TSHR Promotes Proliferation and Invasion of Papillary Thyroid Carcinoma By Regulating NF-κB Signal Pathway

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Abstract

Purpose: To investigate the significance of TSHR and NF-κB P65 expression difference in thyroid papillary carcinoma, and to explore how TSHR affects the biological behavior of PTC cells by regulating NF signaling pathway .

Methods: A total of 87 patients with thyroid papillary carcinoma were collected to investigate the expression of TSHR and NF-κB P65 in PTC by IHC. Tpc-1 cells overexpressing TSHR were constructed and their effects on cell biological behavior were observed by cell counting and Transwell assays. Subsequently, the expression and subcellular localization of key proteins in NF-κB signaling pathway were investigated by western blot and immunofluorescence.

Results: 1. The expression of TSHR in PTC was significantly higher than normal tissues and was only negatively correlated with lymph node metastasis. The NF-κB p65 was also highly expressed in cancer tissues, and was positively associated with tumor size, membrane invasion and lymph node metastasis, and unrelated to gender and age. There was a lightly positive correlation between TSHR and NF-κB p65 expression.

2. The proliferation and invasion ability of TPC-1 cells overexpressing TSHR was suppressed. the expression of P65 showed no change with TSHR in TPC-1 cells, but p-p65 and p-IκB was significantly increased with TSHR overexpressing. Finally, TSHR promoted the p65 to enter into nucleus.

Conclusion: Our study uncovered TSHR may inhibited the proliferation and invasion ability of PTC by NF-κB signaling pathways. Furthermore, we revealed that TSHR may acted as a functional protein in tumor cells without the stimulation of TSH.

Introduction

Thyroid cancer is the most common malignancy in endocrine system, with 567,233 new cases estimated in 2021 and the morbidity is the ninth highest in the world[1], what's more, its annual incidence has more than doubled in the past two decades. Most of this growth is attributed to papillary thyroid carcinoma (PTC), which accounts for > 80% of all thyroid cancers[2]. Although most patients with PTC show good prognosis and are cured by thyroidectomy, with radioiodine (I^{131}) ablation, if necessary, 1–4% of patients at initial diagnosis and 7–23% during follow-up develop distant metastasis[3, 4].

Thyroid-stimulating hormone receptor(TSHR), a G-protein-coupled receptor (GPCR)and glycoprotein receptor, expresses on the membrane of thyroid follicular cells with mission-critical functions in normal physiological conditions. After being activated by thyroid-stimulating hormone (TSH), the TSHR serves a fundamental role in the development of the thyroid gland, as well as regulation of thyroid cell proliferation, differentiation and function[5]. It has been demonstrated that TSHR signaling was required for thyroid carcinogenesis in a mouse model[6], nonetheless, TSH-TSHR signaling has a dichotomous role in thyroid cancer; it can also suppress the capacity of invasion and metastasis of cancer cells and

therefore suppress the lymphatic metastasis[7]. The majority of these previous studies investigated the underlying molecular mechanisms of thyroid cancer growth, however, the role of TSHR in the prognosis, migration, invasion and metastasis of DTC remains unclear.

Nuclear factor (NF)-κB is a vital nuclear transcription factor. It has been demonstrated that NF-κB is associated with many pathological processes, such as malignant tumours, inflammation, and viral infections[8]. It has five subtypes in humans, of which p50 and p65 are the most important subunits[9, 10]. Le, F. etc. found that NF-κB in PTC are positively associated with tumour diameter and lymph node metastasis[11], furthermore, it has been revealed that many molecules regulate the occurrence and development of thyroid cancer through NF-κB pathway[12–14].

To our knowledge, studies on the relationship between TSHR and NF-κB signaling pathway in PTC were rarely reported. Voigt et al. demonstrated a TSH induced activation of the response elements CRE, AP1 and NF-κB, which represented possible further pathways activated by TSH in addition to the classical cAMP and IP pathways[15]. Moreover, our prediction by KEGG suggested that the TSHR pathway may interacted with NF-κB through AC/cAMP/PKA signaling. Therefore, this study intended to investigate the expression and correlation of TSHR and P65, one of NF-κB's key proteins, in PTC tissues, and analyze the clinicopathological parameters of patients. To study further, TPC-1 cell line over-expressed TSHR was used to analyze the expression of p65, P-P65 and P-I κB, and compare the changes of cell proliferation and invasion ability.

Materials And Methods

1. Patients and tissue specimens

The study group comprised 87 PTC patients who underwent radical thyroidectomy at the Yantai Yuhuangding Hospital, Cheeloo College of Medicine, Shandong University from January 2019 to May 2019. Tumor tissues and normal thyroid tissues adjacent to cancer were cryostoraged once removed during the operation. Among these, 22 were male and 65 were female; there were 74 under 55 years old and 13 ≥ 55; the tumor size was ≤ 1cm in 61 cases and > 1cm in 26 cases. All patients were stage □-□, evaluated by AJCC cancer staging system.

