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SOX12 Promotes Thyroid Cancer Cell Proliferation and Invasion by Regulating the Expression of POU2F1 and POU3F1

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Purpose: SOX12 is overexpressed in many cancers, and we aimed to explore the biological function and mechanism of SOX12 in thyroid cancer.

Materials and Methods: We first analyzed the expression of SOX12 in thyroid cancer using data in The Cancer Genome Atlas. Immunohistochemistry and qRT-PCR were performed to identify SOX12 expression in thyroid cancer tissue and cells. Thyroid cancer cells were transfected with small interfering RNA targeting SOX12, and cellular functional experiments, including CCK8, wound healing, and Transwell assays, were performed. Protein expression was examined by Western blot analysis. A xenograft model was developed to evaluate the effect of SOX12 on tumor growth in vivo.

Results: SOX12 expression was increased in thyroid cancer tissue and cells. SOX12 promoted cell proliferation, migration, and invasion and accelerated tumor growth in vivo. The expression of PCNA, Cyclin D1, E-cadherin, Snail, MMP-2, and MMP-9 was affected by SOX12 knockdown. Bioinformatic analysis showed that SOX12 could interact with the POU family. SOX12 knockdown inhibited the expression of POU2F1, POU2F2, POU3F1 and POU3F2, and SOX12 expression showed a positive correlation with POU2F1, POU3F1, and POU3F2 expression in clinical data. POU2F1 and POU3F1 were able to reverse the effect of SOX12 knockdown on thyroid cancer cells.

Conclusion: SOX12 affects the progression of thyroid cancer by regulating epithelial-mesenchymal transition and interacting with POU2F1 and POU3F1, which may be novel targets for thyroid cancer molecular therapy.

Key Words: Thyroid cancer, SOX12, cell proliferation, invasion, POU family

INTRODUCTION

Thyroid cancer is a common tumor of the head and neck and is the most common endocrine system cancer.[1] The incidence of thyroid cancer is about 5% for thyroid tumors and about 1% for systemic malignant tumors.[2] The incidence of thyroid cancer in females is three to four times that in males, making thyroid cancer one of ten cancers considered to be harmful to female health. Although the prognosis of thyroid cancer is good (death rate of about 6.8/100000), about 5% of patients will experience metastatic disease, for which specific and effective treatment is lacking.[3-7] Further exploration of therapeutic targets and targeted drugs will help to improve treatments for this portion of thyroid cancers.

Sox family proteins are a family of conserved transfer factors characterized by a highly conserved high-mobility group (HMG) domain that mediates DNA binding.[8] This HMG domain was first found on the sex determining region Y (SRY) protein involved in male determination in mammals.[9] SOX12 expression has been found to be overexpressed in many kinds of cancers, including gastric cancer, lung cancer, hepatocellular

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carcinoma, colorectal cancer, and renal cancer.[10-12] Excessive expression of SOX12 has been shown to be closely related with cancer cell proliferation, invasion, and migration and to predict poor prognosis.[13-15] Gao, et al.[16] reported that SOX12 knockdown inhibited the growth of multiple myeloma cells by downregulating the Wnt/beta-catenin signaling pathway. Zou, et al.[12] demonstrated that Sox12+ hepatocellular carcinoma cells show strong spheroidizing ability and high cisplatin resistance and easily form distal tumors. Based on The Cancer Genome Atlas (TCGA) dataset, we found that SOX12 is overexpressed in thyroid cancer. Via the STRING database, we found that SOX12 could interact with the POU family, including POU2F1, POU2F2, POU3F1, and POU3F2. However, the biological function of SOX12 in thyroid cancer has not been fully explored.

In this study, we examined the expression of SOX12 in tumor tissue and non-tumor tissue of thyroid cancer patients. The effect of SOX12 knockdown on thyroid cancer cell proliferation, migration, and invasion was also studied. Our study demonstrates that SOX12 is overexpressed in thyroid cancer samples and promotes tumor cell proliferation, migration, and invasion. Additionally, we show that SOX12 is correlated with the POU family, wherein SOX12 accelerates the cell phenotype of thyroid cancer cells by regulating the expression of POU2F1, POU2F2, POU3F1, and POU3F2. We finally show that SOX12 promotes cell proliferation and the invasion of thyroid cancer cells by regulating the POU family.

MATERIALS AND METHODS

TCGA analysis

TCGA analysis of SOX12 expression in thyroid cancer tissues and normal tissues was performed by using UALCAN (http://ualcan.path.uab.edu/analysis.html).

Patients and tissue samples

Clinical specimens were obtained from 87 patients with thyroid carcinoma who underwent surgery at the Shanghai Seventh People's Hospital Affiliated to Shanghai University of Traditional Chinese Medicine from 2017 to 2019. Written informed consent was obtained from all patients involved in the present study. Adjacent non-tumoral thyroid tissues from these patients served as normal controls. No patients underwent radiation therapy or chemotherapy before surgery. The study was approved by the Medical Ethics Committee of Shanghai Seventh People's Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (IRB number: 2017-IRBQYYS-120) and was conducted in accordance with the Declaration of Helsinki.

Cell culture

Human thyroid cancer cell lines (KCT-1, TPC-1, BCPAP, and SW579) and the human thyroid epithelial cell line Htori-3 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in RPMI-1640 (Genom, Hangzhou, China). All cell lines were maintained in a humidified incubator at 5% CO_2 and 37°C.

