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LncRNA HOTAIR Influences the Growth, Migration, and Invasion of Papillary Thyroid Carcinoma via Affection on the miR-488-5p/NUP205 Axis

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Abstract

Objective: The study was aim to investigate the effect of HOX transcript antisense RNA (HOTAIR) on the growth, migration, and invasion of papillary thyroid carcinoma (PTC) and its underlying mechanisms. **Methods:** Cell growth, invasion, and migration was respectively investigated using the MTT assay, trans-well assay, and wound healing assay. The expression of genes and proteins was respectively determined by Western blot analysis and RT-PCR experiments. **Results:** It was demonstrated that high expression of HOTAIR in PTC cells (BCPAP) and tissues resulted in fast tumor growth and poor survival time of the PTC-bearing mice models. Moreover, overexpression of HOTAIR leaded to markedly enhanced proliferation, migration, and invasion of BCPAP cells. Increase the levels of HOTAIR in BCPAP cells signally down-regulated the miR-488-5p levels which was able of inhibiting the growth rate, increasing the apoptosis rate, and decreasing the invasion/migration ability of BCPAP cells. Further studies indicated that HOTAIR promoted BCPAP cell growth, invasion, and migration mainly through regulating the miR-488-5p/NUP205 axis and the levels of Bcl-2 as well. **Conclusion:** HOTAIR promoted the growth, migration, and invasion of papillary thyroid carcinoma mainly through regulating the miR-488-5p/NUP205 axis.

Keywords

lncRNA HOTAIR, miR-488-5p, papillary thyroid carcinoma, cell proliferation, tumor progress

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Introduction

As the most common endocrinal malignant tumor, thyroid cancer has an annually increased global incidence and showed quickly increased morbidity.[1,2] Among all patients who are diagnosed with thyroid cancer, more than 80% of patients are carried with papillary thyroid carcinoma (PTC) named for its papillary histological architecture.[2] Although most patients with thyroid carcinoma achieved a relatively good prognosis than other cancer types, high rate of metastasis and recurrence after routine treatments was observed in a wide range if PTC patients.[3-5] Previous study pointed out that about 20% of PTC patients were diagnosed with metastasis to the regional lymph nodes and resulted in increased mortality.[6]

Long noncoding RNAs (lncRNAs) comprise a very heterogeneous subclass of RNAs that were firstly described by the genome-wide sequencing of cDNA libraries of the mouse genome.[7] Accumulating evidences have demonstrated that lncRNAs play significant roles in the regulation of a variety of biological processes.[8] As one of the most widely studied lncRNAs, lncRNA HOTAIR has been reported was highly expressed in many tumors and its expression level was closely related to the pathological grade, clinical stage and prognosis of some tumors.[9-11] It was previously demonstrated that the biologic function of HOTAIR was validated in human TC cell lines as well and dysregulation of HOTAIR is correlated with metastasis and poor prognosis in patients with TC.[12-14] Aberrant expression of HOTAIR was detected in human TC cells and knockdown of HOTAIR significantly inhibited cell growth and invasion in TPC-1 and SW579 human TC.[12] Additionally,

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previous studies have demonstrated that HOTAIR single nucleotide polymorphisms (SNPs) was associated with the risk of PTC and high expression HOTAIR in PTC tissues resulted in poorer prognosis in general.[15,16] However, the detailed role of HOTAIR in regulation of PTC and its underlying mechanisms are not thoroughly investigated to date.

In general, the lncRNAs participates in the process of tumorigenesis mainly through regulating the expression of multiple microRNAs (miRNAs). For example, the LncRNA ANRIL promoted proliferation, metastasis, and invasion of hepatocellular carcinoma *via* regulating the miR-122-5p expression.[17] Besides, it was reported that the LncRNA CCAT2 regulates the proliferation and invasion of colon cancer cells by affection of the miR-145 level.[18] MicroRNAs (miRNAs) are a class of short non-coding RNAs that can affect tumorigenesis and are closely related to tumor invasion and metastasis.[19] The underlying mechanism of miRNA regulates tumor metastasis and infiltration of tumor cells may lies in that miRNA is able of changing the expression of specifc genes and then affects the connection of tumor cells.[20] miR-488-5p was identified as a tumor suppressor in many cancers, including the acute myeloid leukemia, colorectal cancer, and breast cancer.[20-22] However, the role of miR-488-5p in regulating the progression and metastasis of papillary thyroid carcinoma remain unclear. Therefore, the present study was aimed to evaluate the effect of HOTAIR on tumorigenesis and growth of papillary thyroid carcinoma and the potential relationship between miR-488-5p.

Materials and Methods

Materials

The dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco; Thermo Fisher Scientific, Inc. (Waltham, MA, USA). The 10% heat-inactivated fetal bovine serum (FBS) was achieved from Hyclone (Logan, USA) while the 1% antibiotic/antimycotic solution was from Sigma-Aldrich, Merck KGaA (Darmstadt, Germany). The lentivirus respectively containing HOTAIR, miR-488-5p, si-HOTAIR, si-miR-488-5p and negative control (NC) were purchased from Shanghai Gene Pharma Company (Shanghai, China). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The primary antibodies and horseradish peroxidase conjugated IgG were obtained from BD Bioscience (San Diego, CA, USA). The reagents used here were all analytical grade and obtained from Aladdin reagent co., Ltd. (Shanghai, China) if not mention elsewhere.

Cell Lines and Animals

Human PTC cells (BCPAP) and the normal human thyroid cells (HT-ori 3) were purchased from Cancer Research Center of Chinese Academy of Medical Sciences. Both cells were cultured in DMEM supplemented with penicillin-streptomycin and 10% FBS at 37°C with 5% CO_2.

