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Knockdown of long noncoding RNA SNHG7 inhibits the proliferation and promotes apoptosis of thyroid cancer cells by downregulating BDNF

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Abstract. – OBJECTIVE: Recently, long noncoding RNAs (lncRNAs) have got much attention for their role in tumor progression. LncRNA small nucleolar RNA host gene 7 (SNHG7) was studied in this research to identify how it affects the development of thyroid cancer (TC).

PATIENTS AND METHODS: SNHG7 expression was detected by quantitative Real-time polymerase chain reaction (qRT-PCR) in both TC cells and tissue samples. Pearson's Chi-square test was used to determine the association of SNHG7 expression with several clinicopathological factors. Moreover, colony formation assay, cell proliferation and cell apoptosis assay were conducted. In addition, by performing qRT-PCR and Western blot assay, the underlying mechanism was explored.

RESULTS: SNHG7 expression level was higher in TC samples than that in corresponding ones. The SNHG7 expression was associated with tumor size and TNM stage. Moreover, TC cell proliferation was inhibited, and TC cell apoptosis was induced after SNHG7 was knocked down *in vitro*. Moreover, the mRNA and protein expressions of brain-derived neurotrophic factor (BDNF) were downregulated after knockdown of SNHG7. Furthermore, the expression level of BDNF was positively related to the expression of SNHG7 in TC tissues.

CONCLUSIONS: These results suggested that knockdown of SNHG7 could inhibit TC cell proliferation and induce cell apoptosis via downregulating BDNF, which might be a potential therapeutic target in TC.

Key Words:
Long noncoding RNA, SNHG7, Thyroid cancer, BDNF.

Introduction

Thyroid cancer (TC) originates from follicular or parafollicular thyroid cells. The morbidity of TC exerts an increasing trend in the past decade globally. The incidence of TC is rising by an average of 4.5% annually from 2007 to 2011 in America. Moreover, TC is the eighth most common cancer in China which resulting a substantial burden for both the patients and society[1,2]. Despite the huge development effective treatment, the survival rate of TC is still unsatisfied. TC killed almost 36,000 people in 2010 while it leaded to 24,000 deaths in 1990[3,4]. Therefore, it is urgent to realize the underlying molecular mechanisms of TC and identify new biomarkers for early diagnosis of aggressive TC and targeted treatment.

Long non-coding RNAs (lncRNA) are subtypes of non-protein coding RNAs, which are more than 200 nucleotides in length. Recent studies have indicated that lncRNA serves as a major contributor in carcinogenesis. For example, by regulating vasculogenic angiogenesis, lncRNA MALAT1 is reported to promote tumorigenicity and cell metastasis in gastric cancer[5]. LncRNAAC132217.4 facilitates cell metastasis in oral squamous cell carcinoma *via* regulation of IGF2 expression, which is modulated by KLF8[6]. LncRNA MEG3, depended on p53's transcription, is downregulated in breast cancer, which affects cell proliferation, invasion and migration[7]. In addition, downregulation of lncRNA UCA1 modulated by CRISPR/Cas9 can depress the malignant phenotypes of bladder cancer, and lncRNA UCA1 can be utilized as a novel non-invasive diagnostic biomarker for bladder cancer[8]. However, the clinical role and biological mechanisms of lncRNA small nucleolar RNA host gene 7 (SNHG7) in the development of TC remain unexplored.

In this work, we found out that the expression of SNHG7 was remarkably higher in TC tissues and was associated with tumor size and TNM

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stage. Moreover, knockdown of SNHG7 inhibited the proliferation of TC cells and promoted the cell apoptosis *in vitro*. Moreover, our further researches explored the underlying mechanism about how SNHG7 functioned in TC development.

Patients and Methods

Cell Lines and Clinical Samples

64 TC patients were enrolled for human tissues that received surgery at Shaanxi Provincial People's Hospital from December 2015 to December 2017. Before operation, written informed consent was achieved. No radiotherapy or chemotherapy for any patients before the operation. Tissues got from the surgery were stored immediately at -80°C. All tissues were analyzed by an experienced pathologist. This study conforms as the Ethics Committee of Shaanxi Provincial People's Hospital required.

