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Identification of PTK7 as a promising therapeutic target for thyroid cancer

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Abstract. – OBJECTIVE: To evaluate the possible involvement of PTK7 in the progression of human thyroid cancer and assess its potential effects on the proliferation and apoptosis of thyroid cancer.

PATIENTS AND METHODS: Immunohistochemical (IHC) assays and clinical significance analysis were performed to explore the correlations between PTK7 expression and clinical characteristics of patients with thyroid cancer. Quantitative PCR assays and Immunoblot assays were performed to detect the expression of PTK7 in control or PTK7 shRNA plasmids transfected thyroid cancer cells. MTT assays were performed to detect the effects on the proliferation of thyroid cancer cells. Flow cytometry (FCM) assays were performed to assess the changes in cell apoptosis of thyroid cancer. Additionally, the effects of PTK7 on tumor growth were detected through in vivo tumor growth assays.

RESULTS: PTK7 is highly expressed in human thyroid cancer tissues, and its expression levels are associated with the clinical characteristics, including TNM stage (p=0.015*), and intraglandular dissemination (p=0.024*) of patients with thyroid cancer. PTK7 ablation inhibits cell proliferation and stimulates cell apoptosis of thyroid cancer *in vitro*. Additionally, PTK7 contributes to tumor growth of thyroid cancer cells in mice.

CONCLUSIONS: We demonstrated the involvement of PTK7in the progression of thyroid cancer, and therefore provided a novel and promising therapeutic target for thyroid cancer treatment.

Key Words:
Thyroid cancer, PTK7, Proliferation, Apoptosis, Therapeutic target.

Introduction

Thyroid cancer is the most common endocrine cancer with high morbidity[1,2]. The predominant prevalence subtype of thyroid cancer is papillary thyroid cancer (PTC)[3]. In decades, the main treatment for thyroid cancer is surgical excision combined with radiotherapy and chemotherapy[4]. Although the prognosis of thyroid cancer is an improvement, some thyroid patients still have local infiltration, lymphatic metastasis or distant metastasis in the early stage, which also increases the mortality of patients[5,6]. Similar to a series of tumors, targeted therapy for thyroid cancer has shown great promise in recent years[7]. Therapeutic drugs developed against targets such as EGFR and VEGFR have achieved good therapeutic effects[8]. However, to improve the survival rate of thyroid cancer patients, it is still necessary to develop novel effective therapeutic targets.

Protein tyrosine kinase 7 (PTK7) belongs to the receptor protein tyrosine kinase-like family[9]. PTK7 is a cyto-membrane protein highly expressed in CCRF-CEM cells[10]. Previous studies also demonstrated the involvement of PTK7 in multiple physiological processes, such as tissue morphogenesis and patterning[11,12]. Additionally, PTK7 could serve as regulator in the Wnt and planar cell polarity (PCP) signaling pathway[13,14]. It has also been reported that the mutations in PTK7 could lead to scoliosis and the defects of human neural tube closure[15].

Notably, the potential role of PTK7 in the progression of multiple cancers has been widely revealed[16]. PTK7 was highly expressed in several cancers, such as gastric cancer, lung cancer, and colon cancer[17-19]. In liposarcoma, PTK7 affected cell proliferation and invasion[20]. PTK7 also contributes to cancer stemness of head and neck cancer[21]. Despite its important role in series of tumors, the potential impact of PTK7 on thyroid cancer remains unclear.

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In this study, we found the high expression of PTK7 in human thyroid cancer tissues. PTK7 was correlated with clinical characteristics of thyroid cancer patients. The depletion of PTK7 suppressed the proliferation and stimulated the apoptosis of thyroid cancer cells *in vitro*. We further found that PTK7 promoted tumor growth of thyroid cancer cells in mice. We therefore provided a novel and promising therapeutic target for the treatment of thyroid cancer.

Patients and Methods

Antibodies and Primers

Rabbit anti-PTK7 (1:200 dilution for IHC; 1:1000 dilution for Immunoblot, ab62074, Abcam, Cambridge, UK), Mice anti-β-actin (1:1000 dilution, ab5694, Abcam, Cambridge, UK).

The quantitative PCR primer sequences of PTK7 are as follows: forward, 5'-CAGTTCCTGAGGATTTCCAAGAG-3' and reverse, 5'-TGCATAGGGCCACCTTC-3'; the quantitative PCR primer sequences of GAPDH are as follows: 5'-AACGGATTTGGTCGTATTGGG-3' and reverse, 5'-TCGCTCCTGGAAGATGGTGAT-3'. shRNA plasmids (Ready-to-package AAV) of PTK7 were purchased from the Addgene plc, and the targeted sequences of the shRNA plasmids were as follows:5′- AACATCAAATGGATTGAGGCAGG -3'.

Human Tissue Samples and Analysis

A total number of 79 human thyroid cancer tissues and adjacent tissues measured in this study were collected from patients receiving surgical treatment in the second affiliated hospital of Nanchang University. The clinical characteristics are precisely recorded and listed in Table I.

To explore the possible link between PTK7 expression levels and thyroid cancer, immunohistochemical (IHC) assays were performed. Briefly, sample sections were fixed with 4% paraformaldehyde (PFA) for 30 minutes and subsequently blocked with 2% bovine serum albumin (BSA) for another 30 minutes. Slides were subsequently incubated with PTK7 antibodies at room temperature for 2 hours. Then, the sections were incubated with biotinylated secondary antibody for 1.5 hours, and diaminobenzidine (DAB) was used as a chromogen substrate.

