Original Article: LncRNA XIST promotes the progression of laryngeal squamous cell carcinoma via sponging miR-125b-5p to modulate TRIB2

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ABSTRACT

Introduction: In our study, we investigated the expression of XIST in LSCC cells and tissues and its functional role in cell proliferation, anti-apoptosis, migration and invasion from LSCC cells. Meanwhile, the relationship between XIST, miR-125b-5p, and TRIB2 was also revealed, which may provide a good target for the treatment of LSCC associated with the XIST/miR-125b-5p/TRIB2 axis. **Material and Methods:** Transduced cells (2.5 x 103 cells/100 µl)/well) were prepared for growth analysis and seeded in 96 well plates. Cells were cultured for 24, 48, and 72 h until 10 µl of CCK-8 reagent (DOJINDO, Kumamoto, Japan) was added to each well and cultured for an additional 4 h. Optical density (OD value) was measured at an absorbance of 450 nm using a microplate reader. **Results:** LSCC cell lines (AMC-HN-8 and M4E cells) and nasopharyngeal epithelial cells (NP69 cells) were also selected to examine XIST expression. qRT-PCR analysis data showed higher XIST in LSCC cells compared to NP69 cells. In summary, we found that XIST as an oncogene in LSCC may be an indicator of cancer progression. **Conclusion:** In this study, lncRNA XIST and TRIB overexpression and miR-125b-5p downregulation were observed in LSCC tissues and cells. High-grade XIST often indicates poor prognosis in LSCC patients. Inhibition of XIST slows cell growth and impairs cell metastasis and anti-apoptotic effects. Both XIST and TRIB2 have binding sites for miR-125b-5p, which was predicted for the first time in our study.

Introduction

In the Laryngeal cancer is the second most common squamous cell carcinoma (SCC) in ear, nose, and throat surgery. Laryngeal squamous cell carcinoma (LSCC) accounts for approximately 95% of pathological laryngeal cancer cases, and its incidence has increased in recent years [1-3]. Breathing, speaking, and swallowing are important functions in the body of the lungs, so although people have made efforts to treat the cancer, the pain and death rate of throat cancer is often high [4]. Therefore, new biological markers related to the evaluation and diagnosis of LSCC patients need to be found and more appropriate and appropriate treatments need to be investigated(Fig1).

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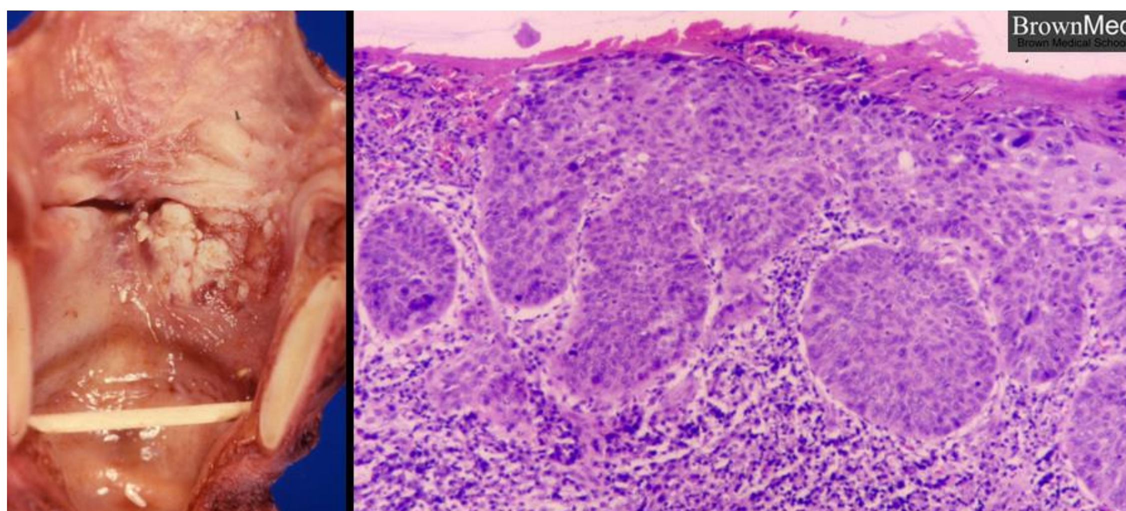

Figure 1: Laryngeal cancer cells

Long non-coding RNAs (lncRNAs) were identified as important cis-trans regulators of gene expression with a length of more than 200 nucleotides, involved in the pathological process of human cancer with LSCC [5-7]. X inactivation specific transcript (XIST) is a novel lncRNA located in the X inactivation center (XIC) that can alter heterochromatin stability, leading to changes in gene expression, thus affecting Cancer disease. XIST-induced chromosomal inactivation is associated with negative selection in fibrosarcoma cells [8]. Meanwhile, previous studies have shown that XIST overexpression is associated with poor prognosis in patients with various cancers, such as breast cancer, prostate cancer pancreas (PC), and brain. However, the role of XIST in LSCC is not clearly defined [9].

