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Thyroid-stimulating hormone receptor affects metastasis and prognosis in papillary thyroid carcinoma

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Abstract. – OBJECTIVE: Although endocrine therapy of papillary thyroid carcinoma (PTC) by inhibiting thyroid-stimulating hormone (TSH) has been used for many years, its mechanism of action is not clear. This study aimed to explore the expression and role of TSH receptor (TSHR) in PTC, to provide a theoretical basis for optimization of endocrine treatment options in PTC.

PATIENTS AND METHODS: Expression of TSHR was tested by immunohistochemistry of tissues from 150 cases of PTC and 21 normal thyroid tissues. Survival analysis was performed by Kaplan-Meier and log-rank analyses, and multivariate analysis was done using a Cox model. The regulatory effects of the TSH-TSHR signal transduction pathway on differentiated thyroid carcinoma cells were explored *in vitro*.

RESULTS: The positive expression rate of TSHR in PTC was 68% (102/150). TSHR expression was an independent factor affecting the prognosis of PTC patients aged > 45 years ($p = 0.006$), and TSHR might have a role in decreasing distant metastasis ($p = 0.024$). *In vitro* experiments showed that up-regulation of TSHR promoted apoptosis of thyroid cancer cells and inhibited metastasis significantly. There was no significant regulatory effect of the TSH-TSHR signal transduction pathway on the proliferation of thyroid carcinoma cells.

CONCLUSIONS: TSHR expression is an independent factor that affects the prognosis of PTC patients, and might decrease distant metastasis in patient aged > 45 years. Up-regulation of TSHR could inhibit metastasis and promote apoptosis in PTC cells.

Key Words:
Papillary thyroid carcinoma, Thyroid stimulating hormone receptor, Prognosis, Metastasis.

Introduction

Thyroid cancer is one of the most common malignant tumors of the head and neck. The occurrence of thyroid malignancy is increased in patients with elevated serum thyrotrophic concentration and/or chronic thyroiditis[1]. Ninety percent of thyroid cancer is differentiated thyroid carcinoma (DTC), including papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma[2-6]. Current treatment strategies for DTC include radical surgery, I^{131} treatment and endocrine therapy by inhibition of thyroid-stimulating hormone (TSH)[4,5,7]. Clinical endocrine therapy based on inhibition of TSH synthesis has been used for many years; however, its effect on prevention of recurrence of PTC is still unclear[6-10].

At present, the theory of endocrine therapy by inhibition of TSH receptor (TSHR) is mainly based on research on the effect of TSH on thyroid cell biology. TSH promotes thyroid cell proliferation through TSHR. TSHR is a common cell membrane receptor in PTC cells, thus theoretically, down-regulation of TSH through suppression of pituitary TSH feedback by oral thyroxin, might inhibit the proliferation of PTC cells, which may reduce tumor recurrence rate[11]. Although endocrine therapy by inhibition of the TSHR pathway has been used in clinical practice for > 30 years, the exact treatment effect is not clear and there is a lack of strong clinical evidence[4,10,12-14]. Cooper et al[10] showed that endocrine treatment by inhibition of TSH is not in-

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dependent of factors affecting patient prognosis. However, endocrine therapy did not affect the 5-year survival rate[12]. According to research by Biondi and Cooper[13], endocrine therapy might only affect some DTC patients with recurrent or progressive disease. In addition, TSH endocrine therapy has some adverse effects including osteoporosis and ischemic heart disease[4,13,15]. Therefore, there is a lack of strong evidence to support the view that TSH endocrine therapy can significantly improve the survival rate of patients with DTC, and the current TSH endocrine therapy lacks a theoretical basis[4,10,12,13]. It is still unknown whether inhibition of TSH synthesis can inhibit PTC tumor growth or distant metastasis.

We recently discovered that expression of TSHR is not significantly up-regulated in DTC, and down-regulation of TSHR only mildly inhibits thyroid cancer cell proliferation, but significantly enhances metastasis[16].

This study was designed to verify the effect and mechanism of endocrine therapy of PTC by inhibition of the TSHR signal transduction pathway. The results will provide a theoretical basis for optimization of endocrine treatment options in PTC.