According to the staining results of IHC assays, we found that PTK7 mainly existed in the cytoplasm of human thyroid cancer tissues. We performed a scoring method as follows. Briefly, the proportion of positive stained cells was graded as follows: 0 = 0% stained cells; 1 represented 1-30% stained cells; 2 indicated 31-70% stained cells; 3 = 71-100% stained cells. The staining intensity was evaluated on a score of 0 (negative), 1 (low), 2 (modest) and 3 (strong). Additionally, the expression levels

Table I. Relationships of PTK7 and clinicopathological characteristics in 79 patients.

Feature	All n = 79	PTK7 expression		χ^2	*p*
		Low n = 18	High n = 61		
Age (year)				2.159	0.142
< 45	28	9	19		
≥ 45	51	9	42		
Gender				3.436	0.064
Male	18	7	11		
Female	61	11	50		
TNM stage				5.940	0.015*
T1-T2	33	12	21		
T3-T4	46	6	40		
Tumor size				1.765	0.184
< 2 cm	25	8	17		
≥ 2 cm	54	10	44		
Lymph node metastasis				0.034	0.854
Yes	38	9	29		
No	41	9	32		
Intraglandular dissemination				5.123	0.024*
Yes	36	4	32		
No	43	14	29		

of PTK7 were detected based on the staining index: score of staining intensity × score of stained cells percentage. Staining index <5 was considered PTK7 low expression, whereas staining index > 5 or = 5 was thought the high expression of PTK7.

Cell Culture and Transfection

The two types of human thyroid cancer cell lines: TPC-1 and KTC-1, were bought from American Type Culture Collection (ATCC, Manassas, VA, USA). TPC-1 cells were cultured in Roswell Park Memorial Insitute-1640 (RPMI-1640) culture medium. KTC-1 cells were maintained in F-12K culture medium with 1% NEAA. All thyroid cancer cells were supplemented with 10% of fetal bovine serum (FBS) at 37°C in a 5% CO_2 incubator.

The control or PTK7 shRNA plasmids were transfected intoTPC-1 and KTC-1 cells by lipofectamine 3000 (L3000015, Thermo Fisher Scientific, Waltham, MA, USA). Stable knockdown clones were screened by lentivirus infection and used for the *in vivo* assays.

Quantitative PCR Assay

To extract total RNA from human thyroid cancer cells. TRIzol (15596026, Invitrogen, Carlsbad, CA, USA) agent was used. Subsequently the RNA was reverse-transcribed by reverse transcriptase (M1701, Promega, Madison, WI, USA). Then, it was reverse transcribed to produce cDNA through synthesis system. After getting the cDNA, quantitative PCR was performed using SYBR Ex Taq kit (638319, TaKaRa, Otsu, Shiga, Japan), and the relative expression of PTK7 was normalized to the expression of GAPDH.

Immunoblot Assays

TPC-1 and KTC-1 were lysed by radio immunoprecipitation assay (RIPA) cell lysis buffer and the total proteins were isolated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) assays. After trans-membrane, the polyvinylidene difluoride (PVDF) membranes were blocked with 5% milk and subsequently incubated with the primary antibodies for the detection of PTK7 and β-actin at room temperature for 2 hours. Then, PVDF membranes were washed with Tris-Buffered Saline and Tween-20 (TBST) and incubated with horseradish peroxidase (HRP)-conjugate secondary antibodies at room temperature for 45 minutes. After washing with TBST buffer for 4 times, interest signals were detected using an enhanced chemiluminescence (ECL) kit.

Cell Proliferation Assays

For colony formation assays, approximately 500 cancer cells were seeded into a 6-well culture plate. The medium was replaced with fresh medium every 3 days. After 2 weeks, cells were fixed with PFA for 30 minutes and stained with 0.1% crystal violet at room temperature for 30 minutes and washed with PBS twice. Then, the colony number was manually counted.

For MTT assays, approximately 1000 cells were added into each well of 96-well plates and maintained for 4 days. Subsequently, cells were incubated with MTT for 4 hours and removed the medium. Subsequently, cells were washed with phosphate-buffered saline (PBS) twice. After washing, 200-μL dimethyl sulfoxide (DMSO) was added into each well to fully dissolve, and the OD value was measured with a microplate reader at 570 nm.

Cell Apoptosis Assays

TPC-1 and KTC-1cells were transfected with control or PTK7 shRNA plasmids and then resuspended, washed twice with PBS. Subsequently cells were incubated with annexin V-FITC and propidium iodide (PI) at 25°C for 20 minutes in the dark. After filtering, samples were analyzed by a FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

Tumor Growth Assay

All animal assay processes were approved by our Institutional Animal Care and Use Committee (IACUC). TPC-1 cells were stably infected with control or PTK7 shRNA lentivirus. About $5X10^5$ control or PTK7-depletion TPC-1 cells were subcutaneously implanted into athymic nude mice. After 2 weeks, tumors were isolated, and the volume of each tumor was measured every 7 days. After 49 days, all tumors were isolated from mice, and the growth curves were calculated.

Statistical Analysis

GraphPad 5.0 software (La Jolla, CA, USA) was used for statistical analysis in this study. All results were represented as mean ± SD. The analysis of correlation between clinical features and PTK7 expression levels was performed by χ^2

analysis. Student's *t*-test was used for statistical comparisons. *Indicates $p < 0.05$ and it represents significant.