Recent studies have shown that many microRNAs (miRNAs) are involved in cancer processes as targets of lncRNAs. In osteosarcoma (OA), not only miR-1277-5p but also miR-137 were identified as binding targets of lncRNA XIST, and they were downregulated in OA tissues and cells. Shen et al. It has also been reported that upregulation of miR-429 caused by loss of XIST in PC cells inhibits epithelial-to-mesenchymal transition (EMT) ability [10-12]. Previous studies have shown that miR-125b-5p is downregulated in esophageal squamous cell carcinoma (ESCC) and negatively regulates HMGA2 expression . Other researchers have identified miR-125b-5p as a potential biomarker for LSCC. However, the potential regulatory role of miR-125b-5p in LSCC and its association with XIST is rarely reported in LSCC [13-15].

Kinase-like protein tribbles homolog 2 (TRIB2) is associated with liver cell survival as an important regulator of the Wnt signaling pathway [16]. Overexpression of TRIB2 is also found in acute myeloid leukemia (AML) cells, and TRIB2 functions as an oncogene by regulating C/EBPα and E2F1 repression. Histological studies of TRIB2 in cancer showed that the development of miR-511 or miR-1297 contributed to the arrest of cell proliferation induced by TRIB2 inhibition in lung adenocarcinoma cells [17-19]. Considering the importance of TRIB2 in cancer, it is important to investigate its role in LSCC.

In our study, we investigated the expression of XIST in LSCC cells and tissues and its functional role in cell proliferation, anti-apoptosis, migration and invasion from LSCC cells. Meanwhile, the relationship between XIST, miR-125b-5p [20], and TRIB2 was also revealed, which may provide a good target for the

treatment of LSCC associated with the XIST/miR-125b-5p/TRIB2 axis [21].

Material and Methods

Statements on ethics and tissue collection

The issue of ethics of tissue and similar tissues is monitored by the Direct Committee at the First People's Hospital of Jining City, Shandong Province. Cancer tissues were collected from 40 patients who signed informed consent after surgery at the First People's Hospital of Jining City, Shandong Province, and were immediately stored at −80 °C. Animal studies were performed at the First Human Hospital of Jining City, Shandong Prefecture [22]. During the experimental study, we used 2% methoxyflurane to euthanize mice in accordance with the regulations of the National Institutes of Health [23-25].

Cell Culture and Transfection

LSCC cell lines (AMC-HN-8 and M4E cells) and nasopharyngeal epithelial cells (NP69 cells) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China).) and 10% fetal bovine serum (FBS; Invitrogen) were maintained in Dulbecco Culture in modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA, USA) at 37 °C, 5% CO and humidified air. Vectors or oligonucleotides (such as small interfering RNA (siRNA) against XIST (si-XIST), has-miR-125b-5p mimic/inhibitor, pcDNA-TRIB2 vector, and all competition controls) were derived from GenePharma (Shanghai, China). .) was developed and transfected into AMC-HN-8 and M4E cells using Lipofectamine® 2000 reagent (Invitrogen). Please see instructions for specific transfer steps. At 48 h post transfection, cells were harvested for subsequent analyses.

RNA isolation and quantitative reverse transcription polymerase (qRT-PCR)

TRIzol Reagent ((Invitrogen) and chloroform were used to isolate total RNA of LSCC tissues or cells, and then the total RNA was precipitated with iso-propanol (VWR International). The RNA precipitation was purified by 70% ethanol and air-dried and then resuspended in sterile water (without nuclease). The concentration of total RNA was detected by an Eon™ Microplate Spectrophotometer (BioTek Instruments, Inc., Winooski, VT). One Step PrimeScript miRNA cDNA synthesis kit (Takara Bio Inc., Dalian, China) was used to carry out the reverse transcription reaction. SYBR® Premix Ex Taq™ II (Takara) was used for PCR on a MiniOpticon™ (Bio Rad, Hercules, CA, U.S.A.). Levels of XIST, miR-125b-5p, and TRIB2 were calculated using the 2−ΔΔCT method and normalized to U6 small nuclear RNA (U6-snRNA) and housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. PCR conditions were: denaturation (30 seconds, 94°C), annealing (30 seconds, 58°C), and extension (30 seconds, 72°C, 30 cycles). Primer sequences are as follows: -3'; TRIB2, forward 5'-CACAAGGTCTACCCCCATCAC-3', reverse 5'-CCCGATACAAGAAACGCAAT-3'; GAPDH, forward 5'-CCAAAATTAGATGGGGCAATGCTGCTGGGG-3', reverse 5'-GTCGATGATGATGATGATGAT-3''; U6, Forward 5′-CTCGCTTCGCAGCACA-3′, Reverse 5′-AACGCTTCACGAATTTGCGT-3′.