Patients and Methods

Patients

We enrolled 150 patients with PTC, who underwent surgery at Sun Yat-Sen University Cancer Center (Guangzhou, China) from January 1990 to December 2013. One hundred and fifty PTC tissue samples and 21 normal thyroid tissues were collected, and the specimens were verified by two pathologists. The 150 PTC patients were aged 10-76 years, with a median age of 38 years, and 93 were female and 57 male, with a ratio of 1.63:1. According to UICC (2002) TNM staging, 102 cases were stage I, 8 stage II, 22 stage III and 18 stage IV. One hundred and one cases had neck lymph node metastases and 2 had distant metastases.

Immunohistochemical Staining

TSH mouse anti-human monoclonal antibody was purchased from Abcam (Cambridge, MA, USA; product number: ab49702), using a solution dilution rate of 1:50. Each paraffin-embedded specimen was cut into 4-μm sections and baked at 1.5 h at 60°C. The slices was deparaffinized with xylene and rehydrated through a graded ethanol series (100% ethanol, 95% ethanol, 80% ethanol, 65% ethanol) to distilled water. Then, the slides were incubated in 3% hydrogen peroxide solution for 15 min at room temperature. The sections were submerged into EDTA antigenic retrieval buffer (0.01 mM, pH 8.0) and microwaved for antigenic retrieval. The sections were treated with 0.3% hydrogen peroxide for 15 min to block the endogenous peroxidase at room temperature, and then were treated with the normal goat serum for 30 min to reduce nonspecific binding. Consequently, the sections were incubated with TSH mouse anti-human monoclonal antibody (1:50). After washing, the sections were incubated with each specific secondary antibody, followed by further incubation with streptavidin-horseradish peroxidase (Zymed, Waltham, MA, USA) at 37°C for 30 min. Diaminobenzidine was used for color reaction, and the antibody was replaced by normal goat serum for negative controls.

We randomly observed five fields, and measured the number of positively stained cells and staining intensity. For positively stained cells, ≤10% was scored as 0, 11%-25% as 1, 26%-50% as 2, and > 51% as 3. Negative staining intensity was scored as 0, weakly positive as 1, positive as 2 and strongly positive as 3; no staining intensity and background has no obvious difference is negative. The final score was sum of the staining intensity score and positive cell number score. The final score was divided into four groups: 0 for negative (–), 1-2 points for weak positive (+), 3-6 for moderately positive (++), and > 6 strong positive (+++)[17]. Results were decided after two repeated observations.

Western Blotting

For extraction of total protein, we removed the medium and washed twice in 1 ml cold PBS, then lysed the total protein for 10 min on ice. Cell and tissue samples were solubilized in SDS lysis buffer, and the protein concentrations were detected by the BCA protein assay kit (Pierce, Rockford, IL, USA). Equal amounts of protein samples (30 μg/lane) were separated by electrophoresis through 9.0% resolving sodium dodecyl sulphate (SDS)-polyacrylamide gel, and then transferred to polyvinylidene fluoride (PVDF) membranes (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Non-specific binding sites were blocked by immersing the membranes in 5% non-fat milk in TBS-T solution (TBS+0.5% Tween-20) for 1 h, and then incubating the membranes with a primary mon-

oclonal antibody for 2 h at room temperature. After washing three times in TBS-T, the membranes were incubated with a secondary antibody (goat anti-rabbit and goat anti-rabbit, respectively, diluted 1:6000 in TBS-T) for 1 h at room temperature. The membranes were washed and proteins were detected by enhanced chemiluminescence. Anti-□-tubulin mouse monoclonal antibody was used to confirm equal loading of lysates (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Image J software (http://imagej.net) was used to analyze the gray levels.

Cell Culture

The cell lines were obtained from The Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences. Cells were maintained in Roswell Park Memorial Institute 1640 (RPMI 1640) medium (Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA), penicillin (100 U/ml), and streptomycin (100 U/ml) at 37°C in a humidified 5% CO_2 incubator.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide Reduction (MTT) Assay

Cells were seeded onto 96-well plates at 2000 cells/well. Each sample had four replicates. The cells were incubated with 0.2% MTT for 4 h at 37°C. One hundred microliters dimethyl sulfoxide (DMSO)/well was added to the culture cells to dissolve the crystals, and cells were counted every day by reading the absorbance at 490 nm.

