LncRNA TUG1 is upregulated and promotes cell proliferation in osteosarcoma

1 Introduction

Long non-coding RNA (LncRNA) is a group of transcribed non-coding RNAs that contain more than 200 nt in length and not encoding any proteins [1,2]. They constitute a major but still poorly characterized part of human transcriptome. They were initially considered as genomic noise. Thanks to the highly thorough sequencing as well as functional technologies in recent years, lncRNAs have been demonstrated to play important roles in basic biological processes [3-5], like DNA replication, gene transcription and epigenetic modification. Recently, we gradually recognized that lncRNAs were closely correlated with human carcinogenesis since they had been proved to be abnormally expressed and act as either oncogenes or tumor suppressors [6-9]. In addition, it is a hot topic to discuss the clinical application of specific lncRNAs as a potential novel class of biomarkers for cancer diagnosis and prognosis [10-12].

Taurine up-regulated 1 (TUG1), a 7.1-kb lncRNA, was a long non-coding RNA that was characterized first two years ago in a paper exploring differential expression of lncRNAs in DNA damage-induced cell death in Hela cells [13]. In the following studies, it was clear that TUG1 was increased in bladder urothelial carcinomas, and promote cell proliferation and apoptosis-inhibition [14]. It exhibited similar high expression and pro-tumorous effects in esophageal squamous cell carcinoma, in which TUG1 had the ability to promote cell migration [15]. However, in other types of cancers, including non-small cell lung cancer [16], TUG1 was reported to be downregulated and inhibits cell proliferation as well as inducing apoptosis. This cancer type-specific activity of TUG1 illustrated the importance of the microenvironment. Although it was already found that in osteosarcoma TUG1 was upregulated [17], the related mechanisms were still lacking. In our study, experiments were designed to access its expression, function and possible signaling pathway that TUG1 regulated.
2 Materials and Methods

2.1 Cell Culture

Human normal osteoblastic cell line hFOB1.19 and human osteosarcoma cell line SaoS2, MG63, U2OS and HOS were ordered from the Cell Line Resource Center, Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences (Shanghai, China) or ATCC (American Type Culture Collection, USA). All these cell lines were cultured in 1640 or DMEM supplemented with 10% fetal bovine serum, penicillin (100 units/ml) and streptomycin (100μg/ml) at 37°C in a humidified 5% CO₂ atmosphere.

2.2 Reagents and Antibodies

Both the control siRNA and LncRNA TUG1 siRNA were purchased by GenePharma (Shanghai, China). The cells were transfected with mimics or inhibitors using Lipofectamine RNAiMAX as described below. PCNA and β-actin antibodies were purchased from Santa Cruz Biotechnology Inc. Phosphated-Akt and total-Akt antibodies were purchased from Cell Signaling Technology Inc.

2.3 Transient Transfection

Transfections were performed using Lipofectamine RNAiMAX Reagent (Invitrogen) as per the manufacturer’s instructions. After 48 hours, cells were harvest for the other experiments.

2.4 Real-Time PCR Analysis

Total RNAs was extracted by TRIZOL from the cell lines of human osteosarcoma. Real-time PCRs were performed using an ABI7900 real-time PCR system (Applied Biosystems, Carlsbad, CA) and the SYBR Premix Ex Taq reagent kit (Takara Bio Inc., Shiga, Japan) using Ct quantization method. Ct value was the number of PCR cycles at which the fluorescence signal exceeded the threshold. ΔCt was the difference in Ct values between the control (GAPDH) and test targets (LncRNA TUG1). ΔΔCt was the difference in ΔCt values between the experimental group and paired control group, which represents the fold change in long non-coding RNA expression. The primers were designed by Primer premier 5.0, and the sequences were as follows:

**TUG1:**

Forward 5’-CTGAAGAAAGGCAA CATC-3’;  
Reverse 5’-GTAGGCTACTACAGGATTG-3’;

**GAPDH:**  
Forward 5’-GTCAACGGATTTGCTGTATT-3’;  
Reverse 5’-AGTCTTCTGGGGTGGCAGTGAT-3’.

2.5 Western Blot

For western blot analysis, in brief, the cell lysate was run on SDS-PAGE in 9% acrylamide gels and transferred onto nitrocellulose membranes. After blocking, blots were incubated with mAb. β-actin was used to normalise protein loading. A total of 30μg of cell lysate was loaded in each lane for western blot analysis.