Inclusion criteria: 1. The final pathology result was papillary thyroid carcinoma; 2. Thyroid hormone and thyroid-related antibody tests performed before surgery were normal.

2. Immunohistochemistical staining and analysis.

Immunohistochemistry (IHC) was performed according to previous methods[6]: 4 μm paraffin-embedded thyroid cancer tissue sections using monoclonal antibodies directed against TSHR (Mouse monoclonal, 1:100, cat. no. ab27974, Abcam) or P65(Rabbit monoclonal, 1: 100 cat. no. ab32536, Abcam)at room temperature for 1 h. The secondary antibody Goat Anti-Mouse IgG H&L (HRP) (cat. no. ZB-2305, ZSGB-

BIO) or Goat Anti-Rabbit IgG H&L (HRP) (cat. no. ZB-2301, ZSGB-BIO) was incubated at room temperature for 15 min. Two senior pathologists were required to assess tissue sections independently. The score was evaluated by estimating the percentage and intensity of tumor cell staining. The scores of positively stained tumor cells were graded as: 0 (no positive tumor or < 5%), 1 (5%-20%), 2 (21-50%), 3 (51–75%) and 4(≥75%). The intensity of tumor cell staining was determined as: 0 (no staining), 1 (light yellow), 2 (yellow brown), 3 (brown). The staining index was calculated as the product of staining intensity multiplied by percentage of positive tumor cells, resulting in scores of 0–1(negative), 2–4(weakly positive), 5–8(positive), and 9–12(strongly positive).

3. Cell culture

We utilized human thyroid cancer cell line TPC1(purchased from Procell Life Science&Technology Co.,Ltd, No.CL-0643), grown in DMEM, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). The cell line was 100% matched to the reported STR profiles in the DSMZ database, and was maintained at 37°C and 5% CO2 culture conditions.

4. Plasmid transfection

cDNAs encoding the TSHR was subcloned into pCDH-CMV-MCS-EF1-Puro (CD510B) vector. Cells at 70% confluence were transiently transfected with plasmid using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Cells were grown for at least 48 hours before harvesting. The efficiency of transfection was determined using Western blotting and RT-PCR to evaluate protein and mRNA expression following cell collection.

5. Western Blot

Cells were lyzed with Cell lysis buffer for Western and IP (Beyotime Institute of Biotechnology, Haimen, China) according to the manufacturer's instructions and the protein concentration was determined using a BCA Protein Assay kit (Pierce; Thermo Fisher Scientific, Inc.) Western blotting was performed using standard methods as described previously[16]. Briefly, 40 ug protein were applied to 12% polyacrylamide SDS gels, separated by SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (GE Healthcare Life Sciences, Chalfont, UK). The membranes were blocked with 5% no-fat milk at room temperature for 1 h, and incubated with anti-TSHR (Mouse monoclonal, 1:1,000, cat. no. ab27974, Abcam) or P65(Rabbit monoclonal, 1: 1000 cat. no. ab32536, Abcam)) primary antibodies at 4˚C overnight. Subsequently, the secondary antibodies Goat Anti-Mouse IgG H&L (HRP) (cat. no. ZB-2305, ZSGB-BIO) or Goat Anti-Rabbit IgG H&L (HRP) (cat. no. ZB-2301, ZSGB-BIO) was used to incubated with the membranes at room temperature for 2 h. Then an enhanced chemiluminescence Amersham ECL Primer kit (cat. no. PK10001, Proteintech) was used to develop the blots according to the manufacturer's protocol. GAPDH was used as the loading control.

6. Quantitative reverse transcription PCR (RT-qPCR)

Total RNA was isolated using TaKaRa MiniBEST Universal RNA Extraction Kit (no. 9767, Takara), and cDNA was produced using EVO M-MLV RT Premix for qPCR kit (code no. AG11706, Accurate

Biotechnology Co. ,Ltd). The relative RNA levels of TSHR was detected with forward primer(5'-TCAAGAAGGTGGTGAAGCAGG-3') and reverse primer(5'-TCAAAGGTGGAGGAGTGGGT-3'). Each sample was examined in triplicates. PCR product specificity was confirmed by the melting-curve analysis

7. Immunofluorescence

After cultured overnight, CAL62 or C643 cells were washed with PBS for three times and then fixed in 4% paraformaldehyde(PFA) for 20 min. Cells were permeabilized with 0.2% Triton X-100 (Sigma-Aldrich), blocked with goat serum and incubated with appropriate primary antibodies(NF-κB p65 Rabbit monoclonal antibody, 1: 50) overnight followed by staining with coraLite488-conjugated secondary antibodies (Goat Anti-Rabbit IgG, 1:100) at 25°C for 1 h. After cells were washed with PBS for four times, nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich). Images were visualized and recorded with a Zeiss LSM780 confocal microscope (Zeiss, Germany).

8. Cell counting

After trypsin digested, a total of 3500 TPC-1 cells per well were seeded in 96-well plates. Cells were cultured for 24 h, 48h and 72h respectively, followed by being added CCK-8 solution 10ul/ well and then incubated at 37°C in a 5% CO2 incubator for 4 h. The stop buffer was added to each well and OD value at 450 nm was measured in a microplate reader.

