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Liver X receptor activation inhibits PC-3 prostate cancer cells via the beta-catenin pathway

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ABSTRACT

Background: Liver X receptors (LXRs) are nuclear receptors family of ligand-dependent transcription factors that play a crucial role in regulating cholesterol metabolism and inflammation. Recent studies show that LXR agonists exhibit anti-cancer activities in a variety of cancer cell lines including prostate. To further identify the potential mechanisms of LXRα activation on prostate cancer, we investigated the effect of LXR agonist T0901317 on PC3 prostate cancer cell and in which activity of beta-catenin pathway involved.
Methods: Prostate cancer PC3 cells were transfected with LXR-a siRNA and treated with LXR activator T0901317. qRT-PCR and western blot were used to detect the LXR-a expression. beta-catenin, cyclin D1 and c-MYC were analyzed by western blot. Cell apoptosis was examined by flow cytometry and Cell proliferation was assessed by Cell Counting Kit-8 assay. Cell migration was detected by Transwell chambers.
Results: Data showed that T0901317 significantly inhibited PC3 cell proliferation as well as invasion and increased apoptosis in vitro. Furthermore, we found that LXRα activation induced the reduction of beta-catenin expression in PC3 cells, and this inhibitory effect could be totally abolished when cells were treated with LXRα. Meanwhile, the expression of beta-catenin target gene cyclin D1 and c-MYC were also decreased.
Conclusions: This study provided additional evidence that LXR activation inhibited PC-3 prostate cancer cells via suppressing beta-catenin pathway.

1. Introduction

Prostate cancer (PCa) is the most frequently diagnosed cancer in old men and also the second leading cause of male cancer death in the Western countries [1]. Although radical prostatectomy and radiation therapy remain the primary choice for localized stage of PCa, there is no effective treatment for patients who develop recurrences or develop into hormone-refractory prostate cancer (HRPC) or those who have metastatic disease at the time of diagnosis. Therefore, identification of novel therapeutic targets for treatment modalities is urgently needed.

Liver X receptors (LXRs) (LXRα and LXRβ), members of the nuclear receptor transcription factor superfamily, are important regulators of cholesterol, fatty acid, and glucose homeostasis that could be activated by endogenous ligands like oxysterols, as well as synthetic agonists such as T0901317 [2]. LXR agonists have exhibited a potent role in treatment of metabolic syndromes, cardiovascular diseases, and murine models of diabetes, atherosclerosis, and Alzheimer'sdisease [3–6]. Recently, evidence also show that LXR agonists exhibit anti-cancer activities in a variety of cancer cell lines including prostate, colon, breast, lung, skin, bone, cervical, ovarian, leukemia and liver [7–9]. In xenograft study, treatment of human prostate cancer LNCaP cells with T0901317 decreased cell growth [10] and delayed the progression of androgen-dependent prostate tumors towards androgen independency in castrated nude mice [11]. Thus, LXR agonist seems to be a promising alternative drug for prostate cancer treatment. However, the regulatory mechanisms of LXR agonist against prostate cancer are still not fully understood. Despite recent studies demonstrated that treatment with the synthetic LXR agonist could downregulate the AKT survival pathway and thus induced apoptosis of LNCaP cells in both xenografted nude mice and cell culture [12]. Moreover, LXR agonist

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may also regulate the carcinogenesis of PCa via the SOCS3 pathway [13]. The concrete characteristics of LXR agonist need further exploration.

Beta-catenin is a crucial mediator of the Wnt pathway, which plays an important role in oncogenesis and embryogenesis [14,15]. The nuclear beta-catenin protein combines with the T cell factor (TCF) family, resulting in expression of the target genes, such as Cyclin D1, MYC and matrix metallopeptidase 7 (MMP7) [14,16]. Previous studies showed overexpression of beta-catenin proteins in metastatic prostate cancer cells in bone [17], and inhibition of beta-catenin could suppress tumor growth in an in vivo xenograft model [18]. In this study, we investigated the effects of nuclear receptor LXRs agonist T0901317 in PC 3 prostate cancer cells and found that LXR activation suppressed beta-catenin transactivation and inhibited cellular proliferation.

2. Materials and methods

2.1. Cell culture and treatments

PC3 cells was obtained from China Center for Type Culture Collection (Wuhan, China) and cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 100 mg/L penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2.

10 μM LXR-a siRNA (Santa Cruz, California) was added to 300 μl siRNA Transfection Medium (Santa Cruz, California), mixed gently, and incubated at room temperature for 20 min. The mixture was then added drop-wise to the plates with gentle shaking. The transfection media were removed after incubation for 24 h at 37 °C, and the cells were transfected again following the same protocol. After another 48 h, the cells were collected for analyzed.

