Long noncoding RNA CASC9 promotes the proliferation and metastasis of papillary thyroid cancer via sponging miR-488-3p

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INTRODUCTION
Thyroid cancer is the most frequent endocrine malignancy. Its incidence is increasing all over the world. About 567,000 new cases were reported worldwide both in men and women in 2018. Thyroid cancer mostly occurs in women and the global incidence in women is three times higher than in men. Although the diagnosis and treatment approaches have improved greatly,
mortality rates from thyroid cancer have changed minimally.\(^1\)\(^2\) Papillary thyroid cancer (PTC) is the major subtype of this cancer, accounting for over 80% of all thyroid cancer.\(^3\)\(^4\) Patients with PTC carried a relatively favorable prognosis; however, a certain part of PTC patients suffered from metastasis to lymph nodes and lungs, leading to the poor clinical outcomes.\(^2\)\(^5\)\(^7\) Studies have demonstrated that genetic and epigenetic alterations play important roles in the development of PTC.\(^8\)\(^9\) However, the underlying molecular mechanisms remain poorly characterized. Therefore, clarifying the mechanisms associated with the pathogenesis of PTC may help to improve the therapeutical strategies for this cancer.

Long noncoding RNAs (lncRNAs) are one important member of noncoding RNAs. They are longer than 200 nucleotides than have limited protein-coding functions. Accumulating evidences indicated that lncRNAs display diverse roles in modulating gene transcription, posttranscription, translation, and epigenetic regulation.\(^10\)\(^11\) LncRNAs are abnormally expressed or dysregulated to mediate multiple human diseases, including cancers.\(^11\)\(^13\)\(^14\) LncRNAs have been demonstrated to participate in the development of PTC. For example, lncRNA ABHD11-AS1 was elevated in PTC, and it augmented the proliferation, migration, and invasion, and suppressed apoptosis of PTC cells by miR-199a-5p/SLC1A5 axis.\(^15\) The expression of lncRNA AB074169 was decreased in PTC, and overexpression of AB074169 caused cell cycle arrest and inhibited tumor growth.\(^16\) LncRNA AB074169 acted as a tumor suppressor in PTC via modulating KHSRP-mediated CDKN1a expression.\(^16\)

Cancer susceptibility candidate 9 (CASC9) is located on human chromosome 8q21.11.\(^17\) It was originally identified as a lncRNA related to esophageal squamous cell carcinoma (ESCC).\(^17\) CASC9 expression was increased in ESCC clinical samples and cells. Downregulation of CASC9 inhibited migration and invasion of ESCC cells.\(^17\) Subsequently, the roles of CASC9 in other cancer types were demonstrated. Studies have found that CASC9 expression was elevated in breast cancer,\(^18\) colorectal cancer,\(^19\) oral squamous cell carcinoma,\(^20\) ovarian cancer,\(^21\) lung adenocarcinoma,\(^22\) gastric cancer,\(^23\) and glioma,\(^24\) and CASC9 functioned as a tumor promotor in these cancers. However, the expression pattern and role of CASC9 in PTC remain unrevealed. In the current study, the expression of CASC9 was examined in human PTC tissues and cell lines. Additionally, the role and mechanism of CASC9 in PTC were investigated by loss-of-function assays both in vitro and in xenograft mice models.

2  | MATERIALS AND METHODS

2.1  | Human tissue collection

A total of 52 pairs of PTC and adjacent normal thyroid tissues (>3 cm from tumor borders) were obtained from patients who underwent thyroidectomies at Peking Union Medical College Hospital and The Mine Hospital of Xuzhou, from December 2016 to May 2018. All patients did not receive any local or systemic treatments before surgery. After confirming by two pathologists independently, collected fresh tissue samples were frozen in liquid nitrogen. All patients enrolled signed informed consent. This experiment was approved by the Research Ethics Committee of Peking Union Medical College Hospital.

2.2  | Cell culture and transfection

Normal human thyroid cell line, Nthy-ori3-1, and two human PTC cell lines (TPC-1 and BCPAP) were used in this study and they were purchased from Shanghai Huiying Biological Technology co., Ltd. TPC-1 and Nthy-ori3-1 cells were cultured in RPMI-1640 medium, and BCPAP cells were maintained in Dulbecco’s Modified Eagle’s medium. Both cell culture medium contains 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. These cells were grown in a cell incubator with 5% CO\(_2\) at 37°C.

