LncRNA PART1 promotes malignant biological behaviors associated with head and neck cancer cells by synergistic action with FUT6

Yanheng Yao  
Anhui Medical University

Jiyuan Shi  
Anhui Medical University

Yunran Gao  
Anhui Medical University

Xiling Xu  
Anhui Medical University

Yuxin Zhang  
Anhui Medical University

Suwen Bai  
The Chinese University of Hong Kong, Shenzhen & Longgang District People's Hospital of Shenzhen Guangdong

Jing Wu  
The First Affiliated Hospital of Anhui Medical University

Juan Du (✉ dujuan@cuhk.edu.cn)  
Anhui Medical University

Research Article

Keywords: Head and neck cancer, LncRNA PART1, FUT6, Apoptosis, Proliferation

Posted Date: October 12th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3418647/v1

License: ☑  This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License

Additional Declarations: No competing interests reported.
Abstract

The aim of this study was to determine the role of lncRNA PART1 in migration, proliferation, and apoptosis in head and neck cancer (HNC) cell lines and its relationship with FUT6 in tumorigenesis and progression. Bioinformatics analysis and qRT−PCR showed that IncRNA PART1 was expressed at low levels in HNC. The results of proliferation, apoptosis, migration and flow cytometry showed that the low expression of IncRNA PART1 inhibited the apoptosis of HN4 cells and promoted the migration and proliferation of HN4 cells. Through bioinformatics analysis, FUT6 was found to be expressed at low levels in HNC and correlated with patient survival. Immunohistochemical, qRT-PCR results showed that FUT6 was underexpressed in tumor tissues and HN4 cells. Cell and animal experiments showed that overexpression of FUT6 could inhibit tumor proliferation and migration. Bioinformatics analysis showed that lncRNA PART1 was positively correlated with FUT6. By qRT−PCR, we observed that after knockdown of IncRNA PART1, both the mRNA and protein expression levels of FUT6 were reduced. The above results indicated that IncRNA PART1 and FUT6 were poorly expressed in HNC and that IncRNA PART1 affected the development of cancer by interacting with FUT6.

1 Introduction

Head and neck cancer (HNC), which includes lip, mouth, nose, oropharynx, throat and nasopharyngeal cancer, causes almost 700,000 new cases and 380,000 deaths worldwide every year [1]. In addition, head and neck squamous cell carcinoma (HNSCC), representing 95% of HNC cases, is a biodiverse and genomic heterogeneous disease originating from the squamous mucosa of the upper digestive tract. Approximately 60% of patients with HNSCC have locally advanced or metastatic disease with low survival [2, 3]. Therefore, the discovery of efficient molecular markers and target proteins related to metastatic proliferation is of great significance for early diagnosis and treatment and may provide a possible therapeutic target for patients with HNC.

Long noncoding RNAs (lncRNAs) longer than 200 nt were initially considered transcriptional noise. However, accumulating evidence suggests that they may play a critical role in various cellular processes ranging from normal development to disease [4]. LncRNA dysregulation leads to malignant biological behaviors such as proliferation, invasion and metastasis to promote tumor progression [5–7]. Moreover, lncRNAs have high tissue specificity and stability and thus have potential as a biomarkers and therapeutic targets [8, 9]. The lncRNA prostate androgen regulates transcript 1 (PART1), which is androgenically regulated in human prostate cancer cells and may play a tumor suppressor role in prostate cancer [10]. Furthermore, downregulation of IncRNA PART1 can promote apoptosis and restrain proliferation of prostate cancer cells by regulating the Toll-like receptor pathway [11]. In bladder cancer, IncRNA PART1 is a tumor-inhibiting factor that helps to inhibit tumor proliferation and cell invasion and promote cell apoptosis [12]. In patients with oral squamous cell carcinoma, patients with high IncRNA PART1 expression survived longer than those with low IncRNA PART1 expression [13]. LncRNA PART1 is poorly expressed in glioma, and its poor expression is negatively correlated with overall patient survival [14]. Nevertheless, the physiological function of IncRNA PART1 in HNC is unclear.
In other aspects, glycosylation, as a universal type of protein modification, is involved in many biological processes. Fucosylation is the most common among the numerous glycosylations. Abnormal fucosylation and overexpression of fucosyltransferases (FUTs), together with catalytic glycoprotein substrates, has been reported to affect cancer cell proliferation [15]. Thirteen FUT genes have been identified in the human genome and are closely related to cancer pathogenesis and progression [16]. The high expression of FUT6 in colorectal cancer could enhance the migration, proliferation, invasion and angiogenesis of colorectal cancer cells *in vivo* and thereby promote tumor growth [17]. Previous studies have shown that high FUT6 expression is an independent poor prognostic factor in patients with acute myeloid leukemia (AML) and that FUT6 may be a therapeutic target for AML patients [18]. Low FUT6 expression was found in breast cancers with high expression of miR-106b. Downregulation of miR-106b expression in human breast cancer cells increased the expression of FUT6, which led to a significant decrease in the invasion, migration, and proliferation of cancer cells[19]. The interesting results of FUT6 in tumor cells suggest that FUT6 may play different roles in different cancer types. Although emerging evidence suggests that the FUT family could lead to cancer development and progression, their role in HNC has not been documented.