Cells Transfection

The BCPAP cells were seeded into 6-well plates and allowed to grown for 80% confluence. Subsequently, cells were respectively infected with various genes-contained lentivirus using the Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) in accordance with the manufacturer's protocol. After 48 h of incubation, all BCPAP cells were collected for further experiments and divided to the following experimental groups: Control group (untransfected BCPAP cells), NC group (negative control siRNA-transfected BCPAP cells), HOTAIR group (HOTAIR transfected BCPAP cells), si-HOTAIR group (si-HOTAIR transfected BCPAP cells), miR-488-5p mimic group (miR-488-5p mimic transfected BCPAP cells), and miR-488-5p inhibitor group (miR-488-5p inhibitor transfected BCPAP cells).

Cell Growth

The collected transfected cells were seeded into 96-well plates at the density of 5×10^3 per well. After respectively incubated the cells for 12 h, 24 h, and 48 h, 20 μL MTT solution was added into each well and allowed to co-incubate with cells for 2 h. Then the optical density (OD) value of each well was determined by a microplate reader.

Colony Formation Experiment

BCPAP cells post transfection were seeded in a 6-well plate at a density of 400 cells/well and cultured at 37°C with 5% CO_2. Then the cells were allowed to consistently culture for 1 week with the culture medium was replaced with fresh ones every 2 days. After that, the BCPAP cells were washed twice with PBS followed by fixation with 4% paraformaldehyde for 30 min. Thereafter, a crystal violet stain was applied to qualitatively analysis the colony formation.

Wound Healing Assay

BCPAP cells post transfection were respectively seeded in 96-well plates at the density of 1×10^4 per well. After incubation for 24 h and the cells were grown to form a confluent cell monolayer, a scratch was made in each well of the plates using 100-μl sterile pipette tip. Then the BCPAP cells were cultured for another 24 h and the migration rate were quantitatively analyzed.

Trans-Well Assay

For transwell assay, the upper chambers were pre-coated with Matrigel (Invitrogen; Thermo Fisher Scientific, Inc.) while the lower chamber were contained by DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Thereafter, 1×10^5 BCPAP cells post transfection were plated on the upper chamber and allowed to grown for 24 h. Then the lower chambers were fixed with 4% paraformaldehyde solution with the upper chambers were swabbed out. After the invasive cells were

stained with crystal violet, invasion ability of the BCPAP cells transfected by various genes or not were quantitatively analyzed.

Cell Apoptosis Assay

After 48 h of transfection, the BCPAP cells post transfection were collected by centrifugation at 1000 g for 5 min with supernatant removed. Then the cells in each tube were incubated with 150 μL binding buffer and 5 μL Annexin-V-FITC and mixed up. After incubation for 15 min under the dark, the mixture of 150 μL binding buffer and 5 μL Annexin-V-FITC was added. The apoptosis rate of cells was determined using the flow cytometry (FACSCanto II, BD Biosciences, San Jose, CA).

Western Blot Assay

Total protein was extracted using the cell lysate followed by quantification by the bicinchoninic acid (BCA; Pierce, Rockford, IL, USA). Then the samples were separated by 10% Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDSPAGE) and transferred to the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After blocking with with 5% skim milk for overnight, the samples were incubated with primary antibodies against NUP205 (1:1,500) and Bcl-2 (1:1,500) at 4°C for 1 h. After washing with PBST, the horseradish peroxidase conjugated IgG (1:3,000) was added and incubated for 24 hour. Finally, the samples were analyzed using the ECL kit (Merck Millipore, Billerica, MA, USA) with β-actin as the internal reference.

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's protocol. The concentrations of different total RNA were determined BCA kit followed by transcription into cDNA (Takara Biotechnology Co., Ltd., Dalian, China) by the PrimeScript RT Master Mix kit (Invitrogen, Carlsbad, CA, USA). Then the PCR amplification reactions were performed using the SYBR green detection method under the help of Bio-Rad iQ iCycler detection system. The specific primer sequences used here were as follows: HOTAIR, F: 5'-GGGTGGCTCACTCTTCTGGC-3', R: 5'- TGGCCTTGCCCGGGCTTGTC-3'; miR-488-5p, F: ACACTCCAGCTGGGTAGCAGCACATCATGG, R: TGGTGTCGTGGAGTCG; NUP205, F: GAAACTTCTGGACATTGAAGGA, R: TGAGGATGGAACTAGGGGAAG; Bcl-2, F: 5'-GCTCAGCCCTGTGCCACCTG-3'; R: 5'-CAGAGGTCGCATGCTGGGGC-3'; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'. Each sample was subjected to a 3-hole replicates and the experiment was repeated twice. The densitometry of relative mRNA expression was performed using the calculation of $2^{-\Delta\Delta Cq}$ and GAPDH was used as an internal control.

Dual-Luciferase Reporter Gene Assay

The potential target site of miR-488-5p was predicted using the Targetscan, miRanda, and Pictar. The sequences of NUP205 3'UTR were inserted into the pGL3 promoter vector followed by transfection of the cells with miR-488-5p mimic or negative control. At 48 hours, luciferase activity was determined using the dual-luciferase reporter assay kit (Promega, Madison, WI, USA).