9. Transwell assays

In transwell assays, 200ul TPC1 cells at 1.5×105/ml transfected with TSHR plasmid were added to upper transwell chambers (pore 8 μm, Corning). The lower wells were filled with 600 μL medium containing 10% FBS. After 24 h of incubation, ESCC cells migrated to the lower wells through pores were stained with 4% PFA and crystal violet solution and counted under a microscope.

10. Statistics

The difference between two groups was examined using the two-tailed unpaired Student's t test or paired Student's t test as indicated. Impacts of TSHR and NF-κB p65 expression in IHC assay were tested by Pearson chi-square test or Fisher's exact probability test. Multivariate analysis was performed by Logistic regression. The correlation between two proteins were tested by Kendall's Tau - B correlation analysis. A P value of less than 0.05 was considered as statistically significant. All analyses were performed with SPSS software package (Version 23.0, SPSS Inc.) or GraphPad Prism (Version 8.0, GraphPad Software, Inc.).

Results

1. TSHR in papillary thyroid carcinoma was over-expressed than normal thyroid tissues

IHC staining showed that TSHR was expressed in both PTC tissues and normal thyroid tissues, and the staining area was mainly distributed in the membrane and cytoplasm of thyroid follicular cells. However, most positive staining area in PTC tissues was in the cytoplasm but not the cell edge, which was different from normal tissues (Fig. 1A-D). In 87 PTC tissues, there were 31 negative TSHR expression cases, 8 weakly positive, 16 positive and 32 strong positive cases. There were 29 cases of negative TSHR expression, 21 weak positive, 16 positive and 21 strongly positive cases in normal thyroid tissues. Chi-square test of the above results showed that the expression level of TSHR in PTC group was higher than that in normal group, which was significantly different (Table 1, $\chi2 = 8.177$, $P = 0.042$).

Table 1
Expression of TSHR in PTC and normal tissues

Groups	No.of cases	Expression intensity				χ2	P value
		Negative	Weakly positive	Positive	Strong positive		
Tumor	87	31	8	16	32		
Normal	87	29	21	16	21	8.177	0.042

Comparing 87 PTC tissues with normal tissues from the same patient, we found the TSHR of PTC tissues was up-regulated in 34 patients(39.1%), down-regulated in 18 patients(20.7%) and others were similar-expressed(40.2%)

2. Expression of NF-κB P65 in PTC was higher than normal thyroid tissue

NF-κB P65 was mainly expressed in cytoplasm and nucleus of thyroid follicular cells(Figure 2A-D). In 87 PTC tissues, there were 38 negative P65 expression cases, 23 weakly positive, 19 positive and 7 strongly positive cases. There were 76 cases of negative P65 expression, 7 weak positive, 4 positive and 0 strongly positive cases in normal thyroid tissues. Chi-square test of the above results showed that the expression level of P65 in PTC group was higher than that in normal group, which was significantly different (Table 2, $\chi2 = 38.907$, $P<0.001$).

Table 2
Expression of NF-κB in PTC and normal tissues

Groups	No.of cases	Expression intensity				χ2	P value
		Negative	Weakly positive	Positive	Strong positive		
PTC	87	38	23	19	7		
Mormal	87	76	7	4	0	38.907	<0.001

Comparing 87 PTC tissues with normal tissues from the same patient, we found the P65 of PTC tissues was up-regulated in 45 patients (51.7%), down-regulated in 3 patients (3.5%) and others were similar-expressed (44.8%).

3. Correlation between TSHR & NF-κB expression and clinicopathologic characteristics of PTC patients

Comparing the relationship between TSHR positive expression and clinicopathological features, univariate analysis showed that TSHR expression was only correlated with lymph node metastasis significantly（p=0.003）,unrelated to gender, age, tumor size and membranous invasion（Table 3）. Multivariate analysis showed that lymph node metastasis was a protective factor for TSHR positive expression after adjusting for tumor size and membranous invasion（OR=0.22（95%CI=0.079-0.628）（Table 4）.

Table 3
Correlation between TSHR expression and clinicopathologic characteristics of PTC patients

Characteristics	No. of cases	No. of positive	Percentage	χ2	P value
Gender					
Male	22	13	59.1	0.358	0.55
Female	65	43	66.2		
Age					
<55	74	48	64.9	0.053	0.817
≥ 55	13	8	61.5		
Tumor size					
≤ 1cm	61	42	68.9	1.97	0.181
> 1cm	26	14	53.8		
Membranous invasion					
Positive	43	26	59.1	1.081	0.299
Negative	44	30	69.8		
Lymphatic metastasis					
Positive	52	27	51.9	8.728	**0.003**
Negative	35	29	82.9		

Table 4
Multivariate analysis between TSHR and clinical factors in thyroid cancer