Flow Cytometry Assay

Apoptosis was measured with the Annexin V-FITC Apoptosis Detection Kit (Invitrogen) and analyzed by flow cytometry (Epics Elite; Beckman Coulter, Brea, CA, USA).

Transwell Assay

One hundred and five cells were placed on the upper layer of a cell-permeable membrane of Matrigel-coated 24-well Boyden chambers (Corning, New York, NY, USA). After 24 h, the non-invading cells were gently removed, and the cells that had invaded the bottom chamber were fixed, stained and counted.

RNAi

siRNA against TSHR was synthesized by GenePharma (Shanghai, China). The cells were plated in six-well plates and transfected with the siRNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Statistical Analysis

The last follow-up time was September 30, 2013. The data were analyzed by SPSS version 19.0 statistical software (SPSS, Chicago, IL, USA). Comparison between groups was performed by $\square^2$ test; survival analysis was conducted using the Kaplan-Meier method and single factor analysis was done using the log-rank test; analysis of prognosis was completed by Cox model. Logistic regression was used for multiple correlation analysis. $p < 0.05$ was considered statistically significant.

Results

TSHR Expression in PTC and Normal Thyroid Tissues

Expression of TSHR did not differ significantly between PTC and normal thyroid tissues. TSHR expression significantly affected the prognosis and distant metastasis in PTC patients aged > 45 years. Up-regulation of TSHR protein did not significantly affect proliferation of PTC, but improved its metastatic capacity significantly.

TSHR expression in Normal Thyroid and Thyroid Carcinoma Tissues

Immunohistochemical staining showed that TSHR was expressed in normal thyroid and thyroid cancer tissues. In normal thyroid tissues, TSHR was expressed mainly on the cell membrane, however, TSHR was mainly located in the cytoplasm (Figure 1A). Western blotting showed that expression of TSHR was positive in 64% of PTC cases (Figure 1B). TSHR expression was detected in 102 of the 150 PTC cases (68%, 102/150) and 6 of the 21 normal thyroid tissues (28.6%, 6/21). TSHR expression was significantly different between PTC and normal thyroid tissue ($p = 0.001$). TSHR expression was not significantly related to age, sex, pathological staging, T staging, N staging, M staging and recurrence in PTC patients ($p > 0.05$) (Table I).

Effect of expression of TSHR on prognosis of PTC

As age is a major independent prognostic factor for well-differentiated thyroid carcinoma, we made stratified prognostic analysis ac-