Tumor Growth Experiments

Ectopic papillary thyroid carcinoma models were established by subcutaneous injection with various 1×10^6 BCPAP cells on the right flanks. Then all mice were raised under the standard condition with free accesses to water and food. For survival investigation, 50 tumor-bearing mice were divided into 5 groups: Control group (mice injected with BCPAP cells), NC group (mice injected with negative control siRNA-transfected BCPAP cells), HOTAIR group (mice injected with HOTAIR transfected BCPAP cells), si-HOTAIR group (mice injected with si-HOTAIR transfected BCPAP cells), and miR-488-5p group (mice injected with miR-488-5p transfected BCPAP cells). Then the survival of each mouse was carefully observed and recorded. Additionally, the tumor growth rate of each mouse was also analyzed by calculation of the tumor volumes every 2 days.

Statistical Analysis

All data were presented as the mean $\pm$ standard deviation and analyzed using the SPSS 19.0 software. Comparisons between groups were performed by Student's t-test with $P < 0.05$ was considered to indicate a statistically significant difference. All experiments in the present study were repeated at least 3 times independently.

Results

High Expression of HOTAIR in PTC Cells and Tissues Resulted in Fast Tumor Growth and Poor Survival Time of the PTC-Bearing Mice Models

The expression of HOTAIR in PTC cells (BCPAP) was firstly detected by RT-PCR experiments and compared with the normal cells (HT-ori 3). As shown in Figure 1A, obvious higher signal of HOTAIR was observed in the BCPAP cells compared with the normal cells. Moreover, 16 pair of PTC tissues and normal control thyroid tissues from animal models were obtained for HOTAIR detection using the RT-PCR method. As shown in Figure 1B, the levels of HOTAIR in PTC tissues was dramatically higher than that in normal control thyroid tissues. These results confirmed a higher expression of

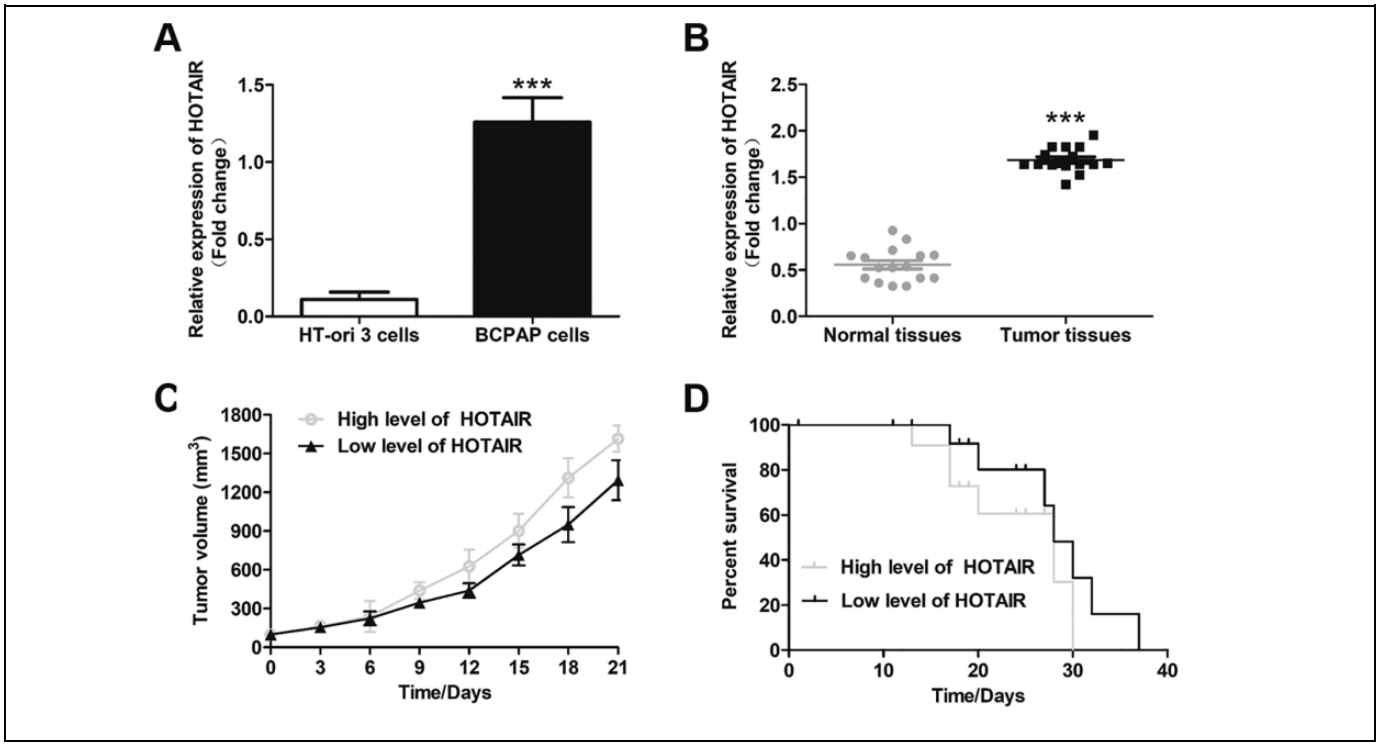

Figure 1. High expression of HOTAIR in PTC cells and tissues resulted in fast tumor growth and poor survival time of the PTC-bearing mice models. (A) The expressions of HOTAIR in PTC cells (BCPAP) and normal control cells (HT-ori 3 cells) detected using the RT-PCR analysis. (B) The levels of HOTAIR in PTC tissues and normal control thyroid tissues analyzed by the RT-PCR assay. (C) Tumor growth rates in the groups of high level of HOTAIR and low level of HOTAIR. (D) Percent survivals of the PTC-bearing mice from the groups of high level of HOTAIR and low level of HOTAIR examined using the Kaplan-Meier curve. ***$P < 0.001$ significantly higher than the normal cells (HT-ori 3) or normal thyroid tissue.