	OR	95%CI	P value
Tumor size	0.692	0.253–1.891	0.473
Membranous invasion	1.870	0.723–4.837	0.197
Lymphatic metastasis			
Positive	Reference	0.077–0.651	**0.006**
Negative	**0.224**		

Table 5 showed that NF-κB P65 was associated with tumor size, membranous invasion and lymph node metastasis, but not with gender or age. Logistic multifactor regression analysis showed that tumor size (OR=7.06;95%CI=1.958-25.463), membranous invasion (OR=3.825; 95% CI=1.38-10.603) and lymph node metastasis (OR=3.236; 95%CI=1.185-8.838) was still an independent influencing factor of P65 protein expression (Table 6). In addition, Kendall's Tau-B method found that there was a positive correlation between TSHR and P65 expression in PTC tissues （Kendall's Tau-B =0.228, P=0.014 < 0.05）.

Table 5
Correlation between NF-κB p65 expression and clinicopathologic characteristics of PTC patients

Characteristics	No. of cases	No. of positive	Percentage	χ2	P value
Gender					
Male	22	16	72.7	3.222	0.073
Female	65	33	50.8		
Age					
<55	74	40	54.1	1.035	0.309
≥ 55	13	9	69.2		
Tumor size					
≤ 1cm	61	27	44.3	12.067	**0.001**
> 1cm	26	22	84.6		
Membranous invasion					
Positive	43	30	69.8	6.248	**0.012**
Negative	44	19	43.2		
Lymphatic metastasis					
Positive	52	36	69.2	8.756	**0.003**
Negative	35	13	37.1		

Table 6
Multivariate analysis between TSHR and clinical factors in thyroid cancer

Characteristics	OR	95%CI	P value
Tumor size			
≤ 1cm	Reference	1.958–25.463	0.003
> 1cm	7.06		
Membranous invasion			
Positive	Reference	1.38-10.603	0.01
Negative	3.825		
Lymphatic metastasis			
Positive	1	1.185–8.838	0.022
Negative	3.236		

4. TSHR suppressed the proliferation and invasion ability of PTC-1 cells

IHC results preliminarily confirmed the increased expression of TSHR in thyroid cancer, and further experiments were conducted to explore the effect of TSHR on thyroid cancer cells. Western blot showed that TSHR protein was almost not expressed in TPC-1 cell lines, (Fig. 3A), so we decided to construct TPC1 cell lines overexpressing TSHR. The TSHR mRNA and protein levels in the three groups of cells transfected with plasmid were shown in Fig. 3B-C, which showed that expression levels in TSHR plasmid group was higher than blank group and vector group. CCK-8 assay revealed that the cell proliferation ability of the overexpression group was significantly decreased compared with the blank group and the vector group (Fig. 3D). In addition, the invasion ability of tumor cells was evaluated by transwell chamber assay, which showed that the average number of cell migration in the overexpression group was significantly weaker than control ($P < 0.001$) (Fig. 3e). These results demonstrated that overexpression of TSHR inhibits the proliferation and invasion of thyroid papillary carcinoma cells.

5. TSHR promote the phosphorylation of P65, and reposition the P65 protein to the nucleus

Verified by tissue, we found a positive correlation between TSHR and NF-κB p65 expression. To further explore the influence of TSHR overexpression on NF-κB signaling pathway, we detected the NF-κB signaling related proteins in 3 groups of cells. As the most important functional subunit, p65 protein is involved in a variety of important life activities of cells, and is the focus of NF-κB family research, as well as the subunit that has attracted the most attention in NF-κB phosphorylation studies, so we detected the

expression of P65 as a representative. Interestingly, no significant changes were observed(Fig. 4A□D). Considering that the activation of NF-κB signaling depends on the conformational change of κB inhibitor protein (IκB) after being phosphorylated, and then the release of the binding κB[17], we assessed the levels of p-P65 and p-I κB. Notably, we observed a significant higher expression in overexpression group than control (Fig. 4B, C, D) ($P < 0.05$). Furthermore, immunofluorescence test revealed that the overexpressed TSHR facilitated the entrance of P65 into nucleus. Taken together, these results proposed an important role that TSHR can promote the phosphorylation of P65 by promoting the phosphorylation of IκB, and reposition the P65 to the nucleus for its function, but has no significant effect on the expression of P65.

Discussion

TSHR, as one of the most important receptors in thyroid cell, performs functions mainly through the Gas-adenylyl cyclase-cyclic AMP (cAMP) and Gaq-calcium pathways[18, 19]., however, it can couple with all four G protein families to activate downstream signaling [20], TSH-TSHR stimulates the expression of various thyroid-specific genes involved in iodide metabolism, such as thyroglobulin (Tg), thyroperoxidase (TPO) and sodium-iodide symporter (NIS), which regulate the iodide uptake of normal or tumorous thyroid cells[21]).

