Introduction

Kidney carcinoma is a main cancer in men and women, accounting for 20%–30% of all cancers in China. Patients who die from kidney cancer are approximately 10–20 million, which has increased the financial burden in developing countries. Kidney cancer was the seventh most common malignancy and accounted for 3.3% of all newly diagnosed cancers in 2012. Renal cell carcinoma (RCC) constitutes approximately 90%–95% of all kidney neoplasms. The estimated economic burden of metastatic RCC was US$1.6 billion (2006 USD) in selected countries. It is a rapidly evolving area of solid tumor oncology. The highest incidence occurs more in the developed countries, and the recent decades have witnessed increasing affluence and technological advancement, especially in the more developed nations. Although there are many treatments such as surgery and chemotherapy for kidney cancer, over the past 30 years, the 5-year progression-free survival (PFS) and overall survival (OS) have remained low. This situation is partially because of the high rate of recurrence and metastasis, which is becoming worse. The majority of kidney cancer patients have been diagnosed the first time with advanced tumor metastasis. The development of kidney cancer through a complicated procedure has a relationship with many genetic variations. Therefore, we need an effective method for diagnosis of kidney cancer, which may lay the foundation for its potential treatment and cure. There are already many signs of progress in the next sequencing technologies from the past years. Some reports have shown that only 10% of the genes were transcribed into coding RNAs, while 90% of the RNAs can be named as the noncoding RNAs (ncRNAs). The ncRNAs were divided into two parts based on their size. The length of small ncRNAs is <200 nt and the length of long ncRNAs is >200 nt.

Cell proliferation in kidney carcinoma is inhibited by IncRNA GASL1

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Abstract

Long noncoding RNA (lncRNA) GASL1 was identified as a novel lncRNA, which plays an important role in the proliferation and apoptosis of cells. This study aimed to compare the expression of GASL1 mRNA in kidney cancer cells and normal cells and detect the biological role of GASL1 in kidney cancer cell line A498. Polymerase chain reaction (PCR) was performed to examine the expression of GASL1 mRNA in kidney cancer tissues, normal tissues, and the cell lines. GASL1 overexpression was achieved in kidney cancer cell lines A498 through transfection. MTT was used to detect the effects of GASL1 overexpression in A498 cells. GASL1 mRNA was significantly overexpressed in adjacent normal tissues compared with renal cell carcinoma. The expression of GASL1 is lower in kidney cancer cell lines than in normal kidney epithelium cell line HREpiC. Overexpression of GASL1 inhibits the proliferation of renal carcinoma cell lines. GASL1 mRNA was down-regulated in kidney cancer tissues and may play a role in kidney cancer cell proliferation.

Keywords

cell proliferation, FCM, kidney carcinoma, lncRNA GASL1, MTTs

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More and more reports have shown that miRNAs can be regarded as oncogenes or tumor suppressor genes.7 In contrast, lncRNAs were regarded as transcriptional, previously. However, lncRNAs could play an important role in many biological signs of progress, including X chromosome inactivation, chromatin remodeling, and transcriptional repression. In this study, a new lncRNA called lncRNA GASL1 will be investigated for its functions in kidney cancer.

Materials and methods
Tissues collection
The specimens were obtained from 30 primary kidney tissues and matched non-tumor tissues at the Department of Department of Uropoiesis Surgical, Shouguang People’s Hospital, from May 2013 to March 2016. The patients were confirmed to be diagnosed with kidney cancer at the Pathology department of thyroid Surgery, Shouguang People’s Hospital. No chemotherapy or radiotherapy was administered before the tissue was collected.

Real-time-polymerase chain reaction for GASL1
Semi-quantitative real-time-polymerase chain reaction (RT–PCR) was carried out using SYBR Green Kit (Life Invitrogen USA) with the ABI 7500 real-time rotary analysis (ABI Life Science). Real-time PCR primers were as follows: GASL1, forward 5-TGTAAACTCCTTCTCGGGGC-3 and reverse 5-GTCTATTTCGGTGAAATTGG-3; GAPDH, forward 5-CGGCGACGACCATTCGAAAC-3 and reverse 5-GAATCGAACCCTGATTCCCCGTC–3. For each run, each reaction was repeated independently at least thrice. RT-PCR was performed under the following conditions: denaturation at 95°C for 5 min, next by 45 cycles of 95°C for 15 s, 60°C for 35 s, and 72°C for the 20 s. The second method was used to calculate the gene expression ratio. The expression of GASL1 was normalized to that of GAPDH.

Cell culture
The human kidney cancer cell lines A498, and kidney epithelium cell line HREpiC were bought from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cell lines were cultured in Dulbecco’s Modified Eagle Medium (DMEM) medium (sigma, USA) and the medium was replenished with 10% fetal bovine serum (FBS), two antibiotics, namely, penicillin and streptomycin (all from sigma, USA), at 37°C in a 5% CO2-95% air atmosphere. Although we culture our cells in the sterilized apparatus/system, to avoid any possibility of fungal and/or bacterial infection during handling, we used these combinations with DMEM because of a long history of using these antibiotics and well-characterized effects; in almost all cell cultures it is added to a 1% concentration. Flow cytometry (FCM) analysis and cell sorting were then carried out directly on EPICS ALTRA Flow Cytosorter (Beckman Coulter, Fullerton, CA, USA). Hoechst 33342 was excited with 100 mW ultraviolet (UV) laser and was detected with 450 band-pass (BP) filter for blue fluorescence and 675 BP filter for red fluorescence. A 610-nm dichroic mirror short-pass (DMSP) filter was used to separate the emission wavelengths. A polygonal live gate in FS-HO blue plot was created to exclude debris and dead cells.

