterone, and cabazitaxel) with different mechanism of actions. Among these 3 drugs, cabazitaxel (Jevtana) is a novel taxoid compound that is a potent microtubule stabilizer like docetaxel and is an important mechanism(s) of action and nonoverlapping toxicity profiles. In the TAX 327 trial, a total of 1,006 men were randomized to receive either docetaxel or mitoxantrone along with oral prednisone in both arms (12, 13). Treatment with docetaxel on a 21-day cycle was associated with an improvement in median overall survival (19.2 vs. 16.3 months, \( P = 0.004 \); refs. 12, 13). Second, Southwest Oncology Group (SWOG) 9916 trial randomized 674 patients to receive either docetaxel and estramustine or mitoxantrone and prednisone (11). As in TAX 327, the SWOG 9916 trial also showed a survival advantage associated with docetaxel therapy (17.5 vs. 15.6 months, \( P = 0.02 \); ref. 11). These studies led to the U.S. Food and Drug Administration (FDA) approval of docetaxel in 2004 and laid the foundation for additional studies of docetaxel for high-risk disease and novel docetaxel-based combinations for CRPCs. More recently, a phase I/IIa study was designed to examine the safety and efficacy of docetaxel in combination with radium-223 chloride for patients with CRPCs with bone metastases and this trial is ongoing (14). Table 1 summarizes some of the significant positive phase III taxane-based clinical trials in CRPCs that are already approved and those under investigation.

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However, the data on the effectiveness of cabazitaxel due to lack of P-gp have only been reported in preclinical models in prostate cancer cell lines. The correlation between P-gp expression and docetaxel resistance remains to be investigated clinically in patients with prostate cancer. Thus, the poor affinity of cabazitaxel for P-gp may or may not be its principal mechanism of action for its enhanced efficacy. Second, the addition of the extra methyl group on cabazitaxel is also thought to enhance its ability to cross the blood–brain barrier, a feature that is uncommon among chemotherapeutic agents. Interestingly, cabazitaxel was shown to be active in both docetaxel-sensitive tumors and those that failed to respond to chemotherapy and was superior to paclitaxel and docetaxel in penetration of the blood–brain barrier in phase I clinical trials (17).

With these observations, cabazitaxel progressed from phase I trial directly to a randomized open-label phase III TROPIC trial in which 755 patients with docetaxel-treated metastatic CRPCs were treated with prednisone and mitoxantrone or cabazitaxel/prednisone (18). The median overall survival was 15.1 months in the cabazitaxel group and 12.7 months in the mitoxantrone group, a 30% reduction in the risk of death (HR, 0.70; P < 0.001; Table 1). Thus, cabazitaxel was the first chemotherapeutic agent to show survival benefit in men with docetaxel-pretreated disease. On this basis, cabazitaxel received FDA regulatory approval in June 2010 for the treatment of patients with metastatic CRPCs whose disease progresses during or after docetaxel treatment. Although mitoxantrone was previously used for the treatment of docetaxel-refractory disease due to its palliative effects, cabazitaxel now offers a new option for patients with CRPCs whose disease progresses during or after docetaxel treatment and has replaced mitoxantrone as the chemotherapeutic treatment of choice after docetaxel treatment. Further assessment of drug safety and quality-of-life benefits imparted by cabazitaxel in combination with different agents is ongoing (19).

The clinical success of the taxanes had led to a search for other drugs that enhance microtubule polymerization. Epothilones (ixabepilone and patupilone) are a new class of microtubule stabilizers that have been investigated in phase II clinical trials in patients with CRPCs and seem to have promising antitumor effects but significant toxicity as well (20, 21). A randomized phase II trial evaluated ixabepilone monotherapy or ixabepilone in combination with estramustine phosphate (EMP) in patients with progressive castrate metastatic prostate cancer (20). PSA decline of at least 50% was noted more frequently in the combination group than the monotherapy group (69% vs. 48%). The rate of tumor regression was also higher in the combination group than the monotherapy group (48% vs. 32%). Despite the encouraging clinical activity, the most common toxic effects in both the groups included significant grade III and IV neutropenia and peripheral neuropathy (20, 21). Future use of epothilones in the treatment of CRPCs will need optimization of the dosing schedule to limit toxicities. Further studies will also be needed to

