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UROLOGIC ONCOLOGY

Urologic Oncology: Seminars and Original Investigations 34 (2016) 432.e15–432.e23

Original article

Enzalutamide inhibits androgen receptor–positive bladder cancer cell growth

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Received 2 February 2016; received in revised form 13 May 2016; accepted 16 May 2016

Abstract

Purpose: Emerging preclinical evidence suggests that androgen-mediated androgen receptor (AR) signals promote bladder cancer progression. However, little is known about the efficacy of an AR signaling inhibitor, enzalutamide, in the growth of bladder cancer cells. In this study, we compared the effects of enzalutamide and 2 other classic antiandrogens, flutamide and bicalutamide, on androgen-induced bladder cancer cell proliferation, migration, and invasion as well as tumor growth in vivo.

Methods: Thiazolyl blue cell viability assay, flow cytometry, scratch wound-healing assay, transwell invasion assay, real-time polymerase chain reaction, and reporter gene assay were performed in AR-positive (e.g., UMUC3, TCCSUP, and 647V-AR) and AR-negative (e.g., UMUC3-AR-short hairpin RNA [shRNA], TCCSUP-AR-shRNA, 647V) bladder cancer lines treated with dihydrotestosterone and each AR antagonist. We also used a mouse xenograft model for bladder cancer.

Results: Dihydrotestosterone increased bladder cancer cell proliferation, migration, and invasion indicating that endogenous or exogenous AR was functional. Enzalutamide, hydroxyflutamide, and bicalutamide showed similar inhibitory effects, without significant agonist activity, on androgen-mediated cell viability/apoptosis, cell migration, and cell invasion in AR-positive lines. No significant effects of dihydrotestosterone as well as AR antagonists on the growth of AR-negative cells were seen. Correspondingly, in UMUC3 cells, these AR antagonists down-regulated androgen-induced expression of AR, matrix metalloproteinase-2, and interleukin-6. Androgen-enhanced AR-mediated transcriptional activity was also blocked by each AR antagonist exhibiting insignificant agonist activity. In UMUC3 xenograft-bearing mice, oral gavage treatment with each antiandrogen retarded tumor growth, and only enzalutamide demonstrated a statistically significant suppression compared with mock treatment.

Conclusions: Our current data support recent observations indicating the involvement of the AR pathway in bladder cancer growth and further suggest that AR antagonists, including enzalutamide, are of therapeutic benefit in AR-positive bladder cancer. © 2016 Elsevier Inc. All rights reserved.

Keywords: Androgen receptor; Bicalutamide; Bladder cancer; Enzalutamide; Flutamide

1. Introduction

Urinary bladder cancer, mostly urothelial carcinoma, is a major public health issue. It was estimated that there were 429,800 new cases of bladder cancer and 165,100 deaths occurred in 2012 worldwide [1]. Despite recent improvements in surgical technique and perioperative care, mortality rates of muscle-invasive bladder cancer remain relatively high [1,2]. In particular, a significant amount of patients with advanced disease fail to respond to conventional nonsurgical treatment, such as systemic chemotherapy or radiotherapy. Therefore, the development of novel treatment options for urothelial cancer is urgently required. Indeed, attempts have been made to identify key molecules responsible for bladder cancer progression, resulting in implementing early phases of clinical trials [3].

There has been a striking imbalance in the incidence of bladder cancer and patient outcomes by sex: men have a

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http://dx.doi.org/10.1016/j.urolonc.2016.05.016
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substantially higher risk of bladder tumor, whereas women tend to have more aggressive tumors [1,4]. Several explanations for this divergence have been proposed, which include sex-related differential exposures to industrial chemicals and cigarette smoke, delays in diagnosis especially in females, and more recently, the difference of sex hormone milieu. In population-based studies, sex disparities in bladder cancer still exist after adjustment for exposure to known carcinogens [5]. We and others have subsequently indicated the involvement of androgens-mediated androgen receptor (AR) and estrogens-mediated estrogen receptor signals in bladder tumorigenesis and cancer progression [6–26]. Specifically, AR activation correlates with promotion of tumor outgrowth. Accordingly, AR inactivation via AR antagonists, such as flutamide and bicalutamide that have been widely used for the treatment of prostate cancer, or knockdown of the receptor results in tumor regression in vitro and in vivo [6,7,9–13,20,23,26]. Thus, an increasing amount of preclinical evidence has suggested that anti-AR therapy is beneficial especially to patients with AR-positive bladder cancer.