The CASC9 interfering plasmid pLVX-CASC9-short hairpin RNA (CASC9 shRNA) was constructed (Target sequence 5'-GCCAGAACACAGTGGAAATGA-3') to downregulate CASC9 expression. And, a scrambled sequence was inserted into pLVX-shRNA1 plasmid, which was used as the control. CASC9 and ADAM9 overexpression plasmid pcDNA3.1-CASC9 and pcDNA3.1-ADAM9 were used to upregulate CASC9 and ADAM9 expression, respectively. miR-488-3p mimic, miR-488-3p antagomir (anti-miR-488-3p), and their controls were purchased from GenePharma. Both PTC cell lines were transfected with the above vectors using Lipofectamine 3000 reagent (Invitrogen) following the manufacturer’s instructions.

2.3  | Cell proliferation analysis

The proliferation of TPC-1 and BCPAP cells was analyzed by Cell counting kit-8 (CCK-8; Beyotime). Briefly, these cells were transfected with designated vectors for 24 hours and then reseeded into 96-well plates at a density of 4 \times 10^3 cells/well (a total of 100 μL). Cell proliferation ability was evaluated at different time points (0, 24, 48, and 72 hours) after planting by adding 10-μL CCK-8 solution. After culturing at 37°C for 30 minutes, a Microplate Reader (Bio-Rad) was used to determine the absorbance at 450 nm.

2.4  | Wound healing assay

Transfected PTC cells were seeded into 6-well plates at 3 \times 10^5 cells/well until approximately 90% confluent. A scratch was made using a 200-μL sterile pipette tip, and...
phosphate buffered saline (PBS) was used to wash the cells. Subsequently, cells were cultured in serum-free medium for another 24 hours, the widths of the scratches were imaged under a microscope, and calculated using ImageJ software.

2.5 | Transwell assay for cell invasion

After 24 hours transfection, 24-well Transwell chambers (8-μm pores; Millipore) with Matrigel (BD) were utilized to perform transwell invasion assay. TPC-1 and BCPAP cells, which were in serum-free medium, were planted in the apical chamber. The basolateral chamber was imbued with 600-μL culture medium containing 20% FBS. These cells were maintained at 37°C for another 24 hours. Cells, which did not traverse the filter, were wiped off using a cotton swab, and cells on the underside of the membrane were fixed with 4% formaldehyde, stained with 0.1% crystal violet, and photographed with a light microscope. The experiments were carried out independently in triplicate.

2.6 | Bioinformatic analysis and dual-luciferase reporter assay

Bioinformatic analysis using Starbase showed that both CASC9 and ADAM9 possess the binding sites of miR-488-3p. CASC9 cDNA was amplified from human PTC tissues and inserted into pGL3 plasmid. Also, the mutant miR-488-3p-binding sequence was introduced to construct the CASC9 mutant plasmid. TPC-1 cells were seeded into 48-well plates, followed by incubation for 24 hours. Then, cells were transfected with CASC9-WT or CASC9-MUT plasmid in combination with miR-488-3p/control mimic using Lipofectamine 3000. After transfection for 48 hours, the relative luciferase activity of each well was analyzed by a Dual-Luciferase Assay Kit (Promega).

2.7 | RNA immunoprecipitation assay

In BCPAP cells, an RNA immunoprecipitation (RIP) assay was developed using an EZ-Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore) with anti-human Ago2 antibody as previously described. The precipitated RNAs were isolated to determine the expression of CASC9 and ADAM9 using real-time PCR analysis.

2.8 | Mouse xenograft model

Twelve female BALB/c nude mice (5- to 6-week-old) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd and housed under specific pathogen-free conditions. All animal procedures were approved by the Animal Care Committee of Peking Union Medical College Hospital. Mice were randomly divided into two groups, and each group contains six mice. For xenograft models, $1 \times 10^7$ BCPAP cells stably transfected with CASC9 shRNA or negative control were subcutaneously injected into the back flanks of mice. The tumor volume was determined every 5 days using the following formula: $(\text{Length} \times \text{Width}^2)/2$. All mice were executed 30 days after implantation. Tumors were collected for further study.

