# TFE3 Regulates the Function of Autophagy-Lysosome to Drive Invasion and Metastasis of Papillary Thyroid Carcinoma

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**Research**

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# Abstract

**Background:** Cumulatively, evidence shows that autophagy plays an important role in the occurrence, development, and metastasis of tumors, and even determines the prognosis of tumors. However, little is known about its role in papillary thyroid carcinoma and TFE3 as a potential oncogene in regulating autophagy lysosome.

**Methods:** Immunohistochemistry and real-time quantitative PCR (qRT-PCR) assays were used to examine the expression of TFE3, P62/SQSTM1 and LC3 in PTC tissues and para-cancer tissues. TFE3, P62/SQSTM1, and LC3 were evaluated using Western blot assay. After inducing TFE3 overexpression by plasmid or TFE3 downregulation by small interfering RNA (siRNA) transfection, MTT, Wound healing assay, Cell migration and invasion assays were used to verify the effects on invasion, migration and autophagy-lysosome related proteins such as P62/SQSTM1 and LC3.

**Results:** The TFE3, P62/SQSTM1, and LC3 in PTC tissues were overexpression than that in para-cancer tissues. Analysis of clinicopathological characteristics in PTC patients showed that high expression of TFE3 was significantly correlated with lymph node metastasis. Overexpression of TFE3 in the PTC cell lines KTC-1 and BCPAP promoted PTC cells proliferation, invasion, and migration. Knockdown of TFE3 had the opposite effect. Furthermore, we found a positive relationship between TFE3 with P62/SQSTM1 and LC3 expression, and silencing TFE3 inhibited P62/SQSTM1 and LC3 expression in PTC cells. Meanwhile, overexpression of TFE3 had the opposite effect.

**Conclusions:** The present study provided the evidence of the underlying mechanisms of TFE3 in inducing the function of autophagy-lysosome of papillary thyroid carcinoma and implicated the potential application of TFE3 as a sensitive marker of PTC lymph node metastasis.


# 1. Introduction

Thyroid cancer (TC) is the most common endocrine malignancy[1]. The incidence of thyroid cancer is increasing rapidly over the past decades[2]. Thyroid cancer has been classified as follicular thyroid cancer (FTC), papillary thyroid cancer (PTC), medullary thyroid cancer(MTC) or anaplastic thyroid cancer (ATC), and PTC accounts for approximately 80% of all thyroid cancers[3]. Although the prognosis of PTC is a better than most other tumors, the prognosis is obviously poor when patients are insensitive to traditional surgery and iodine chemotherapy. Therefore, exploring new biomarkers is of great significance to improve the diagnosis and treatment of PTC

TFE3 is located on the short arm of X chromosome 11.22, and is a member of the microphthalmia family [4]. Recently, the MiT / TFE family were identified as regulators of autophagy. Subsequent studies showed that TFE3 could bind to CLEAR elements, which present in the promoter regions of many lysosomal genes present in the promoter regions of many lysosomal genes, and regulate lysosomal biogenesis in several different cell types [5].

Autophagy is an evolutionary highly conservative catabolic process, which is essential for maintaining cell homeostasis and adapting to various stress situations [6]. Autophagy is primarily a response to the stress of the microenvironment, such as hypoxia, nutrient deficiency, and accumulation of reactive oxygen species (ROS). Dysfunctional autophagy contributes to many diseases, including cancer. Depending on the type and stage of cancer, autophagy can play a positive or negative role in the development of cancer [7]. Thus, understanding the role of autophagy in PTC is crucial for identifying new targets for PTC therapy. Gene Set Enrichment Analysis (GSEA) was used to analyze the enrichment of autophagy and lysosome-related biological functions in PTC. The results showed that the expression of genes involved in autophagy and lysosome-related biological functions was upregulated in the PTC group.

In the present study, we have investigated the expression of the autophagy regulator TFE3 in PTC, and to clarify its role as a potential target in PTC patients.

# 2. Material And Methods

### 2.1 Tissue samples

78 pairs of PTC tissues and para-cancer tissues were acquired from patients who underwent surgical resection at Taizhou Central Hospital between March 2017 and January 2019. Patients with other tumors or tumor history were excluded from this research. No any chemotherapy or radiotherapy was given to patients. The PTC population included 56 females and 22 males, and their age ranged from 18-79 years. All tissue samples were snap-frozen in liquid nitrogen and quickly stored at -80 °C. The clinicopathological characteristics of the PTC patients are listed in Table 1. The patients' clinical stage, tumor stage, and lymph node stage were listed according to the Tumor/Node/Metastasis (TNM) classification[8]. In addition, this research was approved by the Ethics Committee of Taizhou Central Hospital (Taizhou, PRC). Written formal approvals were also obtained from all patients.

### 2.2 Cell lines and cell culture

Two PTC cell lines (KTC-1, BCPAP) were obtained from Chinese Academy of Sciences Cell Bank (Shanghai, PRC). They were cultured in RPMI 1640 (REF:61870036, Gibco, USA) supplemented with 10% fetal bovine serum (REF:10270106, Gibco, USA), 1% Glutamax (REF:35050061, Gibco, USA) , 1% MEM Non-essential Amino Acid Solution (REF:M7145, Sigma, USA) as well as 1% Sodium Pyruvate 100 mM Solution (REF:11360070, Gibco, USA), the following called complete culture medium. Cells were incubated at 37 °C in a humidified atmosphere with 5% CO2 and passaged routinely.

