




LncRNA XIST Promotes Migration and Invasion of Papillary Thyroid Cancer Cell by Modulating MiR-101-3p/CLDN1 Axis

Yong-Liang Du¹ · Yan Liang¹ · Yan Cao¹ · Le Liu¹ · Jian Li¹ · Guang-Qing Shi¹ 

Received: 27 February 2020 / Accepted: 25 June 2020 / Published online: 14 October 2020
© Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

Papillary thyroid carcinoma (PTC) is the most common endocrine malignancy in the world. Long non-coding RNA X-inactive specific transcript (XIST) was found to be upregulated in PTC tissues and cell lines. However, the molecular mechanism underlying PTC metastasis and whether XIST plays a regulatory role in PTC are still largely unknown. qRT-PCR was performed to detect the expression of lncRNA XIST and mRNAs. Western blotting was carried out to detect CLDN1, MMP2, and MMP9. Transwell assay was used to detect migration and invasion. Starbase bioinformatics prediction and luciferase assay were used to validate the relationship of miR-101-3p and XIST or CLDN1. LncRNA XIST was upregulated in PTC tissues and cells. XIST knockdown suppressed migration and invasion of PTC cells. XIST could directly bind with miR-101-3p. Overexpression of miR-101-3p suppressed migration and invasion of PTC cells. CLDN1 was the target of miR-101-3p, and overexpression of CLDN1 can reverse the inhibition of cell migration and invasion by miR-101-3p. What's more, miR-101-3p inhibition and CLDN1 overexpression can reverse the effect of sh-XIST on migration and invasion of PTC cells. XIST promotes migration and invasion of papillary thyroid cancer cells via directly regulating miR-101-3p/CLDN1 axis, which is a novel mechanism of XIST in the regulation of PTC.

Keywords PTC · lncRNA XIST · miR-101-3p · CLDN1

Yong-Liang Du and Yan Liang co-first authors.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10528-020-09985-8>) contains supplementary material, which is available to authorized users.

✉ Jian Li
liyyggoppo@163.com

✉ Guang-Qing Shi
shiguangqingtt@163.com

¹ Nuclear Medicine Department, The Third Xiangya Hospital of Central South University, No.137 Tongzipo Road, Yuelu District, Changsha 410013, Hunan, People's Republic of China

Abbreviations

PTC	Papillary thyroid carcinoma
XIST	X-inactive specific transcript
RAI	Radioactive iodine
NSCLC	Non-small-cell lung cancer
HCC	Hepatocellular carcinoma
CRC	Colorectal cancer
CLDN1	Claudin-1
PBS	Phosphate-buffered saline
RPMI	Roswell Park Memorial Institute
qRT-PCR	Quantitative real-time polymerase chain reaction
MMP	Matrix metalloproteinase
lncRNAs	Long non-coding RNAs
ceRNA	Competing endogenous RNA

Introduction

Papillary thyroid carcinoma (PTC), the most common endocrine malignancy in the worlds, accounts for ~90% of all thyroid cancer (Nguyen et al. 2015). Most patients with PTC have a good prognosis by surgical removal, followed by adjuvant radioactive iodine (RAI) therapy (Rivkees et al. 2011). However, a small fraction of patients with PTC do not respond to RAI therapy or they progress to metastatic disease, resulting in the decline in the survival rate and life quality (O'Neill et al. 2010). Besides, the molecular mechanism underlying PTC metastasis is still largely unknown. Thus, it is of much importance to identify potential biomarkers and therapeutic targets and develop new strategies for treatment of this disease.

Long non-coding RNAs (lncRNAs) constitute a class of noncoding RNAs whose transcripts are >200 nucleotides in length with limited coding potential (Wilusz et al. 2009). They play pivotal roles in the regulation of variety of cellular biological behaviors such as cell proliferation, metastasis, and drug resistance (Bhan et al. 2017). LncRNA XIST, a well-documented lncRNA, has been found to have an aberrant expression and function as an oncogene in multiple cancers. For instance, XIST acts as an oncogene in NSCLC by epigenetically repressing KLF2 expression (Fang et al. 2016), while, XIST regulates PTEN expression and promotes progression of HCC (Chang et al. 2017). Moreover, XIST was also reported to modulate epithelial-mesenchymal transition (EMT) in CRC (Chen et al. 2017). Recently, relative expression of XIST was also found upregulated in PTC tissues and cell lines. However, there is still little know about the regulatory role of XIST in the progression of PTC, which needs further elucidation (Xu and Wang 2018).

MicroRNAs (miRNAs), 22-nt endogenous RNAs, are reported to be involved in regulating multiple physiological biological processes such as tumorigenesis and metastasis (Wu 2010). Increasing evidence from recent studies suggested that miR-101-3p as an anti-cancer molecule was downregulated in a number of cancers, including PTC. For example, miR-101-3p inhibited the tumorigenesis of PTC by targeting USP22. In addition, miR-101-3p was also reported to target CXCL12

to inhibit cellular proliferation and invasion of PTC (Zhao et al. 2016). Based on the bioinformatics analysis, we found that there is a binding site for miR-101-3p on XIST. However, whether XIST could regulate PTC metastasis through sponging miR-101-3p is still unknown.

As a structural constitution in cell–cell adhesion, tight junction has an important role in the maintenance of permeability and integrity of normal epithelial cell barrier. Proteins related to tight junction were also reported to be dysfunction in tumors, indicating that they could play pivotal roles in the metastasis of tumors (Akizuki et al. 2017; Dhanda and Sandhir 2018; Wu et al. 2020). CLDN1 (claudin-1) is a tight junction protein which shows an altered expression pattern in several cancers and is reported to be associated with cancer proliferation, migration, and invasion (Zhao et al. 2016). Recent study also revealed that CLDN1 was upregulated in PTC and could be a potential biomarker of PTC (Zhao et al. 2016). However, little is known about the regulatory mechanism of its expression. Our previous bioinformatics analysis also uncovered that CLDN1 was the target of miR-101-3p. Therefore, whether miR-101-3p could affect the migration and invasion of PTC cells by targeting CLDN1 is needed for further validation.

In this study, we identified that XIST was an oncogene, which was upregulated in PTC and could act as a ceRNA to sponge endogenous miR-101-3p to sequester and inhibit miR-101-3p activity, thereby leading to increased CLDN1 expression, thus regulated PTC invasion and migration. Our findings revealed a novel mechanism underlying XIST in PTC progression and it could serve as a potential biomarker and a therapeutic target for PTC.

