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Original article

Molecular complexity of taxane-induced cytotoxicity in prostate cancer cells

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Abstract

Background: Taxanes are routinely used to treat men with advanced prostate cancer, yet their molecular mode of action is poorly characterized. Taxanes stabilize microtubules and may hence interfere with a plethora of cellular processes, most notably mitosis. However, prostate cancer is typically a slowly growing tumor suggesting that additional processes play a role in the response to taxanes.

Methods: Here, we analyzed the potential effect of taxanes on microtubuli-dependent intracellular transport and signaling processes, specifically, nuclear translocation of the androgen receptor and modulation of the RAS-RAF-MEK-ERK signaling cascade.

Results: We show that the androgen-driven nuclear translocation of the androgen receptor remains virtually undisturbed by docetaxel in prostate cancer cells. However, we found a striking down-regulation of activated ERK1/2 together with enhanced cytotoxicity in both docetaxel or cabazitaxel-treated cells that was comparable to direct MEK kinase inhibition. Remarkably, MEK inhibition alone was less effective in inducing cytotoxicity than taxanes indicating that a down-regulation of activated ERK1/2 may be necessary but is not sufficient for taxane-induced antitumoral effects. In line with this notion, we show in a xenograft mouse model that prostate cancer cells that are resistant to docetaxel overexpress activated ERK1/2. Taken together, our findings underscore that the modulation of ERK1/2 activation, in concert with other mechanisms, plays an important role in taxane-induced antineoplastic effects on prostate cancer cells.

Conclusions: These results suggest at least partially nonoverlapping effects of docetaxel and androgen deprivation therapy and hence help to understand recent clinical findings. A further elucidation of the mode of action of docetaxel would have important implications to optimize current treatment strategies and biomarker development for men with metastatic prostate cancer.

Keywords: Prostate cancer; Docetaxel; ERK1/2

1. Introduction

Prostate cancer is a leading cause of cancer-related morbidity and mortality in men [1]. Despite successful initial treatment, many patients develop disease recurrence and require systemic therapy. Androgen deprivation therapy (ADT) provides effective, but only temporary, tumor control in patients with metastatic prostate cancer. Patients inevitably develop castration resistance, that is, the tumor progresses with androgens at castrate level [2]. Until the introduction of novel antiandrogens [3,4], docetaxel was the only therapy for patients with metastatic castration-resistant prostate cancer (mCRPC) shown to improve survival [5,6]. However, the clinical response to docetaxel is heterogeneous and drug resistance commonly evolves. A related taxane, cabazitaxel, has hence been developed for the use in patients with acquired docetaxel resistance [7].

More recently, 2 large trials have shown that docetaxel also has significant clinical activity in metastatic, castration-naïve patients when combined with ADT. The CHAARTED trial has shown an impressive 17 months advantage in the mean overall survival in patients with high-volume disease treated with the combination of drugs when compared to ADT alone [8]. The STAMPEDE trial showed a median survival of 77 months in

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patients treated with ADT and docetaxel in comparison to 67 months in the ADT alone arm [9]. A third study (GETUG-AFU-15) could not demonstrate an overall survival benefit but showed a more favorable progression-free survival in the ADT plus docetaxel group vs. ADT alone [10].

Although these results suggest a cooperative effect of docetaxel and ADT, the molecular basis of the clinical efficacy of the combination drug regimens is poorly understood.

Docetaxel is an antimitotic taxane that functions through microtubule stabilization. As prostate cancer is typically not characterized by a high mitotic index, it has been suggested that docetaxel-mediated inhibition of intracellular transport processes, in particular, androgen receptor (AR) nuclear translocation, plays an important role in the response to taxane treatment [11]. In this scenario, both ADT and taxanes would both impinge on AR signaling, which is difficult to reconcile with the clinical benefit of a combination therapy.

