Androgen Receptor on the Move: Boarding the Microtubule Expressway to the Nucleus

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Abstract
Recent studies have shown that the microtubule-stabilizing drug paclitaxel, which is commonly used for the treatment of prostate cancer, inhibits signaling from the androgen receptor by inhibiting its nuclear accumulation downstream of microtubule stabilization. This mechanism is independent of paclitaxel-induced mitotic arrest and could provide an alternative mechanism of drug action that can explain its clinical activity. In this review, we highlight the importance of signaling and trafficking pathways that depend on intact and dynamic microtubules, and, as such, they represent downstream targets of microtubule inhibitors. We showcase prostate cancer, which is driven by the activity of the androgen receptor, as recent reports have revealed a connection between the microtubule-dependent trafficking of the androgen receptor and the clinical efficacy of taxanes. Identification and further elucidation of microtubule-dependent tumor-specific pathways will help us better understand the molecular basis of clinical taxane resistance as well as to identify individual patients more likely to respond to treatment. Cancer Res; 72(18); 4611–5. ©2012 AACR.

Introduction
In 2012, prostate cancer will be diagnosed in more than 240,000 men, with approximately 28,000 deaths attributable to prostate cancer (1). Prostate cancer is a heterogeneous disease, driven primarily by androgen receptor (AR) signaling, and has been traditionally treated with androgen deprivation therapy (ADT). Although our understanding of the molecular basis of prostate cancer has significantly increased over the past decade, for men who develop metastases, the principle of ADT is essentially the same as it was first proposed 60 years ago: to interfere with androgen signaling (2). The goal of ADT is to block active AR signaling, either by eliminating the ligand or affecting the receptor directly. Although most patients with prostate cancer are initially sensitive to androgen withdrawal, loss of sensitivity to ADT occurs, leading to the development of castrate-resistant prostate cancer (CRPC; ref. 3). Similarly, even with the introduction of new and more effective therapies that target the androgen axis, such as the CYP17A1 inhibitor abiraterone, which targets the central synthesis of testosterone, and the AR antagonist MDV3100, ADT is not curative (3). The molecular disturbances that contribute to prostate cancer progression in the setting of castrate levels of circulating androgen have been reviewed elsewhere (4–7), but almost universally allow for the continued function of the AR as a transcription factor, resulting in androgen-driven prostate cancer growth. Consequently, targeting the androgen axis has remained a key concept in the development of novel therapeutic strategies.

In 2004, the combination of docetaxel plus prednisone was established as the standard of care for first-line treatment of patients with CRPC, making taxanes the first class of chemotherapy drugs shown to improve survival in CRPC (8, 9). At the cellular level, the taxanes (paclitaxel, docetaxel, and cabazitaxel) bind β-tubulin and stabilize microtubules, resulting in mitotic arrest and cell death (10, 11). Microtubules are dynamic cytoskeletal polymers critically important for several cellular functions, including structural support and the formation of the mitotic apparatus. During cell division, microtubule dynamics increase significantly (4–100 fold) to enable fast "search and chromosome capture" functions required for mitosis (10). Therefore, drugs that stabilize microtubules, like the taxanes, interfere with mitotic cell progression by suppressing microtubule dynamics. This key observation, supported by numerous in vitro studies, has led to the common belief that the clinical activity of taxanes stems from their antimitotic activity (12). However, this mechanism of action has not helped us understand the molecular basis of clinical response and resistance to taxane chemotherapy, as this model applies primarily to rapidly dividing cells and tissues. It is important to emphasize here that patients' tumors have significantly lower rates of cell division than cancer cells growing in vitro. For example, prostate cancer doubles every 33 to 577 days (13, 14), in contrast to the rapidly dividing prostate cancer cells grown in tissue culture with doubling times between 30 to 48 hours (15, 16); therefore, mitotic arrest alone cannot account for the therapeutic benefit of taxane-based chemotherapy (17, 18). Thus, the effects of taxanes on interphase microtubules and the cellular pathways that depend directly on intact microtubules...
could provide an alternate mechanism of action for this class of drugs. Although this notion challenges the existing paradigm of taxanes exerting their clinical activity exclusively through inhibition of mitosis, by shifting the focus to interphase microtubules, it provides a unique opportunity to dissect how these drugs work and why they are not effective in all tumor types and all patients. The question is then raised of what are the functions of interphase microtubules that are critical for the growth and survival of the tumor?