Materials and Method

Tissue Samples

A total of 24 paired PTC and adjacent normal tissues were harvested and confirmed by two independent pathologists from patients during operation at The Third Xiangya Hospital of Central South University. The samples were snap froze in liquid nitrogen immediately after separated from the human body and stored at -80°C . This study was approved by the ethics committee of The Third Xiangya Hospital of Central South University and written informed consent was obtained from each patient.

Cell Cultures

Human thyroid cancer cell lines (NPA87, TPC-1 and KAT-5) and normal human thyroid cell line HT-ori 3-1 were purchased from American Type Culture Collection (ATCC, USA) and were cultured in Roswell Park Memorial Institute (RPMI)-1640 supplemented with 10% fetal bovine serum (FBS, Gibco, USA). Cells in this medium were placed in a humidified atmosphere of 5% CO_2 at 37°C .

Cell Transfection

Has-miR-101-3p mimics was purchased from Genepharma (Shanghai, China). sh-XIST was used to achieve knockdown of lncRNA XIST (Genepharma, China). pcDNA3.1-CLDN1 and empty pcDNA3.1 vector were synthesized and purchased from Geenseed Biotech Co. (Guangzhou, China). sh-XIST, miR-101-3p mimics, miR-101-3p inhibitor, pcDNA3.1-based vectors and their corresponding negative control were transfected into target cells with Lipofectamine 3000 (Invitrogen, USA).

Real-time PCR

Total RNA was isolated with Tri-reagent (Sigma, USA). Then, first-strand cDNA was generated by ImProm-II Reverse Transcription System (Promega, USA). After that, qPCR analysis was then carried out with SYBR Green qPCR assay (Takara, China) and gene-specific primers. GAPDH or U6 were used for normalization, respectively. The $2^{-\Delta\Delta C_t}$ method was used to analyze the relative fold changes. Primers used for qPCR are as follows:

XIST-F 5'-CTCTCCATTGGGTTTAC-3'
XIST-R 5'-GCGGCAGGTCTTAAGAGATGAG-3'
miR-101-3p-F 5'-GCGCGCATACTGTGATA-3'
miR-101-3p-R 5'-GTGCAGGGTCCGAGGT-3'
MMP2-F 5'-ACAAGAACCAGATCACATACAG-3'
MMP2-R 5'-TCACATCGCTCCAGACTT-3'
MMP9-F 5'-TTCCAAACCTTTGAGGGCGA-3'
MMP9-R 5'-CTGTACACGCGAGTGAAGGT-3'
CLDN1-F 5'-CCTCCTGGGAGTGATAGCAA-3'
CLDN1-R 5'-CCAGTGAAGAGAGCCTGACC-3'
GAPDH-F 5'-CCAGGTGGTCTCCTCTGA-3'
GAPDH-R 5'-GCTGTAGCCAAATCGTTGT-3'
U6-F 5'-CTCGCTTCGGCAGCACA-3'
U6-R 5'-AACGCTTCACGAATTTGCGT-3'

Transwell Invasion and Migration Assay

Transwell invasion and migration assay were carried out as described before (Zhou et al. 2020). Briefly, for migration experiment, TPC-1 and KAT-5 cells in RPMI-1640 were applied to the 8 μ m pore 24-well transwell chambers (Corning, USA) for 8 h. For invasion experiment, cells were seeded into each transwell chamber coated with Matrigel (BD Biosciences, USA). 200 μ L serum-free RPMI-1640 were added into the upper chambers, while 10% FBS was added into the RPMI-1640 in the lower chamber. Cells on the upper part were then removed using a cotton swab after

48 h. Cells on the lower part of the membrane were stained with 0.1% crystal violet. The migrated and invasive cells were quantified by photographing 3 independent visual fields under the microscope and wells were repeated in triplicate.

Target Prediction

Bioinformatics algorithms in starbase V2.0 (<http://starbase.sysu.edu.cn>), a bioinformatic website for the prediction of interaction between miRNA and mRNA/lncRNA, was used for the prediction of potential binding sites of miR-101-3p on XIST and CLDN1. miR-Target function mode was set as key parameter to make prediction.

Luciferase Activity Assay

The fragments from XIST and CLDN1 containing the predicted miR-101-3p binding site and mutant sequence, respectively, were amplified by PCR and then sub-cloned to the pmirGLO plasmids (Promega, USA). Luciferase reporter plasmids and miR-101-3p mimics or mimic NC were then co-transfected into HEK-293 cells with Lipofectamine 3000 (Invitrogen, USA). To examine the relative luciferase activity, Dual-Luciferase Reporter Assay System (Promega, USA) was then performed 48 h after transfection.

Western Blot

Cold PBS was used to wash cells and cold IP lysis buffer (ThermoFisher, USA) containing protease inhibitors (Roche, Switzerland) was then used to prepare cell

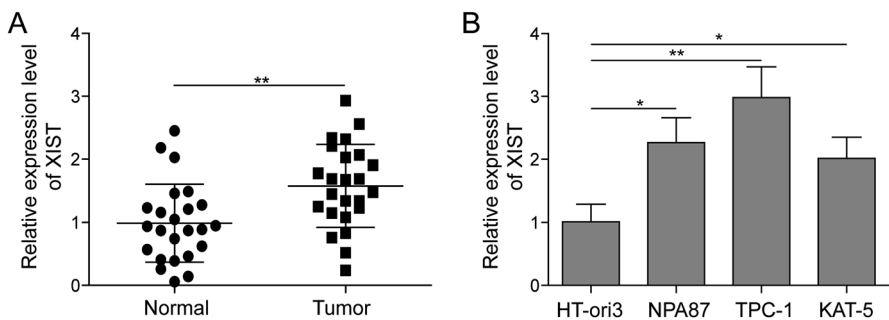


Fig. 1 The expression pattern of lncRNA XIST in PTC tissues and cell lines. **a** The expression level of lncRNA XIST in PTC tissue and adjacent noncancerous tissue samples by qRT-PCR assay ($N=24$). $**P<0.01$. **b** The expression level of lncRNA XIST in human thyroid cancer cell lines (NPA87, TPC-1 and KAT-5) and normal human thyroid cell line HT-ori3 by qRT-PCR assay ($N=3$). $*P<0.05$ and $**P<0.01$. GAPDH was used for normalization