Cell Counting Kit-8 (CCK-8) Assay

Transduced cells (2.5 x 103 cells/100 µl)/well) were prepared for growth analysis and seeded in 96 well plates. Cells were cultured for 24, 48, and 72 h until 10 µl of CCK-8 reagent (DOJINDO, Kumamoto, Japan) was added to each well and cultured for an additional 4 h. Optical density (OD value) was measured at an absorbance of 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

Flow cytometry assay

Annexin V-fluorescein isothiocyanate (using FITC)/propidium iodide (PI) apoptosis detection kit (Immunotech, Marseille, France)

Cells Apoptosis assay. All experimental cells were collected and the concentration was adjusted to 1 × 106 cells/ml. About 200 μl cell suspension was used for assay and was re-suspended in 300 μl binding buffer and gently mixed with 5 μl Annexin V-FITC/PI for 15 min in the dark. FACScan flow cytometer (Becton Dickinson, San Jose, CA, U.S.A.) was used for analysis of apoptosis rate.

Transwell assay

Transfected cells were incubated with serum-free DMEM for 12 h until Transwell assay. Matrigel (Corning Life Sciences, Corning, NY, U.S.A.) was stored at 4°C overnight for preparation. The Transwell chambers were placed into each well of 24 well-plate, respectively. DMEM (500 μl containing 20% FBS) was added to each lower chamber; At the same time, transfected cells were seeded into the upper chamber at a concentration of 1 × 10 cells/100 μl/well (using free DMEM) for migration testing or inoculated into the upper chamber coated with 10 μl of Matrigel. infestation test. After 48 hours of routine operation, remove the Transwell chamber, gently wipe the cells inside, and place in a new 24-well plate containing 4% paraformaldehyde (600 μl). After fixation for 5 min, cells were stained with 0.1% crystal violet for 10 min. Finally, cells at five random locations were counted under an inverted microscope.

Dual Luciferase Reporter Assay

XIST or TRIB2 sequences were synthesized and cloned into the pGL3 dual luciferase reporter vector (Promega, Madison, MI, USA). The new reporter constructs were named pGL3-XIST-WT (XIST wild type), pGL3-XIST-Mut (XIST mutant), pGL3-TRIB2-WT (TRIB2 wild type), and pGL3-TRIB2 -Mut (TRIB2 mutant).). pGL3-XIST-WT and pGL3-TRIB2-WT predicted binding sites for miR-125b-5p. The above reporters were cotransfected with miR-NC or miR-125b-5p into AMC-HN-8 and M4E cells using Lipofectamine® 2000. After 48 hours, relative luciferase activity was determined using a dual luciferase assay kit (Promega). and normalized to Renilla.

Western Blot Analysis

Add RIPA lysis buffer to tissues or cells to remove proteins and measure total protein concentration by the bicinchoninic acid (BCA) method using the BCA kit (Pierce Biotechnology, Inc., Rockford, IL,). UNITED STATES OF AMERICA.). Target proteins were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then the separated proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Bradford, MA, USA) at 100 cm per cm. All membranes were incubated in 5% nonfat milk for 2 h at room temperature and in diluted TRIB2 or β-actin primary antibodies overnight at 4°C. After the membrane was washed twice with phosphate-buffered saline and Tween 20 (PBST), it was sequentially incubated with horseradish peroxidase-labeled goat anti-rabbit secondary antibody for 1 h at 37°C.

The above antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). On a gel imager (Bio-Rad), electrochemiluminescence (ECL; Pierce) was dropped to coat the hybridization membrane for photography. Finally, the test results were analyzed using Quantity One software.

Results

First, XIST expression in 40 pairs of LSCC tissues and adjacent tissues was evaluated using qRT-PCR. As shown in Figure 1A, XIST was significantly upregulated in LSCC tissues compared with adjacent tissues. In addition, analysis of 5-year overall survival by the Kaplan-Meier method showed that high-grade XIST was associated with poor survival (P = 0.0423) (Figure 1B). Expression of XIST correlated with tumor size, TNM stage, tumor differentiation,

and metastasis in LSCC patients. LSCC cell lines (AMC-HN-8 and M4E cells) and nasopharyngeal epithelial cells (NP69 cells) were also selected to examine XIST expression. qRT-PCR analysis data showed higher XIST in LSCC cells compared to NP69 cells (Figure 1C). In summary, we found that XIST as an oncogene in LSCC may be an indicator of cancer progression.