2.9 | Real-time PCR

Trizol reagent (Invitrogen) was used to extract total RNAs from tissues and cultured cells. To analyze the expression of CASC9 and ADAM9, 2-μg total RNA was used for reverse transcription reaction by M-MLV Reverse Transcriptase (Promega), followed by Real-time PCR analysis with SYBR Green Real-time Master Mix (Toyobo). CASC9 and ADAM9 expression levels were measured using the $2^{-\Delta\Delta C_t}$ method normalized to GAPDH. To detect miR-488-3p expression, TaqMan MiRNA Reverse Transcription Kit and TaqMan Human MiRNA Assay Kit (Applied Biosystems) were used as the instructions. miR-488-3p expression was measured with normalization to U6 snRNA.

2.10 | Western blot

Proteins were extracted from tissues and cells with RIPA lysis buffer (Beyotime) including cocktail, a protease inhibitor. Protein concentrations were examined by a protein assay kit (Bio-Rad). Thirty microgram of protein was separated by SDS-PAGE. After that, the protein was electroblotted onto PVDF membranes (Millipore). The membrane was immunoblotted with primary antibodies against ADAM9, epidermal growth factor receptor (EGFR), p-EGFR, Akt, p-Akt, and GAPDH (both from Abcam) overnight at 4°C. After washing with PBS-Tween 20 (PBST), the membrane was incubated with HRP-labeled secondary antibody for 1 hour. Signals were developed with ECL detection reagent (Thermo Fisher). The relative expression level of ADAM9 was calculated by ImageJ software with normalization to GAPDH.

2.11 | Statistical analysis

All in vitro experiments were performed in triplicates independently. Results are presented as mean ± SD. Statistical analyses between groups were performed by Student’s $t$ test or Mann-Whitney $U$ test. Differences more than two groups were analyzed by one-way ANOVA followed by
Bonferroni post hoc test. Chi-squared test was used to assay the relationship between CASC9 and patients’ clinicopathological characteristics. The correlation between CASC9 and miR-488-3p or ADAM9 in PTC tissues was measured by Pearson’s correlation analysis. Data analysis was handled with SPSS19.0 software. Values were considered significant at \( P < .05 \).

3 | RESULTS

3.1 | CASC9 expression is elevated in PTC tissues and cell lines

We firstly measured the expression of CASC9 in 52 PTC tissues by real-time PCR. The results showed that CASC9 expression was higher in PTC tissues than that in adjacent normal thyroid tissues (Figure 1A). The involvement between CASC9 and clinicopathological parameters was further analyzed. We found that higher CASC9 expression was related to large tumor size, advanced stage, or lymph node metastasis. No significant correlation was noted between CASC9 expression and other clinical features, including age, gender, or multifocality (Table 1). Then, CASC9 expression was detected in two human PTC cell lines. As shown in Figure 1B, CASC9 expression was higher in TPC-1 and BCPAP PTC cells than that in normal human thyroid Nthy-ori3-1 cells.

3.2 | CASC9 promotes the proliferation, migration, and invasion of PTC cells

The role of CASC9 in PTC was analyzed by downregulation or overexpression of CASC9. As shown in Figure 2A, the CASC9 shRNA notably decreased CASC9 expression, whereas pcDNA3.1-CASC9 significantly elevated CASC9 expression. CCK-8 analysis displayed that knockdown of CASC9 reduced the proliferation of TPC-1 and BCPAP cells (Figure 2B). The migratory abilities of PTC cells were suppressed after downregulation of CASC9, which was revealed by a wound healing assay (Figure 2C). Transwell assay was conducted to measure the influence of CASC9 on the invasion of PTC cells. Results demonstrated that compared with cells transfected with control shRNA, the number of invasive cells transfected with CASC9 shRNA was decreased (Figure 2D). The results also showed that overexpression of CASC9 facilitated the proliferation, migration,