### 2.3 Cell transfection

The overexpression of TFE3 was achieved by transfection of pcDNA3.1-TFE3. CDNA encoding full-length TFE3 was cloned into pcDNA vector which was sequence confirmed by sanger sequencing. siRNA

targeted to TFE3 (si-TFE3) and the corresponding negative control (si-NC) were used for TFE3 knock down. The siRNA sequences were as follows:

5'- GCAGCUCCGAAUUCAGGAACUTT -3' (sense)

5'- AGUUCCUGAAUUCGG AGCUGCTT -3' (anti-sense)

pcDNA3.1-TFE3 or si-TFE3 was transfected into PTC cells using Lipofectamine®2000 (REF:11668019, Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

### 2.4 Quantitative real-time polymerase chain reaction ( qRT-PCR ) assay

The extraction of total RNA was carried out with TRIzol reagent (RFE:15596018, Invitrogen, USA) and extracted RNA was reverse transcribed into cDNA by RT Kit (REF:K1622, Applied Biosystems, USA) before qRT-PCR. The qRT-PCR was carried out using SYBR® qPCR Mix (REF:A25742, Applied Biosystems, USA) and in an ABI 7500 Fast Real-Time PCR analysis system (Applied Biosystems, USA). All primers used in PCR are listed as follows:

TFE3 (forward): 5'- ACTGGGCACTCTCATC CCTAAGTC -3'

TFE3 (reverse): 5'- TTCAGGATGGTG CCCTTGTTC -3'

β-actin (forward): 5'- TGACGTG GACATCCGCAAAG -3'

β-actin (reverse): 5'- CTGGAAG GTGGACAGCGAGG -3'

β-actin was used as an internal control. The relative quantification of each sample was calculated using $2^{-\Delta\Delta Ct}$ method.

### 2.5 Wound healing assay

Cells were cultured in 6-well plates at a density of 80%, then a 200ul pipette tip was used to create scratches on the cell culture plane, washed three times with phosphate buffer saline (PBS) and cultured in serum-free RPMI 1640 medium. Wounds were observed under the microscope and captured at 0 h and 24 h. The results were analyzed by Image J software (National Institutes of Health, Bethesda, MD, USA), and the rate of cell migration was determined as following: (diameter of original wound − diameter of wound at different times)/ diameter of original wound × 100%.

### 2.6 Cell migration and invasion assays

Cell invasion was performed using a trans well assay. Briefly, PTC cells were trypsinized and suspended in serum-free RPMI 1640 (100μl) and placed in the upper cavity of each Matrigel-coated Transwell insert (REF:3422, Corning, USA). The lower chambers were supplemented with complete culture medium (500μl). After 24 h incubation, cells in the upper chamber were transferred to the membrane of the lower

chamber, which were fixed with methanol and then stained with 0.1% crystal violet. The procedure of the migration experiment was the same as the invasion experiment except that no matrigel was required. Random fields were chosen for imaging, which were performed with Zeiss photomicroscope(Carl Zeiss Meditec, Dublin, CA, USA).

### 2.7 MTT assay

Cell proliferation was performed by using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. KTC-1 and BCPAP cells (3 x $10^3$ cells/well), which were transfected for 24 h, were cultured in 96-well plates for 0, 24, 48 or 72 h. Then, each well was added with 0.1mg/ml MTT (REF:M818, Solarbio, PRC) (50μl) and incubated at 37°C. After 4 h, 150μl of DMSO (REF:D8371, Solarbio, PRC) was added and shook slightly at room temperature for 1 h. The cell viability was measured by the absorbance value of the microplate reader at 570nm.

### 2.8 Western blotting

Total cell protein was extracted by RIPA lysis buffer (REF: 78501, Thermo, USA) and protease inhibitors and diluted with 5X loading buffer to the same concentrations, denatured at 95°C. Then, 20μg denatured protein was subjected to sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis. The protein was electron transferred to Nitrocellulose filter (NC) membrane, the membranes were blocked with 5% BSA (Bovine serum albumin) (REF: A8020, Solarbio, PRC)at room temperature for 1 h, then they were incubated with the primary antibody against TFE3 (1:1000; REF:14779S, Cell Signaling Technology, USA), P62/SQSTM1 (1:1000; REF:184201AP, Proteintech, USA) and LC3 (1:2000; REF:L7543, Sigma-Aldrich, USA) at 4℃ overnight. The membranes were washed three times with phosphate buffer saline containing tween-20 (PBST) and incubated with anti-rabbit (REF:31460) or anti-mouse (REF:31430) secondary antibodies (1:5000; Pierce, Appleton, WI, USA) at room temperature for 1 h. Washed three times with PBST. The protein bands were visualized using enhanced chemiluminescence reagent (REF:32109, Pierce, Appleton, WI, USA) , while β-actin (1:1000, REF:3700, Cell Signaling Technology, USA) as the internal reference, and the relative protein expression was based on the target band/internal control band Gray value ratio calculation.

### 2.9 Immunohistochemistry

Tissue sections were deparaffinized with xylene, then hydrated with alcohol, and antigen retrieval was performed by heating 0.01 sodium citrate buffer in a microwave oven for 15 minutes. The sections were quenched with 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase activity. The nonspecific binding was prevented by adding normal goat serum for blocking for 20 minutes. They were incubated with the primary antibody against TFE3 (1:500; Cell Signaling Technology, USA), P62/SQSTM1 (1:50; Proteintech, USA) and LC3 (1:500; Sigma-Aldrich, USA) for 1 h at room temperature, then added anti-rabbit or anti-mouse secondary antibodies (1:500; Pierce, Appleton, WI, USA)and incubated at room temperature for 1 h. After each treatment, washed 3 times with TBST for 5 minutes each time, and then developed with 3,3-diaminobenzidine. After the sections were counterstained with hematoxylin and

differentiated with hydrochloric acid and alcohol, they were dehydrated, transparent, and fixed. Random fields were chosen for imaging, which were performed with Zeiss photomicroscope (Carl Zeiss Meditec, Dublin, CA, USA).