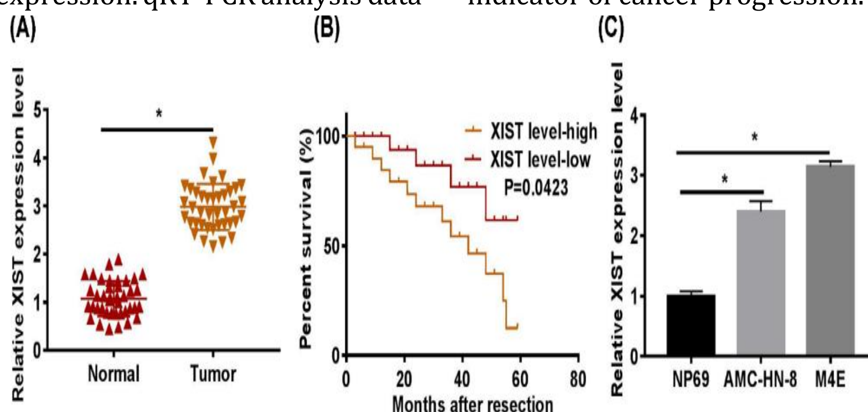

Figure 2: (A) XIST expression in LSCC tissues and matched normal tissues was detected by qRT-PCR (n= 40). (B) Correlation between XIST expression and prognosis of patients with LSCC was analyzed. (C) QRT-PCR analysis of XIST expression was performed in LSCC cells (AMC-HN-8 and M4E cells) and nasopharyngeal epithelial cells (NP69 cells); *P <0.05.

Next, we performed an experimental study to investigate the function of XIST in LSCC cells. Loss of XIST was achieved by transfecting small interfering RNA targeting XIST into AMC-HN-8 and M4E cells for 48 h. QRT-PCR results showed that XIST expression was reduced in LSCC cells (Figure 2A, B). Cell proliferation was then stopped in XIST-inhibited AMC-HN-8 and M4E cells (Figure 2C, D). Flow cytometry results showed that XIST inhibition promoted cell apoptosis (Figure 2E, F). Additionally, knockdown of XIST in AMC-HN-8 and M4E cells reduced cell migration and invasion (Figure 2G,H). In general, XIST plays an oncogenic role in LSCC by inhibiting apoptosis while promoting the growth and metastasis of LSCC cells.

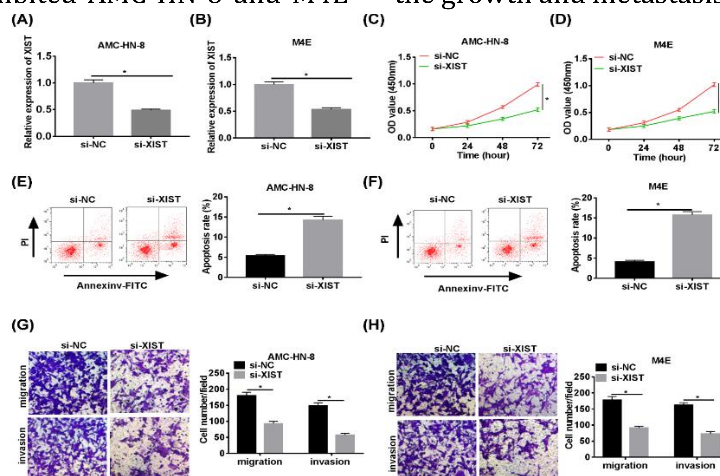

Figure 2: Notes: (A and B) Si-NC or si-XIST was transfected into AMC-HN-8 and M4E cells, and the interference effects were detected by qRT-PCR at 48 h post transfection. (C and D) Cell proliferation was examined by CCK-8 assay and (E and F) flow cytometry was used to detect cell apoptosis. (G and H) Transwell assay was conducted to measure migration and invasion of transfected cells; *P <0.05.

There was an increase in miR-125b-5p and XIST as shown in Figure 3A. To investigate whether miR-125b-5p is the target of XIST, dual luciferase assay was performed by transfecting wild-type and mutant XIST (XIST-WT and XIST-Mut) with miR-NC or miR-125b. simulated entry in AMC-HN-8 and M4E cells, respectively. Luciferase activity was reduced in AMC-HN-8 and M4E cells cotransfected with XIST-WT and miR-125b-5p, but there was no difference between other groups (Fig. 3B,C). The regulatory relationship between miR-125b-5p and XIST was then further investigated in LSCC cells by transfecting si-XIST. Deletion of XIST in AMC-HN-8 and M4E cells resulted in increased miR-125b-5p expression (Figure 3D,E). We then found low levels of miR-125b-5p in LSCC cells and tissues (Figure 3F,G). Generally speaking, miR-125b-5p may be a tumor suppressor from XIST.