**TABLE 1** Correlation between Cancer susceptibility candidate 9 (CASC9) expression and clinicopathological characteristics of 52 PTC patients

| Characteristics          | Number | Low (n = 26) | High (n = 26) | \( P \) |
|--------------------------|--------|--------------|---------------|-------|
| Age                      |        |              |               |       |
| <45                      | 31     | 18           | 13            | .158  |
| ≥45                      | 21     | 8            | 13            |       |
| Gender                   |        |              |               |       |
| Male                     | 17     | 7            | 10            | .375  |
| Female                   | 35     | 19           | 16            |       |
| Tumor size               |        |              |               |       |
| <2 cm                    | 23     | 16           | 7             | .012* |
| ≥2 cm                    | 29     | 10           | 19            |       |
| Multifocality            |        |              |               |       |
| Present                  | 27     | 12           | 15            | .405  |
| Absent                   | 25     | 14           | 11            |       |
| Lymph node metastasis    |        |              |               |       |
| Negative                 | 22     | 16           | 6             | .005* |
| Positive                 | 30     | 10           | 20            |       |
| TNM stage                |        |              |               |       |
| I/II                     | 33     | 21           | 12            | .01*  |
| III/IV                   | 19     | 5            | 14            |       |

*Note: Chi-squared test.*

TNM, Tumor Node Metastasis

\* \( P < .05 \).
and invasion of TPC-1 and BCPAP cells (Figure 2B-D). Herein, the data indicated that CASC9 could promote the proliferation, migration, and invasion of PTC cells.

### 3.3 CASC9 directly binds to and modulates miR-488-3p in PTC cells

Next, the mechanism of CASC9 was determined. The target prediction tool Starbase showed that CASC9 has the potential binding sites for miR-488-3p. The putative binding sequence was revealed in Figure 3A. Their relationship was further confirmed and we found that miR-488-3p mimic dramatically suppressed the luciferase activity of CASC9-WT, but not the luciferase activity of CASC9-MUT compared to the control mimic (Figure 3B). We also found that miR-488-3p expression was remarkably enhanced in TPC-1 and BCPAP cells with CASC9 knockdown (Figure 3C). Moreover, miR-488-3p expression was decreased in human PTC tissues compared to matched para-cancerous tissues (Figure 3D). Importantly, we found that a negative relationship existed between CASC9 and miR-488-3p in tumor tissues (Figure 3E). Also, miR-488-3p expression was decreased in PTC cells compared with Nthy-ori3-1 cells (Figure 3F). These results indicated that CASC9 may function by sponging miR-488-3p.

### 3.4 CASC9 functions via mediating miR-488-3p in PTC cells

The role of miR-488-3p in PTC cells was analyzed. As shown in Figure 4, miR-488-3p mimic inhibited the...
proliferation, migration, and invasion of TPC-1 and BCPAP cells. Contrarily, anti-miR-488-3p promoted these features of PTC cells (Figure 4). These data indicated that miR-488-3p could attenuate the proliferation, migration, and invasion of PTC cells.

We further investigated whether CASC9 functions via mediating miR-488-3p. The results demonstrated that the effects of CASC9 silencing on the proliferation, migration, and invasion of TPC-1 and BCPAP cells were abrogated by anti-miR-488-3p. However, miR-488-3p mimic further strengthened the effect of CASC9 downregulation (Figure 5). In summary, these results suggested that CASC9 facilitated the proliferation, migration, and invasion of PTC cells by negatively regulating miR-488-3p in PTC cells.

3.5 | CASC9 regulates ADAM9-EGFR-Akt signaling by sponging miR-488-3p

This study has shown that miR-488-3p could regulate ADAM9 expression.26 ADAM9 has been found to be responsible for the migration and invasion of thyroid cancer.27 Hence, we further verified the relationship between CASC9 and ADAM9 in PTC. ADAM9 expression was higher in PTC tissues and cells than their controls (Figure 6A,B). There was a positive association between CASC9 and ADAM9 in PTC samples (Figure 6C). An RIP assay with anti-Ago2 antibody revealed that the enrichment of Ago2 on CASC9 was decreased, but enrichment on ADAM9 was elevated after CASC9 downregulation (Figure 6D). Furthermore, miR-488-3p mimic notably decreased ADAM9 expression both in TPC-1 and BCPAP cells. Knockdown of CASC9 reduced ADAM9 expression, which was attenuated by anti-miR-488-3p, but further strengthened by miR-488-3p mimic (Figure 6E,F). Also, both CASC9 knockdown and miR-488-3p mimic could suppress the activation of EGFR and Akt, as demonstrated by deceased phosphorylation of EGFR and Akt. The influences of CASC9 shRNA on the activation of EGFR and Akt were restored by anti-miR-488-3p, whereas enhanced by miR-488-3p mimic (Figure 6F). In addition, the suppressed proliferation, migration, and invasion of PTC cells...
by CASC9 silencing were attenuated by ADAM9 upregulation (Figure 6G-I). These results demonstrated that CASC9 functioned in PTC cells via regulating ADAM9 expression by sponging miR-488-3p.