Results

PTK7 Was High Expression in Human Thyroid Cancer and Associated With the Clinical Features of Patients

To investigate the possible role of PTK7 in the progression of thyroid cancer, we first detected the expression levels of PTK7 in human thyroid cancer tissues and the adjacent tissues through IHC assays. A total number of 79 patients with thyroid cancer who underwent surgical resection in our hospital were included in this assay. According to the results of IHC assays, we found the evident high expression of PTK7 in thyroid cancer tissues compared to adjacent tissues (Figure 1A, B).

We next assessed the clinical significance between PTK7 expression in thyroid cancer tissues and clinical characteristics. 18 patients exhibited low PTK7 expression, whereas 61 showed high expression (Table I). Patient gender, age, tumor size, TNM stage, lymph node metastasis, intraglandular dissemination et al. were analyzed, respectively. According to the results, no significant correlation was found in features such as patient age (p=0.142), gender (p=0.064), tumor size (p=0.184), and lymph node metastasis (p=0.854) between low and high expression PTK7 groups (Table I). Of note, we noticed that PTK7 expression level was significantly correlated with TNM stage (p=0.015*), and intraglandular dissemination (p=0.024*) in thyroid cancer patients (Table I). We, therefore, revealed thatPTK7 expression levels were associated with clinical pathologic features of patients who underwent thyroid cancer.

Collectively, we found that PTK7 was high expression in human thyroid cancer tissues and correlated with the clinical characteristics of thyroid cancer.

PTK7 Depletion Restrains the Proliferation of Thyroid Cancer Cells In Vitro

To explore the possible involvement of PTK7 in the progression of thyroid cancer, the shRNA plasmids targeted PTK7 were used and transfected into two types of thyroid cancer cells, TPC-1 and KTC-1 cells, to deplete its expression. Both quantitative PCR assays and Immunoblot assays showed the transfection of PTK7 shRNA plasmids effectively decrease its expression levels in both TPC-1 and KTC-1 cells, compared to the control shRNA group. (Figure 2A and 2B).

Cell proliferation is critical in cancer progression. Therefore, we performed colony formation assays to investigate whether PTK7 affected the

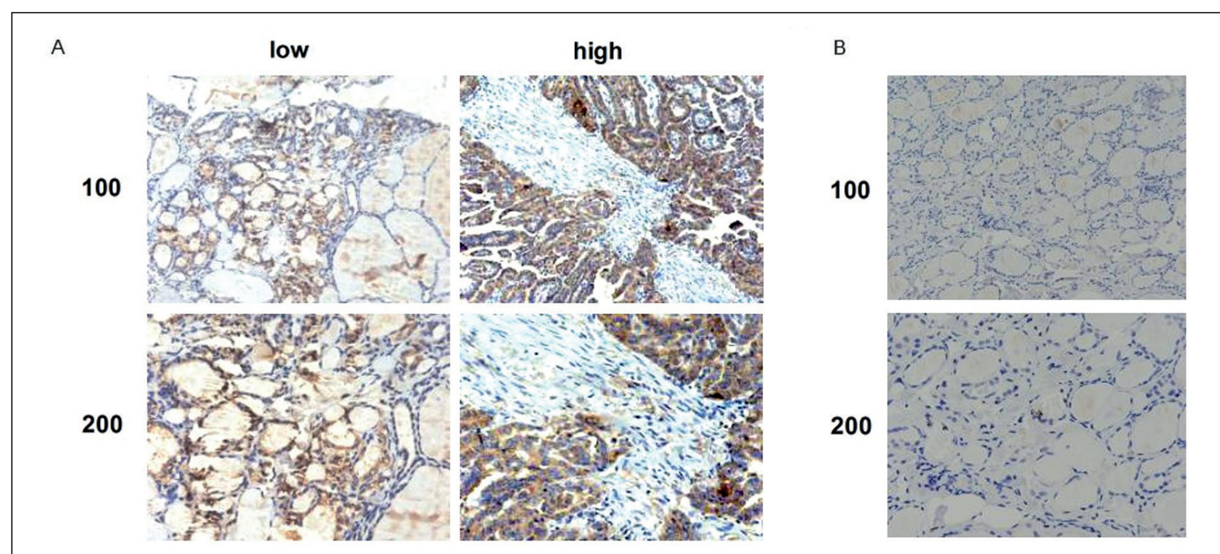

Figure 1. PTK7 was highly expressed in human thyroid cancer tissues. **A**, IHC assays were performed to detect PTK7 expression in human thyroid cancer tissues, and the representative photographs were shown (×100 and ×200 magnification, respectively). **B**, The results of IHC assays exhibited the expression levels of PTK7in corresponding non-tumor tissues (×100 and ×200 magnification, respectively).