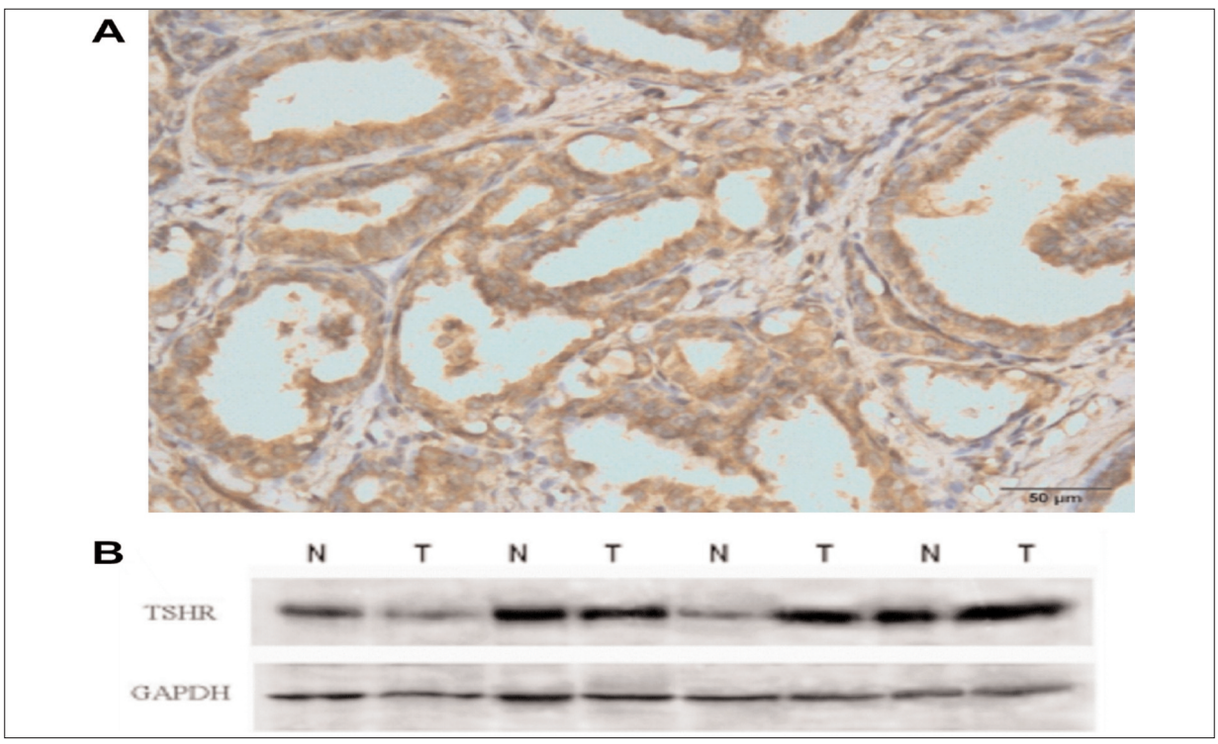

Figure 1. TSHR expression in normal thyroid and PTC tissues. ***A,*** Positive expression of TSHR in PTC (×200). ***B,*** Expression of TSHR in PTC and paired normal tissues.

Table I. Survival analysis results of 150 PTC patients.

Factor	Cases	5-OS (%)	10-OS (%)	χ^2	*p*
TSHR expression					
Negative	68	91	83		
Positive	82	95	86	0.164	0.686
Age					
< 45	97	98	97		
≥ 45	53	87	61	39.765	< 0.01
T stage					
T1+T2	110	99	93		
T3+T4	40	85	62	25.093	< 0.01
N stage					
N0	49	98	89		
N+	101	91	83	1.782	0.182
Distant metastasis					
M0	141	96	84		
M1	9	67	33	31.127	0.000
Tumor residue					
Yes	30	83	69		
No	120	96	89	8.972	0.03
Recurrent laryngeal nerve invasion					
Yes	13	84	62		
No	137	96	87	5.916	0.015
Trachea invasion					
Yes	18	73	55		
No	132	96	90	14.70	< 0.01
Recurrence					
Yes	122	94	85		
No	28	93	84	< 0.01	0.991

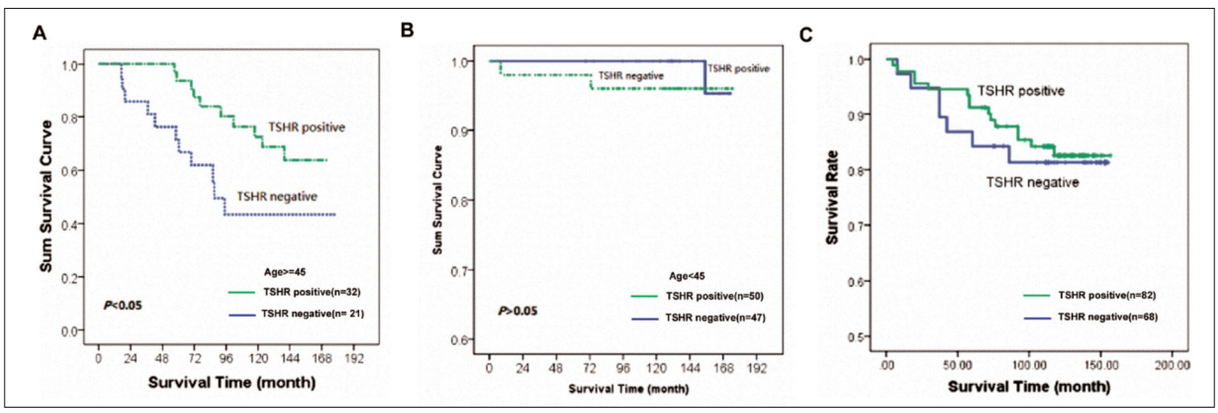

Figure 2. Effect of expression of TSHR on prognosis of patients with PTC. ***A,*** For patients aged > 45 years, TSHR expression affected prognosis of PTC significantly (p = 0.021). ***B,*** For patients aged < 45 years, TSHR expression did not affect prognosis of PTC (p = 0.276). ***C,*** For all 150 PTC patients, TSHR expression did not affect prognosis of PTC (p = 0.686).