Therefore, the functions of IncRNA PART1 and FUT6 were extensively studied in HNC. Here, we showed that IncRNA PART1 was downregulated in HNC. Downregulation of IncRNA PART1 promoted the migration and proliferation of HN4 cells, suppressed the apoptosis of HN4 cells, and decreased FUT6 mRNA expression in HN4 cells. Moreover, in vivo and in vitro studies showed that the overexpression of FUT6 restrained the migration and proliferation of HN4 cells and promoted the apoptosis of HN4 cells. In conclusion, IncRNAs play a significant role in promoting HNC development through their interaction with FUT6, can serve as predictive biomarkers, and provide a range of potential targets for the treatment of HNC.

## 2 Materials and Methods

### 2.1 Cell culture

HN4 cells, a human HNC cell line, were purchased from KINDU (Shanghai, China) and cultured in high glucose DMEM containing penicillin−streptomycin solution and 10% fetal bovine serum (FBS). The human nasopharyngeal epithelial cell line (NP69 cell) was obtained from Fenghui Biological Research (Changsha, Hunan) and cultured in RPMI 1640 medium containing penicillin−streptomycin solution and 10% FBS. The cells were cultured in a 37 °C humidity-controlled incubator with 5% CO2.

### 2.2 Quantitative real time-polymerase chain reaction (qRT-PCR) analysis

Total RNA was extracted from cell or tissue samples with TRIzol reagent (Invitro-gen, USA). The first strand of complementary DNA (cDNA) of FUT6 was transcribed by plus all-in-one 1st Strand cDNA Synthesis SuperMix (JinAn protein, Shanghai). The SYBR green PCR mixture was detected by a Light Cycler 480 machine (Roche, USA). The first cDNA of IncRNA PART1 was transcribed using the LNRCUTE
IncRNA first-strand cDNA Kit (Tiangen, Beijing). PCR amplification was performed following the procedures of 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. The SYBR green PCR mixture was detected by a QuantStudio3 machine (ABI, USA). The relative quantitative expression of IncRNA PART1 and FUT6 was calculated using the \(2^{-\Delta\Delta CT}\) method. The GAPDH was used as an internal reference.

LncRNA PART1-forward: 5'-CTCTGGAAAGCTGAAAGGGCT-3'; LncRNA PART1-reverse: 5'-TGTCCTTTTCCCCCTGGACACA-3'; FUT6-forward: 5'-GCCTTTTAACAAACCACCATAGCT-3'; FUT6-reverse: 5'-GTTGTACATGACCTCTCGGTG-3'; GAPDH-forward: 5'-GGGGTCATTGATGGCAACAATA-3'; GAPDH-reverse: 5'-ATGGGGAAGGTGAAGGTC-3'.

2.3 Cell transfection

The target plasmid of lentiviral vector pCDH-FUT6-MCS-EF1-COPGFP-T2A-PURO or the control plasmid pCDH-CMV-MCS-EF1-COPGFP-T2A-PURO (purchased from General Biology, Anhui) and the package plasmid pSPAX2 and pMD2.G were mixed according to the ratio of pCDH-FUT6-MCS-EF1-COPGFP-T2A-PURO or pCDH-CMV-MCS-EF1-COPGFP-T2A-PURO: pSPAX2: pMD2.G = 4:3:1. The mixed plasmids were transfected into 293T cells with Lipofectamine 3000. After 48 h, the supernatant of cells containing lentivirus was collected. The obtained lentivirus and polybrene (Biyuntian, Shanghai) were added to HN4 cells. Forty-eight hours later, puromycin was added to obtain FUT6-overexpressing cells. Biomics (Shanghai, China) chemically synthesized two small interfering RNAs targeting lncRNA PART1 (si-lncRNA PART1#1 5-CTGTATGAATC GCCA TGAA GACT-3, si-lncRNA PART1#2 5-CCGCTAAACTGGACATTTCAAGA-3). Specific knockdown of lncRNA PART1 was achieved by transfection of lncRNA PART1 siRNA (200 nM) into HN4 cells using Lipofectamine 3000 and Opti-MEM (Invitrogen; Thermo Fisher Scientific). The expression levels of lncRNA PART1 in the total RNA of the transfected cells were determined by qRT-PCR.

