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Long noncoding RNA FOXD2-AS1 accelerates the gemcitabine-resistance of bladder cancer by sponging miR-143

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ABSTRACT

Increasing evidences have proved that long noncoding RNAs (lncRNAs) modulate the tumorigenesis of bladder cancer involved in multiple pathophysiological processes. In the study, we investigate the role of lncRNA FOXD2-AS1 in the gemcitabine (GEM) resistant bladder cancer and explore its potential mechanism. Results showed that lncRNA FOXD2-AS1 was high-expressed in gemcitabine-resistant bladder cancer cells. In vitro experiments, FOXD2-AS1 knockdown suppressed the 50% inhibitive concentration (IC50) of gemcitabine, drug-resistance related genes (MDR1, MRP2, LRP1) expression, invasion and ABCC3 protein expression in gemcitabine-resistant bladder cancer cells (T24/GEM, 5637/GEM). In vivo of xenograft assay, FOXD2-AS1 knockdown inhibited the tumor growth of bladder cancer cells. Bioinformatics program and validation experiments confirmed that FOXD2-AS1 positively regulated ABCC3 protein through targeting miR-143, acting as a competing endogenous RNA (ceRNA). In summary, our results revealed the vital roles of FOXD2-AS1/miR-143/ABCC3 axis in gemcitabine resistance of bladder cancer cells, providing a novel therapeutic strategy for bladder cancer.

1. Introduction

Presently, bladder cancer has acted as one of the most common malignant tumors, accounting for the ninth most common malignancy worldwide [1,2]. In developed country and developing country, although the therapeutic methods for bladder cancer have greatly developed with the progress of technological advance, the mortality rate of bladder cancer patients is still pessimistic [3,4]. Therefore, more attentions should be devoted to the molecular mechanism of bladder cancer, which may help us identify new therapeutic targets.

Long non-coding RNAs (lncRNAs) are a type of non-protein coding transcripts with longer than 200 nucleotides [5]. Emerging evidence has described the important role of lncRNAs gene regulation and cancer progression, including bladder cancer. For example, lncRNA MALAT1 promoted bladder cancer progression by specific suppression of miR-125b and activation of its target genes (Bcl-2, MMP-13), suggesting the important role of MALAT1-miR-125b-Bcl-2/MMP-13 axis [6]. However, the in-depth mechanism of lncRNAs in the tumor pathogenesis still needs sequentially exploration.

Gemcitabine is a deoxycytidine analogue with antitumor activity that is widely used for treatment of several solid tumors, including non-small cell lung cancer, bladder cancer, pancreatic cancer and so on [7,8]. In present study, we investigated and validated the function of lncRNA FOXD2-AS1 in gemcitabine-resistant bladder cancer cells, and discover the potential mechanism in the bladder cancer tumorigenesis.

2. Materials and methods

2.1. Cell lines and culture

Bladder cancer (T24, 5637) cells were purchased from Institute of Cell Biology Chinese Academy of Sciences (Shanghai, China). Bladder cancer cells were cultured in RPMI-1640 Medium (Invitrogen, Carlsbad, CA, USA) adding with 10% fetal bovine serum (FBS, Gibco, NY, USA). Plates were placed at 37 °C with an humidified atmosphere of 5% CO_2.

2.2. Cell counting Kit-8 assay

Cell Counting Kit-8 (CCK-8, Beyotime Inst Biotech, Shanghai, China) assay was performed for the gemcitabine sensitivity assay. The IC50 value was measured according to the manufacturer's instructions. Briefly, bladder cancer cells (5×10^3 per well) were seeded in a 96-well plate and incubated for 24 hour s. Finally, the absorbance was measured at 450 nm using microplate reader.