Cell transfection

Small interfering RNA targeting SOX12 (siSOX12-1: 5'-GCGCGCGAGCCCGGCTGGTGCAAGA-3'; siSOX12-2: 5'-GGTCGCAGCACGAACGGCGGAAGAT-3') and a corresponding negative control (siNC: 5'-ATTGCCTAAGCCTAAGCGTAACGG-3') were purchased from GenePharma Co., Ltd. (Shanghai, China). The siSOX12-1, siSOX12-2, or siNC were transfected into SW579 and TPC-1 cells using Lipofectamine 2000 (Invitrogen, New York, NY, USA) following the manufacturer's instructions. For rescue experiments, SW579 cells were transfected with siSOX12 and then co-transfected with oe-POU2F1 or oe-POU3F1 using Lipofectamine 2000 (Invitrogen, Shanghai, China). After transfection for 24 hours, the expression of SOX12 was determined by qRT-PCR and Western blot. Cells were harvested and subjected to cellular functional experiments.

qRT-PCR

Total RNA from tissues and cell lines were extracted using TRIzol reagent (Invitrogen, New York, NY, USA) according to the manufacturer's protocol. Reverse transcription was performed using 1st-Stand cDNA Synthesis Kits (Xuanya, Shanghai, China). The qRT-PCR was carried out on a Light Cycle 480 system (Roche, Berne, Switzerland) using SYBR Green Premix Ex Taq (Takara, Tokyo, Japan). The primer sequences are shown in Supplementary Table 1 (only online). For each sample, independent experiments were repeated three times. The relative expression levels of mRNA and miRNA were analyzed using the $2^{-\Delta\Delta Ct}$ method.

Cell proliferation assay

The proliferation of SW579 and TPC-1 cells was detected with CCK8 according to the manufacturer's instructions. Briefly, 5×10^4 cells were plated into 96-well plates containing 100 μL of DMEM (with 10% FBS) per well for 1, 2, 3, and 4 days culture at 37°C, 5% CO_2. Cells of each group were plated in five duplicate wells. Then, CCK8 solution (10 μL) was added into each well and allowed to incubate for 4 hours at 37°C. With a multi-well plate reader, the optical density value of each well was obtained at a wavelength of 450 nm.

Wound-healing assay and transwell assay

For the detection of cell migration, SW579 and TPC-1 cells were cultured on a 6-well plate. The SW579 and TPC-1 cells grew to 100% confluence and were scratched with a 20-mL pipette tip. Cell migration pictures were acquired at 0 and 24 hours after the scratch. Transwell analysis was performed with a 24-well microplate (Millipore, Shanghai, China) to evaluate cell

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invasion of SW579 and TPC-1 cells. In serum-free medium, 5×10^4 cells were inoculated into cell culture medium for invasion test. The 1640 medium containing 20% FBS was added into the bottom chamber. After incubation for about 24 hours, the cells on the lower surface of the filter were fixed and examined under a microscope.

Xenograft model experiment

All animal experiments were performed according to institutional and international animal regulations. The animal experiments were approved by the Institutional Animal Care and Use Committee of Shanghai Seventh People's Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (2019-056). TPC-1 cells were transfected with short-hairpin RNA targeting SOX12 (shSOX12: 5'-GCGCGCGAGCCCGGCTGGTGC-3'). Each nude mouse was injected subcutaneously with 0.1 ml of TPC-1 cell suspension (5×10^6 cells/mL.) on the back. The growth of tumors was measured every 7 days. All mice were sacrificed after 28 days, and the solid tumors of the mice were extracted. Tumor sizes were recorded every 7 days, and tumor volume was determined according to the formula: tumor volume (mm^3)=length (mm)×width $(mm)^2/2$.

Western blot

Cells were lysed in 200 μL of lysis buffer. Protein extracts (20 μg) were electrophoresed on 10% sodium dodecyl sulfate polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were blocked with 5% non-fat dried milk in Tris-buffered saline and incubated for 2 hours at room temperature with the primary antibodies for PCNA (dilution: 1:1000, ab92552), Cyclin D1 (dilution: 1:1000, abab40754), E-cadherin (dilution: 1:1000, ab40772), Snail (dilution: 1:1000), MMP-2 (dilution: 1:1000), MMP-9 (dilution: 1:1000), POU2F1 (dilution: 1:1000, ab178869), POU2F2 (dilution: 1:1000, ab179808), POU3F1 (dilution: 1:1000, ab259952), POU3F2 (dilution: 1:1000, ab243045), and GAPDH (dilution: 1:1000, #5174, Cell Sigaling Technology, Danvers, MA, USA), followed by the horseradish peroxidase conjugated secondary antibody. The immunocomplexes were visualized using chemiluminescent horseradish peroxidase kits. GAPDH was used to ensure equivalent protein loading.

Immunohistochemical staining analysis

Tumor tissue or non-tumor tissue sections were deparaffinized by washing over several steps: 1) xylene, 2) 100% ethanol, 3) 95% ethanol, 4) 80% ethanol, and 5) PBS. After washing, the endogenous peroxidase activity was quenched with methanol and H_2O_2 (0.3%) for 5 minutes, and then the slide was blocked in PBS containing 5% bovine serum albumin for 30 minutes and incubated with SOX12 and Ki67 primary antibodies at 4°C overnight (1:100, Solaibao Biotechnology Co., Ltd., Beijing, China). After 24 hours, the sections were incubated with secondary antibody (1:1000; Solaibao Biotechnology Co., Ltd.) for 2 hours.