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### Table 1. Summary of significant positive phase III clinical trials using taxane-based therapies or endocrine therapies in CRPCs that are approved and those under investigation

<table>
<thead>
<tr>
<th>Clinical trial</th>
<th>Treatment arm 1</th>
<th>Treatment arm 2</th>
<th>Prior treatment</th>
<th>HR</th>
<th>Median OS</th>
<th>References</th>
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<tr>
<td>TAX 277</td>
<td>Docetaxel</td>
<td>Mitoxantrone</td>
<td>Chemotherapy-naive</td>
<td>0.78</td>
<td>19.2 vs. 16.3 months</td>
<td>12, 13</td>
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<tr>
<td>SWOG 8916</td>
<td>Docetaxel</td>
<td>Mitoxantrone</td>
<td>Chemotherapy-naive</td>
<td>0.80</td>
<td>17.5 vs. 15.6 months</td>
<td>11</td>
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<tr>
<td>TROPIC</td>
<td>Docetaxel</td>
<td>Mitoxantrone</td>
<td>Chemotherapy-naive</td>
<td>0.70</td>
<td>15.1 vs. 12.7 months</td>
<td>18</td>
</tr>
<tr>
<td>COU-301</td>
<td>Cabazitaxel</td>
<td>Placebo</td>
<td>Prednisone</td>
<td>0.65</td>
<td>14.8 vs. 10.9 months</td>
<td>37</td>
</tr>
<tr>
<td>AFFIRM</td>
<td>Docetaxel</td>
<td>Placebo</td>
<td>Prednisone</td>
<td>0.63</td>
<td>18.4 vs. 13.6 months</td>
<td>39</td>
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</tbody>
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Abbreviations: RDR, reduction in death rate; HR, hazard ratio.
compare these agents head-to-head with cabazitaxel in a post-docetaxel setting.

Effects of MTAs on androgen receptor signaling in prostate cancer

The androgens and the androgen receptors play a critically important role in prostate cancer development and progression (22). Classically, the androgens exert their biologic effects by binding to the androgen receptor, which is a member of the nuclear receptor superfamily that acts as a ligand-dependent transcription factor (22). The binding of androgen to its receptor causes a conformational change in the receptor, followed by dimerization and nuclear translocation of the hormone–receptor complex. Once translocated, the ligand–receptor complex binds to specific androgen response elements (ARE) upstream of the target genes along with coactivator proteins, leading to transcription activation of androgen receptor target genes (22). PSA, a clinical marker routinely used for prostate cancer, is one of the key targets that is directly regulated by the androgen receptor (23). A decline in serum PSA levels is often associated with tumor regression following androgen deprivation therapy. Despite castrate levels of testosterone, nearly all advanced prostate cancers develop evidence of recurrence characterized by an increase in PSA and radiographic progression. This suggests that tumor progression is associated with inappropriately restored androgen receptor function despite sustained androgen ablation (3, 22). The reactivation of androgen receptor in recurrent tumors is hypothesized to occur through multiple mechanisms including androgen receptor amplification, active androgen receptor signaling despite low levels of androgen, androgen receptor mutation, and androgen receptor splice variants (3, 22, 24, 25).

More recently, castration resistance in human prostate cancer was shown to be conferred by frequently occurring alternatively spliced androgen receptor variants such as ARv567es, AR3, and ARv7, which lack the ligand-binding domain (24, 25). However, these variants confer ligand-independent androgen receptor transactivation and produce a constitutively active androgen receptor following castration that plays an important role in prostate cancer progression (24, 25). ARv567es was the most common of the 3 variants identified in 59% of patients with CRPCs in response to androgen deprivation therapy and to abiraterone (25). Thus, owing to the crucial role of androgen receptors in prostate cancer, inhibition of androgen receptor activity is a major therapeutic goal in the management of CRPCs. Recent developments of novel agents targeting the androgen receptor signaling pathway have shown significant clinical activity in patients with CRPCs (26), thus corroborating the importance of androgen receptors as a therapeutic target in CRPCs.