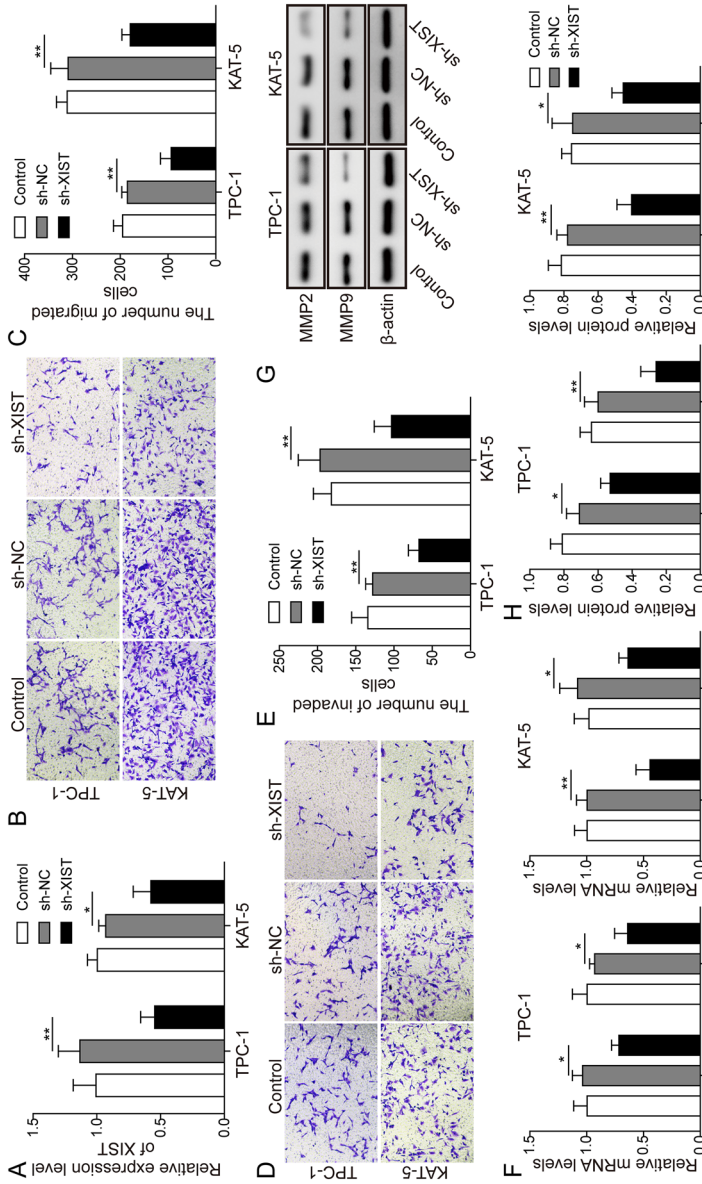


Fig. 2 LncRNA XIST promotes invasion and migration of PTC cells. The PTC cells (TPC-1 and KAT-5) were transfected with sh-XIST and the negative control (sh-NC) for further exploration. **a** The expression level of lncRNA XIST in PTC cells (TPC-1 and KAT-5) after transfected with sh-XIST and the negative control (sh-NC) were measured by qRT-PCR assay. * $P < 0.05$ and ** $P < 0.01$. GAPDH was used for normalization. **b, c** The transwell assays were subjected to measure the effects of XIST knockdown on PTC cells migration. Wells were repeated in triplicate and the migrated cells were quantified per field of view and statistically analyzed. ** $P < 0.01$. **d, e** The transwell assays were subjected to measure the effects of XIST knockdown on PTC cells invasion. Wells were repeated in triplicate and the invaded cells were quantified per field of view and statistically analyzed. ** $P < 0.01$. **f** The relative expression of MMP2 and MMP9 mRNAs after XIST knockdown were assessed by qRT-PCR analysis. * $P < 0.05$ and ** $P < 0.01$. **g, h** The relative expression of MMP2 and MMP9 proteins after XIST knockdown were assessed by western blot analysis. * $P < 0.05$ and ** $P < 0.01$

extracts following the manufacturer's protocol. Proteins samples were separated by 10% SDS-PAGE gel electrophoresis and then transfer to PVDF membranes. After blocking with 5% skim milk, membranes were incubated overnight with specific primary antibodies for CLDN1 (1:1000, Abcam, USA), MMP2 (1:1000, Abcam, USA), MMP9 (1:1000, Abcam, USA) at 4 °C. Optical density method was used to perform quantitative autoradiography using GAPDH (1:5000, Proteintech, USA) as controls. Then, blots were incubated with peroxidase-conjugated anti-mouse or anti-rabbit IgG (1:5000, Proteintech, USA) for 1 h at RT and then developed using a Super Signal West Pico kit (ThermoFisher, USA)

Statistical Analysis

All statistics was analyzed by GraphPad Prism 5 software. Data were presented as the mean \pm standard deviation (SD). Statistical significance between each two groups was evaluated using Student's *t* test and for multiple comparison one-way ANOVA test was carried out, and $P < 0.05$ was considered statistically significant.

Results

LncRNA XIST was Significantly Upregulated in PTC

First, we investigate the expression of XIST in PTC tissue and adjacent noncancerous tissue samples by qRT-PCR assay. Figure 1a shows that XIST was upregulated in PTC tissue samples significantly compared with adjacent normal tissues. Then, the expression level of XIST was further tested in human PTC cell lines NPA87, TPC-1, KAT-5 and normal human thyroid cell line HT-ori 3-1. As displayed in Fig. 1b, XIST was upregulated in all PTC cells.

Knockdown XIST Suppressed Invasion and Migration of PTC Cells

shRNA for specific knocking down XIST in TPC-1 and KAT-5 cells was applied to further exploring the biological role of XIST in PTC, and it successfully down-regulated XIST in both cell lines (Fig. 2a). Meanwhile, transwell assay revealed that decreased XIST significantly inhibited the migration (Fig. 2b, c) and invasion (Fig. 2d, e) ability of TPC-1 and KAT-5 cells. Furthermore, relative expression of MMP2 and MMP9 were assessed by qPCR. Figure 2f shows that MMP2 and MMP9 were decreased after XIST knockdown in both cell lines. While, the protein level of MMP2 and MMP9 were also decreased after XIST knockdown (Fig. 2g, h), confirmed that XIST might regulate PTC cells migration and invasion.