Statistical analysis

Data were analyzed using GraphPad Prism 7 software (Chicago, IL, USA). Each value was acquired from at least three independent experiments. Data are presented as a mean±SD. Comparisons involving two groups were conducted using Student's t-test. Comparisons among multiple groups were conducted with one-way ANOVA with Tukey's post hoc test. Pearson's χ^2 test was used for analysis of the correlation between clinicopathological features and SOX12 expression in thyroid cancer patients. Spearman's correlation analysis was used to evaluate correlations between the expression of SOX12 and POU family proteins in thyroid cancer tissues. $p<0.05$ was considered indicative of statistical significance.

RESULTS

SOX12 is upregulated in thyroid cancer

TCGA data indicated that the expression of SOX12 in thyroid cancer is increased significantly, compared with that in normal tissues ($p<0.01$) (Fig. 1A). Accordingly, we set out to investigate the expression of SOX12 mRNA and protein in thyroid cancer tissues and paired adjacent normal tissues from 87 thyroid cancer patients by qRT-PCR. Immunohistochemistry (IHC) was performed to examine SOX12 protein levels in ten pairs of thyroid cancer tissues and adjacent normal tissues, which were randomly selected. As shown in Fig. 1B and C, both mRNA expression and protein levels of SOX12 were increased significantly in thyroid cancer tissues, compared with adjacent normal tissues ($p<0.01$). Additionally, the expression of SOX12 in thyroid cancer cell lines (KCT-1, TPC-1, BCPAP, and SW579) and normal thyroid cells (Htori-3) was also measured. As shown in Fig. 1D and E, SOX12 was overexpressed in thyroid cancer cell lines. The SW579 and TPC-1 cells exhibited higher expression of SOX12, and they were utilized for SOX12 knockdown and further experiments. Additionally, we analyzed the association of SOX12 expression with clinicopathological parameters in patients with thyroid cancer. As shown in Table 1, high expression of SOX12 was positively associated with tumor stage, tumor size, and American Joint Committee on Cancer (AJCC) stage (8th Edition).

Downregulating SOX12 inhibits cell proliferation and epithelial-mesenchymal transition among thyroid cancer cells in vitro

To further explore the biological effect of SOX12 on the phenotype of thyroid cancer cells, SW579 and TPC-1 cells were transfected with siSOX12-1, siSOX12-2, or siNC. After transfection, SOX12 expression in thyroid cancer cells was examined by qRT-PCR and Western blot analysis (Fig. 2A and B). The results showed that siSOX12-1 and siSOX12-2 were successfully transfected into SW579 and TPC-1 cells. siSOX12-2 showed better transfection efficiency and was therefore used for further experi-

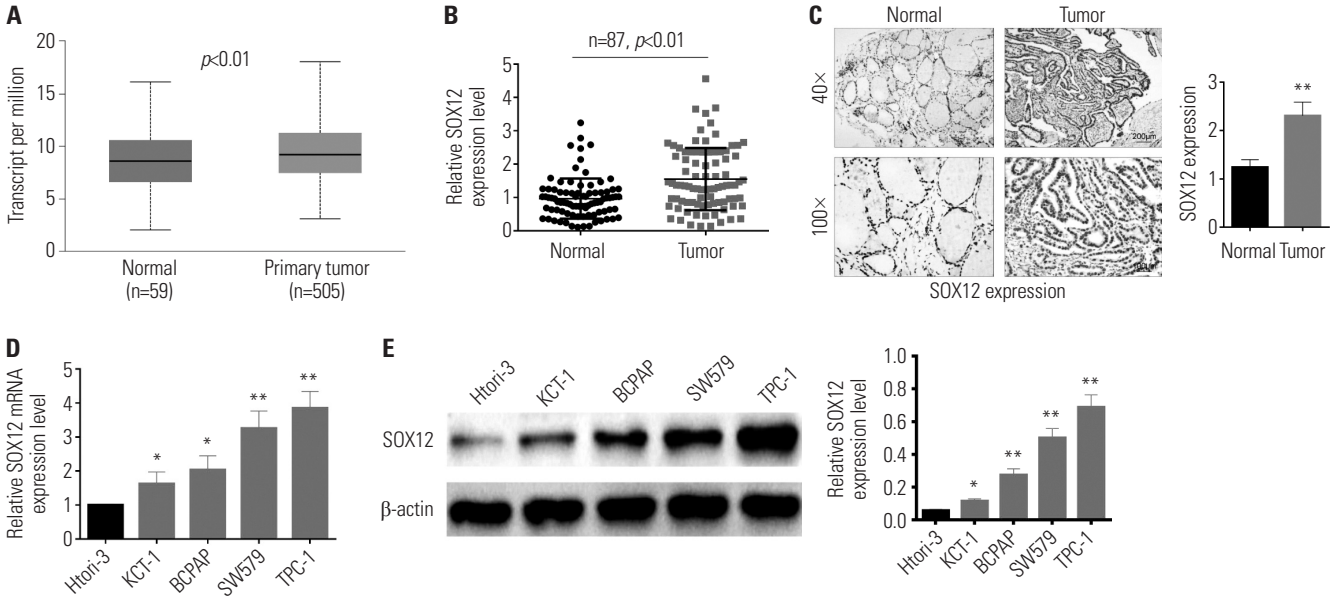

Fig. 1. SOX12 is overexpressed in thyroid cancer tissues and cell lines. (A) SOX12 expression was upregulated in thyroid cancer tissues in the TCGA database. (B) The mRNA expression of SOX12 in thyroid cancer tissues and normal tissues was examined by qRT-PCR. (C) The protein expression of SOX12 in thyroid cancer samples and normal samples was detected by IHC analysis. $^{**}p<0.01$ vs. normal. (D and E) The expression of SOX12 in thyroid cancer cell lines (KCT-1, TPC-1, BCPAP, and SW579) and normal cells (Htori-3) was identified by qRT-PCR and Western blot assays. $^{*}p<0.05$, $^{**}p<0.01$ vs. Htori-3 cells. TCGA, The Cancer Genome Atlas; IHC, immunohistochemistry.