Plasmid construction and cell transfection. Cells were transfected with 100 nM of GASL1-siRNA(GAGTATGTTGGTCACTATAGC), negative control (NC) siRNA, pcDNA3.1-GASL1 expression plasmid, and pcDNA3.1 vector (all purchased from Yearthbio, Changsha, China) using Lipofectaminemax and Lipofectamine 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer’s instructions. PCR was performed to validate whether the GASL1-siRNA and pc-DNA3.1-GASL1 can be transfected into the cell lines.

An shRNA directed against GASL1 (5'-GACGTGTCAGGACCTTCGT) was cloned into the retroviral vector, pRETRO-SUPER. To express GASL1 a 1536bp fragment of GASL1 (based on RACE analysis) was cloned into pEFIRES-P vector.8 For CRISPR/Cas9-mediated deletion pSpCas9(BB)-2A-GFP was used.

MTT assay
A498 cells were balanced and then the cells were plated into 96-well plates (5 × 104 cells/well) and cultured for 0, 24, 48, 72, and 96 h using DMEM medium with 10% FBS at 37°C. The viability of A498 cells was determined using MTT assay. Briefly, following cell culture, 20 µl CCK (sigma USA) was added to each well and the 96 well plates were incubated at 37°C in a humidified 5% CO2 atmosphere for 4 h. The resulting formazan product was dissolved with 100 µl isopropanol and absorbance at 490 nm.
Statistical analysis

Data were expressed as the mean ± standard deviation of three independent experiments. SPSS 19.0 software (IBM Corp, Armonk, NY, USA) was used for statistical analysis. The significant differences between the groups were analyzed using a Student’s t-test or one-way analysis of variance with Tukey’s post hoc test, and P < 0.05 was considered to indicate a statistically significant difference.

Ethical consideration

This study was certified by the Ethics Committee of the Department of Uropoiesis Surgical, Shouguang People’s Hospital. All patients signed the informed consent in this research and it was conducted as per the Helsinki declaration for human volunteers. The reference no. is 1321/IERB/PHS.

Results

We detected the mRNA expression level of GASL1 in 30 pairs of kidney cancer and adjacent tissues. The results showed that the mRNA level of GASL1 is lower in kidney cancer tissue than that in the corresponding normal counterparts as shown in Figure 1(a). We also detect the expression of GASL1 in kidney cancer cells lines A498 and normal kidney epithelium cell line HREpiC by RT-PCR. The results illustrated that the expression of GASL1 in
normal kidney epithelium cell line HREpiC is higher compared with those of kidney cancer cell lines A498 as depicted in Figure 1(b).

Next, we synthesized pc-DNA3.1-GASL1 and pc-DNA3.1. After 48h transfection, the GASL1 expression is up by 8.7-fold in A498 compared with the control cells. The data are shown in Figure 1(c). Moreover, the overexpression of GASL1 inhibits the proliferation of lung cancer cells and promotes cell apoptosis as shown in Figures 2 and 3.
In order to detect whether up- and down-expression of GASL1 can influence cell signal pathway, PCR and Western blot (WB) were performed. The results showed that overexpression of GASL1 can decrease the mRNA and protein level of Rb signal pathway, as represented in Figure 3.

Discussion

LncRNA plays an important role in the development of cancer. The IncRNAs were identified to be associated with proliferation apoptosis and prognosis of renal cancer. The new IncRNA GASL1 was found first in bone cancer. In our study, compared with normal tissue, the expression of GASL1 was lower in kidney cancer. Overexpression of GASL1 inhibits the proliferation of cancer cells and promotes cancer cell apoptosis.

The Rb/E2F pathway is highly regulated and is included in a number of negative feedback loops. For example, the RNA binding protein RBM38, as well as the microRNAs miR-15, miR-16, miR-449a, and miR-449b are all positively regulated by E2F1. Our data indicated that IncRNA GASL1 is a component of a novel negative influence Rb/E2F pathway. In the occurrence and development of cancer that is partially due to a failure to induce cell apoptosis, Bax, as well as Bcl-2, has an important role in regulating apoptosis and cell cycle progression. In our study, overexpression of GASL1 had no effect on the mRNA level of Bax and Bcl-2, but increased the protein level of Bcl-2, indicating that GASL1 may exert its function at the post-transcriptional level. P53 acts as a tumor suppressor protein in many cancers. In lung cancer, the GASL1 interacts with MDM2. Our results show that GASL1 overexpression downregulates the expression of P53 protein. Therefore, we speculated that GASL1 may interact with P53, further regulating the expression of P53.

As a conclusion, in kidney cancer, the expression level of GASL is lower. Whether down-expression of GASL1 does or not, overexpression of GASL1 can influence the proliferation and apoptosis cell signal pathway of lung cancer cells.

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