**Interphase Microtubules as Targets for Taxane Chemotherapy**

In interphase cells, microtubules cover the entire area of the cell’s cytoplasm, originating from the microtubule-organizing center (MTOC), right outside the nucleus, and extending all the way to the plasma membrane, providing ample surface for protein–protein interactions. In epithelial cells, microtubules display an inherent polarity, having their slow-growing minus end embedded in the MTOC and their fast-growing plus end oriented toward the plasma membrane. This polarity is used by microtubule-based motor proteins, moving cargoes either toward the nucleus (dynein) or toward the plasma membrane (kinesins), thereby allowing for the directional flow of signal information within the cell, which ultimately dictates cell function (19–21). All of these qualities make microtubules centralized nodes of dynamic signaling pathways (22), which remain largely unexplored and their therapeutic potential unexploited.

We have focused our efforts into identifying pathways that depend on intact and dynamic microtubules, disruption of which by taxane treatment would be fatal for tumor cell survival. We have shown that the activity of certain transcription factors depends on the chemomechanics of the microtubule cytoskeleton and, therefore, these factors represent downstream targets of taxane activity. These factors include the tumor suppressor p53, which requires intact microtubules and the dynein minus-end–directed motor protein for trafficking and effective nuclear accumulation (23, 24), and the hypoxia-inducible factor-1α (HIF-1α), of which the activity is tightly regulated by microtubule dynamics through microtubule-dependent mRNA trafficking to sites of active protein translation (25, 26). Interestingly, this mechanism does not apply to renal cell carcinoma (RCC), in which HIF-1α regulation is independent of microtubules (27). These results can potentially explain the lack of taxane clinical activity in RCC: identification of the cellular factors that link HIF-1α to microtubules that are missing or are deregulated in RCC can provide a new therapeutic strategy for the treatment of this lethal disease.

Additional proteins of which the translocation is microtubule mediated are the retinoblastoma protein (28), the glucocorticoid receptor (29), and the parathyroid hormone receptor protein (PThRP; ref. 30). Komlodi-Pasztor and colleagues provided a detailed list of other proteins that traffic on or associate with microtubules (14).

In prostate cancer, specifically, we and others have recently shown that taxane chemotherapy impairs AR signaling activity, not through mitosis, but by impairing AR nuclear translocation and inhibiting subsequent transcriptional activation of androgen response element (ARE)–containing target genes (31, 32). Other recent studies showed that paclitaxel-induced inhibition of AR activity is mediated by FOXO1, an AR-suppressive nuclear transcription factor (33), and that docetaxel treatment can downregulate the expression of AR and prostate-specific antigen in prostate cancer cell lines (34).

Prompted by the established clinical activity of taxanes in CRPC, together with the fact that AR continues to drive disease progression despite prior antiandrogen therapies (35), we set out to investigate the role of tubulin and the impact of microtubule-targeting drugs on AR trafficking and signaling.

The results presented in our recent study (31) provide a mechanistic insight for the clinical activity of taxanes in CRPC by revealing an unconventional link between a nuclear transcription factor and the chemomechanics of the microtubule cytoskeleton. As illustrated in Fig. 1, AR associates with microtubules and is trafficked toward the nucleus with the aid of the minus-end–directed motor protein dynein. It is well established that upon ligand binding, AR dimerizes and the ligand–receptor complex translocates to the nucleus (36). However, the mechanism enabling this translocation was previously unknown. The recent studies (31, 32) identify microtubules as the “highway tracks” that enable the rapid and targeted nuclear “delivery” of AR, which is required for its transcriptional activity. What remains to be solved, however, is whether AR binds microtubules directly, remaining tethered in the cytoplasm and associating with dynein only after ligand binding, or whether AR associates with dynein in the cytoplasm and, following ligand binding, the ligand–receptor complex gets recruited to the microtubule for trafficking. Recent data from our laboratory support the first model, as we show that AR association with microtubules is diminished in the presence of ligand (Fig. 2) and that the coprecipitation of AR with dynein is enhanced following ligand stimulation (30). These results suggest that unliganded AR, at steady state, is tethered to the microtubule cytoskeleton and that, upon ligand binding, the complex associates with dynein and is released from the microtubule. In vitro studies using recombinant AR protein and purified microtubules are required to further investigate AR-binding affinity to microtubules. Regardless of direct or indirect binding of AR to the microtubule, dynein’s function is critical for the trafficking not only of AR but also other proteins for which nuclear localization is critical to their respective physiologic roles, such as p53 (23), retinoblastoma protein (28), and PThRP (30). With the implication of dynein in this mechanism, it has also become important to decipher the role of the dynein accessory proteins that mediate cargo recognition specific for AR. This information will enable the development of dynein small-molecule inhibitors, which in combination with taxane chemotherapy, could prove significantly beneficial to patients with CRPC.