Table 1. Associations between SOX12 Expression and Clinicopathological Parameters in Patients with Thyroid Cancer

Characteristics	Number of patients	SOX12 low expression (< median)	SOX12 high expression (≥median)	*p* value
Number	87	42	45	
Ages				0.544
<45 years	43	21	22	
≥45 years	44	21	23	
Sex				0.370
Female	45	23	22	
Male	42	19	23	
Stage (T)				<0.001
T1–T2	41	29	12	
T3–T4	46	13	33	
Stage (N)				<0.001
N0	40	32	8	
N1	47	10	37	
Tumor size				<0.001
<2 cm	42	31	11	
≥2 cm	45	11	34	
AJCC stage				<0.001
I	43	33	10	
II–IV	44	9	35	

ments. Cell proliferation, migration, and invasion were then identified. As presented in Fig. 2C, the proliferation of SW579 and TPC-1 cells was significantly inhibited by siSOX12, compared with siNC, upon CCK8 assay ($p<0.01$). The migration and invasion of SW579 and TPC-1 cells transfected with siSOX12 were also dramatically decreased, compared with cells transfected with siNC ($p<0.01$) (Fig. 2D and E). Moreover, the protein expression of PCNA, Cyclin D1, E-cadherin, Snail, MMP-2, and MMP-9 was evaluated by western blot analysis. The results showed that PCNA, Cyclin D1, Snail, MMP-2, and MMP-9 expression was suppressed and E-cadherin expression was promoted by siSOX12 transfection in SW579 and TPC-1 cells ($p<0.01$) (Fig. 2F).

Silencing SOX12 delays tumor growth in vivo

We then investigated the effect of SOX12 knockdown on tumor growth via a subcutaneous xenotransplant tumor model in nude mice. As exhibited in Fig. 3A, B and C, SOX12 silencing effectively inhibited tumor volume and delayed tumor growth, compared with the control group. We subsequently detected the expression of Ki67 and SOX12 in the tumor tissues by IHC analysis. The results showed that, compared with the shNC group, the expression of Ki67 and SOX12 proteins was markedly decreased in the shSOX12 transfection group (Fig. 3D). The expression of PCNA, Cyclin D1, E-cadherin, Snail, MMP-2, and MMP-9 in xenograft models was also detected by Western blot. The results showed that SOX12 silencing inhibited PCNA, Cyclin D1, Snail, MMP-2, and MMP-9 expression and promoted the E-cadherin expression ($p<0.01$) (Fig. 3E).

SOX12 promotes the expression of POU2F1, POU2F2, POU3F1, and POU3F2

By bioinformatics analysis of protein-protein interactions in the String database (Fig. 4A), we found that SOX12 could interact with POU family proteins (POU2F1, POU2F2, POU3F1, and

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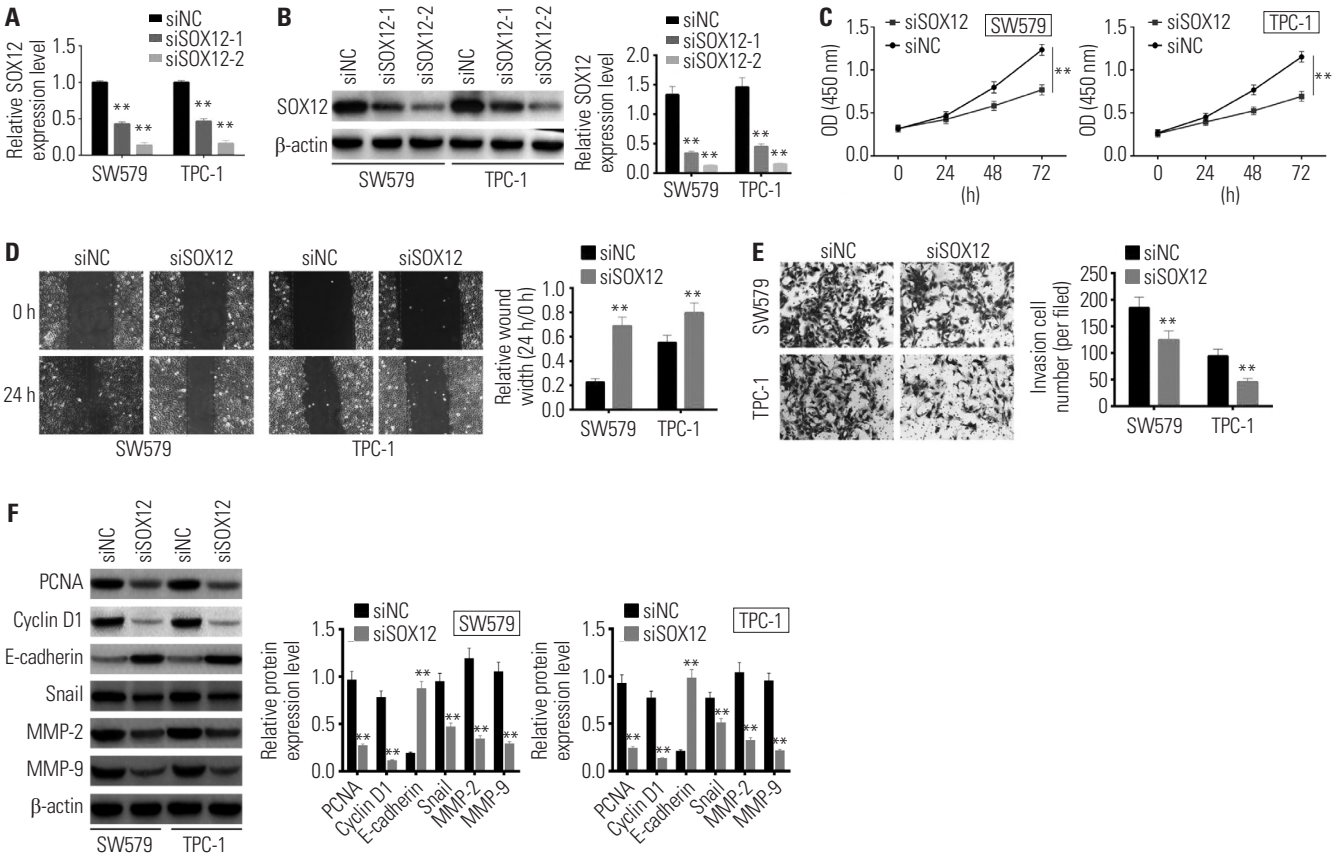