### 2.10 Statistical analysis

All experimental procedures were repeated at least three times. Data were analyzed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 8.0 statistical software (GraphPad Software Inc, La Jolla, CA, USA). Expression association was analyzed with Spearman's correlation analysis. Mann–Whitney U-test was used to compare mean values of more than two groups. All data were expressed as mean ± standard deviation (SD) , and $P$ value < 0.05 indicated statistical significance.

## 3. Results

### 3.1 Autophagy-lysosome is positively correlated with PTC progression

To explore the role of autophagy-lysosomes in the process of PTC, GSEA was used to analyze the enrichment of autophagy and lysosome-related biological functions in PTC. The results showed that the expression of genes involved in autophagy and lysosome-related biological functions was upregulated in the PTC group and the result of functional enrichment was significant (Fig. 1A, B, C, D and E, $P$<0.05). Consistent with the above results, immunohistochemistry assay indicated that the expression of LC3 and P62/SQSTM1 in PTC tissues were significantly higher than that in normal tissues (Fig. 1F and G).

### 3.2 TFE3 expression is overexpressed in PTC

We examined the level of TFE3 in 78 pairs of PTC tissues and para-cancer tissues. The qRT- PCR showed that TFE3 expression was significantly increased in PTC tissues compared with para-cancer tissues (Fig. 2A, B, and C, $P$<0.01). Then, we analyzed the correlation between clinicopathological characteristics and TFE3 expression in PTC patients, the result indicated that high expression of TFE3 was significantly correlated with lymph node metastasis (Table 1, Fig. 2E, $P$<0.05). At the same time, immunohistochemistry assay confirmed that the expression of TFE3 in PTC tissues was higher than that in normal tissues (Fig. 2D).

### 3.3 TFE3 promotes the proliferation of PTC cells

Here, we detected the effect of differentially expressed TFE3 on PTC cell proliferation through MTT assay. As shown in Fig. 3C, compared with the negative control (si-NC), down-regulation of TFE3 expression significantly inhibited cell growth. In keeping with the results of the si-TFE3 group, the pcDNA3.1-TFE3 group demonstrated more obvious cell proliferation ability than pcDNA3.1 group (Fig. 3B, $P$<0.05). These data suggested that TFE3 promoted the proliferation in PTC cells. Assays were repeated in triplicate.

### 3.4 TFE3 accelerates PTC cell migration and invasion in vitro

To detect the role of TFE3 in PTC progression, we conducted a wound healing assay and transwell assay to examine its effects on migration and invasion. PTC cells were transfected with pcDNA3.1-TFE3 or si-TFE3 in 6-well plates. Firstly, the wound healing assay revealed that TFE3 transfection increased the migratory ability (Fig. 4A, B, C, and D, $P<0.05$). Then, by the transwell assay, the result surely manifested that, compared with si-NC group, the cell migration of PTC cells was significantly suppressed in si-TFE3 group, while the number of cells penetrating the lower surface in the pcDNA3.1-TFE3 group was more than that in the pcDNA3.1 group (Fig. 4E and F, $P<0.05$). Consistent with the results of the migration experiment, the invasion experiment showed that cell invasion was noticeably suppressed by TFE3 knockdown and enhanced by TFE3 overexpression (Fig. 4G and H, $P<0.05$). In conclusion, these results indicated that TFE3 might promote migration and invasion behavior in PTC cells. Assays were performed in triplicate.

### 3.5 TFE3 induces autophagy-lysosome in PTC cells

To determine whether autophagy-lysosome is related to TFE3, as shown in Fig. 5C, the expression of LC3 and P62/SQSTM1 in the pcDNA3.1-TFE3 group were higher than in the pcDNA3.1 group. Then, we detected the processing of LC3 I to LC3 II expression in BCPAP and KTC-1 cells transfected with pcDNA3.1-TFE3 or si-TFE3, which is a sign of autophagy[9].The results showed that TFE3 increased the ratio of LC3 Ⅱ/LC3 Ⅰ (Fig. 5A and B, $P<0.05$). Next, we examined the level of P62/SQSTM1 protein. P62/SQSTM1 is selectively incorporated into autophagosomes by simultaneously interacting with LC3 protein[10]. Compared with the pcDNA3.1 group, the pcDNA3.1- TFE3 group showed increased expression of P62/SQSTM1 (Fig. 5A and B, $P<0.05$). Similarly, the si-TFE3 group showed a decrease in P62/SQSTM1 expression compared to the si-NC group. Through Q-PCR experiments, we found that the expression of LC3 and P62/SQSTM1 genes in PTC cells decreased after knocking down TFE3, and overexpression of TFE3, the expression of LC3 and P62/SQSTM1 in PTC cells was increased(Fig. 5C, $P<0.05$). The results above revealed that TFE3 might be able to promote autophagy-lysosome in PTC cells. Assays were performed in triplicate.

## 4. Discussion

PTC is the most common endocrine malignancy, ranked 5th in female tumor incidence[2]. Currently, the treatment of PTC is based on $^{131}$ I radiotherapy and chemotherapy combined with thyroid stimulating hormone (TSH) suppression therapy after surgical resection[11]. However, tumor recurrence and metastasis and the presence of chemotherapy resistance in refractory PTC lead to a decrease in the survival rate of patients with PTC[12].