2.6 MTT Assay

Cellular growth ability was determined by the MTT assay. In brief, 3×10³ U2OS cells/well were plated into 96-well plates. At the indicated time point, 5μl MTT solution (5mg/ml) was added into each well and incubated for 2h at room temperature (RT). Then the reaction was terminated by 100μl DMSO and the absorbance at 590nm was measured on a microplate-reader.

2.7 Statistic Analysis

Data are presented as mean ± sd of at least triplicate experiments. Statistical analyses were performed by using SPSS version 18.0 (SPSS, Chicago, IL) and GraphPad Prism Software (GraphPad Software, Inc., San Diego, CA). For all statistical analyses, a P value of <0.05 was considered statistically significant.

3 Results

Increased expression of TUG1 in osteosarcoma cell lines

Firstly, we applied real-time PCR to quantify the expression of TUG1 in osteosarcoma cell lines. As shown in Figure 1A, when compared to the normal hFOB1.19 cell line, the expression of TUG1 was significantly upregulated in a panel of osteosarcoma cells, including SaoS2, MG63, U2OS and HOS cell lines. This finding was consistent with
the previous conclusion that supported its upregulation in clinical osteosarcoma tissue samples [17].

3.1 TUG1 has a growth-promotion activity in osteosarcoma

Afterwards, TUG1 siRNA was designed and transiently transfected into the U2OS cells to knockdown the endogenous expression of TUG1. The knockdown efficiency was revealed to be effective, as approximately 80% of TUG1 expression was knockdown (Figure 2A). The MTT assay was performed to visualize the proliferation of cells. As shown in Figure 2B, after TUG1 was inhibited, the growth rate of U2OS cells had decreased compared with the cells in the control group. These results confirmed the previous conclusion that regards TUG1 as a potential oncogene via regulating cell proliferation.

3.2 TUG1 modulated the AKT signaling

To further understand the possible mechanisms that govern the pro-growth function of TUG1 in U2OS cells, we detected proliferation-related molecules including proliferating cell nuclear antigen (PCNA) and the well known AKT signaling pathway. As shown in Figure 3, after TUG1 was knocked down, the expression of PCNA was decreased, reflecting the growth inhibition at the molecule level. In addition, we found that the phosphor-AKT (at Ser 473) was also decreased in the TUG1-siRNA group, whereas the total AKT expression was unchanged. Since the phosphorylated AKT was an important transducer of AKT signaling pathway, this result suggested that the oncogenic activity of TUG1 might act through this pathway at least partially.

4 Discussion

Osteosarcoma is the most common primary bone malignant tumor in children and young adults. Although it has small incidence, the high degree of malignancy makes it a second leading cause of cancer-related death in this age group [18]. The 5-year survival rate is about 65% for patients with localized osteosarcoma, however, it decreases to only about 20% for these cases with metastasis [19-20].
Moreover, objective and reliable diagnostic biomarkers and effective targeted therapeutic agents are also lacking. Thus, further elucidating the underlying mechanisms of osteosarcoma is urgently required. According to the recent studies, lncRNAs emerge as a new frontier of translational research from molecular biology to cancer in the clinic.

**Figure 3:** Knockdown of TUG1 reduces survival markers in vitro. U2OS cells were transfected with control siRNA or IncRNA TUG1 siRNA. Forty-eight hours later, cells were harvested for western blotting. Protein level of PCNA, p-Akt (S473) and t-Akt were tested. β-actin was used as the loading control in western blotting. The results showed that inhibited expression of TUG1 suppressed cell proliferation marker PCNA, and also suppressed cell survival marker p-Akt.

In summary, we demonstrated that TUG1 was increased and promoted cell proliferation in osteosarcoma cells. Mechanically, we proved that AKT signaling was involved in this oncogenic regulation. Further studies need to be carried out to verify its pro-tumor activity and clarify the mechanisms in detail.

**Conflict of interest statement:** Authors state no conflict of interest.

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