HOTAIR in PTC cells and tissues than the corresponding normal ones.

Additionally, the effect of HOTAIR on promotion of tumor growth was subsequently investigated on the PTC-bearing mice models. For experiments, 2 kinds of PTC-bearing mice models were firstly established by injection on the right flanks of mice with HOTAIR transfected BCPAP cells (high level of HOTAIR) and HOTAIR non-transfected BCPAP cells (low level of HOTAIR), respectively. As shown in Figure 1C and D, the tumors in the group of high level of HOTAIR ($n = 10$) exhibited obvious faster tumor growth rate than that from the group of low level of HOTAIR ($n = 10$), and resulted in shorter medium survival time. These results together might suggested that overexpression of HOTAIR might acted as a pivotal promoter of tumorigenesis of papillary thyroid carcinoma.

Up-Regulation of HOTAIR Leaded to Rapid Growth, Proliferation, Invasion and Migration of Papillary Thyroid Carcinoma Cells

After transfection of the BCPAP cells with different strategies, the expression of HOTAIR was confirmed by RT-PCR experiments. As shown in Figure 2A, the cells in the group of HOTAIR displayed the highest level of HOTAIR among all groups. In contrast, the cells transfected with si-HOTAIR have the lowest level of HOTAIR compared with other groups. Then the role of HOTAIR in regulation of cell process was determined. As shown in Figure 2B and C, the cells in the group of HOTAIR exhibited the rapidest growth and proliferation rates compared with others. However, the growth and proliferation ability of BCPAP cells was signally down-regulated after transfection with si-HOTAIR. Besides the promotion effect on cell growth and proliferation, the HOTAIR was also demonstrated that played significant role in promotion of invasion and migration of BCPAP cells. As shown in Figure 2D and E, the cells overexpressed with HOTAIR displayed the rapidest invasion and migration rate compared with other groups. In contrast, after knockdown of HOTAIR by si-HOTAIR, the invasion and migration ability of BCPAP cells was dramatically down-regulated.

Increase the Levels of HOTAIR Signally Down-Regulated the miR-488-5p Levels in Papillary Thyroid Carcinoma Cells

As shown in Figure 3A, the expression of miR-488-5p in the HOTAIR transfected BCPAP cells was signally lower than the cells in control group or NC group. However, knockdown of the expression of HOTAIR leaded to an obvious increase of miR-488-5p level. These results indicated that the high level