In the present study, we provide in vitro and in vivo evidence that the notion that taxanes function by blocking AR nuclear translocation may not be universally applicable and that other modes of action may be involved. We show that the down-regulation of ERK1/2 signaling may be such a mechanism that functions most likely cooperatively with other processes to induce cytotoxicity. Our results, together with the clinical findings, suggest at least partially nonoverlapping targets of taxanes and ADT and encourage further studies into the molecular basis of the mode of action of taxanes.

2. Material and methods

2.1. Cell culture and transfections

Human prostate cancer cell lines LNCaP, PC-3, and DU-145 were obtained from CLS Cell Line Service GmbH (Eppelheim, Germany) and maintained according to the distributor's recommendations. For transient transfections, the Neon transfection system (Life Technologies, Carlsbad, CA) was used. According to the manufacturer's instructions, cells were grown to 70% to 90% confluency, harvested and washed with PBS twice. Cells were mixed with 2 μg plasmid DNA in a 100 μl Neon tip before cells were electroporated 2 times with 1250 V for 20 to 30 ms. After transfection, cells were cultured for 24 hours before further experimentation. Plasmids used were pEGFP-C1-AR, pEGFP-C1 empty vector (kindly provided by Michael Mancini via Addgene, Cambridge, MA), pBABE-*KRAS*^{V12} (kindly provided by William Hahn via Addgene), and pBABE empty vector (kindly provided by Karl Münger). The synthetic androgen R1881 was obtained from Sigma. The MEK inhibitors U0126 and selumetinib were obtained from Selleck.

2.2. Fluorescence microscopy

Cells were grown on coverslips and fixed in 4% paraformaldehyde/PBS for 10 minutes at room temperature. After washing with PBS, cells were briefly permeabilized with 1% Triton-X100/PBS and mounted with DAPI (Vector Laboratories, Burlingame, CA).

2.3. Immunofluorescence microscopy

Cells were grown on coverslips, and, after washing with PBS, fixed with ice-cold methanol for 20 minutes at 4°C. Cells were washed with PBS for 2 minutes before blocking with 2% donkey serum and 1% bovine serum albumin in cold PBS for 30 to 40 minutes at 4°C. Afterwards, the coverslips were rinsed with cold PBS and incubated with an antibody against phospho-ERK1/2 T202/Y204 (1:100 in phosphate-buffered saline [PBS]) overnight at 4°C. On the following day, cells were washed with cold PBS for 2 times 2 minutes and then incubated with Alexa Fluor 488 conjugated Donkey anti-Rabbit secondary antibody (Invitrogen) at a 1:750 dilution in PBS for 3 hours at 37°C. Subsequently, slides were washed with PBS for 20 minutes and mounted with DAPI (Vector Laboratories).

2.4. Quantitative image analysis

Images were taken with a Leica DM5000B fluorescence microscope with a DFC425C camera (Leica) using Leica Application Suite (version 4.7) software. Mean fluorescence intensity was calculated using ImageJ (version 10.2).

2.5. Immunoblot analysis

Immunoblotting and immunohistochemistry was performed as previously described [12]. Antibodies used were directed against phospho-ERK1/2 T202/Y204, total ERK (both Cell Signaling), or GAPDH (Santa Cruz).

2.6. Cell viability

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Molecular Probes) was used as read-out for cytotoxicity.

2.7. Mouse xenograft experiments

DU-145 were injected subcutaneously into the flanks of NMRI *nude* mutant mice, and the growth of palpable tumors was monitored followed by a randomized treatment with the pharmacologic agents (vehicle control or 15 mg/kg docetaxel i.v. d 0, 7, 14). Treatment groups consisted of 8 animals per group. To determine therapeutic efficacy, tumors were measured twice weekly and subjected to comprehensive pathologic assessment following sacrifice of the mice. Experiments were carried out until day 17. The study was performed in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the Society of Laboratory Animals (GV SOLAS). All animal experiments were approved by the Committee on the Ethics