Original Article

SNHG15 inhibits proliferation, invasion and migration of oral squamous cell carcinoma by regulating PTEN

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Abstract: Oral squamous cell carcinoma (OSCC) is a type of malignant tumors occurring in head and neck. However, the mechanism underlying OSCC remains unclear. Long non-coding RNAs (lncRNAs) are considered to be involved in the regulation of tumorigenesis and progression. LncRNA SNHG15 is abnormally expressed in various tumors, but its role and mechanism in OSCC have not been elucidated. In this study, oral cancer KB cells were cultured in vitro and divided into 3 groups: control, lncRNA SNHG15 overexpression and lncRNA SNHG15 siRNA groups. pcDNA3.1-SNHG15 plasmid and SNHG15 siRNA were transfected, respectively. Real-time PCR was performed to measure the expressions of lncRNA SNHG15, MMP-9, and MMP-2. MTT colorimetric assay was adopted to test cell survival. Cell migration assay was selected to determine tumor cell migration. Transwell chamber assay was performed to assess cell invasion. Western blot was used to detect PTEN protein expression. The results indicated that transfection of pcDNA3.1-SNHG15 plasmid significantly overexpressed lncRNA SNHG15 level, which promoted tumor cell proliferation, enhanced cell migration and invasion, decreased MMP-9 and MMP-2 expressions, and reduced PTEN protein expression compared with control group (P<0.05). Conversely, transfection of lncRNA SNHG15 siRNA clearly reduced lncRNA SNHG15b level, restrained cell proliferation, attenuated cell migration and invasion, suppressed MMP-9 and MMP-2 expression, and upregulated PTEN protein expression compared with control group or transfection group of pcDNA3.1-SNHG15 plasmid (P<0.05). Therefore, reduction of lncRNA SNHG15 expression in oral cancer KB cell line inhibits cell proliferation, migration, and invasion and maybe through promoting PTEN protein expression.

Keywords: LncRNA SNHG15, oral squamous cell carcinoma, PTEN, proliferation, migration, invasion

Introduction

Oral squamous cell carcinoma (OSCC) is a common malignant tumor and the annual incidence of new cases reaches 300,000, and the incidence and mortality keep rising [1, 2]. Oral cancer is common in the mouth, oropharynx, buccal mucosa, lips, tongue, and other parts, thus can directly infiltrate or spread to surrounding tissues, accompanied by lymph node metastasis, and even with distant metastasis. Therefore, it seriously threatens human health, and brings heavy mental and economic burden [3, 4]. Despite the diversification of oral cancer treatment methods, the current methods mainly rely on surgical treatment, radiotherapy, and chemotherapy. However, due to individualized differences in patients, disease, and TNM staging, the current treatment effect is still poor. The 5-year survival rate and quality of life were not significantly improved [5, 6]. There are many factors in the pathogenesis of oral cancer, involving viral infection, eating habits, genetics, and environmental factors [7, 8]. The biological characteristics of OSCC, include invasiveness, high mortality, and extensive hypoxia, provide significant challenges for the treatment of advanced disease [9].

LncRNAs are long non-coding RNAs with a length of over 200 nt and were initially considered to be the transcriptional “noise”. Non-coding transcripts predominate the human genome [10, 11]. LncRNAs have been thought to participate in tumorigenesis and development by mediating epigenetic, transcriptional, and post-transcriptional regulation. They can be used as tumor biomarkers, which have tis-
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sue sensitivity and can promote tumor growth. They play an oncogene or tumor suppressor role in malignant tumors [12, 13]. LncRNA SNHG15 has been shown to be abnormally expressed in various tumors, such as lung cancer, thyroid cancer, and liver cancer, thus is considered to be one of the markers for tumor prognosis [14-16]. In the present study, we aimed to measure the expression of LncRNA SNHG15 and assess its effect on proliferation, invasion and migration in OSCC cells.

Materials and methods

Main instruments and reagents

The human oral cancer KB cell line (ATCC ® CCL-17TM) was purchased from the US ATCC cell bank (Manassas, VA, USA). DMEM medium, fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Hyclone (San Angelo, TX, USA). Dimethyl sulfoxide (DMSO) and MTT powder were purchased from Gibco (Grand Island, NY, USA). Trypsin-EDTA was purchased from Sigma (St. Louis, MO, USA). PVDF membrane was purchased from Pall Life Sciences (Port Washington, NY, USA). EDTA was purchased from Hyclone. Western blot related chemical reagents were from Beyotime (Nantong, China). ECL reagents were purchased from Amersham Biosciences (Little Chalfont, Buckinghamshire, UK). Rabbit anti-human PTEN monoclonal primary antibody and mouse anti-rabbit peroxidase (HRP)-labeled IgG secondary antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). The Transwell chamber was purchased from Corning (Corning, NY, USA). pcDNA3.1-SNHG15 plasmid and LncRNA SNHG15 siRNA were purchased from Genechem (Shanghai, China). The RNA extraction kit and the reverse transcription kit were purchased from Axygen (Union City, CA, USA). Other commonly used reagents were from Sangon (Shanghai, China). The Labsystem Version 1.3.1 microplate reader was purchased from Bio-rad Corporation (Hercules, CA, USA).