Interestingly, several lines of evidence suggest that taxane-based chemotherapy can inhibit androgen receptor signaling in prostate cancer via its effects on microtubules. Figure 1 is a schematic illustration of some of the emerging mechanistic scenarios of the effects of taxanes on microtubules and androgen receptor signaling in prostate cancer. Kuroda and colleagues recently showed downregulation of androgen receptor and PSA expression in different prostate cancer cell lines following exposure to docetaxel in vitro (27). Conversely, overexpression of androgen receptor in prostate cancer cells resulted in partial abrogation of cytotoxic effects of docetaxel (27). Thus, docetaxel mediates inhibitory effects on the expression of androgen receptors and PSA (Fig. 1). This suggests that the PSA responses observed with docetaxel treatment may represent a combination of cytotoxicity (decrease in tumor cell mass) via its effects on microtubule assembly as well as possible effects on PSA metabolism. Another study by Gan and colleagues showed that taxanes (paclitaxel and docetaxel) can inhibit androgen receptor activity in PTEN-positive CRPC cells by inducing nuclear accumulation of the FOXO1 protein, which is a known androgen receptor–suppressive nuclear factor (Fig. 1). FOXO1 accumulation was shown to increase the interaction between FOXO1 and the androgen receptors in the nucleus (28). Thus, the observed FOXO1-mediated androgen receptor inhibitory effect of paclitaxel in CRPC cells may play an important role in taxane-mediated inhibition of CRPC growth. Moreover, as the tumor suppressor gene, PTEN, which is also an important upstream regulator of FOXO1 is frequently mutated or deleted in advanced CRPCs (29), it was proposed that deregulation of the PTEN/FOXO1 pathway may lead to the development of taxane resistance in prostate cancer. Furthermore, immunohistochemical analysis using tissue microarrays (TMA) of human prostate specimens from docetaxel-treated patients with prostate cancer showed a significant depletion of nuclear androgen receptors, paralleled by an increase in the cytoplasmic androgen receptors and a decrease in PSA immunoreactivity in the individual tissue arrays in response to MTAs (30). Taken together, these studies suggest that chemotherapy with docetaxel not only inhibits cell division but also impairs androgen receptor signaling by interfering with androgen receptor translocation.

More recent studies investigated the contribution of the microtubules on androgen-mediated signaling and the subsequent inhibition of androgen receptor activity following taxane treatment (30–32). Prostate cancer cells treated with paclitaxel followed by the addition of the dihydrotestosterone analogue R1881 had shown significant microtubule bundling associated with a concomitant decrease in androgen receptor nuclear accumulation (31). The profound cytoplasmic sequestration of androgen receptor following paclitaxel treatment suggests a role for microtubules in the shuttling of the receptor from the cytoplasm to the nucleus (31). Furthermore, cells with acquired tubulin mutations, which are known to prevent taxane-induced microtubule stabilization, failed to translocate androgen receptors to the nucleus as expected, confirming a role for microtubules in androgen receptor trafficking (31). More interestingly, the same group also assessed whether taxanes could interfere with nuclear...
translocation of the androgen receptors in human specimens in vivo. Isolation of circulating tumor cells (CTC) from the blood of patients with CRPCs can enable molecular characterization of disease progression without the need of invasive biopsy. Interestingly, perturbation of the microtubule–androgen receptor axis in CTCs isolated from patients with CRPCs receiving taxane therapy (paclitaxel or docetaxel) showed a close correlation with clinical response. For instance, CTCs isolated from a patient who was refractory to paclitaxel treatment exhibited normal organization of the microtubule cytoskeleton with androgen receptors present in both the nucleus and the cytoplasm (31). In contrast, patients who responded well to docetaxel chemotherapy showed bundled microtubules and the androgen receptors was exclusively in the cytoplasm with intense androgen receptor perinuclear staining with a concomitant decline in PSA following chemotherapy (31). Further analysis of 18 additional samples obtained during clinical progression from patients who had more than 25% increase in PSA, 13 (72%) showed nuclear androgen receptor localization whereas among the 17 responders (those having at least a 30% reduction in PSA) or with stable disease, 12 (70.6%) showed cytoplasmic androgen receptor localization (31). These observations show a significant correlation between androgen receptor sequestration and clinical response to taxane chemotherapy (Fig. 1). This suggests that the activity of taxanes in CRPCs is mediated at least in part by inhibiting androgen receptor nuclear transport and signaling via microtubules and that monitoring androgen receptor subcellular localization in the CTCs of patients with CRPC might aid in the prediction of clinical response to taxane chemotherapy and possibly combination therapy as well.