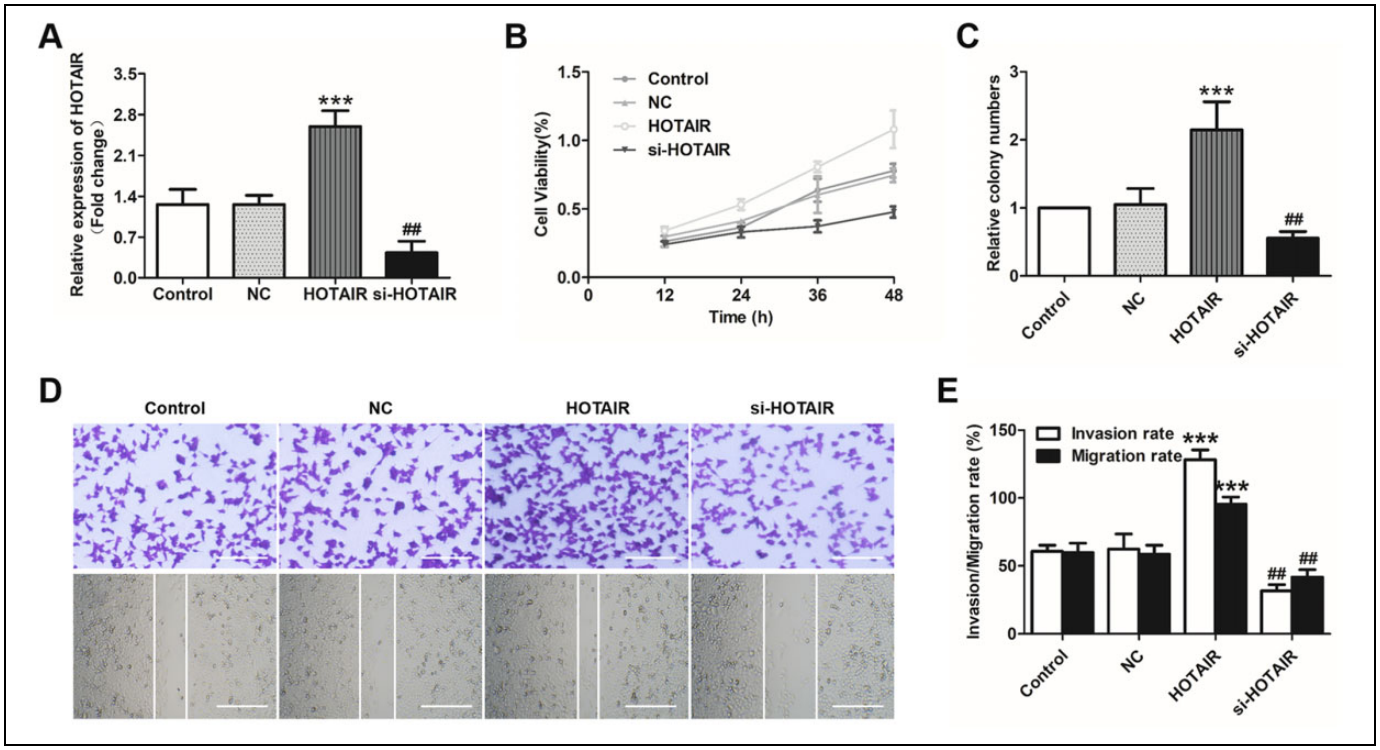

Figure 2. High expression of HOTAIR leaded to rapid growth, proliferation, invasion and migration of papillary thyroid carcinoma cells. (A) Expression of HOTAIR in BCPAP cells post various transfections. (B) Cell viability of BCPAP cells post transfections determined by the CCK-8 assay. (C) Quantitative evaluation of the proliferation rate of BCPAP cells after different transfection. (C) The invasive ability of BCPAP cells after different transfections investigated by the crystal purple staining methods (upper) and the migration ability of BCPAP cells post different transfections determined using the wound healing assay (lower). The bar represents 100 μm. (D) Semi-quantitative evaluation of the invasive ability and migration ability of BCPAP cells post different transfections. ***$P < 0.001$ significantly higher than the control or NC group. $^{\#}P < 0.05$ significantly lower than the control or NC group.

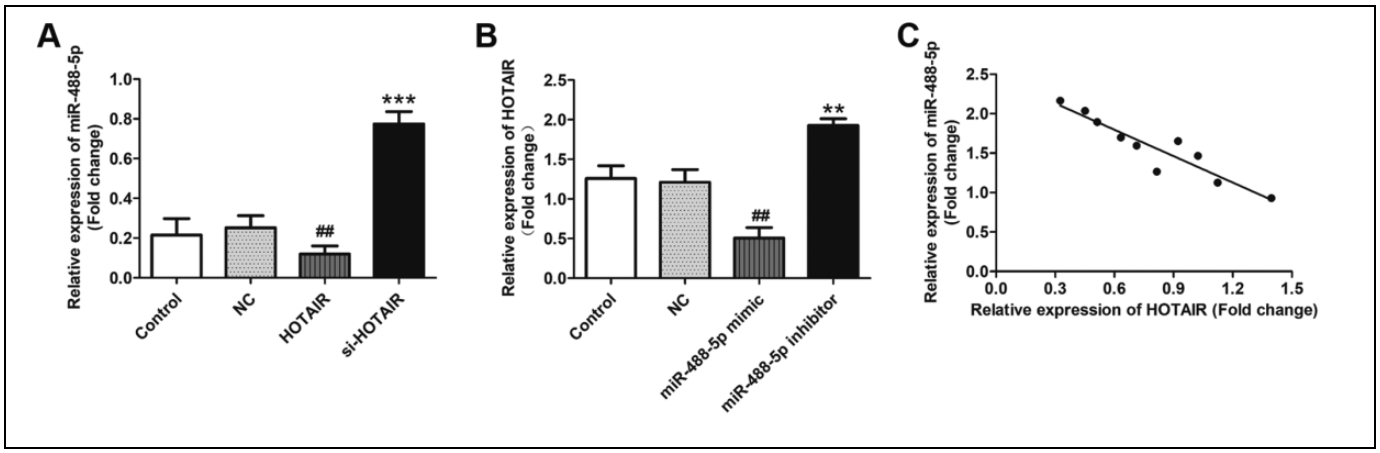

Figure 3. Up-regulation of the levels of HOTAIR signally down-regulated the miR-488-5p levels in papillary thyroid carcinoma cells. (A) Determination of the expression of miR-488-5p in the BCPAP cells after transfected with NC, HOTAIR, and si-HOTAIR. (B) The levels of HOTAIR in the BCPAP cells post transfected with miR-488-5p and anti-miR-488-5p. (C). The correlations between the expression of HOTAIR and miR-488-5p determined by the RT-PCR experiments.**$P < 0.01$, ***$P < 0.001$ significantly higher than the control or NC group. $^{\#\#}P < 0.01$ significantly lower than the control or NC group.

of HOTAIR in BCPAP cells was detrimental to the expression of miR-488-5p. Additionally, transfection of the BCPAP cells with miR-488-5p mimic resulted in significantly down-regulation of HOTAIR levels while not in the miR-488-5p inhibitor transfected cells (Figure 3B). Moreover, the correlations between the expressions of HOTAIR and miR-488-5p detected by RT-PCR was further confirmed using the linear-regression analysis. As shown in Figure 3D, it was obviously