pcDNA3.1-SNHG15 and LncRNA SNHG15 siRNA transfection: The pcDNA3.1-SNHG15 plasmid and LncRNA SNHG15 siRNA were transfected into KB cells. The plasmid sequence of pcDNA3.1-SNHG15 was 5'-TAGGCCACACAAAC-CAATTCATA-3'. The LncRNA SNHG15 siRNA sequence was 5'-AUCUCAUGGGACUGGUGA-3'. The cell density was fused to 70-80% in a 6-well plate. The pcDNA3.1-SNHG15 plasmid and LncRNA SNHG15 siRNA were separately added to 200 μl of serum-free DMEM medium for 15 min incubation. The lipo2000 was mixed with the pcDNA3.1-SNHG15 plasmid and LncRNA SNHG15 siRNA dilution for 30 min incubation at room temperature. After that, they were added to the well together with 1.6 ml serum-free DMEM medium and cultured in a 5% CO₂ incubator at 37°C for 6 hours. The 10% FBS serum DMEM medium was replaced, and the cells were cultured for 48 hours.

Real-time PCR

Total RNA was isolated using Trizol reagent and reversely transcribed to cDNA. The primer sequences were shown in Table 1. Real-time PCR was conducted with conditions as follows: 52°C for 1 min, 35 cycles of 92°C for 30 s, 58°C for 45 s, and 72°C for 35 s. The relative expression of mRNA was calculated by 2^ΔΔCt method using GAPDH as an internal control.

MTT assay

The cells were seeded in 96-well plate at 3000 cells/well and 20 μl MTT was added and incubated for 4 h followed by addition of 150 μl DMSO for 10 min and subsequent measurement of the absorbance value (A) at 570 nm.
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The proliferation rate = A in test group/A in control × 100%.

Transwell assay

Serum-free DMEM medium was replaced according to the kit instruction. After 24 h, the Transwell chamber was coated with a 1:5 50 mg/L Matrigel dilution and air dried at 4°C followed by addition of 500 μl of DMEM medium with 10% FBS to the lower chamber and 100 μl tumor cell suspension to the upper chamber. After 48 hours of culture, the Transwell chamber was washed with PBS. The cells on the membrane were removed, and the membrane was fixed in ice cold ethanol. After staining with crystal violet, cell number on the lower layer was counted.

Cell scratch assay

5 × 10^5 cells were passaged and inoculated into a 6-well plate. A marker pen was placed behind the 6-well plate to evenly draw a horizontal line, which was passed through the hole every 0.5 to 1 cm. At least 5 lines were drawn through each hole. After 24 hours, the tip was used to draw a line as far as possible, followed by further culture in serum-free DMEM medium in a 5% CO₂ incubator at 37°C. The results were observed after 48 hours.

Western blot

Cell protein was isolated using RIPA lysis buffer (150 mM NaCL, 1% NP-40, 0.1% SDS, 2 μg/ml Aprotinin, 2 μg/ml Leupeptin, 1 mM PMSF, 1.5 mM EDTA, 1 mM NaVanadate). After quantification by BCA assay, proteins were separated on 10% SDS-PAGE and transferred to PVDF membrane followed by being blocked and incubated with PTEN (1:1000) primary antibody at 4°C overnight. After washing with PBST, secondary antibody (1:2000) was added and incubated under dark for 30 min followed by being imaged and developed after addition of chemiluminescence reagent for 1 min.

Statistical analysis

Data were processed by SPSS 16.0 software and displayed as mean ± standard deviation (SD) and compared by one-way ANOVA. P<0.05 was considered as statistical difference.

Results

LncRNA SNHG15 expression in KB cells

pcDNA3.1-SNHG15 plasmid transfection significantly promoted the expression of lncRNA SNHG15 compared with the control group (P<0.05). LncRNA SNHG15 siRNA transfection significantly downregulated the expression of lncRNA SNHG15 compared with the control group (P<0.05) (Figure 1).

The impact of IncRNA SNHG15 on KB cell proliferation

MTT assay analysis of the effect of pcDNA3.1-SNHG15 plasmid and IncRNA SNHG15 siRNA transfection on KB cell proliferation showed that upregulation of IncRNA SNHG15 expression by pcDNA3.1-SNHG15 plasmid markedly promoted the proliferation of KB cells compared with the control group (P<0.05). Decreased expression of IncRNA SNHG15 markedly inhibited KB cell proliferation compared...
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with the control group and
LncRNA SNHG15 overexpression group (P<0.05) (Figure 2).

The influence of LncRNA SNHG15 on KB cell migration

It was demonstrated that up-regulation of LncRNA SNHG15 expression markedly promoted the migration of KB cells compared with the control group (P<0.05). Transfection of LncRNA SNHG15 siRNA apparently decreased LncRNA SNHG15 expression and inhibited KB cell migration (P<0.05) (Figure 3).

