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Research Article

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Silencing of aldo-keto reductase 1C3 by siRNA increases chemosensitivity of prostate cancer cells

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ABSTRACT

Aldo-keto reductases 1C3 (AKR1C3) were highly expressed in docetaxel resistant prostate cancer cell lines after evaluating by gene differential expression microarray. This study aimed to investigate the effects of AKR1C3 silencing on the sensitivity of prostate cancer cells to docetaxel treatment. PC-3 cells were transfected with siRNA against AKR1C3 and treated with docetaxel to study the effect of silencing AKR1C3 on the drug sensitivity of the prostate cancer cells. Expression of AKR1C3 in PC-3 cells of various groups was detected by Western blot. Cell viability was examined in the presence or absence of docetaxel using MTT assay. Results demonstrated that silencing of AKR1C3 significantly promoted docetaxel induced cell growth inhibition in prostate cancer cells. Therefore, AKR1C3 silencing enhances chemotherapy of prostate cancer and could be a potential therapeutic target.

Key words: siRNA, AKR1C3, PC-3, prostate cancer, docetaxel

INTRODUCTION

Prostate cancer (PCa) has the highest incidence rate and the second highest mortality rate throughout the world. Most PCa patients who receive hormone treatment exhibit tumor progression and become refractory to androgen deprivation and ultimately progress with castration-resistant prostate cancer (CRPC) within 12-24 months [1,2]. One chemotherapy regimen for CRPC currently recommended by several authoritative guidelines, including the National Comprehensive Cancer Network (NCCN) and the European Association of Urology (EAU) [3,4] is a combined docetaxel and prednisone therapy. Docetaxel does not provide effective biochemical control in all patients, and tumor progression occurs following a median of 6.3 months, resulting in resistance [5]. Therefore, current studies regarding the ability to reverse drug resistance and enhance sensitivity to docetaxel chemotherapy are ongoing.

Aldo-keto reductase 1C3 (AKR1C3) is a member of the aldo-keto reductase protein superfamily [6]. It plays a pivotal role in androgen biosynthesis and in the pre-receptor regulation of AR action within the prostate. Several studies indicate that AKR1C3 is overexpressed in prostate cancer and that expression increases with the progression of the disease [7,8]. AKR1C3 catalyzes the 17-ketoreduction of weak androgen precursors to give testosterone and 5α-dihydrotestosterone and catalyzes the reduction of 5α-androstane-3, 17-dione (5α-Adione) to yield DHT [9]. It also catalyzes the formation of prostaglandin (PG) $F_{2\alpha}$ and 11β-$PGF_{2\alpha}$ from PGH_2 and PGD_2, respectively. $PGF_{2\alpha}$ and 11β-PGF_2 can bind to the prostanoid (FP) receptor, which activates MAPKinase pathways and leads to the phosphorylation and inactivation of the proliferator peroxisome activator receptor gamma (PPARγ) (a pro-proliferative response) [10]. These pro-proliferative signaling molecules can lead to proliferation of tumor cells

[11]. AKR1C3 expression and activity has been implicated in the development of CRPC, making it a rational target [12]. Therefore, we supposed that AKR1C3 inhibition maybe involved in blocking both androgen dependent and independent prostate cancer cell growth.
Docetaxel (DTX) is the first-line chemotherapeutic option for symptomatic CRPC patients who are candidates for chemotherapy [13], which enhances the overall response, clinical remission of the prostate cancer patients [14]. However, resistance can develop through a variety of mechanisms including inhibition of apoptosis and activation of the extracellular signal-related pI3 kinase/Akt survival pathways with the development of metastasis [15]. Recent study indicated that AKR1C3 is highly induced with the *cis*-diamminedichloroplatinum (CDDP) resistance in colon cancer. AKR1C3 participates in the CDDP resistance probably via detoxification of the aldehydes resulting from enhanced oxidative stress [16]. Due to the resistance, it often fails to cure patients, therefore, it is important to identify better or alternative therapeutic strategies that reverse chemotherapy resistance and enhance sensitivity to chemotherapy drugs.

In this study, we used siRNA-mediated selective inhibition of AKR1C3 to investigate the docetaxel sensitivity in PC-3 cells. Furthermore, the effect of AKR1C3 silencing on cancer cell migration was also examined in vitro.

EXPERIMENTAL SECTION

Cells and cell culture

PC-3 prostate cancer cells were provided kindly by Fred Hutchinson Cancer Research Center. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Carlsbad, USA) that was supplemented with 10% fetal bovine serum (FBS) (Hyclone) and 1% penicillin/streptomycin at 37ºC in a humidified atmosphere with 5% CO2. Confluent cells were passaged with trypsin-EDTA (0.05% trypsin and 0.53 mM tetrasodium EDTA) prepared to be transfected by AKR1C3 siRNA.

siRNA transfections

Synthetic 19-nt RNAs were purchased from Thermo Fisher Scientific Inc (Waltham, MA, USA). The AKR1C3 siRNA sequence was 5′-GGU GAG GAA CUU UCA CCA A-3′ and a scrambled oligonucleotide sequence were chemically synthesized used as a negative control. PC-3 cells were seeded into 6 well plates (1×10^6 cells per well) and grown to 70% confluency, replenished in serum-free Optimem 1, then transfected for 4h with a siRNA oligonucleotide pool targeting AKR1C3, using oligofectamine according to manufacturer's instructions. A non-targeting oligonucleotide pool was used at the same concentration as a transfection control. The expression of AKR1C3 was assayed by Western blotting 72 h after transfection.

