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SNORA42 enhances prostate cancer cell viability, migration and EMT and is correlated with prostate cancer poor prognosis

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ABSTRACT

Prostate cancer (PCa) is one of the most common invasive cancers and the second leading cause of cancer-related death in male worldwide, reflecting the needs of diagnostic and prognostic biomarkers for PCa. Emerging evidence has revealed small nucleolar RNAs (snoRNAs) playing a significant role in tumorigenesis and cancer progression. However, there are few reports about snoRNAs in PCa. Here, we found *SNORA42* rather than its host gene (*KIAA0907*) was up-regulated in PCa cell lines. Meanwhile, an obvious up-regulation of *SNORA42* was observed in cancer tissues compared to their adjacent normal tissues. *SNORA42* could be induced by DHT stimulation. Over-expression of *SNORA42* increased prostate cancer cell proliferation and inhibited apoptosis. Importantly, *SNORA42* increased prostate cancer cell migration and invasion. Higher *SNORA42* expression level was found to be correlated with shorter survival in metastatic PCa tissues by Kaplan-Meier survival analysis, but this effect was not found in primary PCa tissues. In conclusion, over-expression of *SNORA42* could have an oncogenic effect on the progression of PCa. *SNORA42* might serve as a prognostic biomarker in PCa.

1. Introduction

Prostate cancer (PCa) is the second leading cause of cancer in male worldwide and the leading cause in developed countries (Torre et al., 2015). Patients with localized PCa could be treated by prostatectomy. However, the majority of PCa deaths were due to metastatic castration-resistant PCa (mCRPC) (Wang et al., 2009). In spite of rapidly developed therapies, mCRPC still remains incurable; it's of urgent need to identify novel regulators to reveal the molecular mechanism of PCa progression.

Recent studies have revealed that noncoding RNAs (ncRNAs), including microRNAs (miRNAs), long noncoding RNAs (lncRNAs) and small nucleolar RNAs (snoRNAs), play an important role in PCa progression (Pickard et al., 2013; Qu et al., 2016; Ramalho-Carvalho et al., 2017). Small nucleolar RNAs are small RNAs of 60–300 nucleotides in length that are predominantly found in the nucleolus (Williams and Farzaneh, 2012). Most snoRNAs are encoded in introns of housekeeping genes that are essential for ribosome biogenesis (Filipowicz and Pogacic, 2002). Furthermore, they play a key role in rRNA modification (Kiss-Laszlo et al., 1996; Decatur and Fournier, 2002). According to types of post-transcriptional modification, there are two major classes of snoRNAs. C/D box snoRNAs associate with 2′-O-ribose methylation, while H/ACA box snoRNAs associate with pseudouridylation (Williams and Farzaneh, 2012). Moreover, growing volume of work has proposed snoRNAs may be important for tumorigenesis or cancer progression. For example, *SNORD50A* and *SNORD50B* directly bind and inhibit K-Ras and are recurrently deleted in human cancer (Siprashvili et al., 2016). In NSCLC, *snoRNA78* promotes the tumorigenesis. In breast cancer, implication of snoRNA U50 is also found (Dong et al., 2009).

In the present study, two public datasets (GSE21034 and GSE45604) were analyzed to identify differentially expressed snoRNAs in PCa. Combining with functional and bioinformatics analysis, we identified *SNORA42* as a key snoRNA in PCa. We presented evidences suggesting that *SNORA42* served as an oncogene in PCa. Furthermore, we found there was a significant increasing of *SNORA42* expression in PCa compared to their adjacent tissues. We found higher expression levels of *SNORA42* were associated with shorter survival time in metastatic PCa but not in primary PCa by using Kaplan-Meier survival analysis. Our results suggested *SNORA42* might be a potential prognosis biomarker for PCa.

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2. Materials and methods

2.1. Microarray data

Microarray data was downloaded from Gene Expression Omnibus (GEO) database (www.ncbi.nlm.nih.gov/geo/) under accession number GSE21034, GSE45604 and GSE23022. 218 tumor samples and 149 matched normal samples were contained in GSE21034. In GSE45604 dataset, the snoRNAs expression levels were measured in 10 normal prostate and 50 prostate cancer samples. Twenty cases of moderately differentiated prostate cancer and corresponding normal tissue were examined in GSE23022 dataset. The statistical significance of the difference may be conveniently estimated by *t*-test snoRNAs having fold changes ≥ 1.5 and P values < 0.05 were selected as of significantly differential expression.

2.2. Cell culture

LNCaP cells were purchased from the American Type Culture Collection (Manassas, USA) which were confirmed by short tandem repeat (STR) analysis. 22RV1, DU145, PC3 and WPMY-1 were obtained from Cell Bank of Chinese Academy of Sciences (Shanghai, China) where they were authenticated by mycoplasma detection, DNA-Fingerprinting, isozyme detection and cell vitality detection. All experiments were carried out with cell lines at passages below 30. The four prostate cancer cell lines were maintained in RPMI 1640 medium (Corning, USA) supplemented with 10% FBS (Hyclone, USA) while WPMY-1 was maintained in DMEM medium (Corning, USA) supplemented with 10% FBS.

2.3. RNA interference and transient transfection

Synthetic *SNORA42* siRNA and its scrambled control negative control (NC) were purchased from GenePharma (Shanghai, China), and used at the concentration of 100 nM. Specific siRNA targeting *SNORA42* was 5′-GTACCCATGCCATAGCAAA-3′ (Mei et al., 2012). Transfection was carried out with Lipofectamine 2000 Transfection Reagent (Life, USA) according to the manufacturer's procedure. The Opti-MEM medium and Lipofectamine 2000 were both purchased from Life Technologies.