2.4 Cell proliferation assay

HN4 cells were cultured in 96-well plates at a density of \(2.0\times10^5/cm^2\) in a 37 °C, 5% CO2 humidity-controlled incubator for 24 h. Cell viability was assessed with the cell count kit-8 (CCK-8) (Vazyme, Nanjing, China). A 10% volume of solution was added to the cells and incubated at 37 °C for 1 h. The amount of formazan dye generated was directly proportional to the number of viable cells, which was detected at an absorbance wavelength of 450 nm and quantified by an automatic microplate reader.

2.5 Immunohistochemistry

The 5 µm thick tissue sections were dewaxed, dehydrated and subjected to antigen repair. The reagents were added dropwise according to the reagent instructions (ZSGB-BIO, Beijing, Universal SP Kit (Mouse/Rabbit Streptomyces Ovalle in biotin assay system, SP-9000)), stained with DAB solution followed by hematoxylin solution, and finally sealed with neutral gum. The tissues were observed turning
a brown–yellow color under the Olympus microscope, and quantitative immunohistochemical analysis was performed with Image-Pro Plus.

2.6 Transferase-mediated dUTP nick-end labeling (TUNEL) assay

The cells were plated on a polylysine-coated slide, and then 4% paraformaldehyde was added to fix the cells for 30 min at 4 °C. After that, according to the instructions of the reagent (Vazyme, TUNEL FITC Apoptosis Detection Kit, A111-01), the cells were stained with a dye. Subsequently, the TUNEL-positive cells were analyzed by fluorescence inverted microscopy (Olympus, Japan), and the apoptosis rate was calculated by Image-Plus 6 software.

2.7 Flow cytometry

After digesting the cells with trypsin, the cells were washed twice with PBS and centrifuged at 2000 rpm and 4 °C for each wash. Then, 70% precooled ethanol was added to the cells and fixed at 4 °C overnight. Subsequently, the fixed cells were stained according to the instructions (C1052, Biyuntien, China), and the cell cycle was tested by flow cytometry (BD/BD FACSCanto II, USA). Data on cell fluorescence were collected to obtain the formation of a univariate karyotype histogram around the fluorescence area signal, and the percentage of nuclei in G1, S and G2/M phases were analyzed by FlowJo software.

2.8 Migration assay

Cells were seeded on 6-well plates until the cells in the wells were confluent, and then the monolayer was scratched with the tip of a sterilized pipette. The medium was then changed to medium without fetal bovine serum. Twelve hours later, the migration of cells along the scratched edges was photographed with a microscope. The results were represented as (S0 − St)/S0, where S0 represents the edge area scratched at the beginning, and St represents the edge area scratched after 12 h.

2.9 Xenograft mouse model of HNC tumor growth

Four-week-old male athymic BALB/C nude mice were purchased from Guangdong Yaokang Biotechnology Co.LTD. The FUT6-overexpressing HN4 cell lines and the control HN4 cell lines were separately mixed with biological matrix adhesive (Corning, USA) at a ratio of 1:1, and then the mixture was subcutaneously injected into the legs of nude mice at a concentration of 5×10^6 M. Tumor growth was recorded every three days. Four weeks later, the nude mice were sacrificed under isoflurane anesthesia. After size and weight measurements, tumors and organs of nude mice were collected and embedded for further study.