Determination of cell proliferation

PC-3 prostate cancer cells were seeded in 96-well plates in quintuplicate with Dulbecco's basal medium plus 10% fetal bovine serum and maintained in culture for 24 h. After transfected with AKR1C3 siRNA and the negative control the cells were treated with DTX (5, 10, 20, 50 or 100 nM) for 24h, 48 h and 72 h, respectively. At the end of incubations, cell medium was removed and 100 μL/well of MTT solution (0.5 mg/mL in PBS) were added. After incubation at 37 °C, 5% CO2 for 4 h, the supernatants were removed carefully, and 150 μl of DMSO was added to each well. Absorbance was measured at 450 nm in a Microplate Reader (Bio-Rad 680). Analysis of the obtained results was done using GraphPad Prism 4 computer program to evaluate cell proliferation rate and cytostatic rate. DTX untreated cells were used as controls.

Western blot analysis

Preparation of total cell lysate and the procedures for Western blot analyses were performed essentially as described previously [17]. Protein samples were separated on 10% polyacrylamide resolving gels with the buffer system and transferred onto nitrocellulose membranes for 2h at 250 mA. Protein binding sites on the nitrocellulose were blocked for 1h at 25°C in 5% (w/v) Marvel/PBS/3% (v/v) Tween-20 (PBST), then incubated overnight at 4°C with AKR1C3 monoclonal antibodies (1:1000 dilution; Invitrogen, California, USA). The membrane was washed 3×10 min in TBST and probed with horseradish peroxidise-conjugated secondary antibodies (Amersham Life Sciences, Buckinghamshire, UK) for 1h at 25°C. Following 3×10 min washes in PBST, bands were detected using enhanced chemiluminescence (ECL+ reagents, Amersham). Densitometric quantification of band intensities was performed using Kodak one-dimensional image analysis software.

Statistical analysis

The data from the experiments were analyzed using the SPSS 18.0 software and expressed in the form of the mean ± SD. The two- tailed Student's t-test was used to analyze the differences in the experiments. Results were considered significant at $p<0.05$ for all analyses.

RESULTS

Effect of AKR1C3 siRNA on expression of AKR1C3 gene in PC-3 cells

To determine the efficiency of the downregulation of AKR1C3 siRNA in PC-3 cells, protein expression levels were determined quantitatively by Western blot analyses. As demonstrated in Figure 1, AKR1C3 protein was strongly expressed in non-transfected and transfected with non-targeting sequence siRNA groups whereas in the cells transfected with specific AKR1C3 siRNA the protein protein rate was 41.3% ± 6.1% compared with the non-targeting control. The results demonstrated that AKR1C3 expression was significantly inhibited at 48 hours after infection with specific AKR1C3 siRNA (p <0.01 compared with control groups).

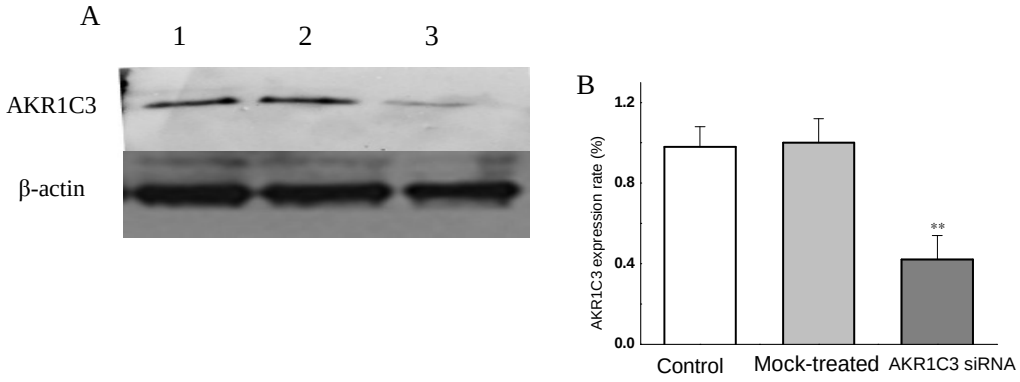

Figure 1. Ribonucleic acid interference inhibited AKR1C3 gene expression in prostate cancer cells. (A) Protein expression of AKR1C3 was detected by Western blotting. Membranes were probed with antibodies for target protein, and expression levels were normalized for loading by probing for β-actin. The blots were developed using an enhanced chemiluminescence kit. (B) **Densitometric analysis was performed using Kodak one-dimensional image analysis software. Lane 1, phosphate-buffered saline; lane 2, siRNA/non-targeting oligonucleotide (mock-treated); and lane 3, siRNA/AKR1C3. ($^{**}P < 0.01$)**