Fig. 2. SOX12 knockdown suppresses the proliferation, migration, and invasion of thyroid cancer cells. (A and B) SW579 and TPC-1 cells were transfected with siNC, siSOX12-1, or siSOX12-2, and the transfection efficiency was examined by qRT-PCR and Western blot assays. (C) Proliferation of SW579 and TPC-1 cells with siNC or siSOX12 transfection was determined by CCK8 assay. (D) Migration of SW579 and TPC-1 cells with siNC or siSOX12 transfection was identified by wound healing assay. (E) Invasion of SW579 and TPC-1 cells with siNC or siSOX12 transfection was identified through transwell assay. (F) Protein expression of PCNA, Cyclin D1, E-cadherin, Snail, MMP-2, and MMP-9 was evaluated by Western blot analysis. $^{**}p<0.01$ vs. siNC group. OD, optical density.

POU3F2). The expression of POU2F1, POU2F2, POU3F1, and POU3F2 in thyroid cancer clinical samples was examined by qRT-PCR. The results showed that the mRNA expression of POU2F1, POU2F2, and POU3F1 was increased in the cancer tissues of thyroid cancer patients (Fig. 4B). There was no significant changes observed in POU3F2 expression between normal and cancer tissues (Fig. 4B). Meanwhile, SOX12 expression showed a positive correlation with POU2F1, POU3F1, and POU3F2 expression in thyroid cancer patients (Fig. 4C). We then examined the protein expression of POU2F1, POU2F2, POU3F1, and POU3F2 in SW579 and TPC-1 cells by Western blot. Fig. 4D shows that knockdown of SOX12 significantly suppressed the expression of POU2F1, POU2F2, POU3F1, and POU3F2, compared with siNC transfection ($p<0.01$).

SOX12 regulates the proliferation, migration, and invasion of thyroid cancer cells by regulating POU2F1 and POU3F1

To determine the underlying regulating mechanism by which SOX12 mediates the oncological phenotype of thyroid cancer cells, rescue experiments were performed. As shown in Fig. 5, SW579 cells were transfected with siSOX12 and then co-transfected with oe-POU2F1 or oe-POU3F1. Cell viability, migration, and invasion were immediately examined. In doing so, we found that siSOX12 significantly inhibited the proliferation, migration, and invasion of SW579 cells, compared with the siNC group ($p<0.05$) (Fig. 5A-C). Meanwhile, oe-POU2F1 or oe-POU3F1 transfection effectively reversed the effect of siSOX12 on the proliferation, migration, and invasion of SW579 cells ($p<0.05$) (Fig. 5A-C). The expression of PCNA, Cyclin D1, E-cadherin, Snail, MMP-2, and MMP-9 was also detected by Western blot. The results showed that siSOX12 silencing inhibited PCNA, Cyclin D1, Snail, MMP-2, and MMP-9 expression, and promoted the E-cadherin expression ($p<0.01$) (Fig. 5D). However, oe-POU2F1 or oe-POU3F1 transfection effectively reversed the effect of siSOX12 on protein expression ($p<0.01$) (Fig. 5D).