On the basis of the evidence that prostate cancer progression remains dependent on androgen receptor activation, with tumor cells generating their own hormones de novo (26), novel hormonal agents such as abiraterone acetate (AA) and enzalutamide (formerly called MDV3100) have recently been developed. Abiraterone acetate is an oral inhibitor of CYP17 that reduces androgen production by inhibiting the steroid synthesis pathway in the testes, adrenals, and in the tumor itself (33). In contrast to abiraterone acetate that abrogates testosterone, enzalutamide is a novel androgen receptor antagonist that prevents translocation of the androgen receptor and inhibits DNA-binding and androgen-regulated expression (34). As taxanes impact androgen receptor signaling, it is important to determine whether taxanes are as active in patients with CRPCs following treatment with abiraterone acetate or enzalutamide or whether these agents would negatively impact taxane benefit. Following promising phase 1 and II clinical trials (35, 36), abiraterone acetate was shown to significantly improve overall
survival (OS) in men with metastatic CRPCs in the post-docetaxel setting in phase III COU-AA-301 trial and COU-AA-302 trial in chemonaive patients (37). The median OS was 14.8 months in the abiraterone acetate arm versus 10.9 months in the placebo arm with a 35% reduction in the risk for death (HR, 0.65; P < 0.001) in the COU-AA-301 trial (Table 1). A more recent study evaluated the response to abiraterone acetate in the postchemotherapy setting in patients with CRPCs whose disease progressed early on docetaxel (38). This study suggests that patients who are refractory to docetaxel do not respond to abiraterone acetate. In addition, patients who were refractory to abiraterone acetate did not respond to docetaxel either, raising the potential for cross-resistance between these 2 agents (38). More recently, 2 large randomized phase III trials were initiated to evaluate the efficacy of enzalutamide in patients with CRPCs in the pre-docetaxel (PREVAIL trial) and post-docetaxel (AFFIRM trial) settings. The AFFIRM trial reported a prolongation in OS in patients with CRPCs who progressed following docetaxel-based chemotherapy (39). The estimated median OS was 18.4 months for enzalutamide-treated patients compared with 13.6 months for placebo-treated men reducing the risk of death by 37% (HR, 0.631; P < 0.001; Table 1). However, comparative data on the efficacy of enzalutamide in a post-docetaxel versus pre-docetaxel setting has not been reported as yet. Enzalutamide was recently approved by the FDA and is marketed as Xtandi for the treatment of metastatic CRPCs.

**Effects of estrogen therapy on microtubules in prostate cancer**

While the role of androgens in the regulation of normal and malignant growth of the prostate has been well-established, increasing evidence suggests that estrogens also significantly contribute to normal prostatic functions as well as to the genesis of prostate cancer. Paradoxically, however, estrogen therapy has previously been a mainstay treatment for advanced prostate cancer (40). The therapeutic effect of estrogen is primarily mediated by suppression of the hypothalamic–pituitary–gonadal axis and by direct effects on the Leydig cells in testis, which together lead to decreased serum levels of testosterone and produces castration-like effects (41). Several decades ago, diethylstilbestrol, a synthetic estrogen, was used as the first standard therapy for prostate cancer treatment (41). Subsequently, diethylstilbestrol diphasate (DESdP), a nontoxic pro-drug form of diethylstilbestrol was also shown to have palliative effects in advanced patients with prostate cancer (42). Administration of estrogenic analogues, diethylstilbestrol and DESdP, showed reproducibly lower levels of serum testosterone to the concentration range observed in castrated patients (41, 42). In addition to their anti-androgenic effects, estrogens also exert direct growth-inhibitory effects by disrupting microtubule formation. Diethylstilbestrol was shown to have concentration-dependent effects on tubulin polymerization, which was associated with the induction of mitotic arrest and subsequent apoptosis in androgen-independent prostate cancer cell lines in vitro (43, 44). Although estrogen therapy was effective for advanced prostate cancer, unfortunately, it was associated with high risk of serious cardiovascular complications, which had strongly limited its clinical use, at least in the United States (40, 45). Since then, several new estrogenic compounds have been evaluated for their potential in the next generation of prostate cancer therapies.