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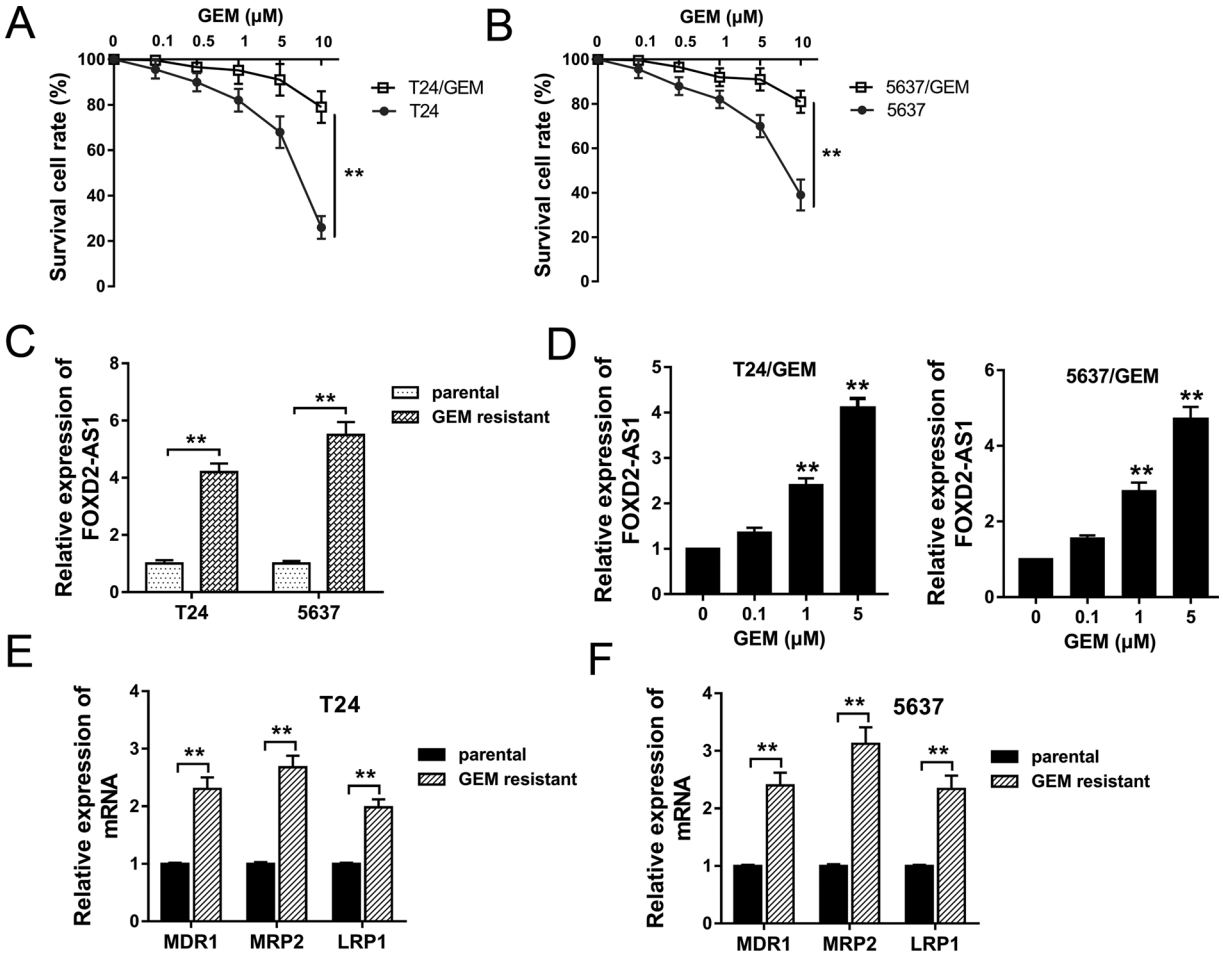

Fig. 1. LncRNA FOXD2-AS1 was over-expressed in gemcitabine-resistant bladder cancer cells. (A, B) Survival cell assay showed the survival rate of gemcitabine-resistant bladder cancer cells (T24/GEM, 5637/GEM) and parental cells (T24, 5637) treated with increasing concentration of gemcitabine (0–10 μM). (C) RT-PCR assay showed the lncRNA FOXD2-AS1 expression in gemcitabine-resistant bladder cancer cells (T24/GEM, 5637/GEM) and parental cells. (D) LncRNA FOXD2-AS1 expression in bladder cancer cells treated with gemcitabine (0, 0.1, 1, 5 μM). (E, F) RT-PCR showed the expression levels of drug resistance related genes (MDR1, MRP2, LRP1) in gemcitabine-resistant bladder cancer cells (T24/GEM, 5637/GEM) and parental cells. Data were expressed as mean ± SD. **P < 0.01 represents statistically difference.

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells and tissue according to specification (Promega, Madison, WI., USA). The concentration and purity of RNA were measured at 260/280 nm using ultraviolet spectrophotometer. cDNA was reversely transcribed from RNA using the Primer-Script one step RT-PCR kit (TaKaRa). Primer sequences were synthesized by Sangon Biotech (Shanghai, China), including FOXD2-AS1, forward, 5′-TGTCTTACACTACAGCCCATTTCCTT-3′, reverse 5′-CTGATACAAGTGAAGCCACATGCG-3′; miR-143, forward, 5′-ACAG ACCGGTACAAGTGCAGA-3′, reverse, 5′-GGTCGGCATACAGCTAAT ACA-3′; GAPDH, forward, 5′-TCCACCACCCTGTTGCTGTA-3′, reverse 5′-ACCACAGTCCATGCCATCAC-3′. Relative RNA expression levels were quantified with SYBR (Applied Biosystems, USA) on 7500 RealTime PCR System (Applied Biosystems, USA). The relative expression was normalized to the expression of GAPDH using $2^{-\Delta\Delta Ct}$ method. The experiment was triplicate.

2.4. Western blot assay

Protein was lysed from bladder cancer cells or tissue samples using protein extraction reagent radio-immunoprecipitation assay (RIPA) (Beyotime, Shanghai, China) added with protease inhibitors cocktail and phenylmethanesulfonyl fluoride (PMSF) (Roche, Switzerland). Protein concentration was quantified by BCA method. Protein was separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membranes (GE Healthcare, UK). Then, the membrane was blocked in 5% no-fat milk and incubated with primary antibodies (anti-ABCC3, 1:1000, Abcam) at 4 °C overnight. Next, the blots were incubated with HPR conjugated secondary antibodies for 1 hour at room temperature. Enhanced chemiluminescence (ECL) chromogenic substrate was used to determine and quantify the integral optical density value of the protein bands. GAPDH acted as the internal control.

2.5. Transwell invasion assay

The invasion of bladder cancer cells were measured using transwell assay. Briefly, bladder cells (5×10^4 per well) were suspended in 200 ml serum free DMEM. Chambers (8 mm, BD Biosciences) were plated with BD BioCoat Matrigel according to the manufacturer's protocol. After incubation, the non-invaded cells on the upper membrane surface were removed with a cotton tip. Then, member was fixed and stained by violet crystalline. Images were gained under an inverted light microscope (Leica Microsystems, Germany) at a magnification of x200.