DISCUSSION

In the present study, we found 1) that SOX12 expression is increased in thyroid cancer tissue and cells, 2) that SOX12 pro-

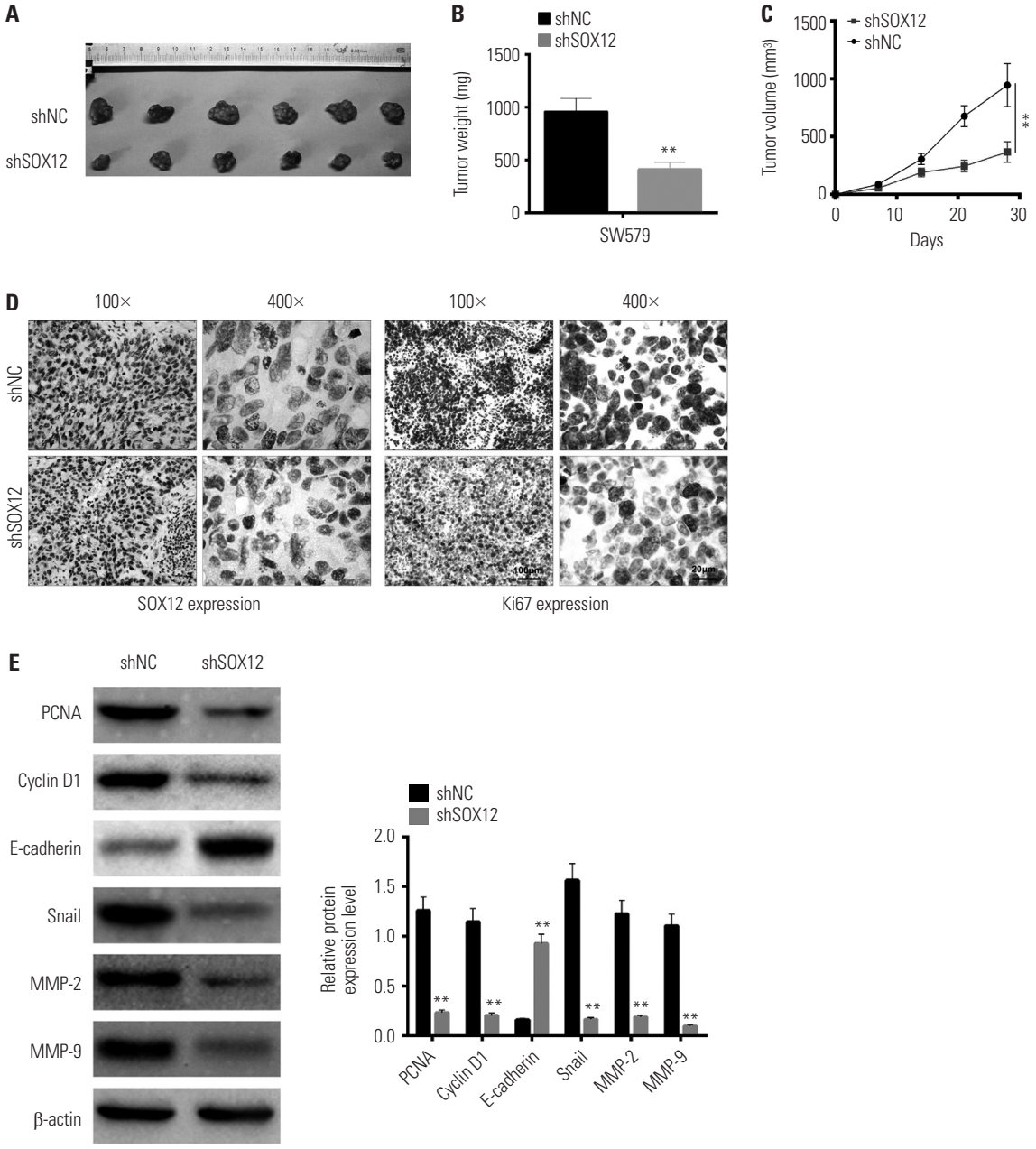

Fig. 3. SOX12 knockdown restrains tumor growth and weight. (A-C) Tumor weights isolated from nude mice in each treatment group were determined on day 28 after injection. Tumor growth curves were established by measuring tumor volume every 7 for 28 days after injection. (D) The expression of SOX12 and Ki67 was examined by IHC analysis. (E) The expression of PCNA, Cyclin D1, E-cadherin, Snail, MMP-2, and MMP-9 in xenograft models was detected by Western blot. **p<0.01 vs. shNC group. IHC, immunohistochemistry.

motes cell proliferation, migration, and invasion in vitro and accelerates tumor growth in vivo, and 3) that SOX12 knockdown inhibits the expression of POU2F1, POU2F2, POU3F1, and POU3F2 and that SOX12 expression shows a positive correlation with POU2F1, POU3F1, and POU3F2 expression in clinical data.

There are many diagnostic methods for thyroid cancer, among which the pathological diagnosis and differentiation of microscopic morphological features are the most commonly used. However, it is still difficult to identify atypical thyroid cancer or micro small cancer by morphology alone. Therefore, the application of molecular biology-assisted diagnosis has become an important identification method, providing a basis for tumor molecular diagnosis and curative effect evaluation. The SOX gene family exerts crucial role in regulating the tumorigenesis and development of several cancers, and several SOX genes act as oncogenes in human cancers. For example, You, et al.[17] reported *SOX5* is expressed excessively in gastric cancer and promotes cell proliferation, metastasis, and epithelial-mesenchymal transition. Sun, et al.[18] demonstrated that *SOX4* pro-