TFE3, which belongs to the MiT/TFE family, is a regulator of autophagy and lysosomal biogenesis[13]. It was first discovered in 2009 that the MiT / TFE family could regulate most lysosomal genes (including promoters encoding hydrolases and lysosomal-related proteins)[14]. Importantly, under stress conditions, complex interactions between MiT / TFE family-dependent autophagy homeostasis pathways and apoptotic processes may occur in cancer cells, which ultimately determine their fate between cell death or

survival[15]. TFE3 simultaneously regulates autophagy induction, lysosomal biogenesis, oxidative metabolism, and oxidative stress, making it play an important role in determining cell fate[16]. Notably, under nonstress conditions, TFE3 interacts with 110 14-3-3 proteins and remains in the cytoplasm. At this time, TFE3 is phosphorylated at Ser321. when affected by stress, TFE3 is dephosphorylated and TFE3/14-3-3 complex is dissociated, while TFE3 is transferred from the cytoplasm to the nucleus to promote autophagy and lysosome biogenesis [5]. TFE3 was found to be fused with papillary renal cell carcinoma (PRCC) on chromosome 1q21.2 [PRCC-TFE3 t(X;1)(p11.2; q21)][17]. Moreover, Fan et al.[18] found that inhibiting MT2-TFE3-dependent autophagy enhances melatonin-induced apoptosis in tongue squamous cell carcinoma. Furthermore, increased TFE3 expression in RCC was associated with poor PFS[19]. In accordance with these results, we found that TFE3 increased PTC cell proliferation, invasion, and decreased apoptosis.

M. Anselmier, a French physiologist, first used the term "autophagy" in a short article describing the effects of fasting on mice published in 1859 [20]. Autophagy, a highly conserved protein degradation pathway from yeast to humans, is essential for clearing protein aggregates and misfolded proteins from healthy cells. Under stress conditions, cells produce a multitude of damaged proteins or organelles, and a double membrane will be produced in the cytoplasm to swallow defective or toxic molecules and organelles to form autophagosomes. Then, autophagosomes fuse with lysosomes and release lysosomal acid enzymes in the vesicles to decompose toxic molecules and other substances, and the resulting products re-synthesize new proteins or organelles[21].The whole process of autophagy involves a variety of evolutionary conserved genes, namely autophagy-related genes (ATGs)[22]. According to the different ways of transporting cellular material to the lysosome, autophagy can be divided into macro autophagy, micro autophagy, and molecular chaperone-mediated autophagy (CMA)[23]. Previous studies demonstrated that autophagy is an important participant in the pathogenesis of many diseases including cancer[24, 25]. Genome-wide association studies found that ATG5 is associated with systemic lupus erythematosus (SLE) in Chinese, indicating that autophagy may be related to the pathogenesis of SLE[26]. Zou et al.[27] found that suppressing autophagy can enhance the chemotherapeutic effects of paclitaxel in cervical cancer cells. However, the relationship between PTC and autophagy has not been fully elucidated.

In this study, 90 cases of PTC and 18 normal samples were selected from the TCGA database for analyzing the mechanism of PTC. Based on GSEA the enrichment of autophagy and lysosomal related biological functions involved in LC3 and P62/SQSTM1 in PTC data was analyzed the results showed autophagy lysosome was positively correlated with thyroid cancer progression. LC3 and P62/SQSTM1 have been widely reported as indicators of autophagy[28, 29]. Meanwhile, TFE3 has been identified as a regulator of autophagy by previous studies[16]. Based on bioinformatics analysis and GSEA data, we furthered validated it in tissue and cells in vitro. We found that TFE3 was significantly higher in 78 PTC tissues than in para-cancer tissues. High expression levels of TFE3 was closely associated with lymph node metastasis. Then, we conducted function assays in KTC-1 and BCPAP cell lines. The results showed that TFE3 enhanced the proliferation, invasion, and migration of PTC cells by regulating autophagy-lysosome, suggesting that TFE3 is a potential sensitive marker in PTC. In this study, we found that

autophagy was induced by TFE3 as evidenced by the upregulation of P62/SQSTM1 protein expression and the ratio of LC3Ⅱ/LC3Ⅰ. Therefore, we hypothesized that TFE3 might positively regulate autophagy lysosome in PTC.

However, the underlying mechanisms of TFE3-mediated autophagy-lysosome and PTC remain unclear. Recent studies have linked the accumulation of ROS to TFE3 activation in the prognosis of cancer[30]. The potential mechanisms of autophagy-lysosome and TFE3 and PTC require deeper research.

# 5. Conclusions

In summary, we detected the expression of TFE3 and its malignant characteristics in PTC. The present study revealed that TFE3 promotes PTC progression through autophagy-lysosome. Moreover, TFE3 as a sensitive marker for lymph node metastasis might be a novel diagnostic and therapeutic target for PTC.

# Abbreviations

Follicular thyroid cancer (FTC)

Papillary thyroid cancer (PTC)

Medullary thyroid cancer (MTC)

Anaplastic thyroid cancer (ATC)

Recombinant Transcription Factor Binding To IGHM Enhancer 3 (TFE3)

Reactive oxygen species (ROS)

Gene Set Enrichment Analysis (GSEA)

Tumor/Node/Metastasis classification (TNM)

Thyroid stimulating hormone (TSH)

Papillary renal cell carcinoma (PRCC)

Autophagy-related genes (ATGs)

Chaperone-mediated autophagy (CMA)

Systemic lupus erythematosus (SLE)

# Declarations

### Ethics approval and consent to participate

This study was approved by Ethics Committee of Taizhou Central Hospital. Written informed consent was obtained from each individual in the study.

### Consent for publication

Not applicable.

### Availability of data and materials

All data for this study are presented in the manuscript.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

All co-authors approved the manuscript.

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Not applicable.

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31. Legends.