Enzalutamide is a synthetic AR signaling inhibitor that not only blocks androgen binding to the AR, with a 5- to 8-fold higher affinity compared with bicalutamide, but also prevents AR nuclear translocation, DNA binding, and coactivator recruitment [27–29]. Because of its mechanisms of action, enzalutamide treatment has demonstrated an unequivocal benefit, over the first generation nonsteroidal antiandrogenic drugs including flutamide and bicalutamide, especially in men with castration-resistant prostate cancer. In contrast, the efficacy of enzalutamide in the growth of bladder cancer cells remains largely unknown. In the current study, we assessed the inhibitory effects of enzalutamide on androgen-induced bladder cancer cell proliferation, migration, and invasion as well as tumor growth in vivo by comparing with those of flutamide and bicalutamide.

2. Materials and methods

2.1. Cell culture and chemicals

UMUC3 and TCCSUP cell lines were originally obtained from the American Type Culture Collection. A 647V cell line was used in our previous studies [6,21,23,26,30]. All these lines were recently authenticated, using GenePrint 10 System (Promega), by the institutional core facility. In addition, stable clones of UMUC3/TCCSUP-AR-short hairpin RNA (shRNA) and 647V-AR were established, using a retrovirus vector (pMSCV/U-AR-shRNA) and a lentivirus vector (pWPI-AR), respectively, in our previous studies [10,16,21,23] (Supplementary Fig. S1). Cells were maintained in Dulbecco's modified Eagle's medium (Mediatech) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100 units/mL) at 37°C in a humidified atmosphere of 5% CO_2. Cells were then cultured in phenol red-free medium supplemented with 5% charcoal-stripped FBS at least 24 hours before experimental treatment. We obtained dihydrotestosterone, flutamide, and hydroxyflutamide from Sigma, and bicalutamide from Santa Cruz Biotechnology. Enzalutamide was provided by Astellas Pharma Global Development and Medivation, Inc.

2.2. Cell proliferation

We used the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay to assess cell viability. Cells (0.5–1 × 10^3) seeded in 96-well tissue culture plates were incubated for 96 hours, and at the end of the culture 10 μL MTT stock solution (5 mg/mL; Sigma) was added to each well with 100 μL of medium and incubated for 4 hours at 37°C. The medium was then replaced with 100 μL dimethyl sulfoxide, followed by incubation for 5 minutes at room temperature. The absorbance was then measured at a wavelength of 570 nm with background subtraction at 655 nm using luminometer (FLUOstar Omega).

2.3. Apoptosis

Flow cytometry was performed in cells (1 × 10^6/10-cm dish) cultured for 24 hours, harvested with trypsin, fixed in 70% ethanol, and stained with propidium iodide buffer (50 μg/mL). Cellular propidium iodide content was measured on a Guava PCA-96 Base System flow cytometer (EMD Millipore) equipped with a green laser at 532 nm wavelength. Data were analyzed, using the Guava Cell Cycle software (EMD Millipore).

2.4. Cell migration

To evaluate the ability of cell migration, a scratch wound-healing assay was performed. Cells at a density of 90%–100% confluence in 12-well plates were scratched manually with a sterile 200 μL plastic pipette tip, cultured for 24 hours, fixed with methanol, and stained with 0.1% crystal violet. The width of the wound area was quantitated, using ImageJ (National Institutes of Health).

2.5. Cell invasion

Cell invasiveness was determined, using a Matrigel (60 μg; BD Biosciences)-coated transwell chamber (8.0 μm pore size polycarbonate filter with 6.5 mm diameter; Corning). Cells (5 × 10^4) in 100 μL of serum-free medium were added to the upper chamber of the transwell, whereas 600 μL of medium containing 5% charcoal-stripped FBS was added to the lower chamber. After incubation for 36 hours at 37°C in a CO_2 incubator, invaded cells were fixed, stained with 0.1% crystal violet, and counted under a light microscope.

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