The therapeutic benefits of estrogen therapy were apparent in combination studies with estramustine. Estramustine is a structural analogue of 17β-estradiol, with unusual pharmacologic properties that are distinct from its estrogenic activities. In addition to its hormonal action, estramustine also acts as a microtubule disruptor that can mediate cytotoxic effects through microtubule disassembly and antimitotic activity (46). Clinical trials with the microtubule inhibitors, estramustine and vinblastine, were shown to induce antitumor response in patients with CRPCs (47). In addition, 2 randomized trial suggested that estramustine increases the efficacy of docetaxel (48, 49). Results of a multicenter randomized phase II trial of docetaxel (arm A) or docetaxel plus oral EMP (arm B) showed more than 50% PSA decline in 40% of the patients with CRPCs in arm A and in 75% of those in arm B (48). The median time to PSA progression was significantly increased to 30 weeks in arm B compared with 20 weeks in arm A (48). Subsequent studies of the impact of the combination of docetaxel-based chemotherapy with EMP on the quality of life showed significant decrease in pain with fewer symptoms in patients with CRPCs who were more likely to achieve a biochemical response (49, 50). Overall, however, the side effects of estramustine continue to limit its routine use in the United States.

2-Methoxyestradiol (2-ME) is another natural metabolite of 17β-estradiol hormone that interferes with tubulin polymerization and has been shown to exert anti-tumor and anti-angiogenic effects in prostate cancer cells lines in vitro and in xenograft models in vivo (51, 52). In the last decade, 2-ME had gained increased attention due to its marked antitumor properties and possible cardiovascular benefits, particularly in patients with taxane-refractory, metastatic CRPCs (53). However, in a recent phase II single-arm open-label study, 2-ME did not show improved clinical outcomes in men with taxane-refractory CRPCs (54). Although 2-ME did not appear to have significant clinical activity as a monotherapy, there is still considerable interest in the clinical evaluation of novel anti-angiogenic agents for prostate cancer. It may be critically important to stratify patients who may best benefit from such agents. Further studies are needed to evaluate the efficacy of 2-ME in combination with conventional MTAs. This may provide patients with more effective options for chemotherapy in the treatment of advanced metastatic disease.
Emerging roles of microtubule regulatory proteins in prostate cancer

Microtubules are regulated by a variety of cellular proteins that modulate their functions and dynamic properties. The intracellular dynamic behavior of microtubules is regulated by a balance in the activities of 2 major classes of microtubule-regulatory proteins, the microtubule-stabilizing proteins, and microtubule-destabilizing proteins. Because of the success of anti-microtubule agents like taxanes in the clinical management of CRPCs, there has been considerable interest generated to identify novel approaches to interfere with the dynamics of the microtubules system. In this section, we discuss some of the important cellular regulators of microtubule dynamics that are attractive targets for prostate cancer therapy and might also serve as prognostic and predictive biomarkers to identify patients responsive to endocrine therapy and/or chemotherapy.

Stathmin. Stathmin (also known as Oncoprotein 18, p18, Lap 18) is the founding member of a family of microtubule-destabilizing proteins that regulates the assembly and disassembly of the mitotic spindle through changes in its cell-cycle–specific phosphorylation (6, 55, 56). The initial evidence for the role of stathmin in the regulation of mitosis came from genetic studies that showed that manipulation of stathmin expression interferes with the progression of cells through mitosis (56, 57). These studies were subsequently followed by the identification of stathmin as a major regulator of the dynamics of polymerization and depolymerization of the microtubules that make up the mitotic spindle (58). Stathmin promotes microtubule depolymerization by 2 distinct mechanisms (6). First, stathmin promotes a catastrophe-promoting microtubule-depolymerizing activity that increases the catastrophe rate at both the plus and the minus ends of the microtubules. The second is a tubulin-sequestering activity that inhibits microtubule polymerization by sequestering tubulin heterodimers and thus depolymerizes the pool of free tubulin heterodimers available for polymerization. Both of these activities of stathmin play a critically important role in the regulation of mitotic and interphase microtubules.