# Tables

Table 1 Correlation between TFE3 expression and clinicopathological characteristics of thyroid cancer patients (n = 78)

| Clinical parameters | Number | TFE3 expression | | *P* value |
|---|---|---|---|---|
| | | Low | High | |
| Age (years) | | | | *P*=0.173 |
| <45 | 42 | 18 | 24 | |
| >=45 | 36 | 21 | 15 | |
| Gender | | | | *P*=1.000 |
| Male | 22 | 11 | 11 | |
| Female | 56 | 28 | 28 | |
| Clinical stage[a] | | | | *P*=0.329 |
| I | 67 | 35 | 32 | |
| II+III | 11 | 4 | 7 | |
| Tumor stage[a] | | | | *P*=0.168 |
| T1+T2 | 73 | 38 | 35 | |
| T3+T4 | 5 | 1 | 4 | |
| Lymph node metastasis[a] | | | | ***P*=0.038*** |
| N0 | 20 | 14 | 6 | |
| N1a+N1b | 58 | 25 | 33 | |

**P* <0.05 was considered significant.

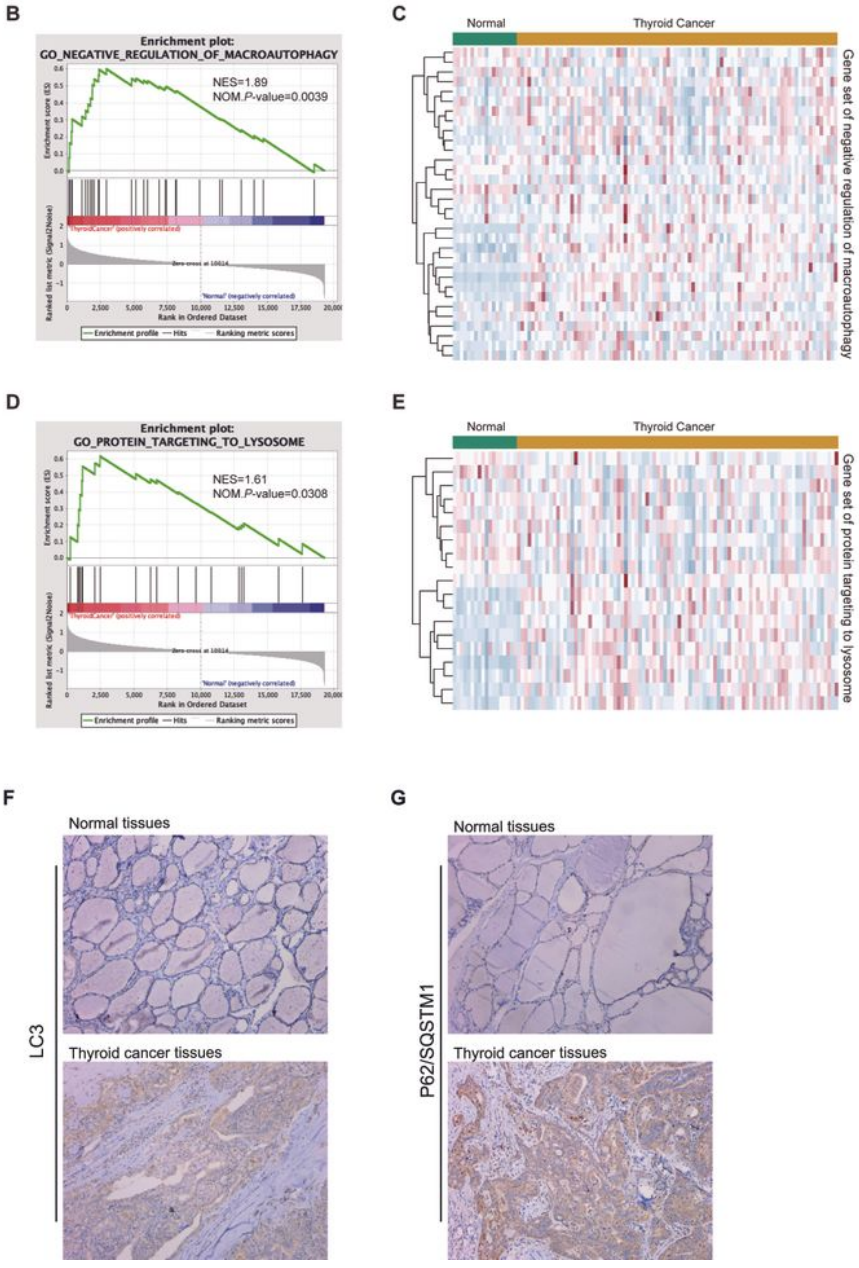
Thyroid carcinoma

[a]According to the AJCC tumor-node-metastases (TNM) staging system（eighth edition）, 2017