**Using the Microtubule–AR Axis to Understand Clinical Taxane Resistance in Prostate Cancer**

The model presented in Fig. 1 provides a basis for understanding clinical taxane resistance in prostate cancer.
Darshan and colleagues (31) showed that perturbation of the microtubule–AR axis is an important determinant of taxane activity, independent of mitosis, as AR cytoplasmic sequestration in circulating tumor cells isolated from patients with CRPC significantly correlated with clinical response to taxane chemotherapy. Taxanes bind β-tubulin, suppress microtubule dynamics, and hyperstabilize the microtubule cytoskeleton by inducing the formation of microtubule bundles, a hallmark of effective drug-target engagement. Microtubule bundling is the first cellular insult that leads to disruption of downstream pathways. This mechanism implies that taxane chemotherapy should be most effective against tumor types in which microtubule-dependent pathways drive tumor progression, like the AR pathway in prostate cancer.

Despite the success of taxanes in CRPC treatment, their efficacy varies from patient to patient, whereas it remains unclear why individual patients respond to paclitaxel but not docetaxel and vice versa, even though these drugs share the same mechanism of action and a common binding site on β-tubulin. In 2010, the docetaxel analogue cabazitaxel was approved by the U.S. Food and Drug Administration for the treatment of patients with CRPC who previously failed docetaxel-based therapy (37). This approval highlights, once again, the activity of this class of drugs in CRPC, while raising the question of finding what the molecular basis of clinical taxane resistance is. According to the model presented here, clinical taxane resistance could arise as a result of the following: (i) impaired drug uptake, potentially due to the presence of P-glycoprotein or other drug transporters; (ii) impaired binding to β-tubulin, possibly due to the presence of tubulin mutations at the drug-binding site or overexpression of βIII tubulin isotype; (iii) the presence of AR mutations or splice variants that do not require microtubule-based transport; and (iv) dysregulation of dynein–cargo interaction.

Figure 1. Proposed model of taxane mechanism of action in prostate cancer. This model represents a novel mechanism of action for taxanes in prostate cancer, which implicates this class of drugs in critical interphase cellular functions such as AR intracellular transport and signaling. In the model, AR associates with microtubules and translocates to the nucleus via the motor protein dynein. This transport is made possible because of the inherent polarity of microtubules, which is recognized by the minus-end-directed motor protein dynein, to transport cargoes toward the nucleus. Taxanes, which bind to and hyperstabilize microtubules, inhibit this trafficking and subsequently prevent AR from reaching the nucleus and activating target genes. This mechanism of action predicts that the combination of a taxane with an inhibitor of AR ligand synthesis (i.e., abiraterone) or with inhibitors of AR-ligand interaction (i.e., MDV3100) would be synergistic in the clinical setting, as there will be inhibition of the AR signaling axis by 2 different but converging pathways. Additionally, the model predicts that a small-molecule inhibitor of dynein would similarly impair AR nuclear accumulation and would also be synergistic in combination with a taxane. Finally, the model suggests that a putative small-molecule inhibitor targeting the interaction between AR and microtubules or dynein could be used therapeutically for CRPC treatment.