3.6 CASC9 downregulation decreases tumor growth in vivo that is related to miR-488-3p/ADAM9 axis

The effect of CASC9 on PTC was validated in xenograft mouse models. CASC9 shRNA significantly reduced CASC9 expression in mice tumor tissues (Figure 7A). Tumor volume was notably reduced in the CASC9 shRNA group compared to the control (Figure 7B). As excepted, downregulation of CASC9 significantly reduced tumor weight (Figure 7C). The expression of miR-488-3p was elevated, while ADAM9 level was decreased in tumor tissues from mice transfected with CASC9 shRNA compared with the control shRNA (Figure 7D,E). These data indicated that CASC9 knockdown decreased tumor growth in mice, which was associated with miR-488-3p/ADAM9 pathway.

4 DISCUSSION

Increasing studies have shown that lncRNAs participate in the initiation and progression of cancers, including PTC.11,28,29 In this study, we found that CASC9 expression was elevated in human PTC tissues and cells. It facilitated the proliferation and metastasis of PTC via modulating miR-488-3p/ADAM9/EGFR-Akt axis.

In recent years, the studies of CASC9 are drawing more and more attention in cancers. Wu et al30 showed that CASC9 expression was increased in ESCC tissues. Its expression was positively involved in tumor size and TNM stage, and its high level predicted poor overall survival of patients with ESCC. Interfering CASC9 suppressed ESCC cell growth and blocked cell cycle G1/S transition in vitro, as well as inhibited tumorigenesis in nude mice.30 It functioned via suppressing PDCD4 expression by recruiting enhancer of zeste homolog 2 and subsequently altering H3K27me3 level in PDCD4 promoter.30 CASC9 also facilitated ESCC metastasis via elevating LAMC2 expression by regulating CREB-binding protein-mediated histone acetylation.31 The expression of CASC9 was increased in ovarian cancer tissues and cells.
and an enhanced level predicted an unfavorable prognosis in ovarian cancer patients. CASC9 accelerated ovarian cancer cell proliferation, invasion, and migration via acting as a competing endogenous RNA (ceRNA) for miR-758-3p to modulate LIN7A expression. CASC9 enhanced breast cancer cell proliferation and metastasis through positively regulating CHK1 via sponging the miR-195/497 cluster or through miR-215/TWIST2 signaling. Elevated CASC9 promoted oral squamous cell carcinoma progression by inhibiting autophagy-mediated apoptosis. However, the influence of CASC9 in PTC remains enigmatic. Hence, this study investigated the role of CASC9 in PTC. We found that CASC9 expression was higher in PTC tissues than in adjacent normal tissues. Elevated CASC9 level was associated with poor clinicopathological features. Also, CASC9 expression was boosted in PTC cells. This suggested that CASC9 may be a regulator in PTC. Our further study demonstrated that knockdown of CASC9 suppressed the proliferation, migration, and invasion of PTC cells in vitro, and inhibited tumor growth in vivo. Whereas CASC9 overexpression elevated the proliferation, migration, and invasion of PTC cells. These results indicated that CASC9 could promote the proliferation and metastasis of PTC.