cording to age. For PTC patients aged < 45 years (younger group) and aged > 45 years (older group), the 10-year overall survival (OS) rates were 97% and 59%, respectively. For PTC patients aged > 45 years, the 10-year OS rates of the TSHR-positive sub-group and TSHR-negative sub-group were 43% and 76% respectively (p = 0.021) (Figure 2A). Single analyses showed that TSHR expression affected prognosis of PTC significantly (p < 0.05) (Table II). Multivariate analysis showed that TSHR expression was an independent factor affecting prognosis of PTC patients (p = 0.006) (Table III). For PTC patients aged < 45 years, the 5-year and 10-year OS rates were 98% and 97%, respectively. The 10-year OS rates of the TSHR-positive sub-group and TSHR-negative sub-group were 100% and 96%, respectively (p = 0.408) (Figure 2B). For all 150 PTC patients, the 10-year OS rates of the TSHR-positive sub-group and TSHR-negative sub-group were 86% and 83%, respectively (p = 0.607) (Figure 2C). Both univariate and multivariate analyses showed that TSHR expression did not affect prognosis of PTC patients aged < 45 years (p > 0.05) (Table III).

Table II. Survival analysis results of PTC patients with age ≥ 45.

Factor	Patients	5-OS (%)	10-OS (%)	χ^2	p
TSHR expression					
Negative	21	76	43		
Positive	32	93	76	5.286	0.021
T stage					
T1+T2	16	88	61		
T3+T4	37	87	60	0.019	0.89
N stage					
N0	21	95	80		
N+	32	81	52	3.121	0.077
Distant metastasis					
M0	47	89	69		
M1	6	50	0	26.765	0.000
Tumor residue					
Yes	20	75	52		
No	33	94	65	1.627	0.202
Recurrence					
Yes	10	86	60		
No	43	90	65	0.046	0.829

Table III. Multivariate results of factors affecting the prognosis of PTC patients.

Factor	B	SE	Wald	p	Exp (B)	Exp (B) 95% CI Lower	Exp (B) 95% CI Upper
Cox analysis results of PTC patients with age ≥ 45							
TSHR expression	-1.204	.558	4.656	.031	.300	.100	.895
N stage	2.629	1.019	6.651	.010	13.853	1.879	102.116
Stage	-2.169	1.011	4.601	.032	.114	.016	.829
Metastasis	1.869	.633	8.717	.003	6.485	1.875	22.432
Tumor residue	-1.965	.882	4.966	.026	.140	.025	.789
Cox analysis results of PTC patients with age < 45							
TSHR expression	2.401	1.507	2.537	.111	11.036	.575	211.792
T stage	-.445	1.007	.195	.658	.641	.089	4.609
N stage	9.803	106.896	.008	.927	18093.276	.000	1.768E95
Stage	1.040	1645.322	.000	.999	2.828	.000	.
Trachea invasion	4.974	467.031	.000	.992	144.609	.000	.
Cox analysis results of 150 PTC patients							
TSHR expression	-.690	.497	1.928	.165	.501	.189	1.329
Age	4.591	1.074	18.266	.000	98.622	12.010	809.844
N stage	2.495	.950	6.904	.009	12.123	1.885	77.974
Stage	-1.892	.926	4.173	.041	.151	.025	.926
Trachea invasion	-1.196	.538	4.952	.026	.302	.105	.867
Metastasis	2.081	.544	14.657	.000	8.014	2.761	23.258
Tumor residue	-1.553	.832	3.481	.062	.212	.041	1.082

Expression of TSHR in Distant Metastasis of PTC

Logistic regression was used to analyze the factors related to distant metastasis in PTC patients. The input variables included: age, TSHR expression, pathological T stage, pathological N stage, stage, extracapsular invasion, cervical anterior muscle invasion, tracheal invasion, recurrent laryngeal nerve invasion, comorbid Hashimoto's thyroiditis, and primary treatment. Stratified analysis according to age showed that TSHR expression was a significant factors related to distant metastasis for PTC patients aged > 45 years, while cancer stage was a significant factor related to distant metastasis for PTC patients aged < 45 years. The results also showed that pathological T stage was the only significant factor related to distant metastasis in all 150 PTC patients. TSHR expression was a significant factor for metastasis in PTC patients aged > 45 years (Table IV).