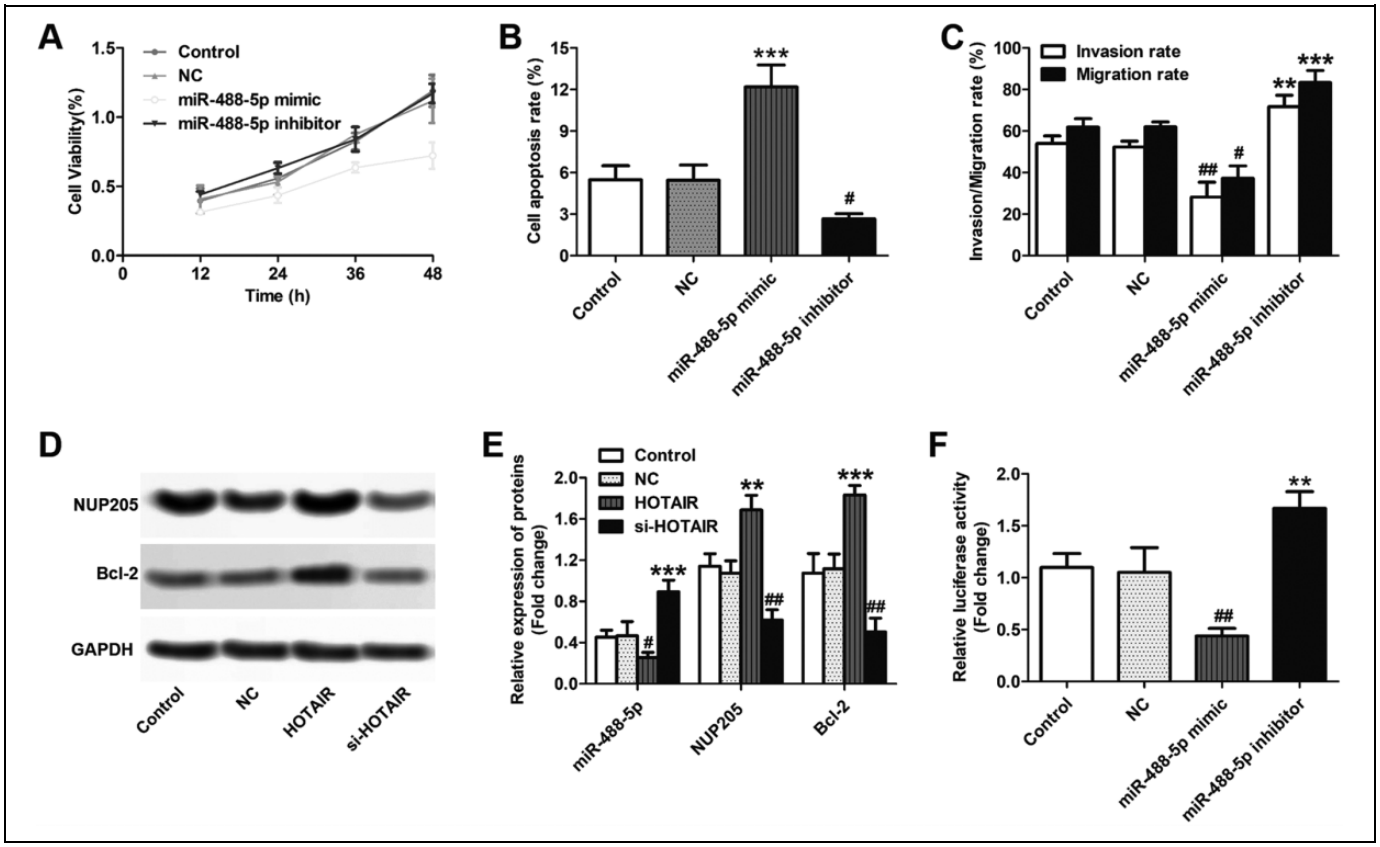

Figure 4. Elevation of miR-488-5p by transfection of si-HOTAIR decreased the growth rate, increased the apoptosis rate, and inhibited the invasion/migration ability of BCPAP cells through regulating the NUP205 and BCl-2 expressions. (A) Cell viability of BCPAP cells after respectively transfected with NC, miR-488-5p, and anti-miR-488-5p. (B) Cell apoptosis rate of the different genes transfected BCPAP cells determined by the Annexin V-FITC/PI double-staining assay. (C) Invasive and migration ability of different genes transfected BCPAP cells respectively determined by the trans-well assay and wound-healing assay. (D) Expression of miR-488-5p, NUP205, and BCl-2 in BCPAP cells post different treatments determined by western-blot assays. (E) Quantitative analysis of the expression of miR-488-5p, NUP205, and BCl-2 in BCPAP cells post different treatments examined by RT-PCR experiments. (F) The role of miR-488-5p expression in regulating the level of NUP205 confirmed by the dual-luciferase reporter gene assay. $^{\#\#}P < 0.005$, $^{\#\#\#}P < 0.005$ significantly lower than the control group. $^{**}P < 0.01$, $^{***}P < 0.001$ significantly higher than the control or NC group. $^{\#\#}P < 0.05$, $^{\#\#}P < 0.01$ significantly lower than the control or NC group.

observed that the expression of miR-488-5p was negatively correlated to the expression of HOTAIR, with increasing the HOTAIR levels signally down-regulated the miR-488-5p level. These results suggested that the HOTAIR affected the cellular process of BCPAP cells through negative regulation of the miR-488-5p level.

Overexpression of miR-488-5p by transfection with si-HOTAIR signally inhibited the growth rate, increased the apoptosis rate, and decreased the invasion/migration ability of BCPAP cells.

As demonstrated above, the expression of miR-488-5p was negatively correlated to the expression of HOTAIR. Therefore, increase the miR-488-5p levels by knock-down of HOTAIR might contribute to the tumor growth inhibition. As demonstrated in Figure 4A, up-regulation of miR-488-5p markedly reduced the growth rate of BCPAP cells, with the OD value of miR-488-5p group was nearly half of the control group. Additionally, transfection of the BCPAP cells with miR-488-5p mimic contributed to higher rate of cell apoptosis compared with the untreated BCPAP cells (Figure 4B). The effect of miR-488-5p on the invasion and migration of BCPAP cells was also studied. As shown in Figure 4C, the cells in the group of miR-488-5p exhibited the weakest capacity of invasion and migration compared with the control group and NC group. These resulted confirmed that increase the levels of miR-488-5p by knock-down of HOTAIR contributed to the inhibition of BCPAP cell growth, invasion, and migration.