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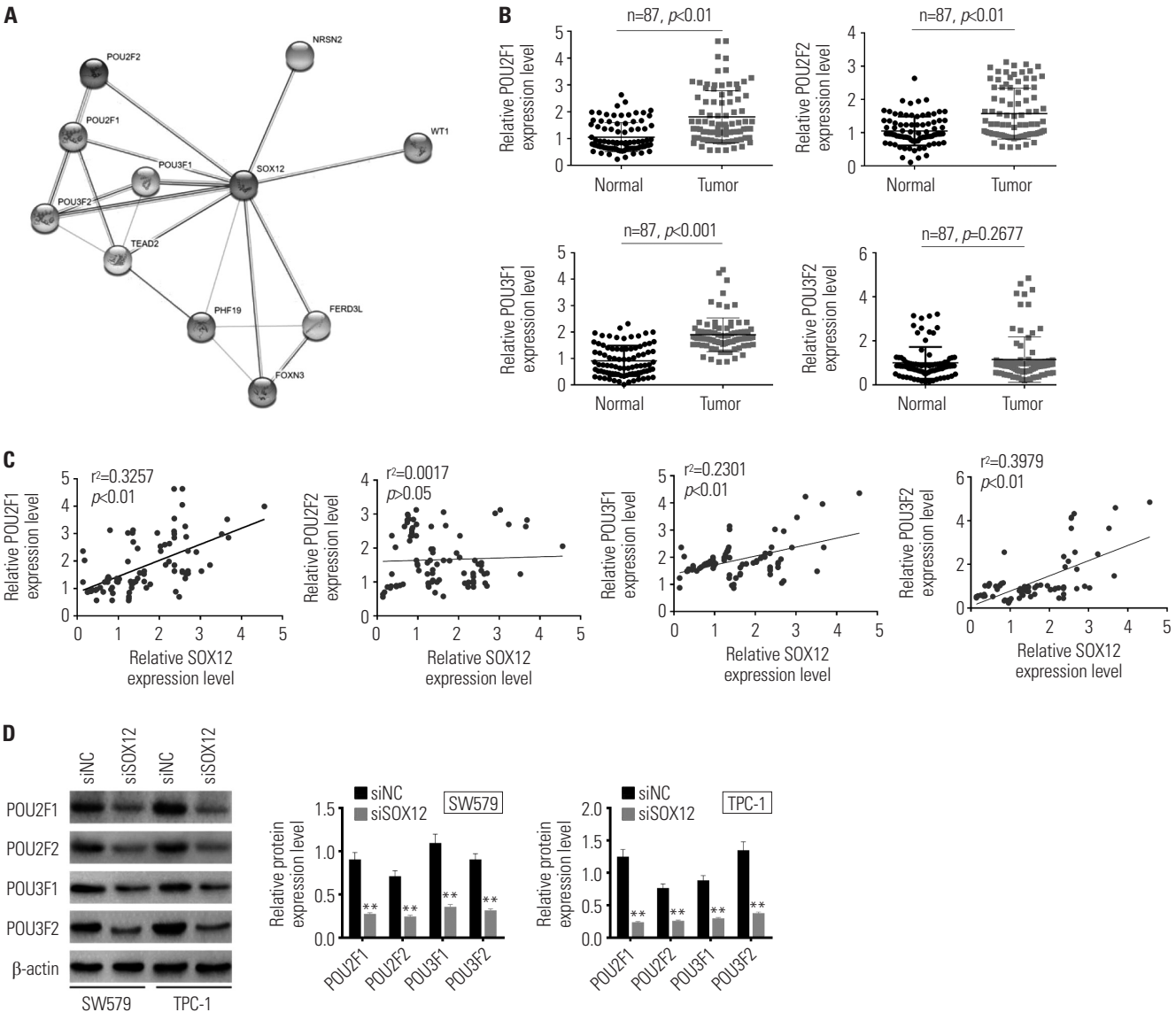

Fig. 4. SOX12 promotes the expression of POU family proteins. (A) Protein-protein interactions were analyzed by String. (B) The mRNA expression of POU2F1, POU2F2, POU3F1, and POU3F2 in thyroid cancer tumor tissues and normal tissues. (C) Correlations between SOX12 expression and POU2F1, POU2F2, POU3F1, POU3F2 expression were analyzed. (D) Protein expression of POU2F1, POU2F2, POU3F1, and POU3F2 in SW579 and TPC-1 cells was examined by Western blot. **$p<0.01$ vs. siNC cells.

motes cervical cancer proliferation by regulating cell cycle and inhibiting cell sensitivity to cisplatin through increasing ABCG2 expression. Tang, et al.[19] indicated that *SOX8* behaves as an oncogene to accelerate the metastasis of triple-negative breast cancer. We also detected other SOX family members in thyroid cancer in the TCGA database. Our results showed that *SOX3*, *SOX2*, *SOX6,* and *SOX9* are downregulated in thyroid cancer. SOX12 appeared to be overexpressed in thyroid cancer. In the present study, we identified the expression, function, and possible mechanism of *SOX12* in TPC. Preliminary studies have reported that SOX12 is overexpressed in many kinds of cancers and accelerates the progression of malignant tumor. Wang, et al.[20] reported that SOX12 expression is increased in lung cancer tissues and predicts poor overall survival and promotes cell proliferation and epithelial-mesenchymal transition. Ding, et al.[21] demonstrated that SOX12 mRNA expression is upregulated in human breast cancer tissues and that SOX12 is critical for cell migration, invasion, apoptosis, and cell cycle arrest by regulating PCNA, CDK2, and Cyclin D1 expression. Du, et al.[11] showed that high SOX12 expression in gastric cancer tissues is associated with metastasis gastric cancer recurrence, metastasis, and poorer patient survival and that SOX12 overexpression promote gastric cancer progression by promoting MMP7 and IGF1 expression. In the present study, we firstly found that both mRNA and protein expression of SOX12 is increased in thyroid cancer tissues. We further analyzed correlations between SOX12 expression and clinicopathological features in thyroid cancer patients and discovered that SOX12