Table IV. Logistic regression results of factors related to distant metastasis in PTC patients.

Factor	B	SE	Wald	p	Exp (B)	Exp (B) 95% CI Lower	Exp (B) 95% CI Upper
Logistic regression results of PTC patients with age ≥ .45							
TSHR expression	-2.730	1.247	4.790	.029	.065	.006	.752
Trachea invasion	-1.943	1.065	3.329	.068	.143	.018	1.155
Logistic regression results of PTC patients with age < 45							
Stage	3.839	1.585	5.871	.015	46.500	2.083	1038.035
Logistic regression results of 150 PTC patients							
T stage	.906	.326	7.722	.005	2.476	1.306	4.692

The input variables included: age, the expression of TSHR, pathologic T stage, pathological N stage, stage, extracapsular invasion, cervical anterior muscles invasion, tracheal invasion, esophageal invasion, recurrent laryngeal nerve invasion, combined with Hashimoto's thyroiditis and primary treatment.

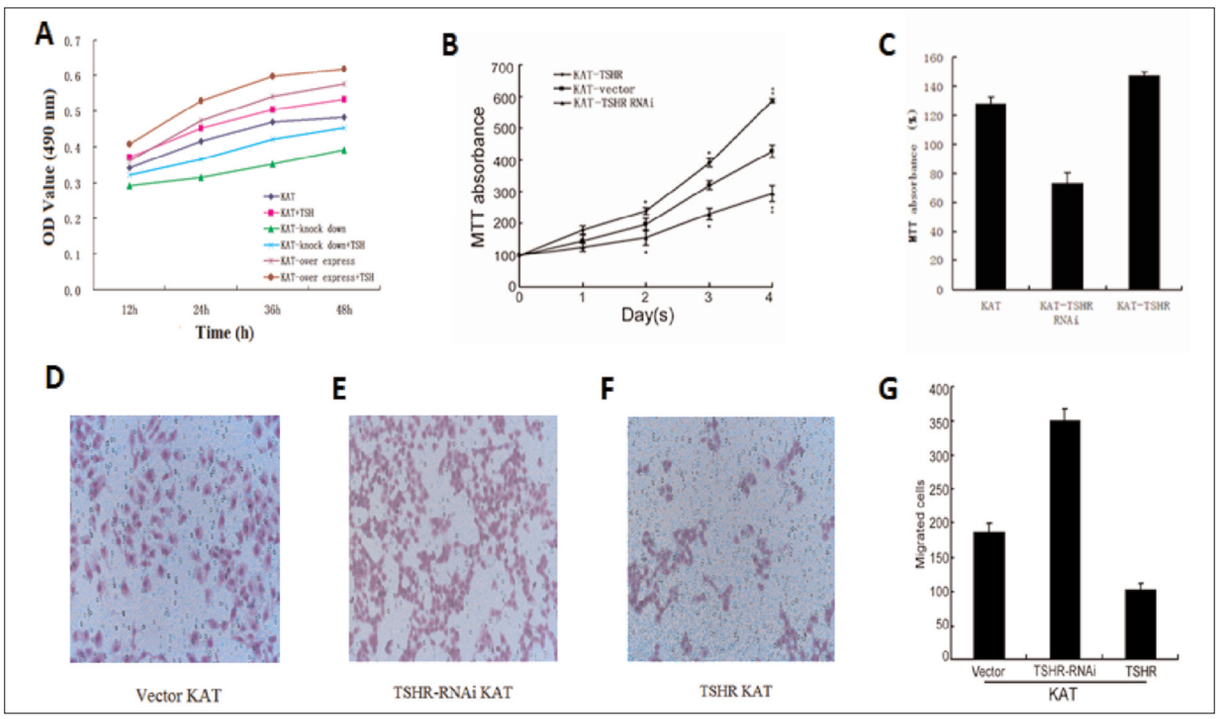

Figure 3. ***A,*** Effect of TSHR on proliferation of thyroid carcinoma cells. MTT assay showed that the TSHR normal cell (KAT), TSHR lower expression cell (KAT knock down) and TSHR over expression cell (KAT over express) was increased by 8.8%, 11.6% and 10.3% respectively after TSH (5 Mu/L) stimulation for 12–48 h ***(A)***. MTT assay showed that up-regulation of TSHR mildly promotes proliferation of KAT-5 PTC cells ***(B*** and ***C)***. Down-regulation of TSHR by RNAi slightly inhibited proliferation of KAT-5 cells ***(B*** and ***C)***. Different levels of TSHR expression on apoptosis of PTC cells: up-regulation of TSHR promoted apoptosis of PTC cells ***(E–H)***. Transwell results showed that up-regulation of TSHR inhibited invasion and metastasis of KAT-5 PTC cells with an inhibition rate of 45.3%, and down-regulation of TSHR significantly promoted invasion and metastasis of KAT cells by 86.7% ***(D-G)***.

Regulation of TSHR Pathway in Cell Proliferation of Thyroid Carcinoma