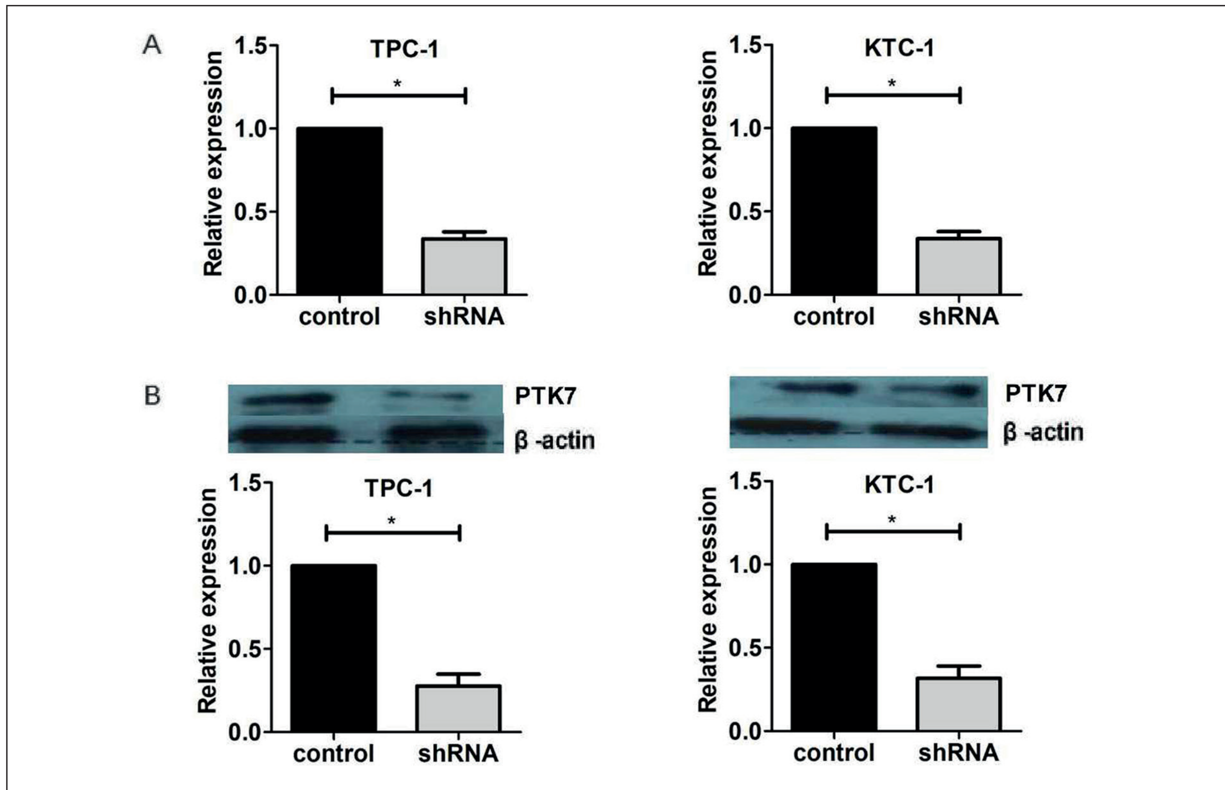

Figure 2. PTK7 expression was effectively reduced in both TPC-1 and KTC-1 cells after the transfection of its shRNA plasmids. **A**, Quantitative PCR assays exhibited the effectively decrease expression of PTK7 after the transfection of its shRNA plasmids in TPC-1 and KTC-1 cells, respectively. **B**, Immunoblot assays exhibited the obvious decrease of PTK7 expression levels following the transfection of its shRNA in TPC-1 and KTC-1 cells. Results are presented as mean ± SD, * $p < 0.05$.

proliferation of thyroid cancer cells. Through colony formation assays, we found that the ablation of PTK7 dramatically suppressed the proliferation of TPC-1 and KTC-1 cells, respectively, with the obvious decrease in colony number (Figure 3A). Consistent with the results of colony formation assays, we found that PTK7 significantly blocked proliferate capacity of thyroid cancer cells through MTT assays (Figure 3B). In conclusion, these results revealed that PTK7 significantly suppressed the proliferation of thyroid cancer cells.

Knockdown of PTK7 Induces Thyroid Cancer Cell Apoptosis

For cancer development, the suppression of apoptosis will greatly fascinate this process. We therefore examined whether PTK7 affected cell apoptosis of thyroid cancer. Through flow cytometry analysis, we noticed that PTK7 ablation significantly contributed to the apoptosis of TPC-1 and KTC-1 cells, with an increased percentage of apoptosis cells (Figure 3C).

PTK7 Promotes Tumor Growth of Thyroid Cancer Cells In Vivo

We then explored whether PTK7 promoted tumor growth of thyroid cancer cells in mice. Tumor growth assays were performed. TPC-1 cells were infected with PTK7 shRNA lentivirus to stably deplete its expression. Control or PTK7 stable depletion TPC-1 cells were injected subcutaneously into nude mice. After 2 weeks, mice tumors were isolated from each group once a week, photographed, and the volume of tumors was measured. After 49 days, all tumors were isolated. Representative images and the growth curve were exhibited in Figure 4A. According to the results, we found the volume of tumors in PTK7 depletion groups was significantly smaller than that in control groups (Figure 4A). In addition, the effective decrease of PTK7expression in PTK7 stable depletion tumor tissue was detected through IHC assays (Figure 4B). In summary, we revealed thatPTK7 fascinates tumor growth of thyroid cancer cells in mice.