High levels of stathmin expression have been found in a wide variety of human malignancies including prostate cancer (6, 59). Interestingly, when biopsy specimens from human prostate tumors were stained with an anti-stathmin antibody, immunoreactivity was seen in poorly differentiated tumors but not in hyperplastic prostate or highly differentiated tumors (59). Thus, it was proposed that the level of stathmin expression may serve as an important prognostic marker for prostate cancer. A more recent retrospective study of 240 radical prostatectomy cases included representative cancer and benign tissue from each prostatectomy specimen in TMAs that were stained with different antibodies including anti-stathmin antibody (60). By univariate analysis, staining with anti-stathmin antibody showed statistically significant outcome predictability (60). Positive staining with anti-stathmin antibody was found to be statistically significant in predicting biochemical failure (60), identifying stathmin as a reliable molecular marker of prognosis in prostate cancer.

In preclinical studies that aimed to target stathmin expression, inhibition of stathmin expression by adenovirus-mediated gene transfer of anti-stathmin ribozyme was shown to mediate significant antiproliferative and antitumorigenic effects in prostate cancer cells in vitro (61), suggesting that stathmin provides an attractive target for prostate cancer therapy. Subsequent evaluation of the therapeutic interactions between stathmin inhibition and different chemotherapeutic agents in prostate cancer showed a synergy, when stathmin inhibition was combined with either a microtubule-interfering drug such as paclitaxel or a topoisomerase inhibitor such as etoposide (62). Furthermore, triple combinations of anti-stathmin therapy with low-dose, noninhibitory concentrations of paclitaxel and etoposide resulted in more profound antiproliferative and antitumorigenic effects in prostate cancer (62). These studies suggest that stathmin inhibition sensitizes prostate cancer cells to chemotherapy. The exact molecular mechanism responsible for the observed synergistic interaction between MTAs and topoisomerases is not clear. Numerous lines of evidence have shown that a deficiency in stathmin decreases the rate of catastrophes and sequestration of tubulin molecules, thereby shifting the equilibrium between the polymerized and unpolymerized tubulin in favor of polymerized tubulin (55, 58, 63). Paclitaxel, on the other hand, stabilizes microtubules by binding to polymerized tubulin (64). Interestingly, unlike paclitaxel, etoposide induces G2 arrest as a result from the rapid inhibition of the activity of p34cdc2, a protein kinase that is critical for the transition from the G2 phase into mitosis in eukaryotic cells of the cell cycle (65). Hence, when prostate cancer cells in which stathmin is inhibited are exposed to taxol and etoposide, cells will have difficulty entering mitosis as a result of etoposide-induced G2 arrest, will have difficulty depolymerizing their spindles as a result of stathmin deficiency, and will have difficulty exiting mitosis, as the polymerized microtubules will be further stabilized by taxol binding (62). This may explain, at least in part, the observed synergy between stathmin inhibition and exposure to paclitaxel and topoisomerases. As paclitaxel and etoposide have been frequently used in the treatment of prostate cancer, combination of these agents with anti-stathmin therapy may provide a superior form of combination therapy that could avoid toxicities associated with the use of chemotherapeutic agents at their maximally tolerated doses.

Tau. Tau is one of the most extensively investigated microtubule-associated proteins that interacts with microtubules of the cytoskeleton. Tau proteins are known to stabilize microtubules and promote tubulin assembly into microtubules (66). Tau is found primarily in the neurons of the central nervous system (CNS) as different isoforms that modulate microtubule stability via changes in its state of phosphorylation (67). While the role of Tau in the
regulation of processes in the CNS is well-documented, tau may also have distinct roles in nonneuronal cells of various tissue origins. Interestingly, numerous studies showed a close correlation between increased expression of tau and increased drug resistance to antmitotic drugs in various cancers, including prostate cancer. Previous studies showed that overexpression of tau in human prostate cancer cell lines correlates with estramustine resistance (68). Comparative analysis of tau expression in estramustine-sensitive and estramustine-resistant prostate cancer cell lines showed higher levels of tau expression in the estramustine-resistant cell line than in estramustine-sensitive prostate cancer cell line (68). The increase in the level of tau in estramustine-resistant cells was attributed to enhanced stability of microtubules as a result of binding of tau to the microtubules. As most MTAs, including estramustine, are known to interfere with microtubule dynamics, it is possible that altered ratios of tau and tubulin may confer a resistant phenotype by modulating the kinetic parameters of microtubule behavior.