LncRNA XIST Directly Bound with miR-101-3p

Accumulated evidences suggested that lncRNAs could function as ceRNA to sponge specific miRNAs to upregulate their downstream target genes, involved in many biological process. To further investigate the specific mechanism of how lncRNA XIST regulated migration and invasion of PTC cells, its potential target miRNAs was predicted by bioinformatics. Figure 3a shows that miR-101-3p was predicted to directly bind with XIST by bioinformatics. To determine whether XIST directly regulates miR-101-3p, luciferase vectors containing wild type or mutant type of XIST were constructed and luciferase reporter assay was performed. Figure 3b shows that miR-101-3p overexpression reduced the luciferase activity of XIST-WT but had no obvious effect on their mutant reporters. Then, qRT-PCR assay was applied to assess the expression of 101-3p in both TPC-1 and KAT-5 cells (Fig. 3c) to confirm the regulation between XIST and miR-101-3p. It showed that XIST knockdown significantly upregulated miR-101-3p.

MiR-101-3p Suppressed Invasion and Migration of PTC Cells

To further explore the biological role of miR-101-3p in PTC, TPC-1 and KAT-5 PTC cells were transfected by miR-101-3p mimics and mimics NC, and miR-101-3p was successfully overexpressed in both cell lines (Fig. 4a). Meanwhile, transwell assay revealed that miR-101-3p overexpression significantly inhibited the migration (Fig. 4b, c) and invasion (Fig. 4d, e) ability of TPC-1 and KAT-5 cells. Furthermore, relative expression of MMP2 and MMP9 mRNA

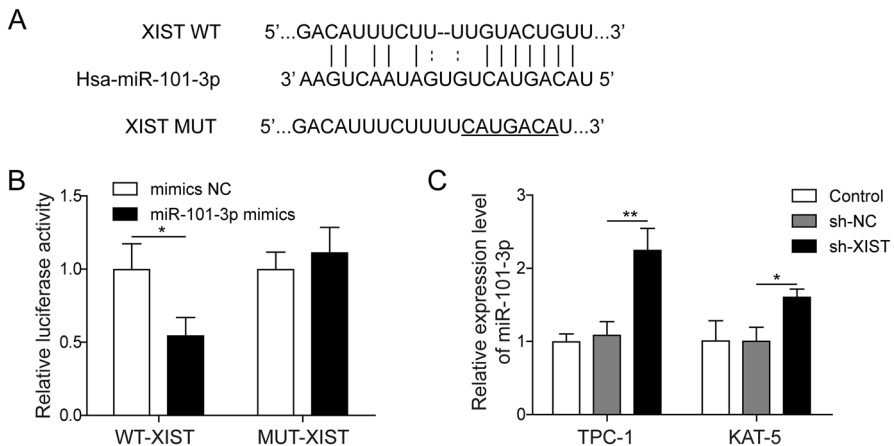


Fig. 3 XIST interacts with miR-101-3p in PTC cells. **a** The predicted binding site between XIST and miR-101-3p. **b** The luciferase activity of XIST-WT and XIST-MUT in HEK-293T cells co-transfected with mimics NC or miR-101-3p mimics. * $P < 0.05$. **c** The expression level of miR-101-3p in TPC-1 and KAT-5 cells after transfected with sh-NC and sh-XIST were detected by qRT-PCR assay. U6 was used for normalization ($N = 3$). * $P < 0.05$ and ** $P < 0.01$