Cell Culture

K1 and TPC-1 (human papillary thyroid cancer cell line), SW579 (human thyroid squamous cell carcinoma cell line) and Nthy-ori 3-1 (normal human thyroid cell line) were provided by the Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, China). Culture medium consisted of 10% fetal bovine serum (FBS; Invitrogen Life Technologies, Carlsbad, CA, USA), penicillin as well as Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Rockville, MD, USA). Besides, cells were cultured in an incubator, which contained 5% CO_2 and was set at 37°C.

Cell Transfection and Grouping

Lentiviral small hairpin RNA (shRNA) targeting SNHG7 was synthesized and then cloned into the pLenti-EF1a-EGFP-F2A-Puro vector (BiosettiaInc., San Diego, CA, USA). Then 293T cells were used for the packaging the viruses, the SNHG7 lentiviruses (sh- SNHG7) and the empty vector (control).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

The total RNA was separated by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). And then the total RNA was reverse-transcribed to cDNAs through reverse Transcription Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). Following are the primers using for qRT-PCR: SNHG7 primers forward: 5′-GTGACTTCGCCTGTGATGGA-3′, reverse: 5′-GGCCTCTATCTGTACCTTTATTCC-3′; Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers forward: 5′-CCAAAATCAGATGGGGCAATGCTGG-3′ and reverse: 5′-TGATGGCATGGACTGTGGTCATTCA-3′. Thermal cycle was as follows: 30 s at 95°C, 5 s for 40 cycles at 95°C, 35 s at 60°C.

Western Blot Analysis

Reagent radioimmunoprecipitation assay (RIPA) (Beyotime, Shanghai, China) was utilized to extract protein from cells. Bicinchoninic acid (BCA) protein assay kit (TaKaRa, Dalian, China) was chosen for quantifying protein concentrations. The target proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, they were incubated with antibodies after replaced to the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Cell Signaling Technology (CST, Danvers, MA, USA) provided us with rabbit anti-GAPDH and rabbit anti-brain-derived neurotrophic factor (BDNF), as well as goat anti-rabbit secondary antibody. Chemiluminescent film was applied for assessment of protein expression with Image J software (NIH, Bethesda, MD, USA).

Colony Formation Assay and Cell Counting Kit-8 (CCK-8)

SW579 (1.5×10^3 cells/well) cells were placed in a 6-well plate. 10 days later, colonies were then fixed with 10% formaldehyde for 30 min and stained for 5 min with 0.5% crystal violet. Next, Canon camera was used for taking photograph of colonies. Next, Image-Pro Plus 6.0 (Silver Springs, MD, USA) was used for data analysis.

96-well plate was used for the culture of TC cells (1×10^3 cells/well). Then we added CCK-8 (10 μL) into these wells at different time. Microplate reader was used for measuring absorbance at 450 nm (Bio-Rad, Hercules, CA, USA).

Flow Cytometric Analysis

Annexin-V-FITC (fluorescein isothiocyanate) apoptosis detection kit (BD Biosciences, Franklin Lakes, NJ, USA) was used for detecting the TC cell apoptosis. Briefly, harvested cells were washed twice using ice-cold PBS. Then, 100 μL of flow cytometry binding buffer was added. After 5 μl of Annexin V/FITC and 5 μL of Propidium Iodide (PI) were mixed, these cells were stained

for 15 min in the dark at the room temperature. Each tube was added with four hundred microliters binding buffer. FACSCalibur flow cytometer was used for analyzing the apoptosis cells (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (SPSS, Chicago, IL, USA) was adopted to conduct the statistical analysis. Data were presented as mean ± SD (Standard Deviation). Chi-square test, Student *t*-text and Kaplan-Meier method were selected when appropriate. $p<0.05$ was considered statistically significant.

Results

SNHG7 Expression Level in TC Tissues

First, qRT-PCR was conducted for detecting SNHG7 expression in 64 patients' tissues and 3 TC cell lines. As a result, SNHG7 was significantly upregulated in tumor tissue samples (Figure 1). As is shown in Table I, the TC patients in the high SNHG7 expression group had larger tumor size and advanced TNM stage ($p<0.05$). In addition, SNHG7 was not associated with age, gender, lymphatic metastasis and extrathyroidal extension ($p>0.05$).