In terms of the relationship between TSHR and thyroid cancer, Qu etc. [22] found that the methylation level of TSHR gene is higher in PTC and closely related to age, lymph node metastasis, clinical stage, and tumor size. In another study, the mutation of TSHR gene was confirmed to be related to lymph node metastasis (LNM) in PTC patients[23]. In expression, the prognostic value of TSHR in thyroid cancer was analyzed. Immunohistochemical analysis of 172 DTC tissues revealed that the lower expression of TSHR was associated with distant metastasis and a poor survival rate[7]. Conversely, it has been revealed that TSHR signaling was required for thyroid carcinogenesis in a mouse model[6], which demonstrated that TSH-TSHR signaling has a bidirectional role in thyroid cancer. In present study, we found TSHR expression in PTC was higher than that in normal tissue, nevertheless, compared in PTC, it was lower in patients with lymph node metastasis and was irrelevant to gender, age and capsular invasion. Comparing PTC tissue with normal tissue from the same patient, we found the TSHR of PTC tissue was up-regulated in 34 patients(39.1%), down-regulated in 18 patients(20.7%) and others were similar-expressed, which demonstrated that TSHR may act as a “inverted U shape” effection in thyroid follicular epithelium: beyond a certain critical point, TSHR may contribute to tumorigenesis of thyroid cancer, however, beyond a higher critical point, TSHR seemed to protect PTC metastasizing. Furthermore, our study showed that TSHR mainly distributed in cytoplasm but rarely in membrane. As a vital functional membrane protein, before located to the membrane correctly and play a physiological role, TSHR should went through post-translational modification processes such as glycosylation and acetylation.

Lu's study[6] found that thyroid cancer cells do not metastasized in the case of TSHR knockout, suggesting that TSHR mediated growth signals are essential for thyroid cancer metastasis. However, Liu et al.[7] found that patients with low TSHR expression were more likely to have metastatic lesions, and the

decreased metastasis ability of thyroid cancer cells expressing TSHR might be realized by inhibiting the process of EMT. This further confirms the "inverted U" curve role of TSHR in thyroid cancer cells previously proposed. The results in this study demonstrated that the proliferation and invasion ability of thyroid cancer cells were significantly decreased after overexpression of TSHR. Considering that TSH was not added in our medium, it suggested that TSHR might affect the biological behavior of TPC-1 cells through other pathways, not entirely dependent on the signaling cascade that occurs when TSH binds to it.

Recently, the close association between inflammation and malignant tumors has attracted the attention of many researchers. NF-κB signaling pathway is involved in various immune and inflammatory responses, and also involved in regulating the expression of multiple genes related to cell proliferation and differentiation. In inactive state, NF-κB binds as a dimer to an inhibitory protein known as a κB inhibitor (IκB) and is located in the cytoplasm in an inactive form[24]. There are two major activation ways of NF-κB: the classical pathway mainly activates the dimer composed of p65, P50, C-REL and RelB subunits. The noncanonical way starts with CD40, RANK, LTβR and BAFF triggering Ikkα phosphorylation. The ultimate goal of both approaches is to phosphorylate and activate dimer proteins and then transfer them into the nucleus to regulate the transcription of target genes[25]. In this research, P65 and IκB were selected as representative proteins of this pathway for further investigation. As far as we know, the abnormal of NF-κB pathway has been found to be associated with various of malignant tumors, and the expression of related protein is abnormal in colon cancer, breast cancer, melanoma and ovarian cancer[26].

Pyo et al.[27] demonstrated that nuclear expression of NF-κB P65 was significantly correlated with tumor size (1cm), lymph node metastasis, and extra-glandular invasion. In Zhou's study[27], TPC-1, BCPAP, PCCL3 and PTC3-5 cell lines were cultured with pyrrolidone dithiocarbamate (PDTC), a selective inhibitor of NF-κB, followed by significant reduction of the proliferation and migration ability. Therefore, it is reasonable to believe that the activation of NF-κB signaling pathway may promote the growth and aggressiveness of papillary thyroid carcinoma. Results in current study showed that, after over-expressed TSHR, the NF-κB p65 phosphorylation activated, however, TPC − 1 cell's proliferation and invasive ability declined. The reason for this may be the complex internal environment of tumor cells in vivo has an irreplaceable effect on them, which doesn't exist in vitro cell experiments. It may also be that the inhibitory effect of overexpressed TSHR on tumor cell growth and invasion through other pathways exceeds the promoting effect of NF-κB signaling pathway activation, which needs further study.

As one of the G-protein-coupled receptor family on the surface of cell membrane, previous studies usually focused on elucidating the TSH-stimulated signaling pathways involved in the proliferation and differentiation of thyroid cells, but in fact, there were few studies on the downstream signaling pathways of TSHR except the cAMP/PKA pathway. Results from Lu et al.[28] demonstrated that thyroid hormone-mediated genes involving the transcription factor NF-κB regulate adipogenesis and lipolysis during development.