2.10 Statistical analysis

Statistical analysis with t tests was performed using GraphPad Prism V.8 software (GraphPad Software, San Diego, California) to calculate the mean ± SEM of the indicated number of samples. A two-sided value of \( p < 0.05 \) was considered statistically significant.
3 Results

3.1 Low expression level of lncRNA PART1 in head and neck cancer

By analyzing the genetic information of HNC tumor tissues and their corresponding adjacent normal tissue specimens in the HNC database (HNCDB), the results showed that the expression level of lncRNA PART1 in HNC tumor tissues was markedly reduced compared with that in their counterparts (Fig. 1A). Furthermore, we performed qRT–PCR analysis of tumor tissues and adjacent normal tissues from five HNC patients to determine the expression levels of lncRNA PART1 (Fig. 1B). These results showed that lncRNA PART1 plays an important role in HNC.

3.2 The role of low expression of lncRNA PART1 in HN4 cells.

A specific siRNA (si-lncRNA PART1) was synthesized to reduce the expression level of lncRNA PART1. As shown in Fig. 2A, the expression of lncRNA PART1 was notably downregulated after si-lncRNA PART1 transfection compared to the control group. The results indicated that our knockdown system of lncRNA PART1 was effective in inhibiting the expression of lncRNA PART1. The effect of the lncRNA PART1 expression level on apoptosis was evaluated by TUNEL assay. Apoptosis of HN4 cells was significantly reduced when lncRNA PART1 was knocked down (Fig. 2B). Moreover, the cell cycle was detected by flow cytometry. As expected, the proportion of cells in G0/G1 phase was notably decreased, and the proportion of cells in S phase was markedly increased after transfection of si-lncRNA PART1 (Figs. 2C and D). Knockdown of lncRNA PART1 enhanced cell proliferation. The CCK-8 method was applied to detect the proliferation activity of HN4 cells. The results indicated that the proliferation vitality in the si-lncRNA PART1 transfection group was notably increased in comparison with the control group (Fig. 2E). Moreover, the results of the scratch experiment showed that cell migration in the si-lncRNA PART1 group was notably increased in comparison with the control group (Fig. 2F). These results indicated that the low level of lncRNA PART1 could enhance the migration and proliferation and inhibit the apoptosis of HN4 cells; therefore, the expression level of lncRNA PART1 may play a crucial role in the progression of HNC.

3.3 FUT6 expression in HNC and the predicted relationship with lncRNA PART1

Coexpressed genes (CO-GENES) of lncRNA PART1 and the differentially expressed genes (DEGs) between HNC tumor tissues and corresponding adjacent normal tissues in the Human Cancer Metastasis Database (HCMDB) were further studied. As shown in Fig. 3A, 82 genes were found in the intersection of CO-GENES and DEGs. The KEGG signaling pathways for these 82 differentially expressed genes were analyzed, and then the first signaling pathway with a P < 0.01 was selected (Fig. 3B). There were 13 genes in this signaling pathway in the HCMDB. The results showed that 8 genes (FUT6, FUT2, ADH7, ATP6V0A4, CYP2C18, GALNT12, GCNT3, and GMDS) had significantly lower expression in patients with HNC (Fig. 3B).
In addition, to investigate the role of these eight genes in the overall survival of patients with HNC, the relevant data in the HCMDB were further analyzed. The Kaplan–Meier survival curve showed that the FUT6 level was remarkably correlated with the overall survival status of HNC patients. The lower the expression level of FUT6 in patients with HNC, the lower the overall survival rate (Fig. 3D). Furthermore, the comparison of the expression level of FUT6 in normal tissues and HNC tissues was studied by immunohistochemistry. The results indicated that the expression level of FUT6 was obviously lower in HNC tumor tissues than in normal tissues (Figs. 3E-F). The mRNA levels of FUT6 in HN4 cells and NP69 cells were measured by qRT–PCR. As indicated in Figs. 3G, the mRNA expression levels of FUT6 were clearly lower in HN4 cells than in the control group. The differentiated level of FUT6 showed that it may play a vital role in the occurrence and development of HNC.