The effect of LncRNA SNHG15 on KB cell invasion

Transwell assay was selected to detect the effect of pcDNA3.1-SNHG15 plasmid and LncRNA SNHG15 siRNA on invasion of KB cells. It was revealed that upregulation of LncRNA SNHG15 expression after pcDNA3.1-SNHG15 plasmid transfection significantly enhanced KB cell invasion compared with the control group (P<0.05). Transfection of LncRNA SNHG15 siRNA obviously declined the expression of LncRNA SNHG15 and restrained KB cell invasion compared with the control group (P<0.05) (Figure 4).

The impact of LncRNA SNHG15 on MMP-2 and MMP-9 expressions in KB cells

Transfection of pcDNA3.1-SNHG15 plasmid markedly elevated the expressions of MMP-2 and MMP-9 in KB cells (P<0.05). Transfection of LncRNA SNHG15 siRNA apparently suppressed the expressions of MMP-2 and MMP-9 in KB cells (P<0.05) (Figure 5).

Figure 2. The impact of LncRNA SNHG15 on KB cell proliferation. *P<0.05, compared with control. **P<0.05, compared with LncRNA SNHG15 overexpression group.

Figure 3. The influence of LncRNA SNHG15 on KB cell migration. A. Cell scratch assay detection of the impact of regulating LncRNA SNHG15 on KB cell migration. B. Migration ability analysis. *P<0.05, compared with control. **P<0.05, compared with LncRNA SNHG15 overexpression group.
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**Figure 4.** The effect of lncRNA SNHG15 on KB cell invasion. A. Transwell assay detection of the impact of regulating lncRNA SNHG15 on KB cell invasion. B. Migration ability analysis. *P*<0.05, compared with control. #P<0.05, compared with lncRNA SNHG15 overexpression group.

**Figure 5.** The impact of lncRNA SNHG15 on MMP-2 and MMP-9 expressions in KB cells. *P<0.05, compared with control. #P<0.05, compared with lncRNA SNHG15 overexpression group.

**The influence of IncRNA SNHG15 on PTEN expression in KB cells**

Transfection of pcDNA3.1-SNHG15 plasmid significantly downregulated PTEN expression in KB cells (P<0.05). Transfection of IncRNA SNHG15 siRNA obviously enhanced PTEN expression in KB cells compared with the control group (P<0.05) (Figure 6).

**Discussion**

LncRNA regulates cell growth, proliferation, cell cycle, and apoptosis and involves in the occurrence and development of human diseases [17]. In recent years, the role of lncRNA in tumors has attracted the attention of scholars. Numerous studies found that lncRNA has the potential of a therapeutic target as a transcriptional and post-transcriptional regulator, and can be used as one of the prognostic indicators of tumors [18, 19]. Some lncRNAs were revealed to be abnormally expressed in oral cancer, such as lncRNA HO-TAIR, which is the first lncRNA to be found to have a reverse regulatory function in oral cancer [20]. Because of the rich blood supply and lymphoid tissues around the oral tissues, oral cancer is prone to metastasis as an early target. The survival rate is low and the treatment is difficult. Therefore, finding a lncRNA for oral cancer can help to clarify the mechanism and potential therapeutic targets [21]. LncRNA SNHG15 is abnormally expressed in various tumors [13-16]. Therefore, this study analyzed the role of lncRNA SNHG15 in oral cancer cells. It was confirmed that transfection of pcDNA3.1-SNHG15 plasmid overexpressed
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In KB cells, lncRNA SNHG15 overexpression promoted tumor cell proliferation, migration, and invasion ability. Conversely, transfection of lncRNA SNHG15 siRNA significantly reduced lncRNA SNHG15 expression, inhibited cell proliferation, and decreased cell migration and invasion. It was suggested that up-regulation of lncRNA SNHG15 can promote the development and progression of oral cancer. On the contrary, downregulation of lncRNA SNHG15 can inhibit tumor proliferation, invasion and migration.

Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes containing zinc ions involved in the development and progression of tumors. MMP-2 and MMP-9 are gelatinases and their activation is closely related to tumor metastasis and infiltration [22, 23]. It was found that overexpression of IncRNA SNHG15 promoted MMP-2 and MMP-9 expression, while transfection of IncRNA SNHG15 siRNA markedly reduced MMP-2 and MMP-9 expression. PTEN gene has phosphatase activity and regulates several cell growth signaling pathways, such as PI3K/AKT. PTEN protein can inhibit the development of tumors by antagonizing the activity of phosphorylase, such as tyrosine kinase [24, 25]. This study proved that overexpression of IncRNA SNHG15 inhibited PTEN expression, whereas downregulation of IncRNA SNHG15 expression promoted PTEN expression. It was indicated that IncRNA SNHG15 participates in oral cancer progression by regulating PTEN, MMP-2, and MMP-9. In further research, it is necessary to deeply analyze the expression of IncRNA SNHG15 in tumor tissues of patients with oral cancer, and its correlation with clinicopathological features, which provides a reference for clinical application of IncRNA SNHG15 as a therapeutic target.

Conclusion

Reduction of IncRNA SNHG15 expression in oral cancer KB cells inhibits proliferation, migration, and invasion of these cells.

Disclosure of conflict of interest

None.

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References


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