MTT results showed that the TSHR normal cell (KAT), TSHR lower expression cell (KAT knock down) and TSHR over expression cell (KAT over express) after TSH (5 Mu/L) stimulation for 12-48 h were increased by 8.8%, 11.6% and 10.3%, respectively. TSH did not significantly promote cell proliferation (Figure 3A). We studied the influence of expression of TSHR on cell proliferation of thyroid carcinoma. In the KAT-5 PTC cell line, up-regulation of TSHR protein only mildly promoted proliferation (Figure 3B and 3C). Transwell results showed that up-regulation of TSHR inhibited invasion and metastasis of KAT-5 PTC cells with an inhibition rate of 45.3%, and down-regulation of TSHR significantly promoted invasion and metastasis of KAT cells by 86.7% (Figure 3D-3G).

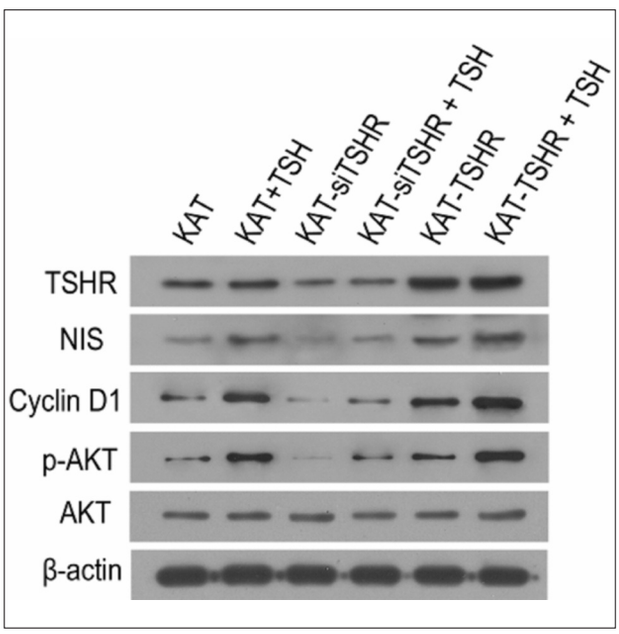

Figure 4. Relationship between TSHR expression and expression of cyclin D, AKT and NIS in KAT-5 PTC cells.

Effect of TSHR Up-Regulation on PTC Cell Apoptosis

To clarify the effect of TSHR on cell biological characteristics of PTC, we examined the effects of different levels of TSHR expression on apoptosis of PTC cells, and flow cytometry showed that up-regulation of TSHR promoted apoptosis of PTC cells.

Effect of TSHR Down-Regulation on Thyroid Cancer Cell Metastasis

Transwell experiments showed that up-regulation of TSHR significantly inhibited metastasis of PTC cells, and down-regulation of TSHR promoted metastasis of PTC cells.

Effects of TSHR Expression Level on Cyclin D, AKT and NIS

To clarify the role of TSHR in the biological behavior of PTC, we examined the relationship between TSHR expression and expression of cyclin D, AKT and NIS in KAT-5 PTC cells (Figure 4). Up-regulation of TSHR correlated with over-expression of cyclin D and p-AKT, however, there was no relationship between expression of TSHR and NIS. Up-regulation of TSHR promoted mild proliferation of PTC cells, but it did not alter iodine uptake ability of thyroid cancer cells (Figure 4).