In a recent study[29], TSH stimulated PKC-mediated nuclear NF-κB p65 subunit expression in rat thyroid cell lines TPTL-5 and PCCl3. CHIP and knockdown assay indicated that P65 was the nuclear effector of TSH action, which induced transcriptional expression of thyroid differentiation markers. This suggested that NF-κB plays a key role in TSHR-induced follicular differentiation. Voigt et al[15]. used luciferase reporter gene analysis and found that the accumulation of NFκB mediated luciferase in COS7 cells was significantly increased after TSH stimulation, suggesting a possible interaction between the two signaling pathways. Above results were similar to those in this study, but different from above results, the phosphorylation level of P65 was significantly increased after TSHR overexpression, and the nucleation of P65 was more significant. We hypothesized that this might be caused by the phosphorylation and degradation of inhibiting protein IκB, so the expression of phosphorylated IκB protein was further detected. Western blot results showed that the expression of P-I κB was significantly increased in the TSHR overexpression group.

Conclusion

Combined with the results, we found that TSHR and NF-κB P65 expression were increased in PTC tissues compared with normal tissues, but decreased TSHR expression may be associated with lymph node metastasis in thyroid cancer patients, and NF-κB P65 expression may be associated with tumor growth, lymph node metastasis, and membranous invasion. Further cell assay showed that TSHR overexpression may promote the proliferation and invasion ability of papillary thyroid carcinoma cells without TSH, and this process may be closely related to NF-κB signaling pathway, the specific mechanism remains to be further studied.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Yantai Yuhuangding Hospital.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

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Figures

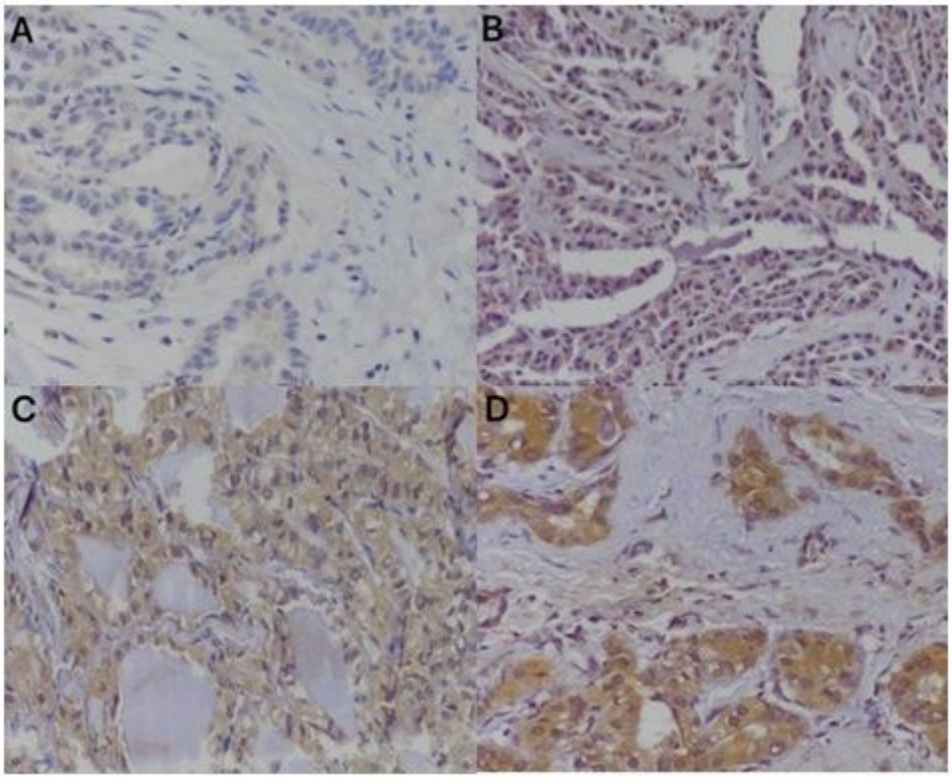

Figure 1

Representative images of different expression intensities IHC staining of TSHR 200×（A） Negative staining（B）Weakly positive staining（C）Positive staining（D）Strong positive staining