LncRNAs participate in various biological process mainly via acting as ceRNAs. The mechanism of CASC9 in PTC was mainly focused on the ceRNA regulation. miR-488-3p was found to be an important tumor suppressor in various cancers. In this study, we found that miR-488-3p expression was decreased in PTC tissues and cells. miR-488-3p mimic suppressed, whereas anti-miR-488-3p promoted the proliferation, migration, and invasion of PTC cells, which demonstrated that miR-488-3p played tumor-suppressing roles in PTC. Next, we further investigated the relationship between CASC9 and miR-488-3p. Bioinformatic analysis showed that CASC9 has the putative binding sites for miR-488-3p, and dual-luciferase reporter assay verified their direct combination. And, CASC9 could negatively regulate miR-488-3p expression in PTC cells. As expected, miR-488-3p expression was negatively related to CASC9 in PTC tissues. In addition, anti-miR-488-3p significantly reversed

**FIGURE 5** Cancer susceptibility candidate 9 (CASC9) functions via negatively regulating miR-488-3p. TPC-1 and BCPAP cells were transfected with sh-CASC9 and miR-488-3p mimic/anti-miR-488-3p or transfected with sh-NC/sh-CASC9 only. A, CCK-8 was used to assay cell viability. B, Wounding healing analysis for cell migration. C, The invasion of these cells under different treatment was determined by transwell. *P < .05 vs the sh-NC group, #P < .05 vs the sh-CASC9 group.
the suppressed proliferation, migration, and invasion of PTC cells by CASC9 downregulation. And, miR-488-3p mimic further strengthened the effects of CASC9 silencing on PTC cells. In general, these data suggested that CASC9 functioned in PTC via interacting with miR-488-3p.

Members of ADAM family are closely associated with a variety of biological events, like ectodomain shedding, cell proliferation, adhesion, migration, and invasion. ADAM9, as an important member of this family, is widely distributed in human tissues and involved in various biological processes. ADAM9 is highly expressed in multiple cancers and is related to aggressive tumor phenotypes and poor clinical outcomes. ADAM9 has been found to be responsible for the growth and metastasis of thyroid cancer. We found that ADAM9 expression was higher in PTC tissues and cells than their controls. ADAM9 was demonstrated to be a target of miR-488-3p in non-small cell lung cancer. We also confirmed that miR-488-3p could regulate ADAM9 expression in PTC cells. Therefore,
we further determined the association between CASC9 and ADAM9. Our results showed that ADAM9 expression was positively related to CASC9 in human PTC tissues. An RIP assay with anti-Ago2 antibody and expression analysis demonstrated that CASC9 acted as a ceRNA for miR-488-3p to elevate ADAM9 expression. We also found overexpression of ADAM9 partially reversed the suppressed proliferation, migration, and invasion of PTC by CASC9 silencing. Additionally, this study found downregulation of CASC9 in mice notably increased miR-488-3p, but decreased ADAM9 expression in tumors. Although we did not further evaluate the regulatory mechanism among CASC9, miR-488-3p, and ADAM9 in vivo using restore experiments, considering the detailed regulatory mechanism among them has been verified in PTC cells, our results were sufficient to demonstrate that CASC9 regulated the malignant phenotypes of PTC via miR-488-3p/ADAM9 pathway. In general, these results suggested that CASC9 promoted the malignant properties in PTC by sponging miR-488-3p to relieve its inhibition on ADAM9 expression, leading to the activation of EGFR-Akt signaling.

In conclusion, our study proved for the first time that elevated CASC9 expression promoted the proliferation and metastasis of PTC. The data described a novel mechanism of CASC9 in cancers. CASC9 facilitated the aggressive phenotypes of PTC by regulating miR-488-3p/ADAM9 pathway. This study will increase our understanding of the progression of PTC and may provide a novel target for the treatment of this disease.

CONFLICT OF INTEREST
The authors have no conflict of interest.