Cells transfected with LXR-a siRNA were treated with 3uM LXR activator T0901317 (Alexis Biochemicals, San Diego, CA, USA) for 48 h. Cells without T0901317 treatment were used as controls.

2.2. qRT-PCR

Total RNA was extracted with Trizol reagent (Invitrogen, CA), and single-stranded cDNA was generated with the cDNA synthesis kit (Takara, Kyoto, Japan) according to standard protocols. Real-time PCR was performed using SYBR Green master mix kit (Applied Biosystems, USA). Human GAPDH gene was used as endogenous control. The PCR primer sequences were as follows: LXR-α, 5′-CATGCCTACGTCTCCATCCA-3′ and 5′-CGGAGGCTCACCAGTTTCA-3′; GAPDH, 5′-TCAACGACCACTTTGTCAAGCTCA-3′ and 5′-GCTGGTGGTCCAGGGGTCTTACT-3′. All reactions were performed in triplicate. Data were analyzed using the $2^{-\Delta\Delta Ct}$.

2.3. Western blot

Cell lysates were prepared. Total cellular proteins (50 μg) were subjected to SDS-PAGE, and transferred to nitrocellulose membranes (Amersham, USA). Specific polyclonal antibody against LXR-α, beta-catenin, cyclin D1, c-MYC (Cell Signaling, Boston, USA) diluted in TBS-T containing 5% nonfat milk was used to detect indicated proteins. The appropriate horseradish peroxidase (HRP) conjugated IgG was used as secondary antibody. Antibody on membrane was visualized by enhanced chemiluminescence (Pierce, Rockford, IL, USA). Western blot for β-actin was used as an internal sample.

2.4. Proliferation assay

The cellular proliferation of transfected cells was measured by Cell Counting Kit-8 (dojindo, Japan) assay. Briefly, 10 μl CCK-8 resolution was added to each well of 100 μl medium. Absorbance was measured at 450 nm on automatic ELISA reader (Model 680, Biorad, Marnes-La-Coquette, France). All determinations were carried out in triplicate and repeated three times.

2.5. Apoptosis analysis

Cells treated with T0901317 for 24 h were harvested and stained with FITC-conjugated Annexin V and propidium iodide (PI) (Invitrogen, Paisley, UK) according to manufacturer instructions. All Annexin V positive cells were considered apoptotic and their percentage was calculated among the total number of cells. Cells taking the vital dye PI were considered dead. Samples were analyzed by flow cytometry using FACS Calibur (BD Biosciences, Mississauga, Canada) and FlowJo software.

2.6. Cell invasion and migration assay

The ability of cells in vitro invasion and migration assays were performed in Transwell chambers (Corning, New York, NY, USA) following the manufacturer's instructions. In the upper chambers, 5×10^5 cells were cultured in serum-free medium on 8 μm porous polycarbonate membranes, which were coated with diluted matrigel basement membrane matrix for invasion assay or with none for migration assay. The lower chambers were added with RPMI-1640 medium containing 10% FBS. After incubation for 24 h, non-invading cells remaining on the upper surface of the filter were removed, and the cells that migrated to the underside of the membrane were fixed with 4% paraformaldehyde and stained with Giemsa (Sigma). Cells were calculated in 10 randomly selected fields under 200 × magnification and expressed as the average number of cells/field of view. These data were represented as the average of the three independent experiments.

2.7. Statistical analysis

Statistical analyses were performed using SPSS version 13.0 (SPSS, Chicago, IL, USA). Values were expressed as the mean ± standard deviation. Statistically significant differences between groups in each assay were determined by ANOVA, or student's *t*-test. $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Knockdown of LXR-α expression in PC3 cells by LXR-α siRNA

The LXR-α siRNA and negative control siRNA were transfected into the PC3 prostate cancer cell line respectively. To test the knockdown efficiency, expression of LXR-α at both mRNA and protein levels was examined by Real-time RT-PCR and western blot. Data showed LXR-α mRNA and protein expression were suppressed greatly after RNA interference (Fig. 1). It is displayed that LXR-α was significantly down-regulated by LXR-α siRNA in PC3.

3.2. The effects of biological behaviors of PC3 cells treated with LXR activator treatment

PC3Cells transfected with LXR-α siRNA were treated with 3uM LXR activator T0901317 for 48 h. The results showed cells treated with control siRNA and LXR activator significantly reduced cellular proliferation rate and immigration as well as increased apoptosis rates ($P < 0.05$), compared to cells without T0901317 treatment and (or) LXR-α siRNA (Fig. 2).