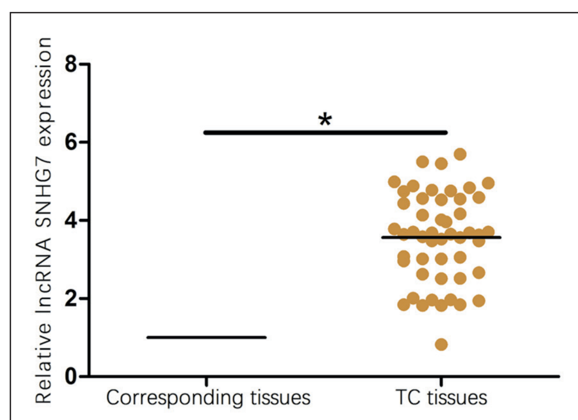

Figure 1. Expression levels of SNHG7 were increased in TC tissues. SNHG7 expression was significantly increased in the TC tissues compared with corresponding tissues. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

Knockdown of SNHG7 Inhibited Cell Growth in TC Cells

SNHG7 level of TC cells was higher than that of Nthy-ori 3–1 (normal human thyroid cell line) (Figure 2A). According to SNHG7 expression in TC cells, we chose SW579 TC cell line for the knockdown of SNHG7. Then, qRT-PCR was utilized for detecting the SNHG7 expression (Figure 2B). Moreover, the outcome of CCK-8 assay also revealed that after SNHG7 was knocked down in

Table I. Correlation between SNHG7 expression and clinicopathological characteristics in TC patients.

Characteristics	Patients	Expression of SNHG7		*p*-value
		Low group	High group	
Total	64	28	36	
Age (years)				0.393
≤ 55	35	17	18	
> 55	29	11	18	
Gender				0.614
Male	32	15	17	
Female	32	13	19	
Tumor size				0.033
≤ 2 cm	27	16	11	
> 2 cm	37	12	25	
Extrathyroidal extension				0.469
Yes	31	15	16	
No	33	13	20	
TNM stage				0.003
I-II	28	18	10	
III-IV	36	10	26	
Lymphatic metastasis				0.659
No	34	14	20	
Yes	30	14	16	

$p<0.05$ is considered as statistically significant.