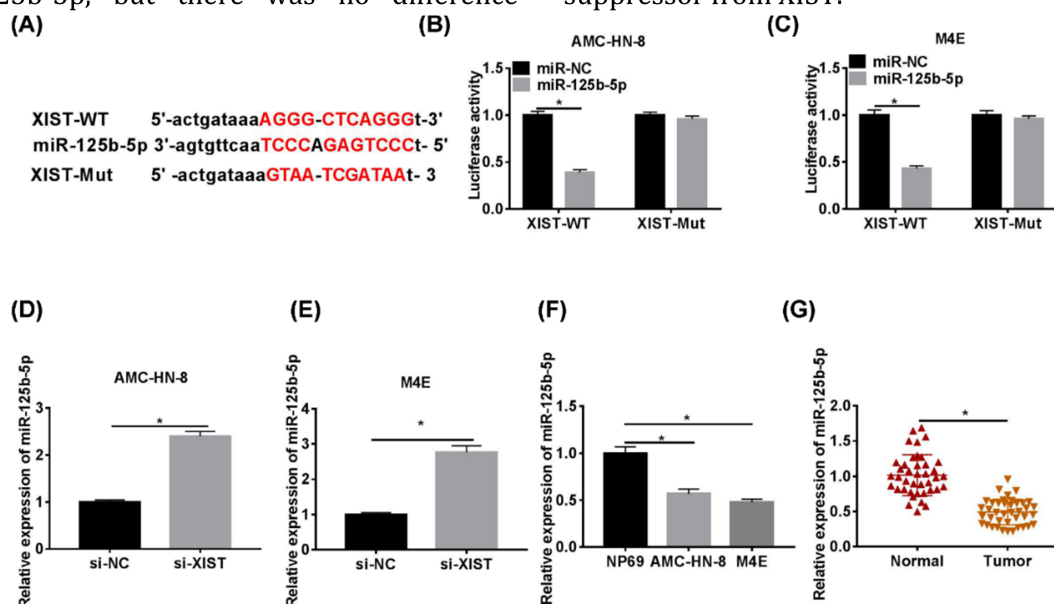

Figure 3: Notes: (A) The binding site alignment of miR-125b-5p was predicted in XIST. (B and C) Luciferase activity was measured by Dual-luciferase reporter assay in cells co-transfected with pGL3-XIST-Wt or pGL3-XIST-Mut vectors and miR-NC or miR-125b-5p. (D and E) The relationship between miR-125b-5p and XIST was detected by qRT-PCR in LSCC cells. (F and G) The relative expression of miR-125b-5p was examined by qRT-PCR in LSCC cells and tissues (n= 40); *P <0.05.

Discussion

In this study, we identified lncRNA XIST as a novel marker of laryngeal squamous cell carcinoma (LSCC), and XIST overexpression is closely associated with the poor prognosis of LSCC patients [26-28]. In previous reports, high levels of XIST in LSCC were implicated in vitro and in vivo, which promoted tumor growth and reduced apoptosis [29]. Similar experiments were performed in our study and we found that XIST was significantly increased in AMC-HN-8 and M4E cells as well as in LSCC tissues. In addition, higher XIST expression generally represents lower 5-year survival in LSCC patients according to Kaplan-Meier analysis. These findings are consistent with the report by Xiao et al., in addition, silencing the XIST gene will slow down cell proliferation and weaken the resistance to apoptosis, migration, and invasion of LSCC cells, further determining the effect of XIST on LSCC [30-32].

Recently, miR-92b has been investigated and validated as a target inhibitor of XIST in hepatocellular carcinoma (HCC). Similarly, Xing et al. reported that exosomal miRNA-503 accumulation by deletion of XIST led to

inhibition of tumor metastasis, providing a therapeutic advantage to the breast [33-35]. These studies show that miRNA is associated with lncRNA XIST. MiR-125b-5p expression is increased in hepatitis B virus (HBV)-infected HCC. However, in contrast, a decrease in miR-125b-5p was detected in LSCC cells, and miR-125b-5p regulates cellular glycolysis and growth by directly targeting hexokinase-2 (HK2), an important enzyme in the glycolysis process. Using online software [36-38], we found that miR-125b-5p, the target of XIST, was negatively expressed in LSCC cells (AMC-HN-8 and M4E cells) and tissues corresponding to Luqman cells. In our study, si-XIST) was delivered to LSCC cells and caused significant upregulation of miR-125b-5p, thus inhibiting the growth and metastasis of LSCC cells [39-41]. Meanwhile, anti-miR-125b-5p expression can effectively abrogate XIST loss. These data suggest that the promoting effect of XIST on LSCC progression is mediated by direct inhibition of miR-125b-5p expression [42].