Downregulation of AKR1C3 expression increases chemosensitivity

To examine whether downregulation of AKR1C3 could sensitize prostate cancer cells to docetaxel, a first-line chemotherapeutic drug for castration resistant prostate cancer, PC-3 cells were transiently transfected with AKR1C3 siRNA or control siRNA for 48 hours and treated with docetaxel for an additional 24 hours at different concentrations including 0, 5, 10, 20, 50 and 100 nM. The tumor cell viability was assessed with MTT assay. The results revealed that silencing AKR1C3 prior to docetaxel treatment significantly potentiated the inhibitory effects of docetaxel on cell proliferation, compared to the non-targenting oligonucleotide treated and docetaxel alone treatment groups.

DISCUSSION

In the present study, we used the AKR1C3 siRNA to study the effects of down regulation of AKR1C3 expression on PC-3 sensitivity to docetaxel. Our results showed that AKR1C3 siRNA treatment caused a specific and profound decrease of AKR1C3 protein expression that was associated with decreased cell growth whereas increased docetaxel sensitivity.

RNAi is emerging as a powerful technique characterized by its high efficiency, high specificity, and low toxicity. RNAi is the sequence-specific gene silencing mediated by siRNAs, which are produced from double-stranded RNA of exogenous or endogenous origin by Dicer, a member of the RNase III protein family. The resulting siRNAs are about 21-23 nucleotides long and are then incorporated into the RNA-induced silencing complex, which then targets and cleaves mRNA, containing a sequence identical to that of the siRNA [18, 19]. siRNA has a number of theoretical and practical advantages over antisense oligonucleotide techniques, partly because of the greater resistance of siRNA to nuclease degradation [20]. Therefore, according to the previous clues that AKR1C3 may be involved in prostate cancer drug resistance, we study the biologic function of AKR1C3 by siRNA assay on prostate cancer cells sensitivity to docetaxel treatment.

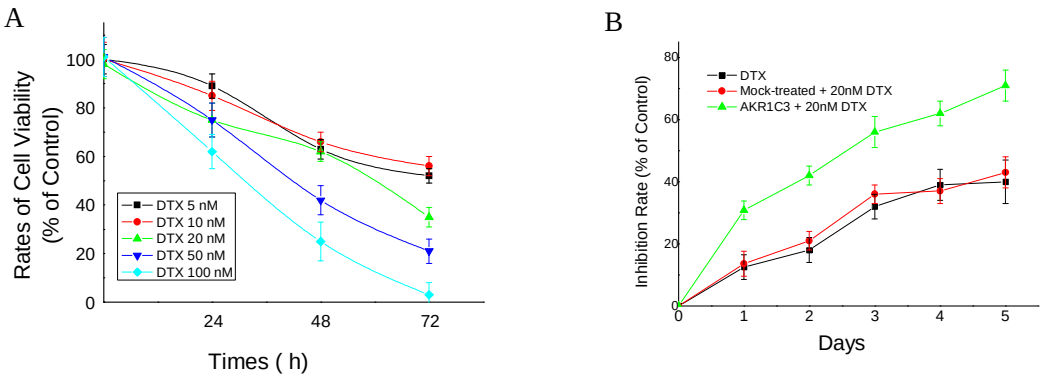

Figure 2. Susceptibility of PC-3 cells to different concentrations of docetaxel or to 20nM docetaxel after silencing AKR1C3. (A) Cells were treated with increasing concentrations of docetaxel and cell viability was assayed using MTT assays at different time point. (B) Cells were transfected with AKR1C3-targeted siRNA or non-targeting sequence of siRNA, then treated with 20 nM docetaxel and cell viability was assayed by MTT assays. Statistically significant differences relative to control were determined using two- tailed Student's t-test. Values represent mean ± SD (*n* = 6)

Docetaxel is used as first line anticancer drug in prostate cancer therapy, however resistance usually develop through a variety of mechanisms including inhibition of apoptosis and activation of the extracellular signal-related pI3 kinase/Akt survival pathways with the development of metastasis. Our data showed that the increasing sensitivities of doxetaxel in prostate cancer cells are followed by AKR1C3 down-regulation. AKR1C3 has been shown to be involved in cisplatin resistance in colon cancers and is overexpressed in prostate cancer cells which may be involved CRPC [21]. The upregulation of AKR1C3 could contribute to intracellular synthesis of androgen receptor (AR) ligands and stimulation of cancer cell proliferation through AR signaling [22,23]. In this study, our data indicated down-regulating AKR1C3 might cooperate with docetaxel to decrease cell survival and increase cell apoptosis, which may lead to enhanced drug sensitivity.

Our data suggest that the AKR1C3 gene can be regarded as a promising target gene in genetic therapy for prostate cancer cells and the use of AKR1C3 siRNA for cancer therapy deserves additional investigation. The development of inhibitors targeting AKR1C3 and combined with docetaxel treatment could represent a promising approach for effective antitumor therapy.

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