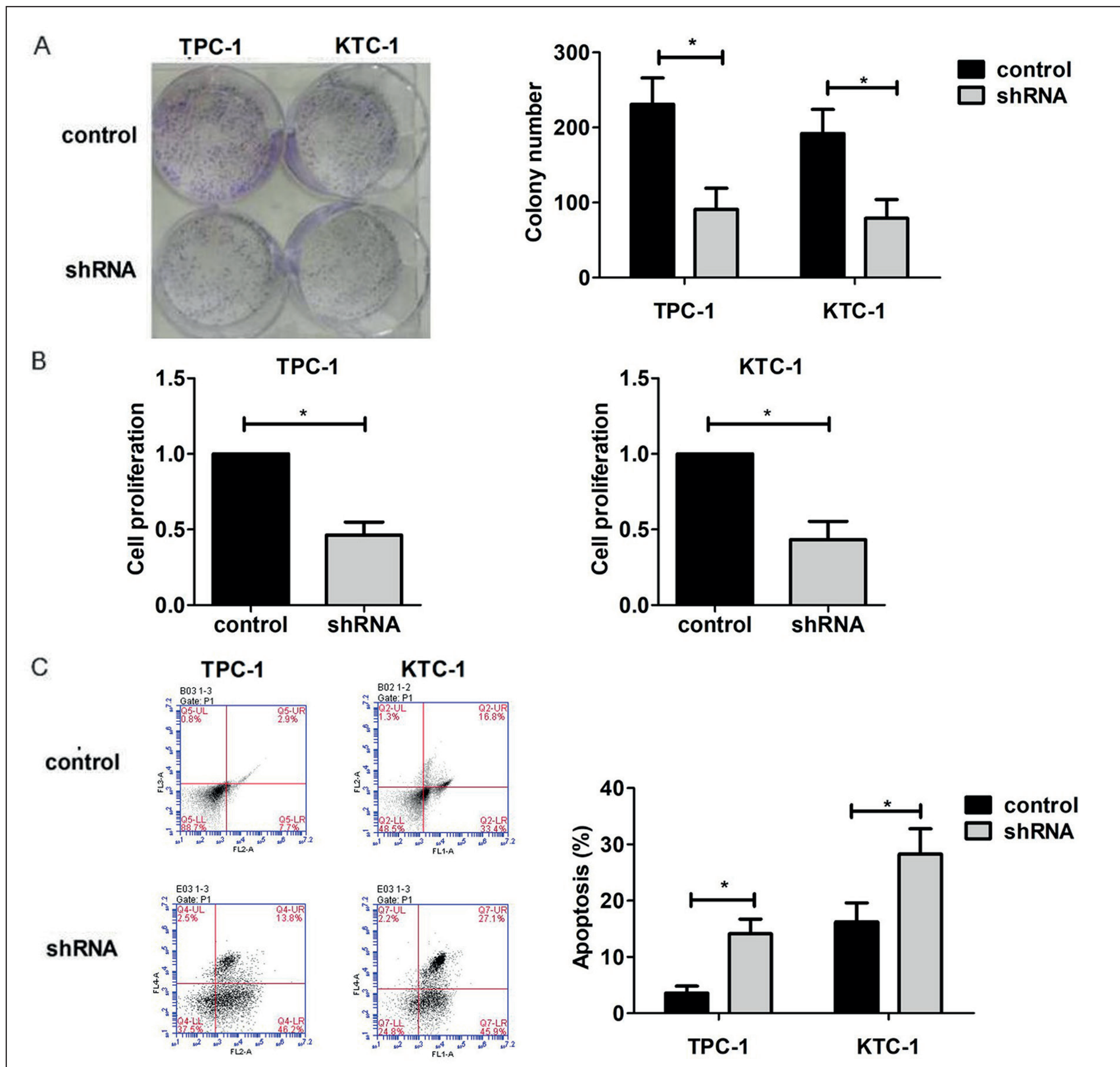

Figure 3. PTK7 fascinates cell proliferation and inhibits cell apoptosis of thyroid cancer. **A**, Colony formation assays were performed in TPC-1 and KTC-1 cells, and the number of colonies was manually counted. **B**, MTT assays were conducted using TPC-1 and KTC-1 cells transfected with control or PTK7 shRNA plasmids, and the OD value at 570 nm wavelength was measured. **C**, FCM assays were performed using TPC-1 and KTC-1 cells transfected with control or PTK7 shRNA plasmids, and the percentage of apoptosis cells was compared between control and PTK7 depletion groups. Results are presented as mean ± SD, * $p < 0.05$.

Discussion

Although the traditional treatment methods for thyroid cancer have significantly improved, high invasiveness and recurrence rates result in low quality of life in patients with thyroid cancer[8,22]. To combat this disease more effectively, we still need to further understand its pathogenesis, and find more effective therapeutic targets[4]. A large number of studies have reported that multiple members of the receptor protein tyrosine kinase-like family could extensively affect the progression and development of cancer[23]. Here we identified a receptor protein tyrosine kinase-like protein, PTK7, which was highly expressed in human thyroid cancer tissues. Through the analysis of tumor tissues from 79 patients who underwent thyroid cancer, our data further proved that PTK7