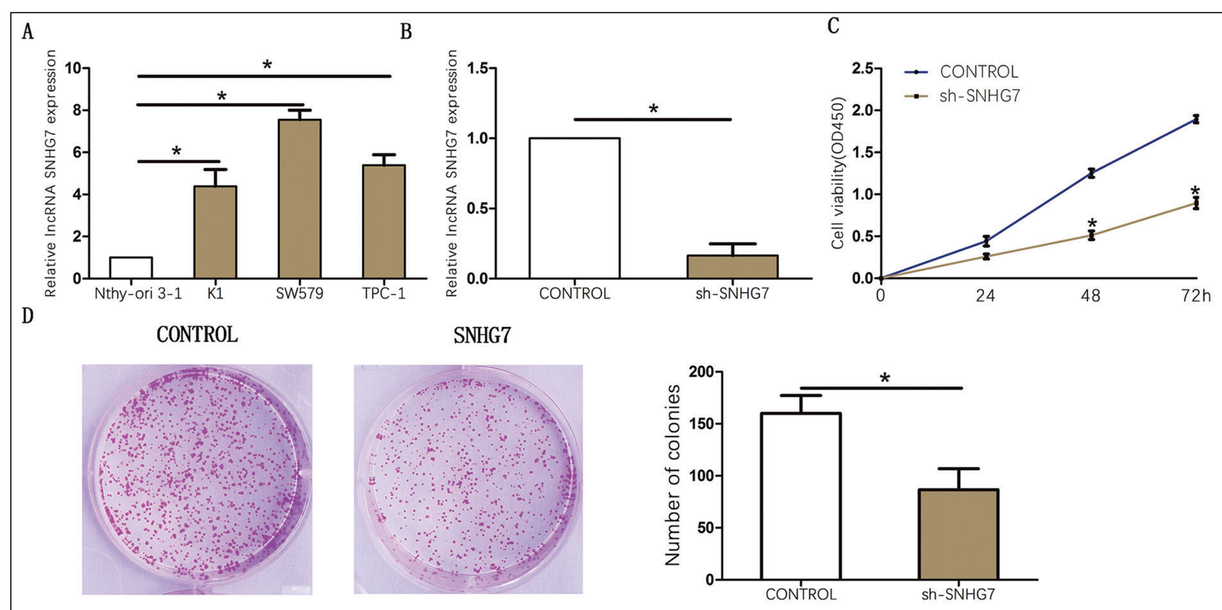

Figure 2. Knockdown of SNHG7 inhibited TC cell proliferation. ***A,*** Expression levels of SNHG7 relative to GAPDH were determined in the human TC cell lines and Nthy-ori 3-1 (normal human thyroid cell line) by qRT-PCR. ***B,*** SNHG7 expression in TC cells transduced with SNHG7 lentiviruses (sh-SNHG7) and the empty vector (control) was detected by qRT-PCR. GAPDH was used as an internal control. ***C,*** CCK-8 assay showed that cell proliferation of TC cells was significantly inhibited *via* knockdown of SNHG7 in TC cells. ***D,*** Colony formation assay showed that knockdown of SNHG7 significantly decreased cell colonies in TC cells. The results represented the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$, as compared with the control cells.

TC cells, the cell proliferation was remarkably suppressed (Figure 2C). The results of colony formation assay revealed that after SNHG7 was knocked down, the colonies of TC cells were significantly decreased (Figure 2D).

Knockdown of SNHG7 Promoted Cell Apoptosis in TC Cells

The outcome of cell apoptosis assay revealed that after SNHG7 was knocked down in TC cells, cell apoptosis rate of SW579 TC cell was remarkably increased (Figure 3).

The Interaction Between BDNF and SNHG7 in TC

QRT-PCR results showed that compared with the BDNF level in empty vector (control) group, expression level of BDNF in TC cells was lower in SNHG7 lentiviruses (sh-SNHG7) group (Figure 4A). Western blot assay found out that after

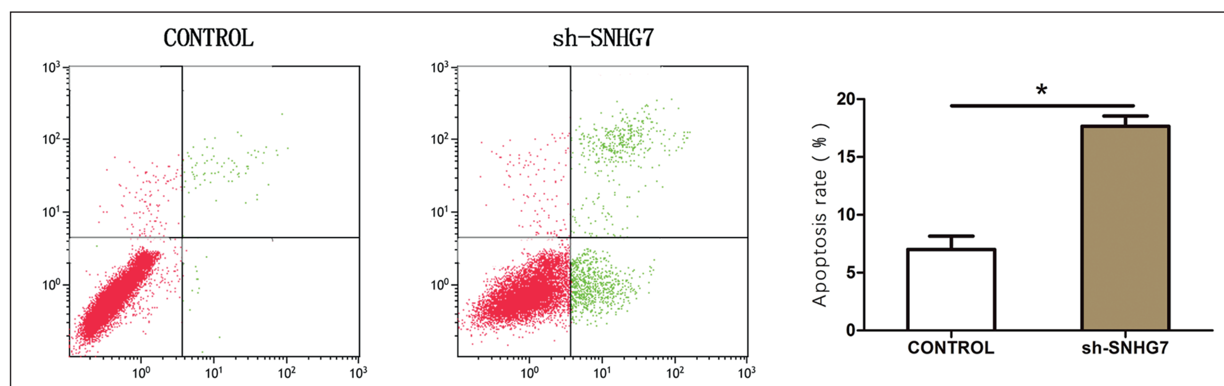

Figure 3. Knockdown of SNHG7 promoted TC cell apoptosis. Apoptosis assay showed that cell apoptosis rate of TC cells was significantly promoted *via* knockdown of SNHG7 in TC cells. The results represented the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$, as compared with the control cells.