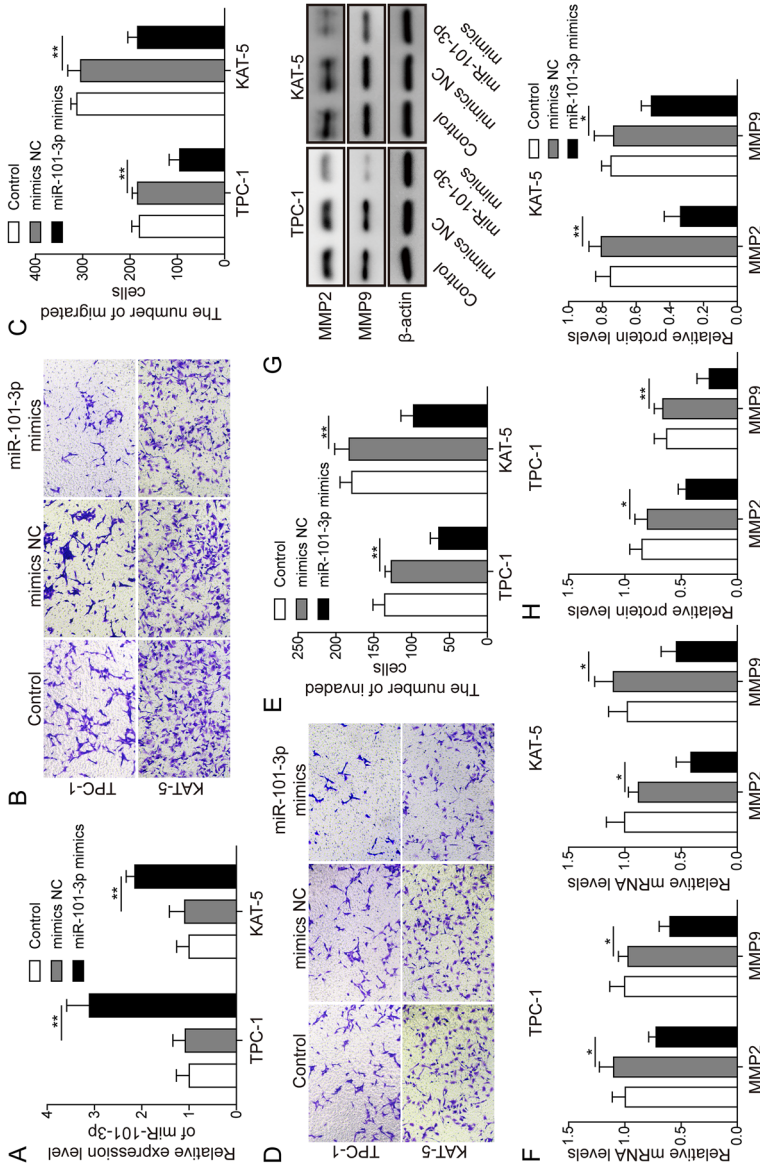


Fig. 4 miR-101-3p inhibits migration and invasion of PTC cells. The PTC cells (TPC-1 and KAT-5) were transfected with mimic NC and miR-101-3p mimic for further exploration. **a** The expression level of miR-101-3p in PTC cells TPC-1 and KAT-5 transfected with NC and miR-101-3p mimic by qRT-PCR assay (N=3). ***P*<0.01. U6 was used for normalization. **b, c** The transwell assays were subjected to measure the effects of miR-101-3p overexpression on PTC cell migration. Wells were repeated in triplicate and the migrated cells were quantified per field of view and statistically analyzed (N=3). ***P*<0.01. **d, e** The transwell assays were subjected to measure the effects of miR-101-3p overexpression on PTC cell invasion. Wells were repeated in triplicate and the invaded cells were quantified per field of view and statistically analyzed (N=3). ***P*<0.01. **f** The relative expression of mRNAs of MMP2 and MMP9 after miR-101-3p overexpression were assessed by qRT-PCR analysis (N=3). **P*<0.05. **g, h** The relative expression of MMP2 and MMP9 proteins after miR-101-3p overexpression were assessed by western blot analysis (N=3). **P*<0.05 and ***P*<0.01

were assessed by qPCR analysis. Figure 4f shows that MMP2 and MMP9 were decreased after miR-101-3p overexpression. While, the protein level of MMP2 and MMP9 were also decreased after miR-101-3p overexpression (Fig. 4g, h). These results indicated that miR-101-3p regulated PTC cells migration and invasion.

CLDN1 Directly Bound with MiR-101-3p

To further investigate the specific molecular mechanism of how miR-101-3p regulated invasion of PTC cells, and ceRNA network consisting of lncRNA, miRNA, and its target gene, we validated its potential target mRNA by bioinformatics. Figure 5a shows that miR-101-3p was predicted to directly bind with CLDN1 3'UTR by bioinformatics. To determine whether CLDN1 was the target of miR-101-3p, luciferase vectors containing wild type or mutant type of CLDN1 was constructed and luciferase reporter assay was performed. miR-101-3p overexpression significantly reduced the luciferase activity of CLDN1-WT in HEK-293T cells but had no obvious effect on their mutant reporters (Fig. 5b). qRT-PCR assay was then applied to assess the expression of CLDN1 in both TPC-1 and KAT-5 cells (Fig. 5c) to further confirm the regulation between CLDN1 and miR-101-3p. The results showed that CLDN1 mRNA was significantly downregulated by miR-101-3p overexpression. Similarly, CLDN1 protein was also significantly downregulated by miR-101-3p overexpression (Fig. 5d, e). Taken together, CLDN1 was validated to be the direct target of miR-101-3p.

MiR-101-3p Inhibited Migration and Invasion of PTC Cells Through CLDN1

As shown in Fig. 6a, b, CLDN1 was downregulated when cells were transfected with miR-101-3p mimics, while CLDN1 overexpression could upregulate CLDN1. Then, we verify the downstream molecular mechanism of miR-101-3p regulating migration and invasion of PTC cells. Transwell assay revealed that miR-101-3p overexpression significantly inhibited the migration of both cell lines, while, CLDN1 overexpression could rescue this phenomenon (Fig. 6b, c). Similarly, miR-101-3p overexpression also significantly inhibited the invasion of both cell lines, while, CLDN1 overexpression could rescue this phenomenon (Fig. 6d, e). And the expression of CLDN1, MMP2, and MMP9 were downregulated by miR-101-3p overexpression. Upregulation of CLDN1 can reverse the inhibited role of miR-101-3p mimic on the expression of CLDN1, MMP2, and MMP9 (Fig. 6f, g). Taken together, these results indicated that miR-101-3p regulated migration and invasion of PTC cells through CLDN1.

XIST Knockdown Inhibited Migration and Invasion of PTC Cells by CLDN1

The expression level of CLDN1 was detected in TPC-1 and KAT-5 cells after transfected with sh-NC and sh-XIST, and Fig. 7a shows that CLDN1 was