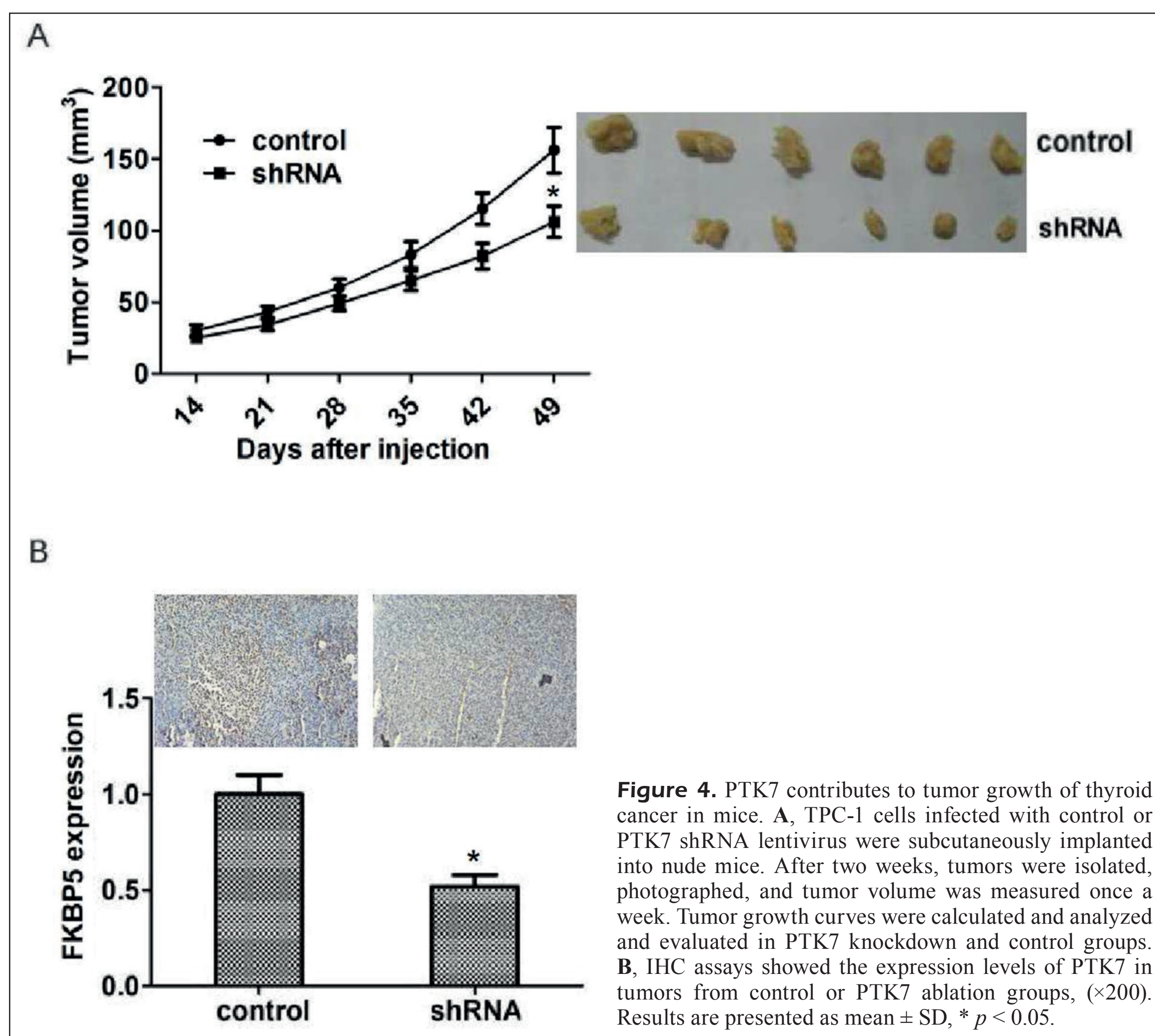

Figure 4. PTK7 contributes to tumor growth of thyroid cancer in mice. **A**, TPC-1 cells infected with control or PTK7 shRNA lentivirus were subcutaneously implanted into nude mice. After two weeks, tumors were isolated, photographed, and tumor volume was measured once a week. Tumor growth curves were calculated and analyzed and evaluated in PTK7 knockdown and control groups. **B**, IHC assays showed the expression levels of PTK7 in tumors from control or PTK7 ablation groups, (×200). Results are presented as mean ± SD, * $p < 0.05$.

was associated with clinical-pathological characteristics including TNM stage (*p*=0.015*), and intraglandular dissemination (*p*=0.024*). Our results also demonstrated the involvement of PTK7 in the progression of thyroid cancer. We, therefore, identified PTK7 as a novel therapeutic target for the treatment of thyroid cancer.

The critical role of PTK7 has been widely revealed in multiple types of cancers[11,17]. High expression of PTK7 was also identified in multiple cancers such as lung cancer and colon cancer[17,18]. Similarly, here we noticed that PTK7 was highly expressed in human thyroid cancer tissues, suggesting the critical role of PTK7 in thyroid cancer progression. Through colony formation assay, MTT assay, and FCM assay, we found PTK7 significantly affected the proliferation and apoptosis of thyroid cancer cells *in vitro.* Consistent with these data, we also found PTK7 promoted tumor growth of thyroid cancer cells *in vivo.* Similarly, Shin et al[24] showed that the depletion of PTK7 inhibited cell proliferation, migration, and invasion via targeting ERK, JNK, p38, Akt and FAK in esophageal cancer. Additionally, a miRNA, miR2055p, regulated the proliferation, migration and invasion of colorectal cancer cells via targeting PTK7[25]. PTK7 was also correlated with the poor prognosis of glioma and mediated the expression of Id1 in CD44-high glioma cells[26]. This study is the first to confirm the important role of PTK7 in the progression of thyroid cancer. We also found that PTK7 not only regulates tumor cell proliferation, but also affects apoptosis, which is different from the effects of PTK7 on

other tumors. However, the detailed molecular mechanisms still need further study.