### 3.4 The relationship of lncRNA PART1 with FUT6 and the role of FUT6 in HN4 cells

The coexpression of FUT6 and IncRNA PART1 was studied based on the starBase V3.0 database, and FUT6 was positively correlated with IncRNA PART1 (Fig. 4A). si-lncRNA PART1-2 was selected for qRT–PCR analysis to detect the mRNA level of FUT6 after IncRNA PART1 knockdown. The mRNA level of FUT6 was obviously reduced in the si-IncRNA PART1 group in comparison with the control group (Fig. 4B). The TUNEL results showed significantly enhanced apoptosis in FUT6-overexpressing HN4 cells (Fig. 4C). The CCK-8 assay indicated that the proliferation activity of FUT6-OE HN4 cells significantly decreased (Fig. 4D). The cell cycle was further studied by flow cytometry. As shown in Figs. 4E-F, the proportion of G0/G1 phase obviously increased and S phase significantly decreased in FUT6-OE HN4 cells in comparison with the control group, indicating the diminished proliferation ability of HN4 cells overexpressing FUT6. Moreover, the scratch experiment suggested that the cell migration ability was significantly reduced for FUT6-OE HN4 cells in comparison with the control group (Fig. 4G). Generally, IncRNA PART1 may interact with FUT6 to regulate the proliferation, apoptosis and migration of HN4 cells. The overexpression of FUT6 in HN4 cells promoted the apoptosis of HN4 cells and reduced their proliferation and migration.

### 3.5 The role of FUT6 overexpression in head and neck cancer cell growth in vivo

The above in vitro studies indicated that the overexpression of FUT6 might have antitumor effects. In vivo experiments on subcutaneous tumor formation in nude mice were performed. FUT6-OE HN4 cells or control HN4 cells transfected with an empty plasmid were subcutaneously injected into the hind limbs of BALB/c-Nu mice. Tumor growth in nude mice was observed and recorded every 3 days, and tumors were collected on the 28th day. As shown in Figs. 6A-C, the results showed that FUT6 overexpression notably impeded tumor growth, with decreased tumor weight and volume. The expression level of FUT6 in tumors was further studied by immunohistochemical analysis, which showed that the overexpression group had a higher level than the control group. It was also suggested that the expression of PCNA proliferating protein decreased in comparison with the control group (Figs. 5D-E). These in vivo results indicated that the overexpression of FUT6 blocks tumor growth.
4 Discussion

LncRNA PART1 and FUT6 play critical roles in tumorigenesis and cancer progression. In this study, we found that IncRNA PART1 was less expressed in HNC tissues than in adjacent normal tissues. When IncRNA PART1 was expressed at low levels, the results showed enhanced cell proliferation and migration as well as decreased cell apoptosis in HN4 cells, suggesting that IncRNA PART1 may play an important role in HNC. Interestingly, FUT6 was found to have lower expression levels in patients with HNC through bioinformatics studies. Moreover, the survival rate was found to positively correlate with the FUT6 expression level, which indicates that the FUT6 gene is vital in HNC patients. Immunohistochemistry revealed that FUT6 expression was decreased in HNC tissues in comparison with normal tissues. Furthermore, the results of qRT–PCR demonstrated that the mRNA expression levels of FUT6 were obviously lower in HN4 cells than in NP69 cells. The influence of FUT6 overexpression on the apoptosis, proliferation and migration of HN4 cells was further explored. The results of the TUNEL assay showed that FUT6 overexpression notably increased the apoptosis of HN4 cells. Both flow cytometry and CCK-8 assay showed that the proliferative activity of HN4 cells was notably decreased after the overexpression of FUT6. In addition, the scratch experiment suggested that the migration ability of FUT6-overexpressing HN4 cells was notably weakened. These results indicate that FUT6 overexpression can promote the apoptosis of HN4 cells and inhibit their migration and proliferation. In addition, bioinformatics studies on the coexpression of FUT6 and IncRNA PART1 based on the StarBase v3.0 database found that FUT6 and IncRNA PART1 were positively correlated. When IncRNA PART1 was knocked down in HN4 cells, both the mRNA levels of FUT6 decreased, demonstrating that IncRNA PART1 could regulate FUT6. Therefore, the influence of IncRNA PART1 on the proliferation, apoptosis and migration of cancer cells may function by regulating the level of translation and transcription of FUT6.

LncRNAs are involved in multiple physiological processes and play as a crucial role in the development of various diseases, including cancer [20]. IncRNA PART1 is prostate androgen regulatory transcript 1, which was first discovered as a new gene to be expressed mainly in the prostate and regulated by androgens in human prostate cancer cells [10]. It has also been reported that downregulating IncRNA PART1 could regulate the Toll-like receptor pathway, which inhibits the proliferation of prostate cancer cells and promotes the apoptosis of prostate cancer cells. Studies on colorectal cancer showed that the expression level of IncRNA PART1 was increased in cancer tissues and cells, and the proliferation and metastatic capacity of colorectal cancer cells were reduced after downregulation [21]. Our studies showed that IncRNA PART1 was underexpressed in patients with HNC, and its low expression could promote cancer cell migration and proliferation and inhibit cancer cell apoptosis.