Tribbles homolog 2 (TRIB2) belongs to the Tribbles pseudokinase and plays an important role in the etiology of many cancers. Researchers demonstrated that TRIB2 expression is elevated in breast cancer (CRC) and that TRIB2 overexpression relies in part on direct regulation of p21 expression and modulation of AP4 expression to promote growth cell growth and delay CRC cell senescence. In another study, activation of TRIB2 in lung adenocarcinoma cells could be reduced by increasing miR-206/miR-140, thus attenuating the invasion and growth of cells. We found that miR-125b-5p binds to the 3'UTR of TRIB2 and low TRIB2 expression was detected in LSCC cells with upregulated expression of miR-125b-5p. There is also high expression of TRIB2 mRNA and protein in LSCC cells and tissues [43-45]. A similar role of TRIB2 in cancer was discovered by Xin et al. Additionally, our rescue experiments showed that upregulation of TRIB2 reversed the effects of miR-125b-5p overexpression on LSCC cell proliferation [46], anti-apoptosis, and metastasis. Mechanistically, TRIB2 expression was reduced in si-XIST-treated cells, whereas deletion of miR-125b-5p clearly abolished this effect [47-49]. Additionally, carcinogenic effects of XIST were detected in mouse xenograft models. These results therefore indicate that silencing XIST down-regulates TRIB2 expression via miR-125b-5p in sponge LSCC cells. However, due to limitations, we will complete the relevant in vivo experiments in the future [50].

Conclusion

In this study, lncRNA XIST and TRIB overexpression and miR-125b-5p downregulation were observed in LSCC tissues and cells. High-grade XIST often indicates poor prognosis in LSCC patients. Inhibition of XIST slows cell growth and impairs cell metastasis and anti-apoptotic effects. Both XIST and TRIB2 have binding sites for miR-125b-5p, which was predicted for the first time in our study. Downregulation of XIST increased the expression of miR-125b-5p, while upregulation of miR-125b-5p reversed the inhibitory effect of LSCC cells treated with XIST knockdown. On the other hand, miR-125b-5p negatively regulates TRIB2 and reverses the induction of XIST on TRIB2 expression in LSCC cells. Our results provide a reliable XIST-miR-125b-5p-TRIB2-associated therapy for LSCC.

References

[1] R. Azhough et al., Pilonidal sinus surgery with mechanical drilling technique. Indian Journal of Surgery. **2020**;82:824-7. [Crossref], [Google Scholar], [Publisher]

[2] N. Forghani, et al., Fulminant Guillain-Barre syndrome associated with SARS-CoV-2: A case series. J Clin Images Med Case Rep. **2022**;3(1):1626. [Crossref], [Google Scholar], [Publisher]