Notably, in some types of cancers, PTK7 was significantly downregulated, including metastatic melanoma and ovarian carcinoma[27,28]. This is completely different from what we found. We revealed that the expression of PTK7 in thyroid cancer was significantly up-regulated, which, combined with the study results in other tumors, suggested the complexity of PTK7 in the regulation of tumor development.

In addition to its effect on tumors, PTK7 also affects a variety of other physiological and pathological processes[15]. PTK7 has been implicated in the pathogenesis of scoliosis and neural tube defects[12,13]. PTK7 plays a key role in the regulation of Wnt and PCP signaling pathways known to regulate development and homeostasis, and the defects of PTK7 are probably of clinical relevance[29,30]. As was known, both PCP and WNT signaling pathways could widely affect tumor progression[31,32]. We next should explore whether PTK7 might be involved in the regulation of thyroid cancer development through the PCP and WNT signaling pathways.

Conclusions

Collectively, our current findings declare that PTK7 is highly expressed in human thyroid cancer tissues, and its expression levels are correlated with the clinical characteristics of patients with thyroid cancer. PTK7 ablation results in the inhibition of cell proliferation and the induction of cell apoptosis *in vitro*. Additionally, PTK7contributes to the growth of thyroid cancer in mice. These results help to provide a novel and promising therapeutic target for the treatment of thyroid cancer in the future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval and Consent to Participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The animal study was carried out in accordance with the guidelines approved by the Animal Experimentation Ethics Committee of the Second Affiliated Hospital of Nanchang University. The protocol was approved by the Committee, and all efforts were made to minimize suffering.

Authors' Contribution

Fei Duan, Jie Tang, and Fang-Li Kong carried out the experiment of molecular biology and drafted the manuscript. Hui-Wen Zou and Bao-Liang Ni carried out the animal experiment. Bao-Liang Ni and Ji-Chun Yu participated in the design of the study and performed the statistical analysis. All authors read and approved the final manuscript.

References

1) Nakaya T, Takahashi K, Takahashi H, Yasumura S, Ohira T, Ohto H, Ohtsuru A, Midorikawa S, Suzuki S, Shimura H, Yamashita S, Tanigawa K, Kamiya K. Spatial analysis of the geographical distribution of thyroid cancer cases from the first-round thyroid ultrasound examination in Fukushima Prefecture. Sci Rep 2018; 8: 17661.
2) Soheylizad M, Khazaei S, Jenabi E, Delpisheh A, Veisani Y. The relationship between human development index and its components with thyroid cancer incidence and mortality: using the decomposition approach. Int J Endocrinol Metab 2018; 16: e65078.
3) Wu F, Li F, Lin X, Xu F, Cui R, Zhong J, Zhu T, Shan SK, Liao XB, Yuan L, Mo Z. Exosomes increased angiogenesis in papillary thyroid cancer microenvironment. Endocr Relat Cancer 2019; 26: 525-538.
4) Agrawal VR, Hreno J, Patil T, Bowles DW. New therapies for anaplastic thyroid cancer. Drugs Today (Barc) 2018; 54: 695-704.
5) Calo PG, Pisano G, Medas F, Marcialis J, Gordini L, Erdas E, Nicolosi A. Total thyroidectomy without prophylactic central neck dissection in clinically node-negative papillary thyroid cancer: is it an adequate treatment? World J Surg Oncol 2014; 12: 152.
6) Sabra MM, Sherman E, Tuttle RM. Prolongation of tumour volume doubling time (midDT) is associated with improvement in disease-specific survival in patients with rapidly progressive radioactive iodine refractory differentiated thyroid cancer selected for molecular targeted therapy. Clin Endocrinol (Oxf) 2019; 90: 617-622.
7) Jaber T, Waguespack SG, Cabanillas ME, Elbanan M, Vu T, Dadu R, Sherman SI, Amit M, Santos EB, Zafereo M, Busaidy NL. Targeted therapy in advanced thyroid cancer to resensitize tumors to radioactive iodine. J Clin Endocrinol Metab 2018; 103: 3698-3705.
8) Ton GN, Banaszynski ME, Kolesar JM. Vandetanib: a novel targeted therapy for the treatment of metastatic or locally advanced medullary thyroid cancer. Am J Health Syst Pharm 2013; 70: 849-855.
9) Banga SS, Ozer HL, Park SK, Lee ST. Assignment of PTK7 encoding a receptor protein tyrosine kinase-like molecule to human chromosome 6p21.1-->p12.2 by fluorescence in situ hybridization. Cytogenet Cell Genet 1997; 76: 43-44.
10) Liu Z, Chen W, Han Y, Ouyang J, Chen M, Hu S, Deng L, Liu YN. A label-free sensitive method for membrane protein detection based on aptamer and AgNCs transfer. Talanta 2017; 175: 470-476.

11) Golubkov VS, Prigozhina NL, Zhang Y, Stoletov K, Lewis JD, Schwartz PE, Hoffman RM, Strongin AY. Protein-tyrosine pseudokinase 7 (PTK7) directs cancer cell motility and metastasis. J Biol Chem 2014; 289: 24238-24249.

12) Bin-Nun N, Lichtig H, Malyarova A, Levy M, Elias S, Frank D. PTK7 modulates Wnt signaling activity via LRP6. Development 2014; 141: 410-421.

13) Hayes M, Gao X, Yu LX, Paria N, Henkelman RM, Wise CA, Ciruna B. ptk7 mutant zebrafish models of congenital and idiopathic scoliosis implicate dysregulated Wnt signalling in disease. Nat Commun 2014; 5: 4777.