Discussion

We found that expression of TSHR in PTC and normal thyroid tissues was significantly different (68% *vs*. 28.6%, $p < 0.05$). Wang et al[18] reported that expression of TSHR in thyroid cancer was higher than in normal thyroid tissue. Expression of TSHR protein in PTC, nodular goiter and normal thyroid tissue was 91.07%, 70.97% and 27.27%, respectively[19], which is consistent with our results.

In order to investigate whether TSHR is involved in the effect of TSH on cell proliferation of thyroid carcinoma, we first examined the expression of TSHR in PTC. The results showed differences of TSHR protein expression in TPC and normal thyroid tissues. We further studied the effect of TSH on cell proliferation, and showed that TSH did not significantly stimulate TPC cell proliferation. Some researchers have found that bovine TSH does not promote proliferation of TPC cells[20]. We also studied the effect of TSHR expression on cell proliferation and showed that, in PTC KAT-5 cells with high expression of TSHR, up-regulation of TSHR only mildly stimulated proliferation. These results suggest that stimulation of the TSH–TSHR signaling pathway does not significantly change cell proliferation in thyroid cancer. Also, our results showed that expression of TSHR was not related to T stage in PTC. Such results indicate that TSHR does not have a significant role in cell proliferation of PTC.

Lymph node metastasis of central compartment in PTC is common. We also studied the effect of TSHR on the invasiveness and metastatic ability of PTC cells. Our results indicated that up-regulation of TSHR inhibits invasion and metastasis, and down-regulation of TSHR promotes invasion and metastasis. Our clinical results also showed that high TSHR expression was significantly related to a low rate of distant metastasis, and a significant factor for distant metastasis of PTC patients.

Consequently, TSHR might have a role in decreasing distant metastasis in PTC patients, especially in those aged ≥ 45 years. Until now, the effect of TSHR on invasion and metastasis has not been reported. To clarify the effect of TSHR on cell biological characteristics of PTC, we examined the effects of different levels of TSHR expression on apoptosis of PTC cells. The results showed that up-regulation of TSHR may promote apoptosis in PTC.

To detect the effect of TSHR on the biological characteristics and function of PTC at the molecular level, we examined the effects of different levels of TSHR expression in KAT-5 PTC cells. TSHR did not stimulate expression of cyclin D, AKT and p-AKT significantly, nor did it affect expression level of NIS. These results indicate that TSHR may stimulate DTC cell proliferation, but does not affect iodine uptake in thyroid cancer cells.

Our study also revealed that TSHR expression was not an independent prognostic factor in PTC patients aged < 45 years.

Cooper et al[10] studied the survival status of 683 patients with differentiated thyroid cancer after oral administration of thyroxin, which showed that endocrine therapy by inhibition of TSH is not an independent prognostic factor, and may only benefit a small number of patients with high risk. Sugitani[12] conducted a prospective study of 433 cases of PTC, and showed that endocrine treatment by inhibiting TSH did not influence 5-year survival. In addition, TSH endocrine therapy has some adverse effects. If serum TSH level is re-

duced to below 0.5 Mu/L by oral administration of thyroxin, the incidence of senile osteoporosis and heart disease increases[4,13,15]. Therefore, before the application of endocrine therapy, we should evaluate whether patients will benefit from the treatment. However, there is no strong evidence to support that endocrine therapy can significantly increase survival rate in patients with PTC[4,10,12,13], which indicates that the current endocrine therapy still lacks a theoretical basis.

Our work also showed that TSHR expression affects the prognosis of PTC significantly and TSHR expression is an independent prognostic factor in patients aged > 45 years, and TSHR might have a role in decreasing distant metastasis, especially in patients aged ≥ 45 years. However, TSHR does not have a significant role on cell proliferation in PTC. Therefore, our results support the conclusion that TSH endocrine therapy can significantly increase survival in some patients with TSHR-positive PTC[21]. The effect of endocrine therapy on inhibition of TSH is still controversial. Further basic and clinical research is needed to verify the effect of TSH endocrine treatment in PTC.

Conclusions

TSHR expression is an independent factor affecting the prognosis of PTC and might have a role in decreasing distant metastasis in patients aged > 45 years. Up-regulation of TSHR could inhibit metastasis and promote apoptosis in PTC cells.

Acknowledgements

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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