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Figure 2. Ligand treatment decreases the association of AR with microtubules. A microtubule cosedimentation assay using whole-cell lysate from PC3:mCh-tub cells transfected with GFP-AR[wild-type (wt)] was carried out in the presence or absence of the synthetic dihydrotestosterone analogue (R1881, 10 nmol/L). Briefly, a total of 1 mg of precleared cell extract [high-speed supernatant (HSS)] from the transfected cells was incubated for 30 min with 10 μmol/L exogenous purified bovine brain tubulin and subjected to a cycle of polymerization with 20 μmol/L paclitaxel at 37°C. The samples were centrifuged at 100,000g to separate the microtubule polymers [warm pellet (WP)] from the soluble tubulin dimers [warm supernatant (WS)], resolved by SDS-PAGE and immunoblotted for the presence of AR and tubulin. Note that R1881 treatment decreases the amount of AR protein that cosediments with the microtubule polymer, as can be seen by the shift from 85% to 44% of AR in the WP (%P = 100′ WP/(WP + WS)). Protein quantification was done using ImageJ (NIH) software. Tubulin was detected as microtubule polymers in the WP fraction in both conditions. HSP, high-speed pellet.

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For the first possibility, limited studies have not suggested a significant correlation between P-glycoprotein expression and response to taxane treatment in patients with prostate cancer (38). Similarly, tubulin alterations, such as β-tubulin mutations or altered isotype expression, have not been associated with response to taxane-based therapy in CRPC either (39). Conversely, alterations of AR have been extensively studied in CRPC, albeit not in the context of taxane resistance. Recent studies have shown the presence of alternatively spliced AR variants, such as ARV567 and AR-V7, which arise following castration (40–43). These variants lack the ligand-binding domain, are insensitive to ADT, and are constitutively active in the nucleus, which allows for continuous AR transcriptional activity. The ARV567 variant was shown to be present in 59% of patients with CRPC and to arise in response to ADT or to the newer AR-targeted therapies, such as abiraterone (44). The frequency of this molecular alteration in CRPC makes it imperative to determine whether these variants are under microtubule control, similar to wild-type AR, and whether they would respond to taxane treatment. Our model predicts that any AR variant lacking the microtubule- or dynein-binding domain would be insensitive to taxane treatment and, thereby, has the potential to serve as a predictive biomarker of clinical taxane activity. Finally, for the dysregulation of dynein–cargo interaction, our model predicts that any aberration and/or mutation in the dynein motor protein that impairs cargo (AR) recognition and/or transport should lead to taxane resistance.

Therapeutic Implications and Perspectives

The model presented herein suggests that simultaneous targeting of different pathways that inhibit AR signaling may result in greater or more durable antitumor effects. Specifically, combination of a taxane, which interferes with AR nuclear translocation, with an inhibitor of androgen synthesis (e.g., abiraterone) or an inhibitor of AR–ligand interaction, such as MDV3100, which also inhibits AR nuclear accumulation (by yet-to-be defined mechanism; ref. 45), could yield enhanced therapeutic efficacy. To this end, a phase I trial is currently testing the combination of docetaxel and abiraterone in patients with CRPC. More importantly, understanding the precise mechanisms by which prostate cancer cells circumvent AR signaling inhibition will allow development of novel, more targeted therapies. For instance, the development of a small-molecule inhibitor of dynein, or of the dynein–AR interaction, could be added to the space of CRPC therapy. Similarly, the recent identification of an N-terminal–targeted AR inhibitor, which has the potential to target both AR wild-type and variants as the N-terminal domain is conserved (46, 47), should be synergistic in combination with a taxane. A deeper understanding of the mechanisms used by a prostate cancer cell to bypass AR inhibition as well as the cellular factors that regulate AR signaling in CRPC is required to develop approaches that will allow men to live with metastatic prostate cancer beyond the 1 to 2 years typically associated with responses to chemotherapy after ADT.

Summary

In summary, our group’s work highlights the importance of microtubule-dynein–dependent trafficking for transcription factors, such as AR, that require rapid and targeted nuclear translocation upon specific stimuli. In addition, this work challenges the existing paradigm whereby the clinical activity of taxanes in prostate cancer is attributed solely to the drugs’ antimitotic effects and it highlights the therapeutic importance of the signaling events that are impaired downstream of drug-induced microtubule disruption.

Disclosure of Potential Conflicts of Interest

D.M. Namus and P. Giannakakou are paid consultants for Sanofi-Aventis. No potential conflicts of interest were disclosed by the other author.

Authors’ Contributions

Conception and design: D.M. Namus
Acquisition of data: M. Thadani-Mulero, P. Giannakakou
Analysis and interpretation of data: P. Giannakakou
Writing, review, and/or revision of the manuscript: M. Thadani-Mulero, D. M. Namus, P. Giannakakou

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