Based on bioinformatics analysis, one gene from the fucosyltransferase family, FUT6, was discovered to be significantly associated with the overall survival status of patients with HNC. Fucosylation is one of the most important types of glycosylation in cancer [22]. FUT6 in this study is a biosynthetic enzyme in the FUT family, which can be used to catalyze the conversion of fucose from guanosine diphosphate β-L-fucose transfer to various sugar substrate receptors of oligosaccharides, glycoproteins and glycophospholipids [23]. In total, 13 FUT genes have been found in the human genome, which are closely
related to pathological processes such as cancer occurrence and tumor progression. For example, FUT4 is involved in the metastasis and proliferation of breast cancer and can be used as a potential biomarker for the diagnosis and prognosis of breast cancer [24]. FUT5 and FUT6 are highly expressed in colorectal cancer and promote tumor growth by promoting the proliferation, invasion, migration and angiogenesis of colorectal cancer cells in vivo [17]. Low expression of FUT6 has been found in breast cancers with high expression of miR-106b. Downregulation of miR-106b in human breast cancer cells can increase FUT6 expression leading to a significant decrease in the migration, invasion, and proliferation of cancer cells [19]. In this study, we found that the expression of FUT6 was decreased in HNC. The lower the expression of FUT6 was, the lower the overall survival rate of patients with HNC. The migration and proliferation ability of HN4 cells was reduced, and apoptosis was enhanced after the overexpression of FUT6 in HN4 cells. Similarly, in vivo experiments also demonstrated that the proliferative capacity of tumor cells decreased after the overexpression of FUT6. Therefore, FUT6 may be an effective target to prevent the development and metastasis of HNC.

Generally, lncRNAs can regulate gene expression within the nucleus at both the epigenetic and transcriptional levels or at the posttranscriptional and translational levels in the cytoplasm [25]. In addition, lncRNA is reported to be involved in various biological processes, including sponging miRNA to translate mRNA, maintaining protein stability, regulating glucose metabolism and cell signal transduction [26–28]. In tongue squamous cell carcinoma tissues and cell lines, it was found that lncRNA PART1 had a low expression level and miR-503-5p had a high expression level. Further studies found that lncRNA PART1 could play a tumor suppressive role in tongue squamous cell carcinoma by targeting miR-503-5p [29]. LncRNA have also been reported to regulate mRNA stability or translation and affect cellular signaling cascades. For example, lncRNA Staufen-1 was demonstrated to mediate mRNA attenuation by interacting with the double-stranded RNA in the 3’UTR of mRNA [30]. Markus et al. deeply studied the lncRNA TINCR as a critical lncRNA required for somatic histodifferentiation by interacting with a series of differentiated mRNAs [31]. LncRNA-p21 was also reported to selectively inhibit the translation of these mRNAs by interacting with JUNB and CTNNB1 mRNA [32]. In our studies, lncRNA PART1 was found to be positively correlated with FUT6. When lncRNA PART1 was knocked down in HN4 cells, both the mRNA expression levels of FUT6 were reduced, indicating that lncRNA PART1 could regulate the translation and transcription of FUT6. In other words, lncRNA PART1 may affect the proliferation, apoptosis and migration of HNC cells through the regulation of FUT6.

**Declarations**

**Data Availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Funding**
This work was supported by grants from the National Natural Science Foundation of China [grant number 81972539 and 82273391] and the Longgang Medical and Health Science and Technology Project [grant number LGKCYLWS2022004].

Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

Acknowledgments

The authors would like to thank all the reviewers who participated in the review and all those who participated in this study. YHY conceived and designed the study. YHY, MHW, JYS and YRG performed the experiments. SWB, XLX and YXZ performed the bioinformatics analysis. YHY wrote the manuscript. JW, BGW and JD reviewed the data and revised the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Tumor and normal tissue samples were collected with informed consent from patients at the First Affiliated Hospital of Anhui Medical University (Protocol code 20190340). A portion of the tissue samples was made into wax blocks for immunohistochemical analysis, and another portion was frozen in liquid nitrogen for further analysis. The protocol for the use of the mice was approved by the Animal Experimentation Ethics Committee of Anhui Medical University (protocol code 20190327).