[3] K. Shahsavarinia, et al., Diagnostic value of red blood cell distribution width in patients with organophosphate poisoning in emergency department of Sina hospital in 2019. J Clin Images Med Case Rep. **2022**;3(2):1634. [Crossref], [Google Scholar], [Publisher]
[4] S. Eskandari, et al., Relationship between patent foramen ovale and COVID-19 in patients admitted to an intensive care unit. Revista Espanola De Cardiologia (English Ed). **2021**;74(8):725. [Crossref], [Google Scholar], [Publisher]
[5] P. Jalali, et al., GUCA2A Dysregulation as a Promising Biomarker for Accurate Diagnosis and Prognosis of Colorectal Cancer. **2023**. [Crossref], [Google Scholar], [Publisher]
[6] SM. Haghdoost, MK. Gol, The necessity of paying more attention to the neurological and psychological problems caused by the COVID-19 pandemic during pregnancy. Health. **2020**;3(4). [Crossref], [Google Scholar], [Publisher]
[7] MK. Gol, D. Aghamohamadi., Effect of massage therapy with and without elastic bandaging on pain, edema, and shoulder dysfunction after modified radical mastectomy: a clinical trial. International Journal of Women's Health and Reproduction Sciences. **2020**;8(1):73-8. [Crossref], [Google Scholar], [Publisher]
[8] MK. Gol, et al., Design and psychometrics cultural competence questionnaire for health promotion of Iranian nurses. Journal of education and health promotion. **2019**;8. [Crossref], [Google Scholar], [Publisher]
[9] K. Hashemzadeh, et al., Effects of Foot Reflexology on Post-sternotomy Hemodynamic Status and Pain in Patients Undergoing Coronary Artery Bypass Graft: A Randomized Clinical Trial. Crescent Journal of Medical & Biological Sciences. **2019**;6(4). [Google Scholar], [Publisher]
[10] M. Khanbabayi Gol, et al., Frequency ratio of carpal tunnel syndrome in women with breast cancer treated with lymphedema in Tabriz medical education centers; 2018-2019. The Iranian Journal of Obstetrics, Gynecology and Infertility. **2020**;22(12):62-8. [Crossref], [Google Scholar], [Publisher]
[11] L.H Anvar, et al., The study of rs324420 (C385A) polymorphism of the FAAH gene of the endocannabinoid system in patients with epilepsy and ADHD. Epilepsy Research, **2023**. 192: p. 107100. [Crossref], [Google Scholar], [Publisher]
[12] A. Mobed, et al., Biosensors technology for anti-epileptic drugs. Clinica Chimica Acta, **2022**. 533: p. 175-182. [Crossref], [Google Scholar], [Publisher]
[13] S. Charsouei, S., et al., Evaluation of Three- and Five-Year Disease-Free Survival in Patients With Cervical Cancer With Spinal Cord Metastasis Under Treatment With External Radiotherapy+ Brachytherapy. International Journal of Women's Health & Reproduction Sciences, **2022**. 10(2). [Google Scholar], [Publisher]
[14] M. Zamani Esfahlani, et al., Oral Levothyroxine Prophylactic Administration on Bleeding and Hemoglobin Changes During and after Total Hip Arthroplasty in Patients with a History of Ischemic Heart Disease: A Randomized Clinical Trial. Trauma Monthly, **2023**. 28(3): p. 805-814. [Google Scholar], [Publisher]
[15] K. Komlakh, et al., Designing a Guideline for Elective and Emergency Spinal Cord Surgeries During the COVID-19 Pandemic. Trauma Monthly, **2022**. 27(Especial Issue (COVID-19 and Emergency Medicine)): p. 1-7. [Crossref], [Google Scholar], [Publisher]
[16] S. Charsouei, et al., Effects of COVID-19 pandemic on perceived stress, quality of life, and coping strategies of women with breast cancer with spinal metastasis under chemotherapy. Int. J. Women's Health Reprod. Sci, **2021**: p. 55-60. [Crossref], [Google Scholar], [Publisher]

[17] S.M. Haghdoost, and M.K. Gol, The necessity of paying more attention to the neurological and psychological problems caused by the COVID-19 pandemic during pregnancy. Health, **2020**. 3(4). [Crossref], [Google Scholar], [Publisher]

[18] M.K. Gol, and D. Aghamohamadi, Effect of massage therapy with and without elastic bandaging on pain, edema, and shoulder dysfunction after modified radical mastectomy: a clinical trial. International Journal of Women's Health and Reproduction Sciences, **2020**. 8(1): p. 73-8. [Crossref], [Google Scholar], [Publisher]

[19] M.K. Gol, A. Dorosti, and M. Montazer, Design and psychometrics cultural competence questionnaire for health promotion of Iranian nurses. Journal of education and health promotion, **2019**. 8. [Crossref], [Google Scholar], [Publisher]

[20] K. Hashemzadeh, M. Dehdilani, and M.K. Gol, Effects of Foot Reflexology on Post-sternotomy Hemodynamic Status and Pain in Patients Undergoing Coronary Artery Bypass Graft: A Randomized Clinical Trial. Crescent Journal of Medical & Biological Sciences, **2019**. 6(4). [Google Scholar], [Publisher]

[21] M. Khanbabayi Gol, M. Eidy, and M. Zamani Esfahlani, Frequency ratio of carpal tunnel syndrome in women with breast cancer treated with lymphedema in Tabriz medical education centers; 2018-2019. The Iranian Journal of Obstetrics, Gynecology and Infertility, **2020**. 22(12): p. 62-68. [Crossref], [Google Scholar], [Publisher]

[22] N. Shahidi, F. Mahdavi, and M.K. Gol, Comparison of emotional intelligence, body image, and quality of life between rhinoplasty candidates and control group. Journal of Education and Health Promotion, **2020**. 9. [Crossref], [Google Scholar], [Publisher]

[23] S. Jiang, et al., A meta-analysis review of the effect of Zn-doped synthetic polymer materials on bone regeneration. Journal of Drug Delivery Science and Technology, **2022**: p. 103792. [Crossref], [Google Scholar], [Publisher]

[24] V Tajiknia, S Hassani, H Seifmanesh, A Afrasiabi, H Hosseinpour, J. Obstetrics Gynecology and Reproductive Sciences, **2021** 5 (9) [Crossref], [Google Scholar], [Publisher]

[25] SM Shushtarian, M Reza soltani, MJ Namazi, Journal of Advanced pharmacy Education& Research. **2020**;10:(s2) [Google Scholar], [Publisher]