HOTAIR Promoted BCPAP Cell Growth, Invasion, and Migration Mainly Through Regulating the miR-488-5p/NUP205 Axis

It was previously demonstrated that the NUP205 was the potential target gene of miR-488-5p.[14] Therefore, the effect of HOTAIR on the regulation of miR-488-5p levels and NUP205 levels in BCPAP cells was both investigated. As shown in Figure 4D and E, after transfection of cells with HOTAIR, the expression of miR-488-5p was dramatically down-regulated

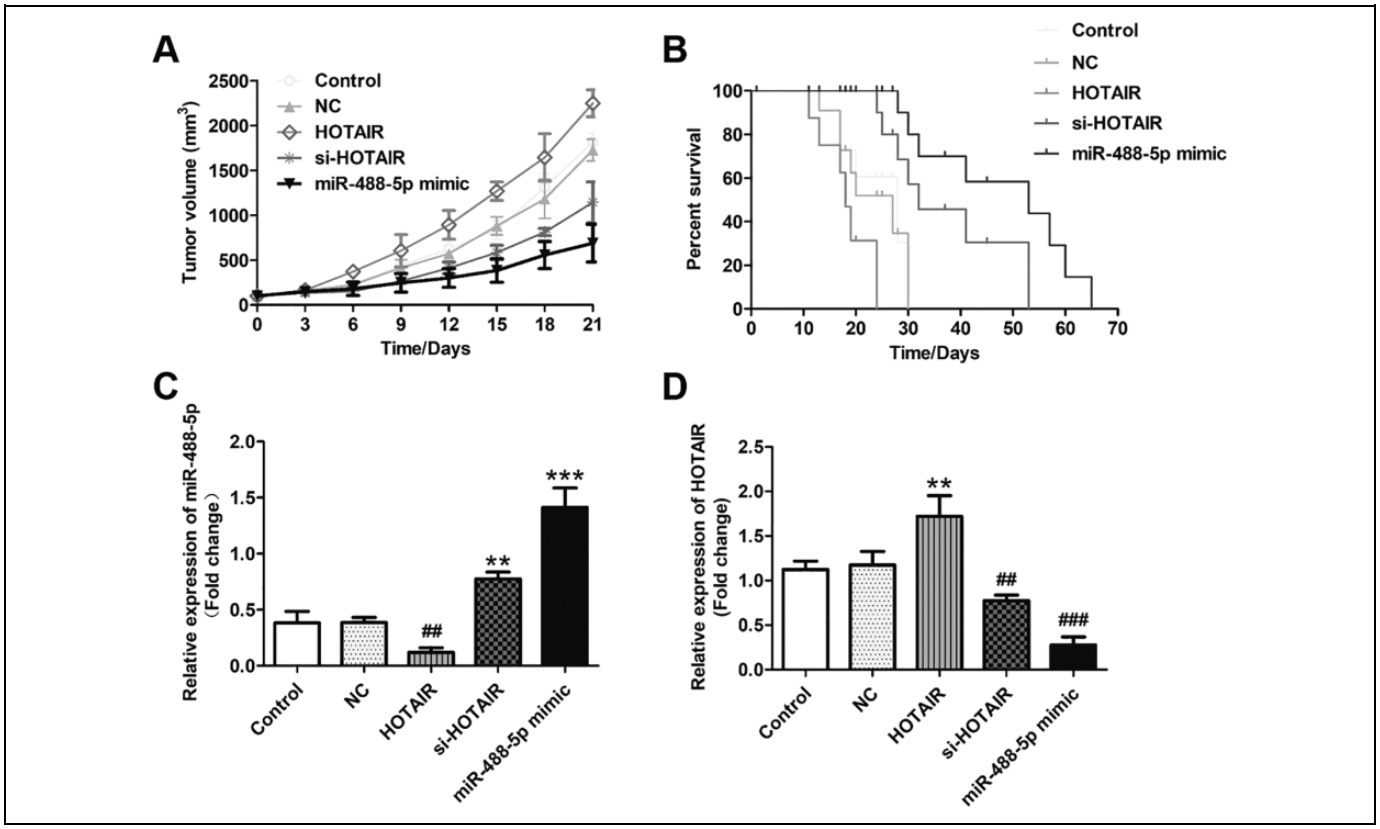

Figure 5. Increase the HOTAIR levels contributed to rapid growth of PTC by negative regulation of the miR-488-5p expression. (A) Tumor growth of papillary thyroid carcinoma-bearing mice after respectively treated by NC, HOTAIR, si-HOTAIR, and miR-488-5p. (B) Medium survival time of the tumor-bearing mice received various treatments. Quantitatively analysis of the levels of HOTAIR (C) and 488-5p (D) in tumor tissues by RT-PCR experiments. $^{\#\#}P < 0.005$, $^{\#\#\#}P < 0.005$ significantly lower than the control group. $^{**}P < 0.05$, $^{***}P < 0.001$ significantly higher than the control or NC group.

while the expression of NUP205 was signally increased. In contrast, elevation of the miR-488-5p levels in BCPAP cells by knock-down of HOTAIR significantly decreased the NUP205 levels. These results confirmed that the HOTAIR promoted BCPAP cell growth, invasion, and migration mainly through regulating the miR-488-5p/NUP205 axis. Additionally, knock-down of HOTAIR in BCPAP cells contributed to obviously down-regulated levels of Bcl-2 as well, indicated the aberrant expression of HOTAIR favorable for BCPAP cell survival. For further confirmation, the dual luciferase reporter assay was performed. As results shown that overexpression of miR-488-5p dramatically weakened the luciferase activity of the NUP205 vector ($p < 0.05$) without attenuating that of the mutant vector (Figure 4E). These results confirmed that the HOTAIR promoted BCPAP cell growth, invasion, and migration mainly through regulating the miR-488-5p/NUP205 axis.