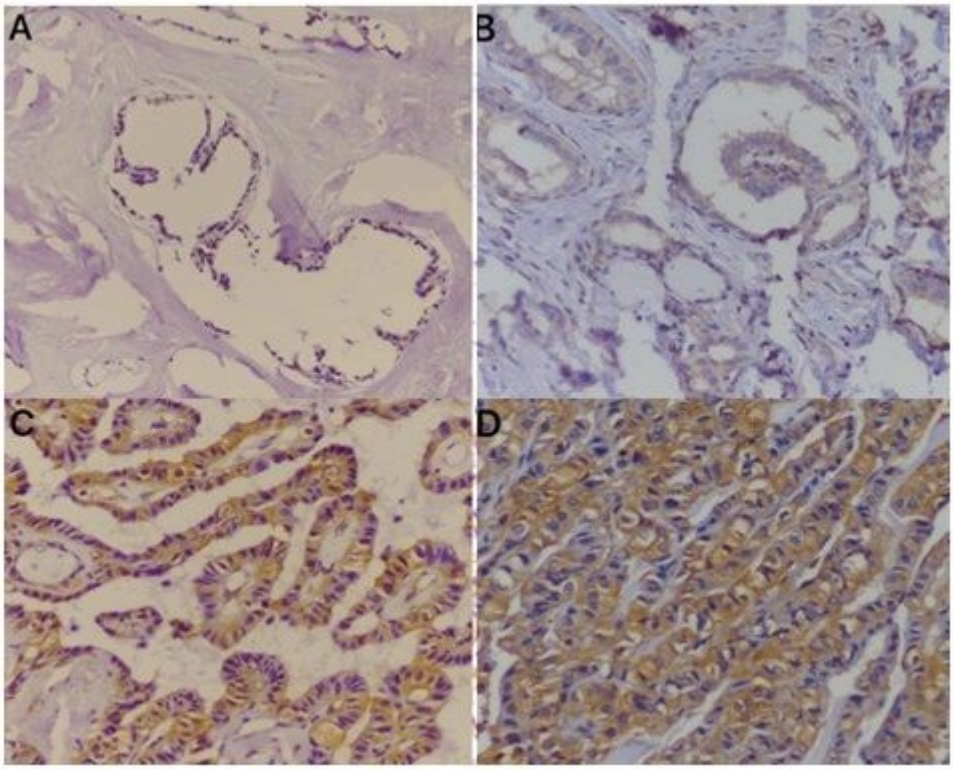

Figure 2

Representative images of different expression intensities IHC staining of TSHR 200×（A） Negative staining（B）Weakly positive staining（C）Positive staining（D）Strong positive staining