# Figures

A

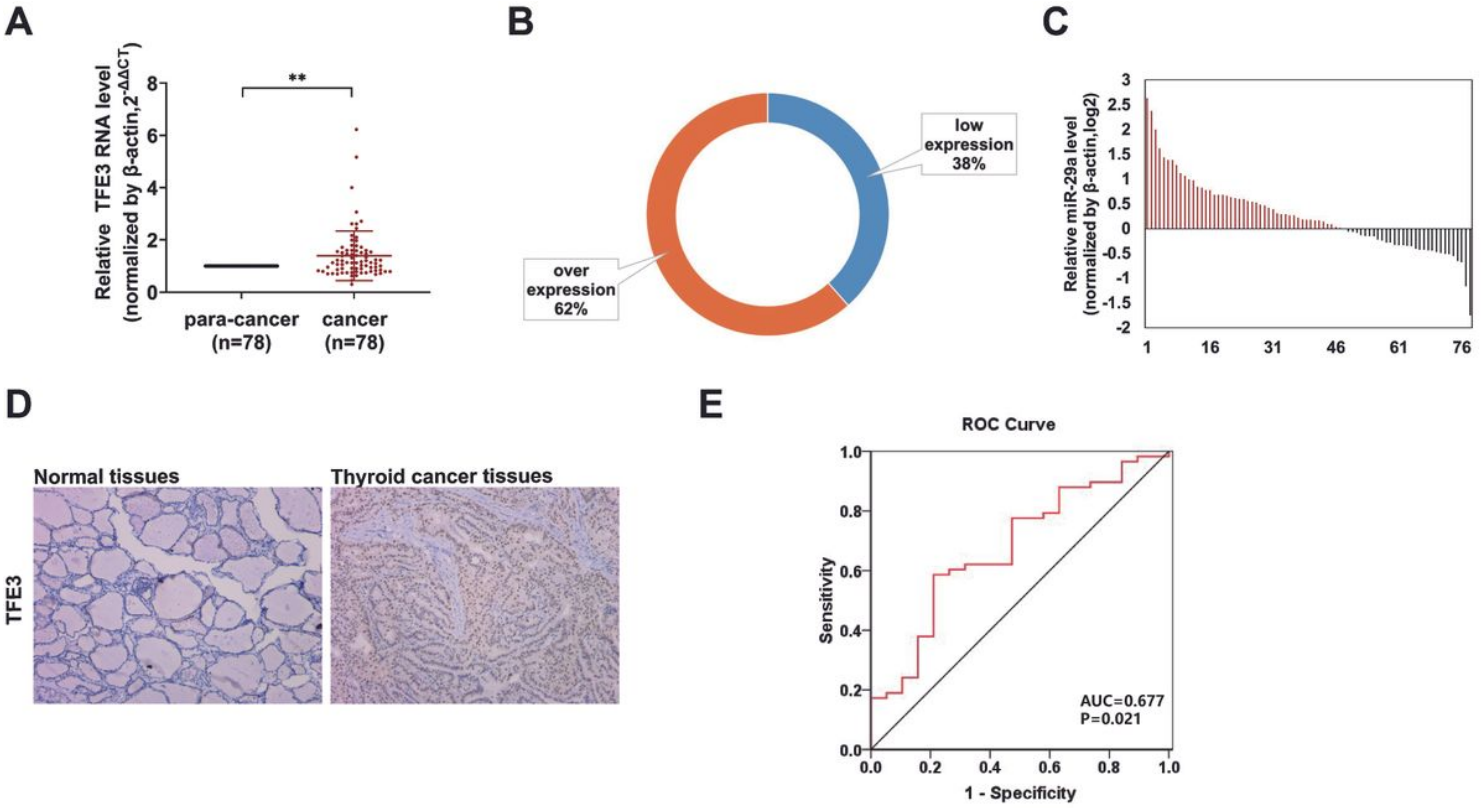
| GO Term | Size | NES | NOM.*P*-value |
|---|---|---|---|
| GO NEGATIVE REGULATION OF MACROAUTOPHAGY | 32 | 1.8898067 | 0.003921569 |
| GO PROTEIN TARGETING TO LYSOSOME | 19 | 1.6136397 | 0.030864198 |
| GO PROTEIN LOCALIZATION TO LYSOSOME | 35 | 1.6637899 | 0.012727273 |
| GO GOLGI TO LYSOSOME TRANSPORT | 8 | 1.6946115 | 0.014492754 |
| GO REGULATION OF AUTOPHAGY OF MITOCHONDRION | 39 | 1.6154093 | 0.029940119 |
| GO NEGATIVE REGULATION OF AUTOPHAGY | 79 | 1.5461751 | 0.03024911 |
| GO NEGATIVE REGULATION OF AUTOPHAGY OF MITOCHONDRION | 9 | 1.5578619 | 0.045833334 |




## Figure 1

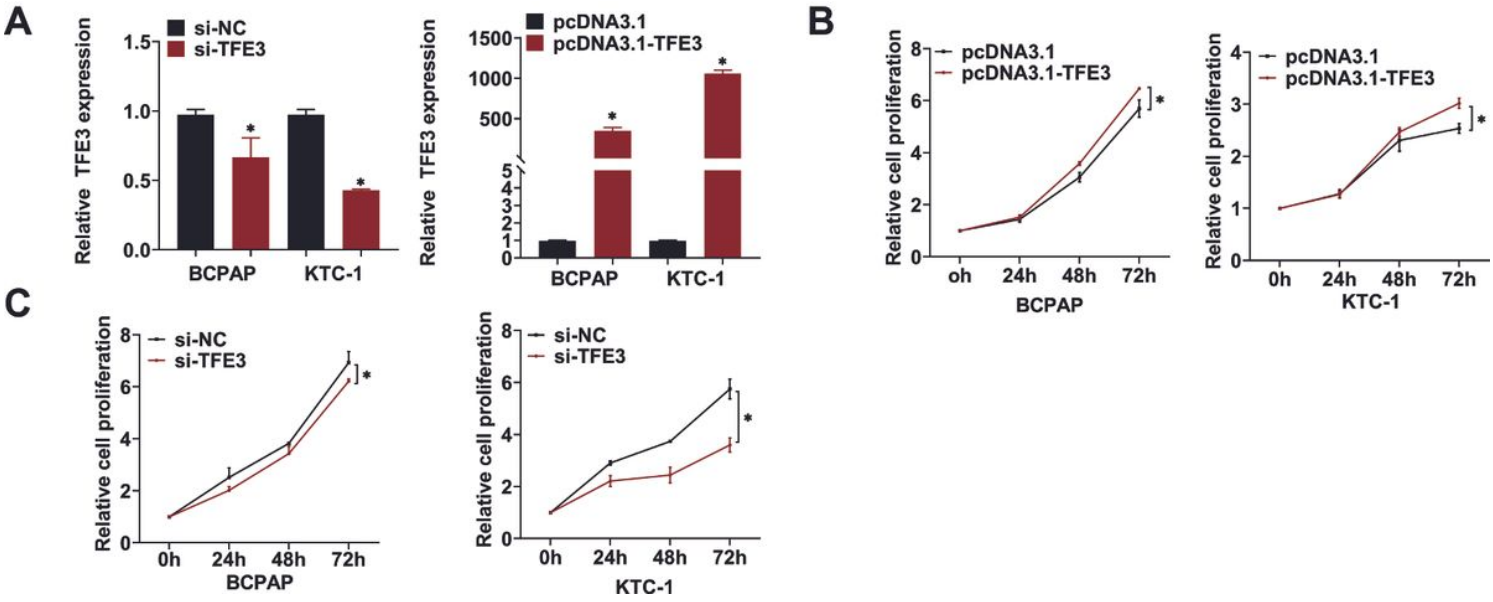
Autophagy was positively correlated with thyroid cancer progression. Gene set enrichment analysis (GSEA)was showed that the expression of genes involved in autophagy and lysosome-related biological functions was upregulated in the thyroid cancer. A. GSEA of data revealed that human thyroid cancer specimens have elevated expression of autophagy–lysosome genes compared with normal thyroid tissue. NES, normalized enrichment score; Nom., nominal. B. GSEA showing correlation between thyroid