2.4. Subcellular fractionation

Approximately 3×10^6 cells grown on 10 cm dishes (Corning), trypsinised, washed in cold 1x PBS and centrifuged (1200 rpm, 5 min). Pellets were lysed in 1 mL hypotonic lysis buffer (10 mM HEPES pH 7.9, 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM β-mercaptoethanol, 0.075% NP-40, 1x Murine RNase inhibitor, 1x protease/phosphatase inhibitor cocktails, Roche) and incubated for 15 min at 4 °C with rotation. Nuclei were pelleted by centrifugation (1200 rpm, 4 °C) for 15 min. The cytoplasm was collected from the supernatant. Nuclei was washed three times in 800 μL PBS and collected as pelleted nuclear fraction. Fractionated cytoplasmic and nuclear lysates were confirmed by localization of GAPDH and Histone 3.1, respectively.

2.5. RNA isolation and real-time qPCR

Tissue samples were grinded into powder in liquid nitrogen until the tissue block was not visible, and then 1 mL Trizol was added into the powder. Total RNA was extracted from tissue samples and PCa cells using Trizol reagent (Life Technologies, CA, US) according to the provided instructions by the manufacturer. Reverse transcription was performed according to the manual of NovoScript Reverse Transcriptase (Novoprotein Scientific Inc. China). Specific primers for mature *SNORA42* were purchased from GenePharma (Shanghai, China). Relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Each sample was run in triplicate to ensure quantitative accuracy. After the reverse transcription, 500 ng of the complementary DNA was used for subsequent qRT-PCR reaction. For nuclear and cytoplasmic lysates, *U6* and *GAPDH* were used as reference controls for the normalization of *SNORA42* and *KIAA0907*, respectively. The primer sequences were as follows: *U6*: 5′-CGCTTCGGCAGCACATATACTAA-3′ (forward), 5′-TATGGAACGCTTCACGAATTTGC-3′ (reverse); *GAPDH*: 5′-AGCCACATCGCTCAGACAC -3′ (forward), 5′-GCCCAATACGACCAAATCC-3′ (reverse); *KIAA0907*: 5′−CCCTACGGAGTACCAAGCATAG-3′ (forward), 5′-CAGGAGCAGCAGGAATAAAAGGA-3′ (reverse); *SNORA42*: 5′-TGGATTTATGGTGGGTCCTTCTCTG-3′ (forward), 5′-CAGGTAAGGGGACTGGGCAATGGTT-3′ (reverse).

2.6. SNORA42 over-expression construct

The intact sequence of *SNORA42* RNA was amplified from reserve transcription product by PCR, then inserted into pcDNA3.1 (+) (Invitrogen) vector, and the sequence was confirmed by DNA sequencing. The primers for amplification were 5′-TAAGCTTGGTACCGAGCTCGTAGGATCCTGGTAATGGATTTATGGTGGGT-3′ (forward primer) and 5′- TGCTGGATATCTGCAGAATTTAAAGCTTCACTGTGCAACCCCCTTCAGTGCT-3′ (reverse primer).

2.7. Cell proliferation assay

Cell proliferation was assessed by Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Japan) in octuplicate according to the manufacturer's instructions. Absorbance was measured at 450 nm with Microplate Reader ELx808 (Bio-Tek, USA). The absorbance at 615 nm was used as a reference.

2.8. 5-ethylnyl-2′-deoxyuridine (EdU) incorporation assay

DU145 and PC3 cells were incubated with 5-ethynyl-20-deoxyuridine (EdU) for 4 h after cell transfection. Detection of EdU was performed as directed in the Cell-Light™ EdU Apollo 567 In Vitro Kit (Ribobio, China). After two washes with PBS, the cells were incubated with 50 μL of 4% paraformaldehyde in PBS for 15 min at room temperature. Cells were treated with 100 μL of 0.5% TritonX-100 for 10 min, and then incubated with 100 μL of 1X Apollo staining solution for 30 min. The DNA contents of the cells in each well were stained with 100 μL of Hoechst 33,342 for 30 min and visualized under a fluorescence microscope.

2.9. Cell cycle and apoptosis assay

Cells were harvested 48 h after transfection. For cell cycle assay, cells were incubated with 0.03% triton X-100 and propidium iodide (PI) (50 ng/mL) for 15 min; the percentages of cells in different phases of cell cycle were measured with a FACS calibur flow cytometer (BD, USA) and analyzed with ModFit software (Verity Software House, USA). For apoptosis assay, cells were assayed with FITC Annexin V Apoptosis Detection Kit (BD, USA) and analyzed by flow cytometry.

2.10. Western blotting analysis

Cells were lysed in RIPA buffer (Boston Bioproducts) supplemented with protease inhibitors (Complete, EDTA-free; Roche Diagnostics) and PMSF (Calbiochem). Lysates were separated on a 10% or 15% acrylamide gel and subjected to western blot analysis. Immunoblots were incubated overnight at 4°C with the following primary antibodies: anti-procaspase3 (Proteintech, UK), anti-p17(Proteintech, UK), anti-CDH1(Proteintech, UK), anti-CDH2 (Proteintech, UK), anti-VIM (Proteintech, UK), anti-ZEB1 (Proteintech, UK), anti-Histone H3.1 (Proteintech, UK), anti-GAPDH (Proteintech, UK) and anti-Actin antibodies (goat polyclonal; Santa Cruz Biotechnology; 1:2000). Goat anti-