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REFERENCES
1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
2. Cabanillas ME, McFadden DG, Durante C. Thyroid cancer. *Lancet*. 2016;388:2783-2795.
3. Cancer Genome Atlas Research N. Integrated genomic characterization of papillary thyroid carcinoma. *Cell*. 2014;159:676-690.
4. Yu K, Wang T, Li Y, et al. Niclosamide induces apoptosis through mitochondrial intrinsic pathway and inhibits migration and invasion in human thyroid cancer in vitro. *Biomed Pharmacother*. 2017;92:403-411.
5. Lee YC, Na SY, Park GC, Han JH, Kim SW, Eun YG. Occult lymph node metastasis and risk of regional recurrence in papillary thyroid cancer after bilateral prophylactic central neck dissection: a multi-institutional study. *Surgery*. 2017;161:465-471.
6. Yang L, Sun R, Wang Y, et al. Expression of ANGPTL2 and its impact on papillary thyroid cancer. *Cancer Cell Int*. 2019;19:204.
7. Ito Y, Ishikawa H, Kihara M, et al. Control of lung metastases and colon polyposis with lentivinib therapy in a patient with cribriform-morular variant of papillary thyroid carcinoma and an APC gene mutation: a case study. *Thyroid*. 2019;29(10):1511-1517.
8. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. *Nat Rev Cancer*. 2013;13:184-199.
9. Kowalska A, Walczyk A, Kowalik A, et al. Increase in papillary thyroid cancer progression is accompanied by changes in the frequency of the BRAF V600E mutation: a single-institution study. *Thyroid*. 2016;26:543-551.
10. Rammarine VR, Kobelev M, Gibb EA, et al. The evolution of long noncoding RNA acceptance in prostate cancer initiation and progression, and its clinical utility in disease management. *Eur Urol*. 2019;76(5):546-559.
11. Jiang MC, Ni JJ, Cui WY, Wang BY, Zhuo W. Emerging roles of IncRNA in cancer and therapeutic opportunities. *Am J Cancer Res*. 2019;9:1354-1366.
12. Roy S, Awasthi A. Emerging roles of noncoding RNAs in T cell differentiation and functions in autoimmune diseases. *Int Rev Immunol*. 2019;38(5):232-245.
13. Chang W, Wang J. Exosomes and their noncoding RNA cargo are emerging as new modulators for diabetes mellitus. *Cells*. 2019;8:853.
14. Hosseini E, Bagheri-Hosseinabadi Z, De Toma I, Jafarisani M, Sadeghi I. The importance of long non-coding RNAs in neuropsychiatric disorders. *Mol Aspects Med*. 2019;70:127-140.
15. Zhang X, Tong H, Ding YU, et al. Long noncoding RNA ABHD11-AS1 functions as a competing endogenous RNA to regulate papillary thyroid cancer progression by miR-199a-5p/SLC1A5 axis. *Cell Death Dis*. 2019;10:620.
16. Gou Q, Gao L, Nie X, et al. Long noncoding RNA AB074169 inhibits cell proliferation via modulation of KHSRP-mediated CDKN1a expression in papillary thyroid carcinoma. *Can Res*. 2018;78:4163-4174.
17. Pan Z, Mao W, Bao Y, Zhang M, Su X, Xu X. The long noncoding RNA CASC9 regulates migration and invasion in esophageal cancer. *Cancer Med*. 2016;5:2442-2447.
18. Zhang J, Wang Q, Quan Z. Long non-coding RNA CASC9 enhances breast cancer progression by promoting metastasis through the mediation of miR-215/TWIST2 signaling associated with TGF-beta expression. *Biochem Biophys Res Comm*. 2019;515:644-650.
19. Luo K, Geng J, Zhang Q, et al. LncRNA CASC9 interacts with CPSF3 to regulate TGF-beta signaling in colorectal cancer. *J Exp Clin Cancer Res*. 2019;38:249.
37. Ueno M, Shiomi T, Mochizuki S, et al. ADAM9 is over-expressed in human ovarian clear cell carcinomas and suppresses cisplatin-induced cell death. Cancer Sci. 2018;109:471-482.

38. Wang J-J, Zou JX, Wang H, et al. Histone methyltransferase NSD2 mediates the survival and invasion of triple-negative breast cancer cells via stimulating ADAM9-EGFR-AKT signaling. Acta Pharmacol Sin. 2019;40:1067-1075.

39. Fisher KE, Jani JC, Fisher SB, et al. Epidermal growth factor receptor overexpression is a marker for adverse pathologic features in papillary thyroid carcinoma. J Surg Research. 2013;185:217-224.

40. Han J, Zhang M, Nie C, et al. miR-215 suppresses papillary thyroid cancer proliferation, migration, and invasion through the AKT/GSK-3beta/Snail signaling by targeting ARFGEF1. Cell Death Dis. 2019;10:195.

41. Zhang Y, Sui F, Ma J, et al. Positive feedback loops between NrCAM and major signaling pathways contribute to thyroid tumorigenesis. J Clin Endocrinol Metab. 2017;102:613-624.

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