Recent studies indicate that the level of tau expression can also influence paclitaxel sensitivity (69). Low level of tau expression was associated with increased sensitivity to paclitaxel (69). Tau can bind to β-tubulin on both the inner and the outer surfaces of microtubules and it can bind at the same site as paclitaxel on the inner surface of microtubules. Thus, it was proposed that tau compromises the efficacy of MTAs by competing with the drugs for the microtubule-binding site. The clinical significance of tau expression and taxane sensitivity has been extensively investigated in patients with different cancers. For instance, reduced expression of tau was associated with a favorable response to paclitaxel in gastric cancer cases (70). Similarly, low expression of tau was associated with higher rates of pathologic complete response (pCR) to preoperative paclitaxel chemotherapy in patients with breast cancer (BrCa; ref. 71). A subsequent study investigated the prognostic and predictive values of tau expression in estrogen receptor–positive primary breast cancer in 3 patient cohorts that included patients who received no systemic adjuvant therapy, patients who received adjuvant endocrine therapy but no adjuvant chemotherapy and patients who received preoperative paclitaxel containing chemotherapy (72). This study reported an inverse correlation between endocrine sensitivity and chemosensitivity.

High tau expression was significantly associated with no recurrence at 5 and 10 years (P = 0.005 and P = 0.05, respectively) in patients treated with tamoxifen, indicating a predictive value for endocrine therapy. On the other hand, tau expression was significantly lower in patients who achieved pCR to paclitaxel containing chemotherapy (P < 0.001). Thus, this study suggested that high tau expression in estrogen receptor–positive breast cancer could indicate an endocrine sensitivity but no chemosensitivity. In contrast, low tau expression could identify a subset of patients that have poor prognosis with endocrine therapy alone and may benefit from adjuvant taxane-containing chemotherapy. The predictive value of tau expression remains to be determined in prostate cancer. Figure 2 illustrates the role of microtubule-regulatory proteins and their link to hormonal and chemotherapy.

Stathmin-Tau as biomarkers of chemosensitivity and endocrine sensitivity. While stathmin overexpression has been linked to reduced sensitivity to taxanes, inhibition of stathmin results in chemosensitization of prostate cancer patients (73). This study showed that high tau expression in estrogen receptor–positive breast cancer could indicate an endocrine sensitivity but no chemosensitivity. In contrast, low tau expression could identify a subset of patients that have poor prognosis with endocrine therapy alone and may benefit from adjuvant taxane-containing chemotherapy. The predictive value of tau expression remains to be determined in prostate cancer. Figure 2 illustrates the role of microtubule-regulatory proteins and their link to hormonal and chemotherapy.

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Figure 2. Schematic illustration of the role of microtubule regulatory proteins and their link to hormonal and chemotherapy. The dynamics of microtubule polymerization and depolymerization is regulated by microtubule-stabilizing factors such as MAP–tau and microtubule-destabilizing factors such as stathmin. Stathmin and tau have opposite but complementary roles in the dynamic regulation of microtubules. The level of expression of stathmin and tau seems to play a critically important role in the modulation of chemosensitivity and endocrine sensitivity as indicated. MT, microtubule.
cancer cells following paclitaxel treatment (62). The level of stathmin expression has also been shown to correlate with efficacy of endocrine therapy (73). Although much of these data have been generated in patients with breast cancer and not in prostate cancer, these observations are still of considerable clinical interest as breast cancer and prostate cancer are closely related diseases that share many similarities in terms of the biologic and pathologic processes. Low stathmin staining intensity could predict favorable outcome in patients on endocrine monotherapy and could identify a low-risk group for which 5 years of endocrine therapy is sufficient (73). In contrast, high stathmin expression could possibly serve as a marker for endocrine resistance (73, 74). A recent study used a 2-biomarker model that examined stathmin expression in conjunction with tau to determine their prognostic value in a large cohort of patients with primary breast cancer (75). When stratified by hormonal receptor status, low stathmin correlated with receptor positivity showing improved survival whereas high stathmin expression predicted worse overall survival (HR, 1.48; \( P = 0.06 \)). Survival analysis showed 10-year survival of 53.1% for patients with high stathmin expression versus 67% for low expressers (\( P < 0.003 \)). The ratio of tau to stathmin expression showed a positive correlation to disease-free survival (HR, 0.679; \( P = 0.0053 \)) with a 10-year survival rate of 65.4% for patients who had a high ratio of tau to stathmin versus 52.5% 10-year survival rate for those with a low ratio (\( P = 0.0009 \)). This study revealed that the ratio of tau to stathmin was an independent predictor of overall survival (HR, 0.609; \( P = 0.008 \)). Although these studies suggested that low stathmin and high tau are associated with increased microtubule stability and better prognosis in breast cancer, it did not include a taxane-treated cohort to evaluate the prognostic value of either stathmin or tau/stathmin ratio in response to taxane therapy. While the connection between stathmin-tau as biomarkers of chemosen-sitivity and endocrine sensitivity is interesting, some of these studies measured only one of the microtubule-regulatory proteins and not both. Therefore, these clinical observations may not be internally consistent. Nonetheless, the above clinical studies on stathmin and tau are intriguing in breast cancer, and it would be of interest to confirm the relevance of these observations in patients with CRPCs. Further studies on stathmin and tau are needed to better assess the response to endocrine therapy and chemotherapy.