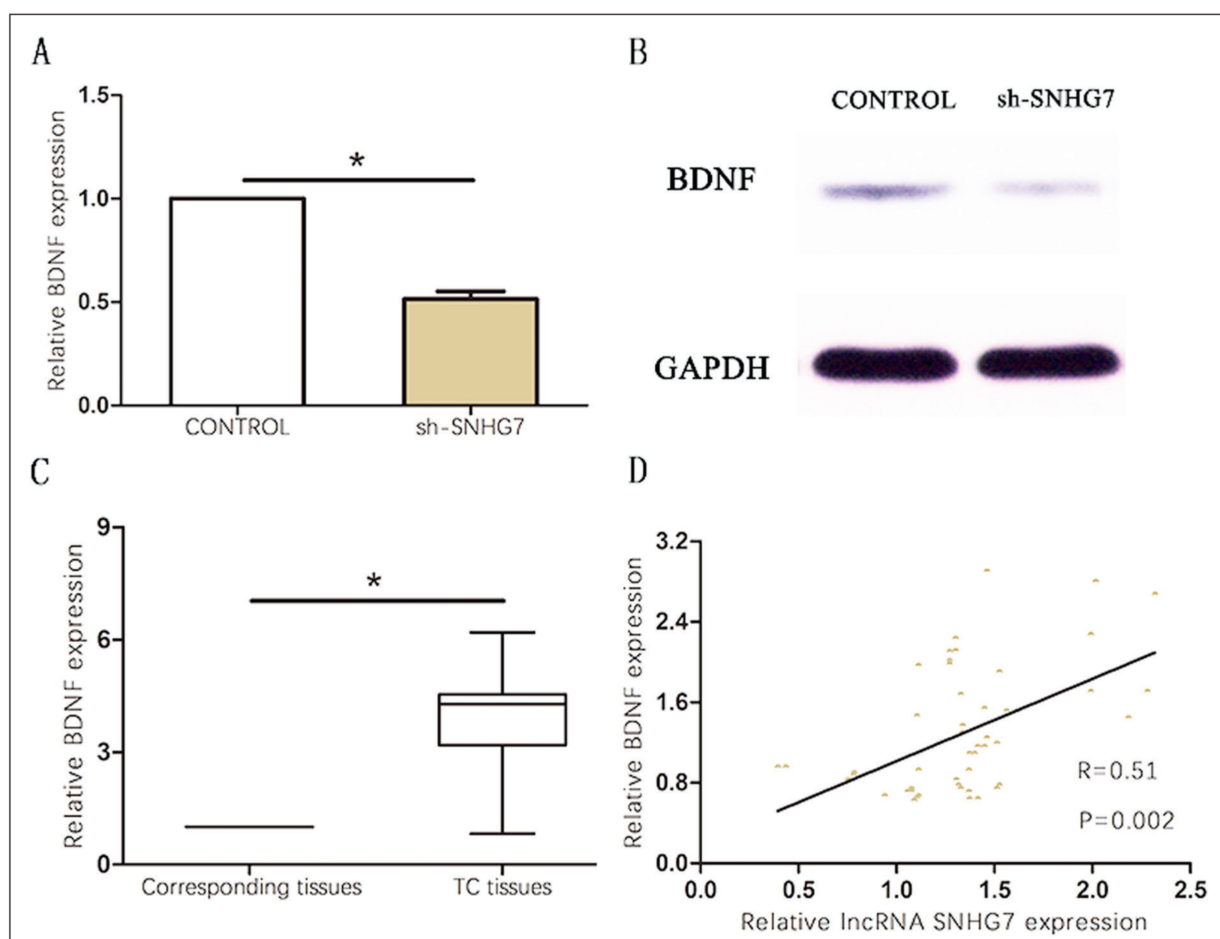

Figure 4. Interaction between SNHG7 and BDNF. ***A,*** qRT-PCR results showed that BDNF expression was lower in SNHG7 lentiviruses (sh-SNHG7) compared with the empty vector (control). ***B,*** Western blot assay revealed that BDNF protein expression was decreased in SNHG7 lentiviruses (sh-SNHG7) compared with the empty vector (control). ***C,*** BDNF was significantly upregulated in TC tissues compared with corresponding tissues. ***D,*** The linear correlation between the expression level of BDNF and SNHG7 in TC tissues. The results represented the average of three independent experiments. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