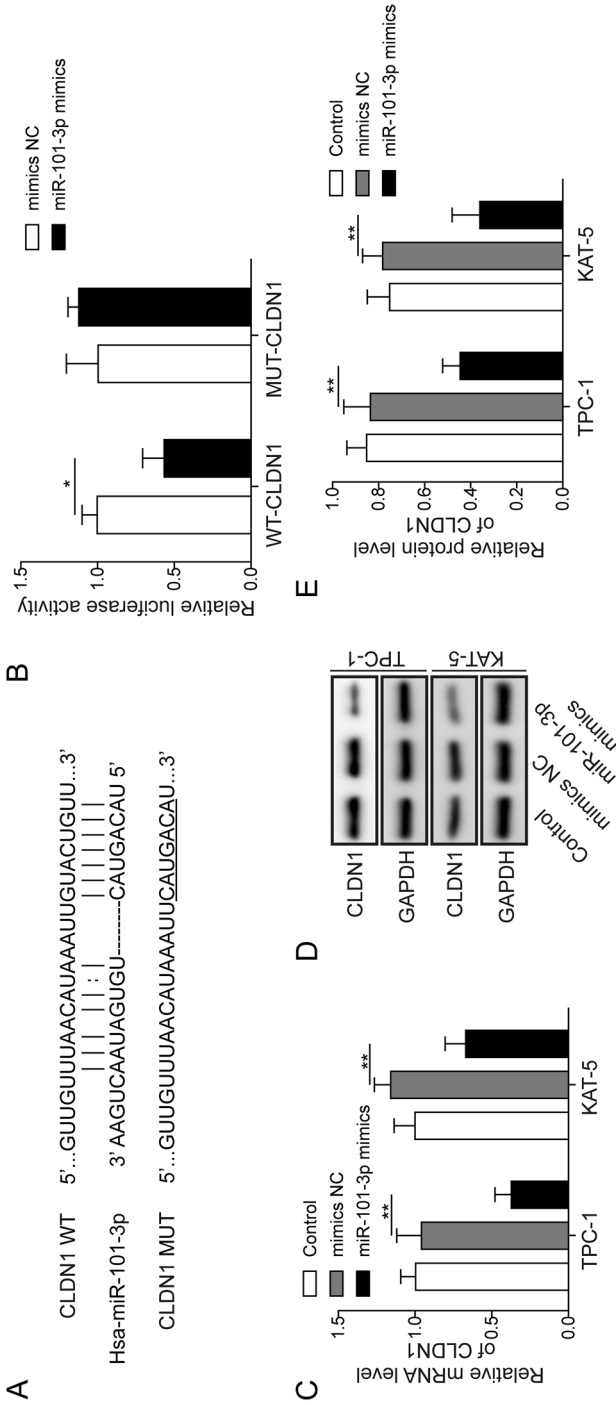


Fig. 5 CLDN1 interacts with miR-101-3p in PTC cells. **a** The predicted binding site between CLDN1 and miR-101-3p. **b** The luciferase activity of CLDN1-WT and CLDN1-MUT in HEK-293T cells transfected with mimics NC or miR-101-3p mimics. $*P < 0.05$. **c** The relative expression level of CLDN1 mRNA in TPC-1 and KAT-5 cells transfected with NC mimics and miR-101-3p mimics by qRT-PCR assay. GAPDH was used for normalization. $**P < 0.01$. **d**, **e** The relative expression level of CLDN1 protein in TPC-1 and KAT-5 cells transfected with NC mimics and miR-101-3p mimics by WB assay. GAPDH was used for normalization. $**P < 0.01$

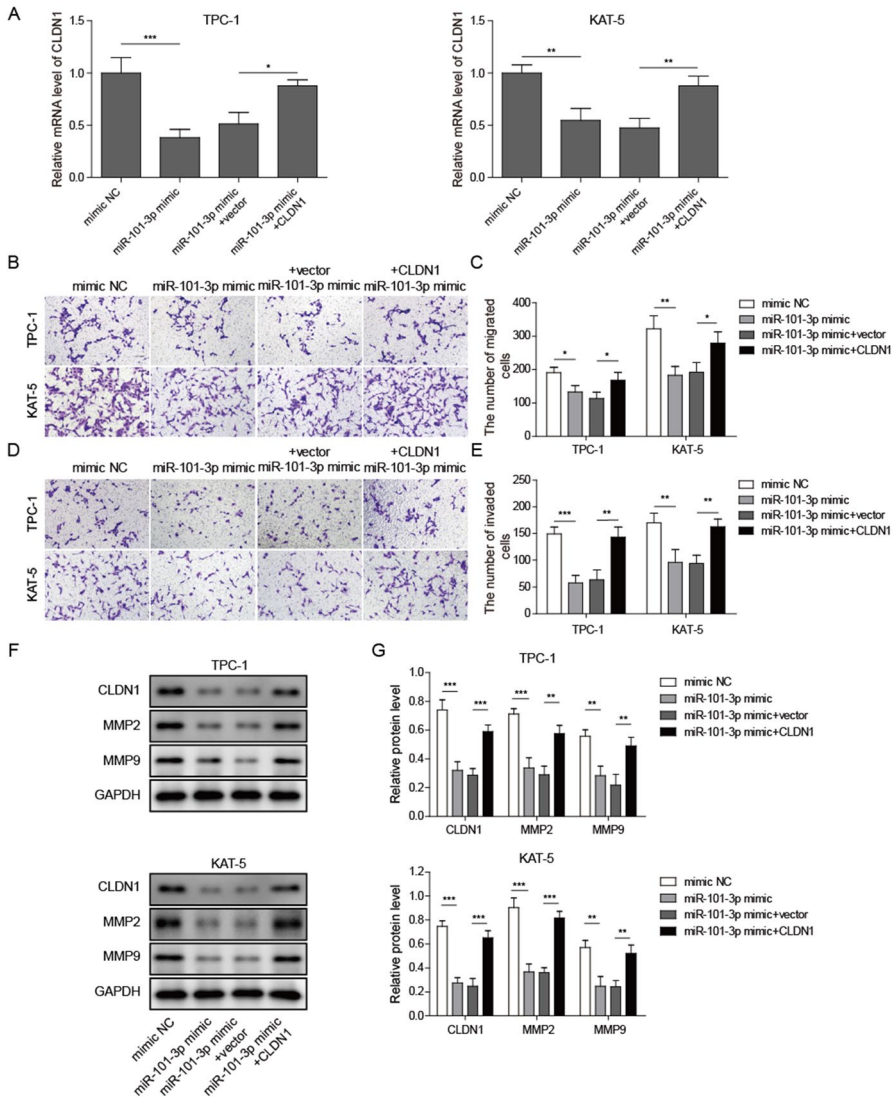


Fig. 6 miR-101-3p mimics inhibits invasion and migration of PTC cells through CLDN1. **a** The expression level of CLDN1 after transfection by qRT-PCR assay. GAPDH was used for normalization ($N=3$). $*P<0.05$, $**P<0.01$ and $***P<0.001$. **b, c** The transwell assays were subjected to measure PTC cell migration after transfection. Wells were repeated in triplicate and the migrated cells were quantified per field of view and statistically analyzed. $*P<0.05$ and $**P<0.01$. **d, e** PTC cell invasion was measured after transfection. Wells were repeated in triplicate and the invaded cells were quantified per field of view and statistically analyzed ($N=3$). $**P<0.01$ and $***P<0.001$. **f, g** The expression of CLDN1, MMP2, and MMP9 in PTC cells were detected by WB assay. GAPDH was used for normalization. $**P<0.01$ and $***P<0.001$

downregulated in sh-XIST group while miR-101-3p inhibition or CLDN1 over-expression could rescue this phenomenon. To further verify the downstream