cancer and the macroautophagy gene signature. C. Upregulation of macroautophagy genes in thyroid cancer relative to matched normal tissue.D. GSEA showing correlation between thyroid cancer and the lysosome gene signature. E. Upregulation of lysosome genes in thyroid cancer relative to matched normal tissue. F,G. Immunohistochemistry assay showed an increase in P62/SQSTM1 and LC3 expression in thyroid cancer tissues compared with the normal tissues.



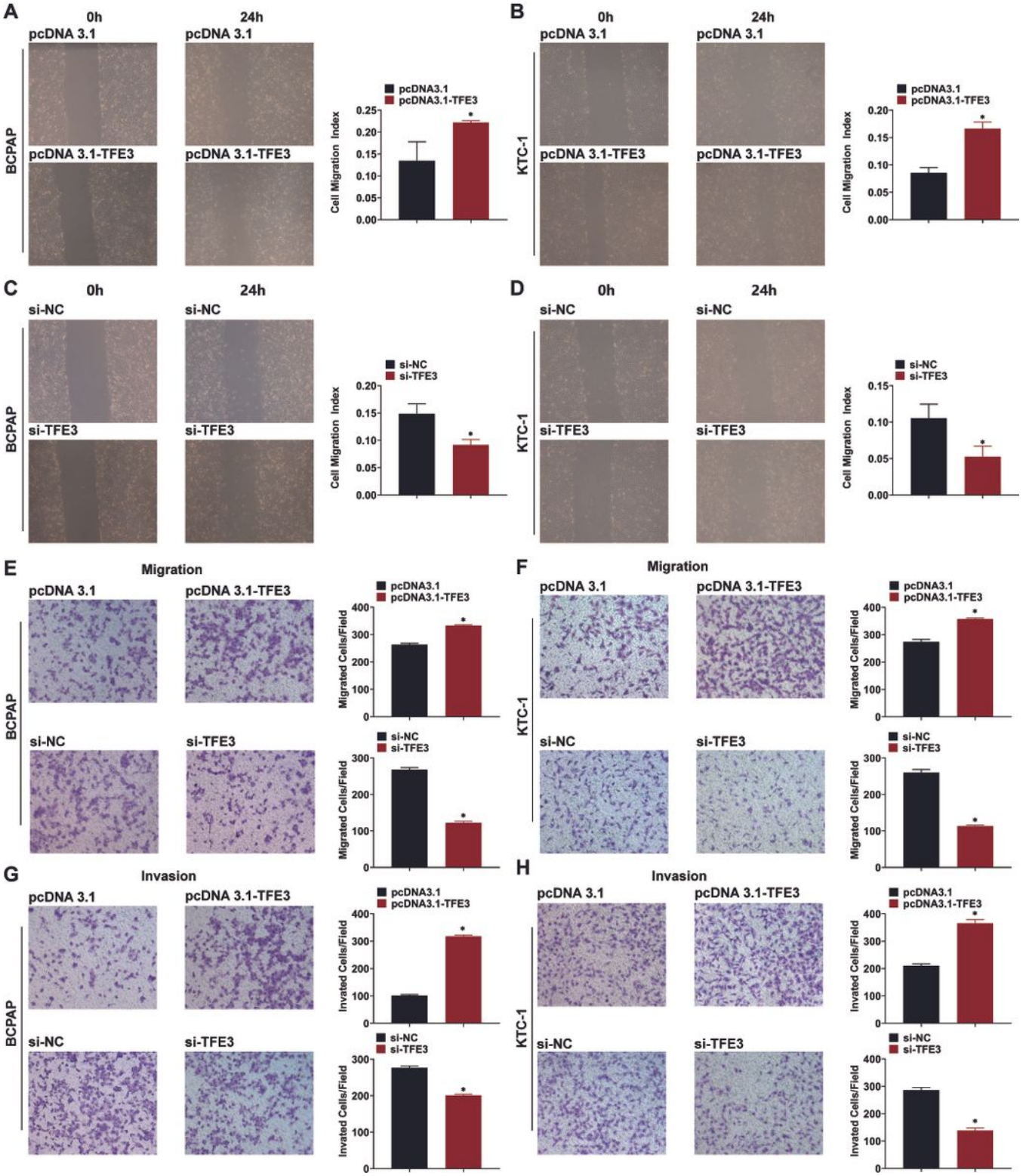

## Figure 2

TFE3 was overexpressed and closely associated with lymph node metastasis in PTC. A,B,C. TFE3 mRNA expression was significantly higher in PTC than in normal thyroid tissues. D. Immunohistochemistry assay showed an increase in TFE3 gene expressions in PTC tissues compared with the normal tissues (**P<0.01).E. the ROC curve of TFE3 about lymph node metastasis in PTC.