References


Figures
Figure 1

Low expression of lncRNA PART1 in head and neck cancer (A) Expression of IncRNA PART1 in HNC data from the HNCDB (http://hncdb.canceromics.org/). (B) The relative IncRNA PART1 expression levels were detected by using qRT-PCR in HNC tissues (n=5) and adjacent normal tissues (n=5). Values represent means ± SEMs. ***p<0.001, compared with the control group. HNC, head and neck cancer. Ctrl, control group.
Low expression of lncRNA PART1 inhibited apoptosis of HN4 cells and promoted cell proliferation and migration (A) Transfection efficiency of si-lncRNA PART1 was determined by qRT-PCR (n=3). (B) A TUNEL assay was used to detect the apoptosis rate of HN4 cells with transfected with si-lncRNA PART1 (n=3). Scale bar=100 μm. (C) A CCK-8 assay was used to detect the effect of si-lncRNA PART1 on HN4 cell proliferation (n=6). (D) Representative cell cycle images and (E) Summary data showing that low
expression of lncRNA PART1 promotes proliferation and increases the number of cells in the S phase (n=5). (F) Representative photos showing cell migration of HN4 cells transfected with si-lncRNA PART1 and the ratios of cell migration (n=5). β-tubulin or GAPDH was used as a loading control. Values represent means ± SEMs. *P<0.05, **P<0.01, compared with control group. si-lncRNA PART1, siRNA-LncRNA PART1. Ctrl, control group.

Figure 3.
FUT6 expression in head and neck cancer and the predicted relationship with IncRNA PART1 (A) Venn diagram showing the intersection of the predicted IncRNA PART1 correlation genes and HNC differential genes. (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The top 15 KEGG pathway terms enriched in the intersection of IncRNA PART1 correlation genes and HNC differential genes. (C) Eight genes in the TCGA database were expressed at low levels in patients with HNC. (D) Kaplan–Meier survival plot of FUT6 related to OS. Downregulated samples are in blue, while upregulated samples are in red. Survival months are shown along the x-axis. Overall survival rates are shown along the y-axis. (E) Protein expression of FUT6 in HNC tissues (n=5) and adjacent normal tissues (n=5) analyzed by immunohistochemistry. The expression of FUT6 was significantly lower in HNC tissues. Scale bar: 50 μm. (F) Quantification of FUT6 protein expression levels in HNC tissues and adjacent normal tissues. (G) The mRNA level of FUT6 in NP69 cells and HN4 cells was measured by qRT–PCR (n=3). Values represent means ± SEMs. **P<0.01, compared with the control group. HNC, head and neck cancer. Ctrl, control group.
LncRNA PART1 interacts with FUT6 and the role of FUT6 in HN4 cells (A) The correlation between LncRNA PART1 and FUT6 was identified and analyzed using StarBase V3.0. (B) The mRNA level of FUT6 in HN4 cells was measured by qRT–PCR after si-LncRNA PART1 transfection (n=6). (C) A TUNEL assay was used to detect the apoptosis rate of HN4 cells with overexpressing of FUT6 (n=3). Scale bar=100 μm. (D) A CCK-8 assay was used to detect the effect of FUT6 overexpression on HN4 cell proliferation (n=6). (E)
Representative cell cycle images and (F) summary data showing that the overexpression of FUT6 inhibits proliferation and decreases the number of cells in the S phase (n=4). (G) Representative photos showing cell migration of HN4 cells with overexpressing of FUT6 and the ratios of cell migration (n=5). β-tubulin or GAPDH was used as a loading control. Values represent means ± SEMs. *P<0.05, **P<0.01, ***P<0.001, compared with the control group. FUT6-OE, overexpression of FUT6. Ctrl, control group.

Figure 5.
The role of FUT6 overexpression in head and neck cancer cell growth *in vivo* (A) Tumors derived from HN4 cells with stable overexpression of FUT6 and control HN4 cells were collected (n=6). (B-C) Tumor volume and weight were observed in the two groups. (D) Protein expression of FUT6 and PCNA in FUT6-OE tumor tissues (n=3) and control tumor tissues (n=3) analyzed by immunohistochemistry. Scale bar: 50 μm. (E) Quantification of FUT6 protein and PCNA protein expression levels in FUT6-OE tumor tissues and control tumor tissues. Values represent means ± SEMs. **P<0.01, ***P<0.001, compared with the control group. FUT6-OE, overexpression of FUT6. Ctrl, control group.