Conclusion

The rapidly changing landscape of therapeutic options during the past 2 years has marked a new era in the clinical management of CRPCs. The classical paradigm of the clinical activity of taxanes through exclusive inhibition of mitosis has dramatically changed with the discovery that taxanes can induce interphase microtubule bundling, a cellular insult that can impair nuclear translocation of the androgen receptor and thus inhibit transcription of the ARE-containing target genes (30–32). These observations reveal for the first time an unconventional link between the microtubule and the endocrine signaling pathways, highlighting the therapeutic importance of microtubule-mediated androgen receptor signaling events that are impaired downstream of drug-induced microtubule bundling (30–32). These findings suggest that the microtubule and the endocrine signaling pathways interact with each other and can strongly impact clinical outcome by markedly inhibiting prostate cancer cell growth. While microtubules can serve as dynamic highway tracks for androgen receptor trafficking to enable rapid and targeted nuclear delivery for androgen receptor transcriptional activity, perturbation of the microtubule–androgen receptor axis may prove to be an important determinant of taxane activity. The intersection of the androgen receptor and the microtubule functions may have important clinical implications in terms of providing unique opportuni-ties to better understand the molecular basis of taxane resistance in CRPCs and to identify patients who would benefit from such a treatment. Furthermore, as the andro-gen receptor splice variants can contribute to CRPCs (24, 25), it would be of interest to determine whether the alternatively spliced androgen receptor variants are also modulated by the microtubule signaling pathway in a manner similar to the wild-type androgen receptor and whether they would respond to taxanes.

Recent advances in the molecular understanding of the microtubule and the androgen receptor signaling pathways have led to the development of not only novel MTAs such as cabazitaxel but also hormonal agents such as abiraterone and enzalutamide, which have now been placed at the forefront in the therapeutic armamentarium for the treatment of patients with CRPCs. Combination of taxanes with abiraterone or enzalutamide is attractive and may have better clinical outcomes, as such a combination would inhibit the microtubule–androgen receptor axis by 2 different but converging pathways. However, it would be critically important to determine the optimal role and timing of these agents, either given sequentially or in combination. Unfortunately, there is no validated gene signature that correlates with individual treatment response in prostate cancer. The identification of novel biomarkers might help to identify patients who would benefit most from each treatment and to determine the appropriate drug to use in a given situation. In this regard, microtubule regulatory proteins, such as stathmin and tau, may provide powerful predictive and prognostic tools to identify patients who would benefit from endocrine therapy and/or taxane-containing chemotherapy.

Despite the recent landmark developments in the therapeu-tic options for patients with CRPCs, it is still essential to build on developing molecular-targeted therapies for prostate cancer. Compelling results from preclinical studies on targeting microtubule regulatory proteins such as stathmin (61, 62) are intriguing and suggest that future development of targeted therapies holds great promise.
The discovery of synergism between taxanes and anti-stathmin therapy in prostate cancer is particularly attractive, as both agents target the microtubule pathway (62). Such molecular-targeted therapies may be designed in combination with taxanes at much lower doses than at their maximum tolerated doses. This would avoid toxicities associated with the use of multiple chemotherapeutic agents at full therapeutic doses. With the growing discoveries of novel drugs under investigation and the identification of novel molecular targets, application of combinatorial strategies might be the key to the future development of effective regimens in CRPCs.

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