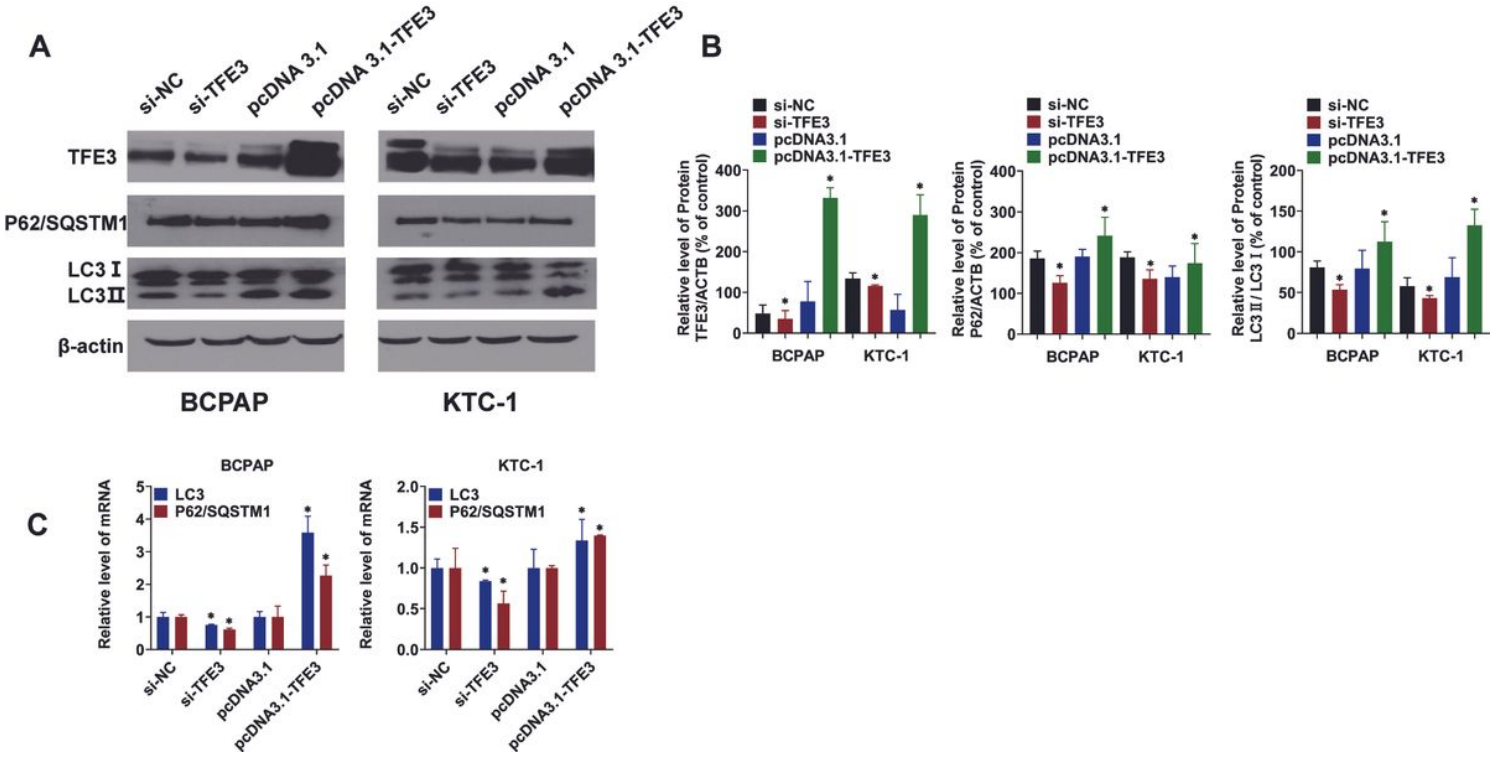

## Figure 3

TFE3 promotes proliferation of PTC cells. A. After TFE3 transfection, the expression of TFE3 was detected by real-time PCR . B. MTT assay showed overexpression of TFE3 significantly increased the proliferation of BCPAP and KTC-1 cells （*P<0.05）, C. MTT assay showed knockdown of TFE3 decreased the proliferation of BCPAP and KTC-1 cells （*P<0.05).




## Figure 4

TFE3 accelerates PTC cells migration and invasion in vitro. A,B. Wound healing assay showed that overexpression of TFE3 increased thyroid cancer cells migration. C,D. silencing of TFE3 reduced PTC cells migration. E, F, G and H. Transwell assay indicated that overexpression of TFE3 increased the migratory and invasive ability of PTC cells ,while knockdown of TFE3 decreased the migratory and invasive ability of PTC cells (*P<0.05).




## Figure 5

TFE3 induces autophagy-lysosome in PTC cells. A ,B. After transfected with pcDNA3.1-TFE3 or si-TFE3, BCPAP and KTC-1 cells showed significant changes in TFE3 protein level. Overexpression of TFE3 increased the expression of P62/SQSTM1, the opposite results were observed in BCPAP and KTC-1 cells transfected with si-TFE3. The ratio of LC3Ⅱ/LC3Ⅰin BCPAP and KTC-1 cells increased significantly after pcDNA3.1-TFE3 transfection, and the opposite results appeared after si-TFE3 transfection . C. After knocking down TFE3, the expression of LC3 and P62/SQSTM1 in PTC cells was decreased, and overexpression of TFE3, the expression of LC3 and P62/SQSTM1 in PTC cells was increased (*P<0.05).