[26] SM Bagheri, S Hassani, A Salmanipour, GSC Advanced Research and Reviews, **2022** 11 (3), 101-105 [Crossref], [Google Scholar], [Publisher]

[27] SH AH Maleki,A Gholami, M Mohammadi, A Farhiudian, Journal of pharmaceutical Negative Results, **2022** 13 (10), 4137-4158 [Crossref], [Google Scholar], [Publisher]

[28] SAY Ahmadi; et al, Formerly Current Pharmacogenomics Journal.**2020**, 17(3): 197-205 [Crossref], [Google Scholar], [Publisher]

[29] SAK AH Jalali, AH Maleki, S Hassani, E Khoshdel, The seybold Report Journal, **2023** 18 (05), 999-1022 [Google Scholar], [Publisher]

[30] S Sayad; et al, Archive of Oncology Journal.**2020** ;26(1): 6-9 [Crossref], [Google Scholar], [Publisher]

[31] S Sayad; et al, Annals of Research in Antioxidants Journal.**2019**; 4(2) [Crossref], [Google Scholar], [Publisher]

[32] S Moshar, MR Soltani, MJ Namazi, Journal of Advanced pharmacy Education & Research.**2020**; 10:(s2) [Google Scholar], [Publisher]

[33] S Hassani, M Rikhtehgar, A Salmanipour, GSC Biological and Pharmaceutical Sciences, **2022** 19 (3), 248-252 [Crossref], [Google Scholar], [Publisher]

[34] S Habibollahi Khaled hamid,M Eghbalnejad Mofrad, SMA Alavi, MJ Namazi, Journal of Advanced pharmacy Education & Research. **2021**;10 185-187 [Google Scholar], [Publisher]

[35] MR AH Jalali, S Hassani, S Albuzyad, A Moaddab, The seybold Report Journal, **2023** [Google Scholar], [Publisher]

[36] M.E Akbari; et al, International Journal of Cancer Management.2016;9(6) [Crossref], [Google Scholar], [Publisher]

[37] FB AH Jalali,S Hassani, A Zare,T Ziaadini, The seybold Report Journal, **2023** 18 (04), 1634-1653 [Google Scholar], [Publisher]

[38] F Safari, H Safari, Eurasian Journal of Chemical, Medicinal and Petroleum Research, **2022** 1 (2), 150-154 [Google Scholar], [Publisher]

[39] F Rokhtabnak, S Sayad, M Izadi, SD Motlagh, P Rahimzadeh, Anesthesiology and Pain Medicinem **2021** 11 (5) [Crossref], [Google Scholar], [Publisher]

[40] F HYSENI; et al, The Importance of Magnetic Resonance in Detection of Cortical Dysplasia, Curr Health Sci Journal. **2021**; 47(4): 585–589 [Crossref], [Google Scholar], [Publisher]

[41] F Beiranvandi, Z Kuchaki, A Zare, E Khoshdel, A Jalali, Journal of Pharmaceutical Negative Results, **2022**, 4417-4425 [Crossref], [Google Scholar], [Publisher]

[42] E. Ghaibi, et al., Eurasian Journal of Chemical, Medicinal and Petroleum Research, **2022**, 1, 33-39. [Google Scholar], [Publisher],

[43] E Harizi; et al, Radiology Case Reports **2022** 17 (11) 4152-4155 [Crossref], [Google Scholar], [Publisher]

[44] E Kola, A Gjata, I Kola, et al., Radiology Case Reports, **2022** 17 (3), 1032 [Crossref], [Google Scholar], [Publisher]

[45] E Kola, A Gjata, I Kola, A Guy, J Musa, et al., Radiology Case Reports, **2021** 16 (11), 3191-3195 [Crossref], [Google Scholar], [Publisher]

[46] E Ghasemi, AH Fathi, S Parvizinia., J Iran Dent Assoc **2019**; 31 (3):169-176 [Crossref], [Google Scholar], [Publisher]

[47] D Nakuci, E Kola, E Horjeti, I Kola, B Shaipi, J Musa, A Guy, M Alimehmeti, Archives of Clinical and Medical Case Reports, **2020** 4 (5), 760-765 [Google Scholar], [Publisher]

[48] D Nakuci, E Kola, E Horjeti, I Kola, B Shaipi, J Musa, A Guy, M Alimehmeti, Archives of Clinical and Medical Case Reports, **2020** 4 (5), 760-765 [Google Scholar], [Publisher]

[49] A. Mobed et al., Biosensors technology for anti-epileptic drugs. Clinica Chimica Acta. **2022**;533:175-82. [Crossref], [Google Scholar], [Publisher]

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