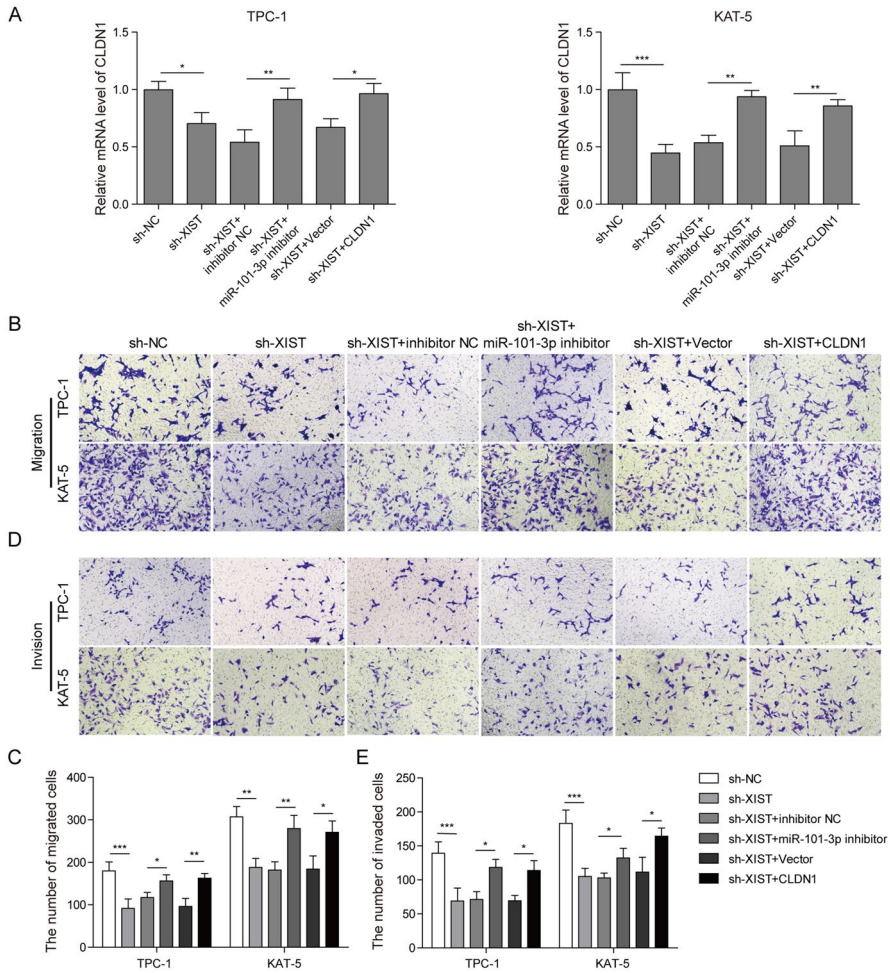


Fig. 7 XIST knockdown inhibits invasion and migration of PTC cells through CLDN1. **a** The expression level of CLDN1 in TPC-1 and KAT-5 cells were measured by qRT-PCR assay. GAPDH was used for normalization ($N=3$). $*P<0.05$, $*P<0.01$ and $***P<0.001$. **b, c** The transwell assays were subjected to measure PTC cell migration after transfection. Wells were repeated in triplicate and the migrated cells were quantified per field of view and statistically analyzed. $*P<0.05$, $**P<0.01$ and $***P<0.001$. **d, e** The transwell assays were subjected to measure PTC cell invasion after transfection. Wells were repeated in triplicate and the invaded cells were quantified per field of view and statistically analyzed ($N=3$). $*P<0.05$, $**P<0.01$ and $***P<0.001$

molecular mechanism of XIST regulating migration and invasion of PTC cells, transwell assay revealed that XIST knockdown significantly inhibited the migration of both cell lines, while, miR-101-3p inhibition or CLDN1 overexpression could rescue this phenomenon (Fig. 7b, c). Similarly, XIST knockdown also significantly inhibited the invasion of both cell lines, while, miR-101-3p inhibition or CLDN1 overexpression could rescue this phenomenon (Fig. 7d, e). Taken

together, these results indicated that XIST regulated migration and invasion of PTC cells through miR-101-3p/CLDN1 axis.

Discussion

PTC has been reported to have an excellent prognosis after treatment and with low mortality. However, there are still some small percentage patients who suffer from more aggressive PTC characterized by metastasis recurrence (Grant 2015). As the biological behavior of PTC varies widely, it has not yet been identified an ideal genetic marker for PTC prognosis. Therefore, to find new biomarkers and clarify the biological and molecular mechanisms for PTC progression is quite critical. Increasing study has revealed that lncRNAs could play regulatory roles in many physiological and pathological processes of PTC (Sawada 2013). For instance, lncRNA n384546 was reported to promote PTC progression and metastasis through miR-145-5p/AKT3 axis (Sawada 2013). LncRNA NORAD promoted PTC progression through targeting miR-202-5p (He et al. 2019). While, XIST can sponge miR-141 to promote proliferation and invasion in PTC (Xu and Wang 2018). In consistent with previous study, lncRNA XIST expression was found to be upregulated in PTC tissues and cell lines here. XIST knock-down could inhibit cell migration, and invasion of PTC cells, further suggesting a potential novel target for PTC treatment.

MiR-101-3p was reported to be a tumor-suppressive miRNA and is lowly expressed in several cancer types. MiR-101-3p was reported to involve in many biological process such as cell apoptosis, proliferation, migration, and invasion (Qiu et al. 2018). Recent study showed that miR-101-3p was significantly down-regulated in PTC tissue and thyroid cancer cell lines, and that the downregulated miR-101-3p was associated with lymph node metastasis (Wang et al. 2014). It could also inhibit the tumorigenesis of PTC through targeting USP22 (Zhao et al. 2016). In our study, miR-101-3p was verified to bind with XIST through luciferase assays and XIST could regulate cell migration and invasion of PTC cells through sponging miR-101-3p. These results elucidated the molecular mechanism of PTC progression regulated by lncRNA XIST through a ceRNA axis.

CLDN1, a tight junction protein, whose aberrant expression was reported to be associated with cancer proliferation, migration, and invasion (Kwon 2013). Previously, It has been reported that CLDN1 promotes metastasis of esophageal squamous carcinoma through AMPK/STAT1/ULK1 signaling pathway (Wu et al. 2020). Besides, it is also associated with invasion of oral squamous cell carcinoma cells (Oku et al. 2006). High expression of CLDN1 in PTC was reported and it was in associated with regional lymph node metastasis of PTC (Nemeth et al. 2010). In this study, we revealed for the first time that CLDN1 was the target of miR-101-3p through luciferase assays. Moreover, XIST could upregulated CLDN1 through sponging miR-101-3p, thus promoted the migration and invasion of PTC cells. Taken together, these results proved that XIST acted as a ceRNA and regulated metastasis of PTC cells through miR-101-3p/CLDN1 axis.