Increase the HOTAIR Levels Contributed to Rapid Growth of PTC by Negative Regulation of the miR-488-5p Expression

Finally, the effect of HOTAIR on the growth of PTC was evaluated *in vivo*. A shown in Figure 5A and B, the mice in the HOTAIR group exhibited the rapidest growth rate, which resulted in the shortest medium survival time (26 days). After knockdown of the expression of HOTAIR by transfection of si-HOTAIR, the tumor growth rate was dramatically down-regulated and the survival time of PTC-bearing mice was prolonged to 46 days. Additionally, up-regulation of the miR-488-5p levels in PTC resulted in the slowest tumor growth rate and longest survival time (53 days). What's more, expressions of HOTAIR and miR-488-5p in PTC tissues were respectively detected post the tumor growth assay. It was revealed that the tumor tissues of the mice, which achieved the shortest medium survival time, exhibited the highest level of HOTAIR and the lowest level of miR-488-5p (Figure 5C and D). In contrast, tumor tissues of mice from the group of si-HOTAIR have obvious higher level of miR-488-5p and lower level of HOTAIR than the control or NC group. Similar to above, the miR-488-5p treated mice displayed the lowest expression of HOTAIR than others.

Discussion

In recent years, increasing evidence suggested that the HOTAIR is highly expressed in a wide array of tumor types.[12,15] Multiple studies have demonstrated that the HOTAIR played significant

roles in the regulation of a variety of biological processes.[14,12] The favorable role of HOTAIR in TC or PTC has been demonstrated as well with dysregulation of HOTAIR is closely correlated with metastasis and poor prognosis in patients with TC or PTC.[12-14] As consistent with previous reports, our study confirmed that the HOTAIR was highly expressed in papillary thyroid carcinoma cells and tissues. In contrast, significant lower levels of HOTAIR was detected in normal control thyroid cells or tissues. What's more, we also demonstrated that high expression of HOTAIR in PTC resulted in fast tumor growth rate and poor prognosis.

As a worthy biomarker for diagnosis and prognosis of cancer, lncRNAs has been reported with ability of promoting tumor progress through regulating the growth, proliferation, migration, and invasion of tumor cells.[23,24] Although the favorable role of HOTAIR in PTC has been demonstrated, the detailed effect of HOTAIR on the growth of PTC was not yet thoroughly investigated. In the present study, the detailed role of HOTAIR in regulation of the cellular process of papillary thyroid carcinoma cells was studied. The obtained results revealed that up-regulation of HOTAIR levels in BCPAP cells dramatically enhanced the proliferation rate, migration ability, and invasive numbers of PTC cells. Additionally, high level of HOTAIR in PTC tissues leaded to rapid tumor growth rate and short medium survival time. Such conclusion could be further confirmed by the fact that knocking down of the HOTAIR expression leaded to significant inhibition on the activity of BCPAP cells and papillary thyroid carcinoma tissues.

Generally, lncRNAs promotes tumor progress mainly through affecting the coding of RNA molecules through the indirect way.[25,26] MiR-488-5p is a recurrence-related exosomal miR that has potential effect on inhibition tumorigenesis and tumor progress.[20-22] Previous study demonstrated that knockdown of SNHG1 was able of suppresses cell proliferation, metastasis, and invasion of acute myeloid leukemia by regulating the miR-488-5p expression.[27] However, whether there is correlations between the expressions of miR-488-5p and HOTAIR in PTC remains unclear. Therefore, in the present study, the role of HOTAIR in regulation of the miR-488-5p levels in PTC was evaluated. It was confirmed that high level of HOTAIR in BCPAP cells or papillary thyroid carcinoma tissues was detrimental to the expression of miR-488-5p. However, knockdown of the HOTAIR expression leaded to an obvious increase of miR-488-5p level. Contrary results could be observed with increasing the miR-488-5p levels in papillary thyroid carcinoma cells and tissues dramatically weakened the levels of HOTAIR.

As demonstrated previously, overexpression of miR-488-5p resulted in considerable antagonism effect on the invasion and metastasis of breast cancer.[28] Our studies also confirmed that up-regulation of miR-488-5p was closely related to the significant decrease of proliferation rate, migration ability, and invasive numbers of BCPAP cells. Additionally, transfection of the BCPAP cells with miR-488-5p resulted in obvious higher rate of cell apoptosis compared with the control cells. These results were further confirmed by *in vivo* experiments with the miR-488-5p treated mice exhibited the longest survival time and tumor growth rate. More importantly, underlying mechanisms were further investigated and it was revealed that up-regulation of HOTAIR resulted in down-regulation of miR-488-5p and in turn increased the NUP205 and the Bcl-2. These results indicated that the HOTAIR promoted the growth, invasion, and migration of PTC mainly through regulating the miR-488-5p/NUP205 axis and the levels of Bcl-2 as well.

In conclusion, the present study demonstrated that the HOTAIR cloud be a worthy biomarker of papillary thyroid carcinoma with high expression of HOTAIR was detected in tumor tissues or cells. Additionally, HOTAIR plays significant role in promotion of growth, invasion, and migration of PTC through specifically silencing the miR-488-5p expression and up-regulation of NUP205 and Bcl-2 as well. Knocking down of the HOTAIR levels might contributed to the inhibition of the activity of PTC.

Authors' Contributions

Feng Xia, Wei Xia, and Xudong Yu were responsible for the conception and design of the study. Feng Xia and Wei Xia were responsible for the collection of data, data analysis, interpretation and manuscript writing. Xudong Yu revised the manuscript.

Availability of Data and Materials

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

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Statement

Our study was approved by the guidelines that was approved by the ethics committee of Hubei Maternal and Children's Hospital. (approval no. SYXK2017-0065).

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