14) Linnemannstons K, Ripp C, Honemann-Capito M, Brechtel-Curth K, Hedderich M, Wodarz A. The PTK7-related transmembrane proteins off-track and off-track 2 are co-receptors for Drosophila Wnt2 required for male fertility. PLoS Genet 2014; 10: e1004443.

15) Shnitsar I, Borchers A. PTK7 recruits dsh to regulate neural crest migration. Development. 2008; 135: 4015-4024.

16) Zhang H, Wang A, Qi S, Cheng S, Yao B, Xu Y. Protein tyrosine kinase 7 (PTK7) as a predictor of lymph node metastases and a novel prognostic biomarker in patients with prostate cancer. Int J Mol Sci 2014; 15: 11665-11677.

17) Lin Y, Zhang LH, Wang XH, Xing XF, Cheng XJ, Dong B, Hu Y, Du H, Li YA, Zhu YB, Ding N, Du YX, Li JY, Ji JF. PTK7 as a novel marker for favorable gastric cancer patient survival. J Surg Oncol 2012; 106: 880-886.

18) Chen R, Khatri P, Mazur PK, Polin M, Zheng Y, Vaka D, Hoang CD, Shrager J, Xu Y, Vicent S, Butte AJ, Sweet-Cordero EA. A meta-analysis of lung cancer gene expression identifies PTK7 as a survival gene in lung adenocarcinoma. Cancer Res 2014; 74: 2892-2902.

19) Meng L, Sefah K, O'Donoghue MB, Zhu G, Shangguan D, Noorali A, Chen Y, Zhou L, Tan W. Silencing of PTK7 in colon cancer cells: caspase-10-dependent apoptosis via mitochondrial pathway. PLoS One 2010; 5: e14018.

20) Patel RB, Li T, Liao Z, Jaldeepbhai JA, Perera H, Muthukuda SK, Dhirubhai DH, Singh V, Du X, Yang J. Recent translational research into targeted therapy for liposarcoma. Stem Cell Investig 2017; 4: 21.

21) Yu B, Wu K, Wang X, Zhang J, Wang L, Jiang Y, Zhu X, Chen W, Yan M. Periostin secreted by cancer-associated fibroblasts promotes cancer stemness in head and neck cancer by activating protein tyrosine kinase 7. Cell Death Dis 2018; 9: 1082.

22) Khan ZA, Mehta S, Sumathi N, Dhiwakar M. Occult invasion of sternothyroid muscle by differentiated thyroid cancer. Eur Arch Otorhinolaryngol 2018; 275: 233-238.

23) Kurihara M, Koda H, Aono H, Sugimoto I, Sakurai Y, Sano T, Saito Y, Murayama S, Mori M. Rapidly progressive miliary brain metastasis of lung cancer after EGFR tyrosine kinase inhibitor discontinuation: an autopsy report. Neuropathology 2019; 39: 147-155.

24) Shin WS, Gim J, Won S, Lee ST. Biphasic regulation of tumorigenesis by PTK7 expression level in esophageal squamous cell carcinoma. Sci Rep 2018; 8: 8519.

25) Chen S, Wang Y, Su Y, Zhang L, Zhang M, Li X, Wang J, Zhang X. MiR2055p/PTK7 axis is involved in the proliferation, migration and invasion of colorectal cancer cells. Mol Med Rep. 2018; 17:6253-6260. DOI: 10.3892/mmr.2018.8650. PMID: 29488611.

26) Liu Q, Zhang C, Yuan J, Fu J, Wu M, Su J, Wang X, Yuan X, Jiang W. PTK7 regulates Id1 expression in CD44-high glioma cells. Neuro Oncol 2015; 17: 505-515.

27) Lhoumeau AC, Martinez S, Boher JM, Monges G, Castellano R, Goubard A, Doremus M, Poizat F, Lelong B, de Chaisemartin C, Bardin F, Viens P, Raoul JL, Prebet T, Aurrand-Lions M, Borg JP, Gonçalves A. Overexpression of the promigratory and prometastatic PTK7 receptor is associated with an adverse clinical outcome in colorectal cancer. PLoS One 2015; 10: e0123768.

28) Wang H, Li G, Yin Y, Wang J, Wang H, Wei W, Guo Q, Ma H, Shi Q, Zhou X, Wang J. PTK7 protein is decreased in epithelial ovarian carcinomas with poor prognosis. Int J Clin Exp Pathol 2014; 7: 7881-7889.

29) Hayes M, Naito M, Daulat A, Angers S, Ciruna B. Ptk7 promotes non-canonical Wnt/PCP-mediated morphogenesis and inhibits Wnt/beta-catenin-dependent cell fate decisions during vertebrate development. Development 2013; 140: 1807-1818.

30) Martinez S, Scerbo P, Giordano M, Daulat AM, Lhoumeau AC, Thome V, Kodjabachian L, Borg JP. The PTK7 and ROR2 protein receptors interact in the vertebrate WNT/Planar cell polarity (PCP) pathway. J Biol Chem 2015; 290: 30562-30572.

31) Harb J, Lin PJ, Hao J. Recent Development of Wnt signaling pathway inhibitors for cancer therapeutics. Curr Oncol Rep 2019; 21: 12.

32) Humphries AC, Mlodzik M. From instruction to output: Wnt/PCP signaling in development and cancer. Curr Opin Cell Biol 2018; 51: 110-116.