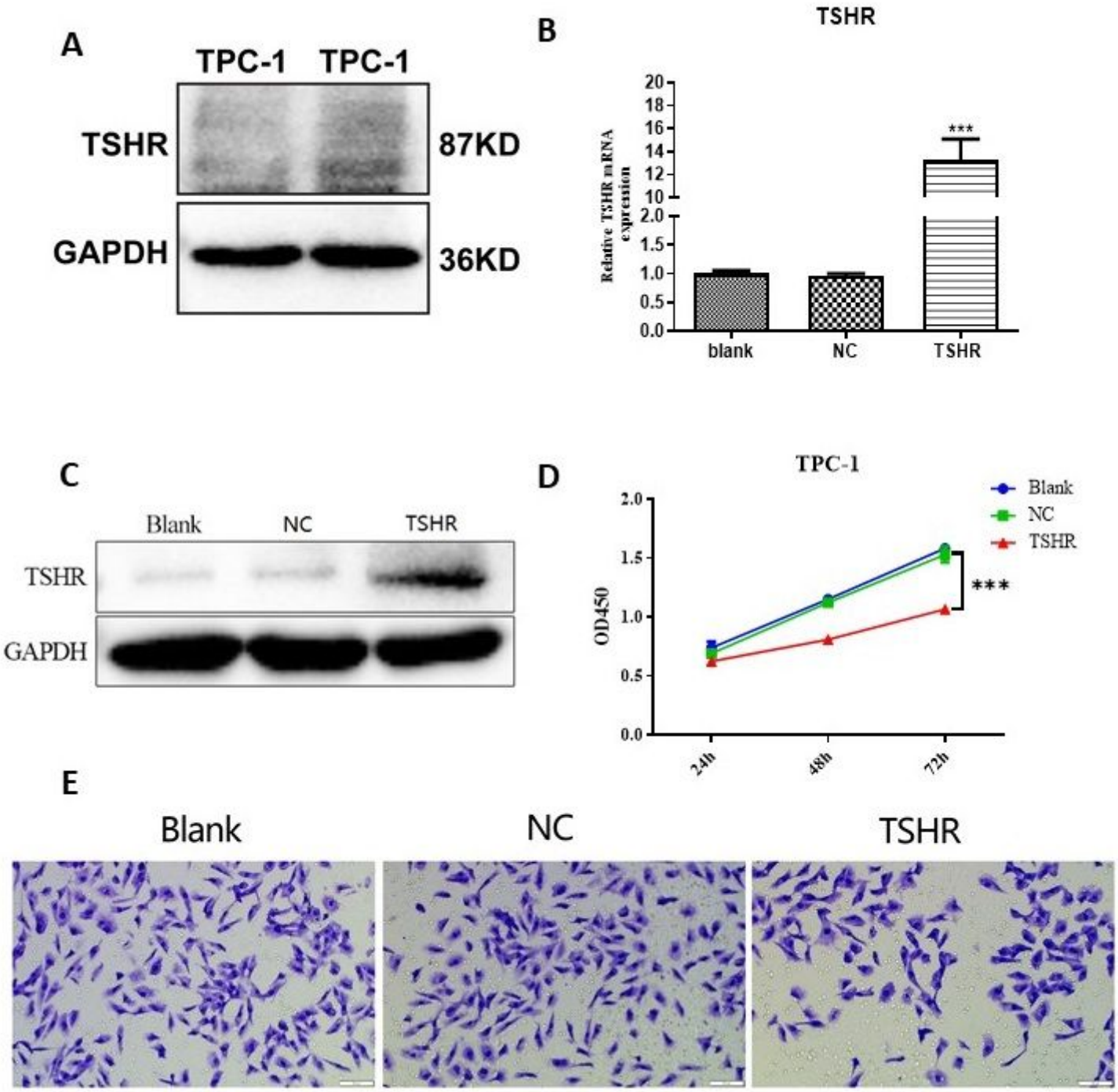

Figure 3

Effect of TSHR overexpression on TPC-1 cell line. A: Expression of TSHR in wild type TPC-1 cells；B： Comparison of TSHR mRNA expression in each groups after plasmid transfection；C：Comparison of TSHR expression in each groups after plasmid transfection；D：Representative growth curve of TPC-1 cells overexpressed for TSHR measured by CCK8 assay；E：Transwell results of TSHR differentially expressed cells

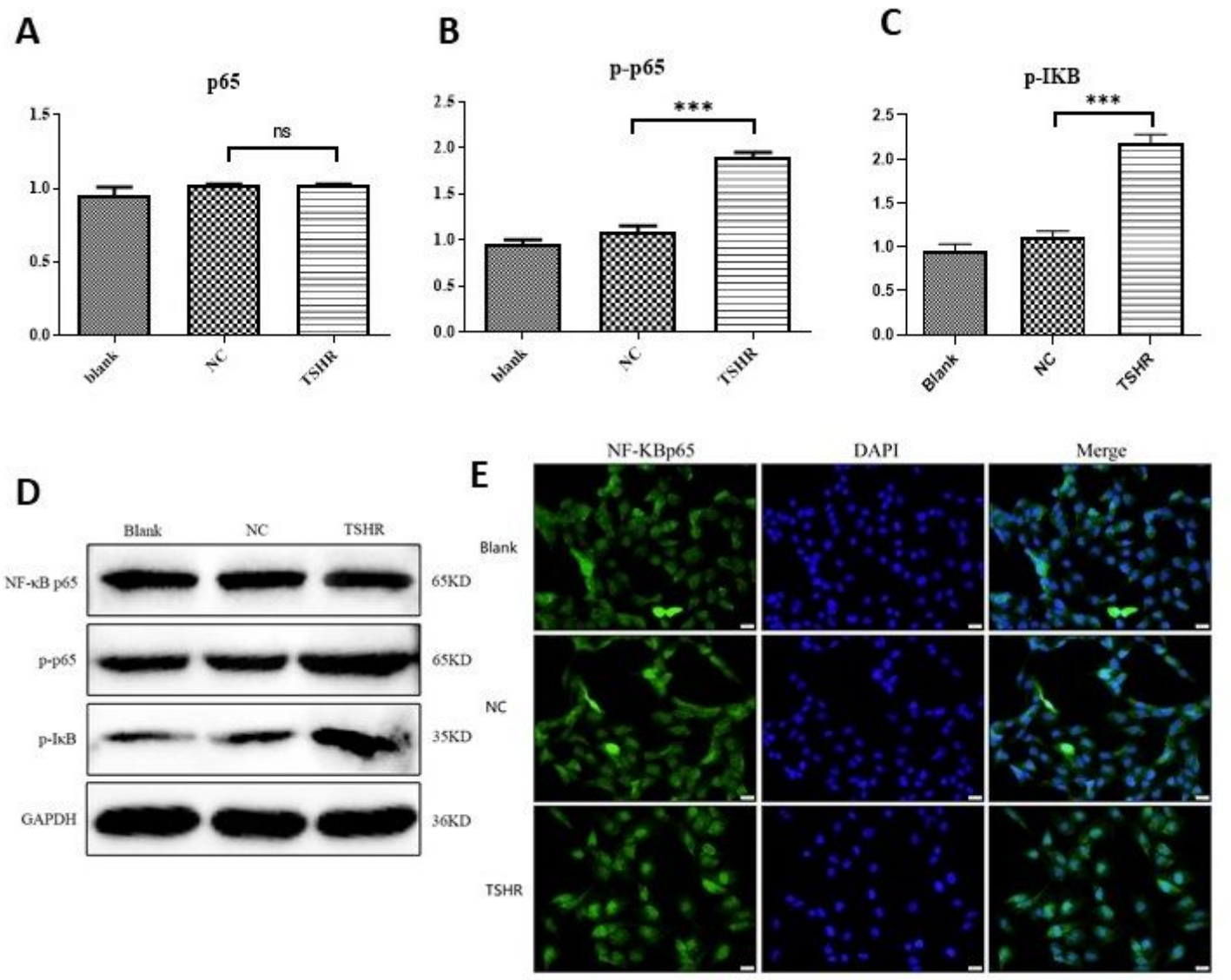

Figure 4

Effects of TSHR overexpression on the expression of key proteins in NF-κB signaling pathway. A- D were the expression levels of P65, p-P65 and p-I κB protein, respectively；E：Subcellular localization of p65 protein by immunofluorescence.