SNHG7 was knocked down, BDNF could be downregulated at protein level (Figure 4B). We further found that BDNF expression of TC tissues was significantly higher compared with that of corresponding tissues (Figure 4C). Correlation analysis demonstrated that BDNF expression level positively correlated to SNHG7 expression in cancer tissues (Figure 4D).

Discussion

A plenty of lncRNAs have been revealed to play an important role in oncogenesis and progression of TC. For instance, overexpression of lncRNA ANRIL facilitates cell invasion and cell metastasis in thyroid cancer *via* TGF-beta/Smad signaling pathway[9]. LncRNA NR_036575.1 acts as an oncogene in thyroid cancer by contributing to the cell proliferation and cell migration, which could be applied as a potential biomarker and therapeutic target[10]. LncRNA ENST00000537266 and ENST00000426615 are important regulators of cell proliferation and cell motility in papillary thyroid cancer, which can help to understand the pathogenesis of TC[11]. In addition, through regulating miRNA-214 expression, lncRNA NEAT1 facilitates malignant progression of thyroid carcinoma[12]. Moreover, lncRNA MEG3 inhibits cell migration and cell invasion in thyroid carcinoma through targeting of Rac1[13].

Small nucleolar RNA host gene 7 (SNHG7) is located on chromosome 9q34.3 with a length of 2176 bp, which promotes cell proliferation, cell

invasion and migration in many cancers[14]. For example, lncRNA SNHG7 facilitates the epithelial-to-mesenchymal transition and tumor proliferation in osteosarcoma by regulating miR-34a Signals[15]. LncRNA SNHG7 is upregulated in colorectal cancer and is found out to be negatively related to the prognosis of colorectal cancer by regulating PI3K/Akt/mTOR pathway[16]. LncRNA SNHG7 promotes cell proliferation and cycle progression in prostate cancer through miR-503/Cyclin D1 pathway[17]. In addition, lncRNA SNHG7 is overexpressed in bladder cancer, which enhances the cell proliferation and inhibits cell apoptosis of bladder cancer[18].

In this study, we find that SNHG7 was upregulated both in TC samples and cells. The SNHG7 expression was associated with tumor size and TNM stage. Besides, after SNHG7 was knocked down, TC cell proliferation was observed to be inhibited. Moreover, after SNHG7 was knocked down, TC cell apoptosis was found to be promoted. Above results indicated that SNHG7 promote tumorigenesis of TC and might act as an oncogene.

Brain-derived neurotrophic factor (BDNF) has been reported to participate in the pathophysiology of nervous system, which is a member of the neurotrophic superfamily. While, recent studies have indicated that BDNF also plays a crucial role in tumor growth and invasion in many cancers. For instance, activated by STAT3, BDNF promotes cell proliferation in human non-small-cell lung cancer *via* TrkB signaling pathway[19]. BDNF could induce cell migration and invasiveness in SKOV3 ovarian cancer[20]. In addition, dual inhibition of autophagy and BDNF/TrkB may offer a potential therapeutic target for colorectal cancer[21]. Moreover, BDNF signaling pathway plays an important role in the mechanism related to early recurrence in triple negative breast cancer and BDNF-TrKB signaling may affect the prognosis of patients with triple negative breast cancer[22].

In the present study, BDNF expression could be downregulated after knockdown of SNHG7. Moreover, BDNF expression in TC tissues was positively related to SNHG7 expression. All the results above suggested that SNHG7 might promote tumorigenesis of TC *via* targeting BDNF.

Conclusions

Above data identified that SNHG7 was remarkably upregulated in TC patients. Besides, SNHG7 could enhance TC cell growth and inhibit cell apoptosis through targeting BDNF. These findings suggest that SNHG7 may contribute to therapy for TC as a candidate target.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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