However, there are still some limitations of this study. This novel molecular mechanism of metastasis of PTC cells was revealed by *in vitro* experiments, which means in the future, further verification in mice model was needed to prove its potential of therapeutic targets. In conclusion, our study provided the first clue showing the regulatory role of XIST in PTC through miR-101-3p/CLDN1 axis, providing new insights into the mechanisms underlying PTC malignancy and suggesting a new potential therapeutic target.

Acknowledgements This research project is funded by the Fundamental Research Funds for the Central Universities of Central South University (2019zzts823).

Author Contributions Y-LD: Conceptualization; YC: Data curation; JL: Formal analysis; G-QS: Funding acquisition; YL: Investigation; Y-LD: Methodology; G-QS: Project administration; LL: Resources; JL: Software; YC: Supervision; YL and LL: Validation; Y-LD: Visualization; Y-LD: Roles/writing—original draft; G-QS: Writing—review & editing. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the ethics committee of The Third Xiangya Hospital of Central South University and written informed consent was obtained from each patient.

References

- Akizuki R, Shimobaba S, Matsunaga T, Endo S, Ikari A (2017) Claudin-5, -7, and -18 suppress proliferation mediated by inhibition of phosphorylation of Akt in human lung squamous cell carcinoma. *BBA Mol Cell Res* **1864**:293–302
- Bhan A, Soleimani M, Mandal SS (2017) Long noncoding RNA and cancer: a new paradigm. *Cancer Res* **77**:3965–3981
- Chang S, Chen B, Wang X, Wu K, Sun Y (2017) Long non-coding RNA XIST regulates PTEN expression by sponging miR-181a and promotes hepatocellular carcinoma progression. *BMC Cancer* **17**:248
- Chen DL, Chen LZ, Lu YX, Zhang DS, Zeng ZL, Pan ZZ, Huang P, Wang FH, Li YH, Ju HQ, Xu RH (2017) Long noncoding RNA XIST expedites metastasis and modulates epithelial-mesenchymal transition in colorectal cancer. *Cell Death Dis* **8**:e3011
- Dhanda S, Sandhir R (2018) Blood-brain barrier permeability is exacerbated in experimental model of hepatic encephalopathy via MMP-9 activation and downregulation of tight junction proteins. *Mol Neurobiol* **55**:3642–3659
- Fang J, Sun CC, Gong C (2016) Long noncoding RNA XIST acts as an oncogene in non-small cell lung cancer by epigenetically repressing KLF2 expression. *Biochem Biophys Res Commun* **478**:811–817
- Grant CS (2015) Recurrence of papillary thyroid cancer after optimized surgery. *Gland Surg* **4**:52–62
- He H, Yang H, Liu D, Pei R (2019) LncRNA NORAD promotes thyroid carcinoma progression through targeting miR-202-5p. *Am J Transl Res* **11**:290–299
- Kwon MJ (2013) Emerging roles of claudins in human cancer. *Int J Mol Sci* **14**:18148–18180
- Nemeth J, Nemeth Z, Tatrai P, Peter I, Somoracz A, Szasz AM, Kiss A, Schaff Z (2010) High expression of claudin-1 protein in papillary thyroid tumor and its regional lymph node metastasis. *Pathol Oncol Res* **16**:19–27
- Nguyen QT, Lee EJ, Huang MG, Park YI, Khullar A, Plodkowski RA (2015) Diagnosis and treatment of patients with thyroid cancer. *Am Health Drug Benefits* **8**:30–40
- Oku N, Sasabe E, Ueta E, Yamamoto T, Osaki T (2006) Tight junction protein claudin-1 enhances the invasive activity of oral squamous cell carcinoma cells by promoting cleavage of laminin-5

- gamma2 chain via matrix metalloproteinase (MMP)-2 and membrane-type MMP-1. *Cancer Res* 66:5251–5257
- O'Neill CJ, Oucharek J, Learoyd D, Sidhu SB (2010) Standard and emerging therapies for metastatic differentiated thyroid cancer. *Oncologist* 15:146–156
- Qiu J, Zhang W, Zang C, Liu X, Liu F, Ge R, Sun Y, Xia Q (2018) Identification of key genes and miRNAs markers of papillary thyroid cancer. *Biol Res* 51:45
- Rivkees SA, Mazzaferri EL, Verburg FA, Reiners C, Luster M, Breuer CK, Dinauer CA, Udelsman R (2011) The treatment of differentiated thyroid cancer in children: emphasis on surgical approach and radioactive iodine therapy. *Endocr Rev* 32:798–826
- Sawada N (2013) Tight junction-related human diseases. *Pathol Int* 63:1–12
- Wang C, Lu S, Jiang J, Jia X, Dong X, Bu P (2014) Hsa-microRNA-101 suppresses migration and invasion by targeting Rac1 in thyroid cancer cells. *Oncol Lett* 8:1815–1821
- Wilusz JE, Sunwoo H, Spector DL (2009) Long noncoding RNAs: functional surprises from the RNA world. *Genes Dev* 23:1494–1504
- Wu W (2010) MicroRNA: potential targets for the development of novel drugs? *Drugs R D* 10:1–8
- Wu J, Gao F, Xu T, Li J, Hu Z, Wang C, Long Y, He X, Deng X, Ren D, Zhou B, Dai T (2020) CLDN1 induces autophagy to promote proliferation and metastasis of esophageal squamous carcinoma through AMPK/STAT1/ULK1 signaling. *J Cell Physiol* 235:2245–2259
- Xu Y, Wang J (2018) Long noncoding RNA XIST promotes proliferation and invasion by targeting miR-141 in papillary thyroid carcinoma. *Onco Targets Ther* 11:5035–5043
- Zhao H, Tang H, Huang Q, Qiu B, Liu X, Fan D, Gong L, Guo H, Chen C, Lei S, Yang L, Lu J, Bao G (2016) MiR-101 targets USP22 to inhibit the tumorigenesis of papillary thyroid carcinoma. *Am J Cancer Res* 6:2575–2586
- Zhou XY, Liu H, Ding ZB, Xi HP, Wang GW (2020) lncRNA SNHG16 promotes glioma tumorigenicity through miR-373/EGFR axis by activating PI3K/AKT pathway. *Genomics* 112:1021–1029

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.