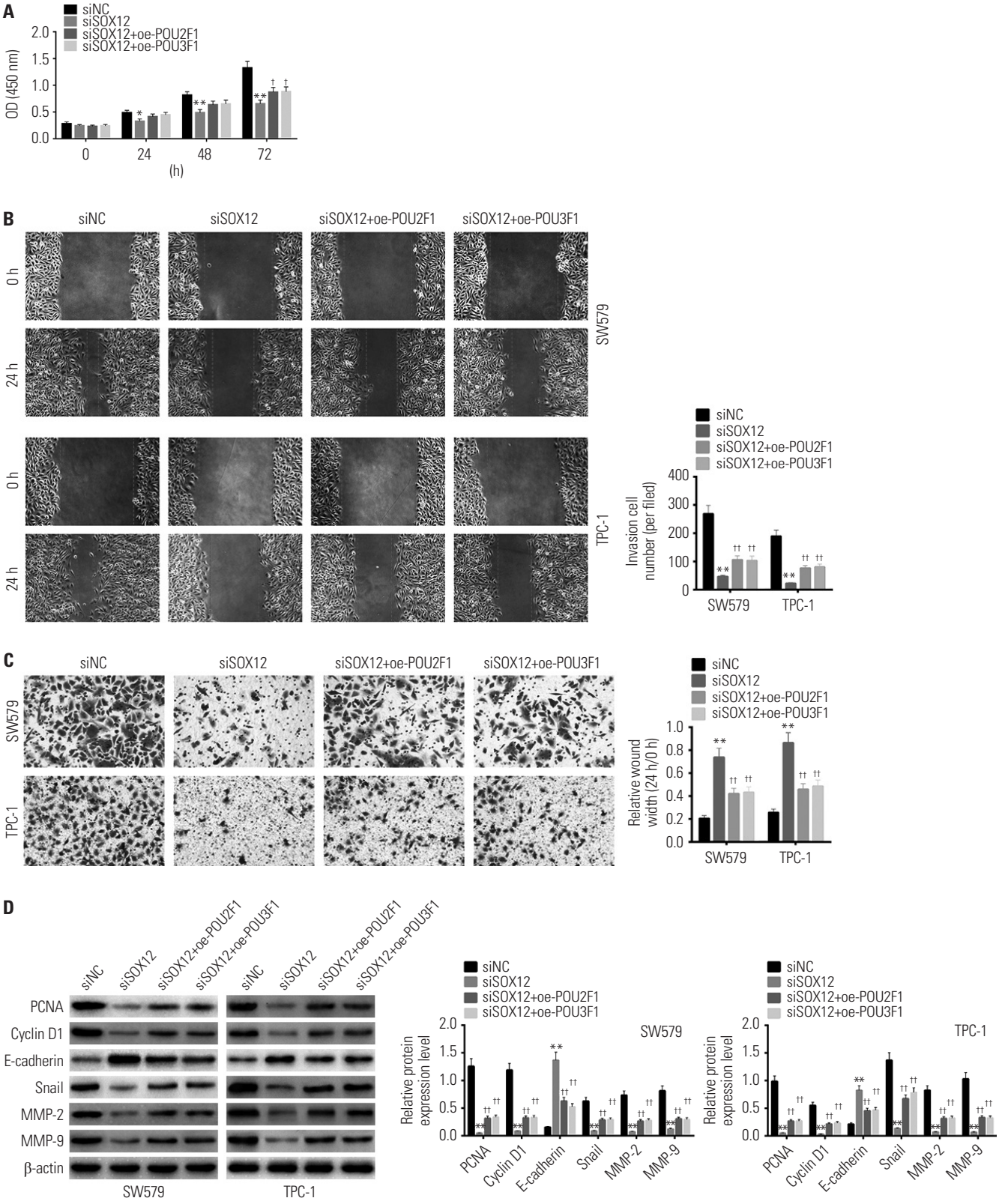

Fig. 5. SOX12 regulates the proliferation, migration, and invasion of thyroid cancer cells by regulating POU2F1 and POU3F1. SW579 cells were transfected with siSOX12 and then co-transfected with oe-POU2F1 or oe-POU3F1. (A) Proliferation of SW579 cells was determined by CCK8 assay. (B) Migration of SW579 cells was identified by wound healing assay. (C) Invasion of SW579 cells was identified through transwell assay. (D) The expression of PCNA, Cyclin D1, E-cadherin, Snail, MMP-2, and MMP-9 was determined by Western blot. *p<0.05, **p<0.01 vs. siNC cells; †p<0.05, ††p<0.01 vs. siSOX12 cells. OD, optical density.

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expression is related with tumor stage and tumor size in thyroid cancer patients.

Compared with normal cells, tumor cells exhibit high proliferative, migratory, and invasive abilities.[22] Accordingly, we performed cellular functional experiments to assess the effect of SOX12 expression on thyroid cancer cells. We found that SOX12 knockdown inhibited cell proliferation, migration, and invasion. The expression of PCNA, Cyclin D1, E-cadherin, Snail, MMP-2, and MMP-9 was also determined. PCNA and cyclin D1 are two well-known biomarkers for tumor cell proliferation, both of which are expressed in the G1 phase of the cell cycle.[23] PCNA is a necessary factor for DNA synthesis, and its positive expression in the nucleus is closely related to cell proliferation.[24,25] Cyclin D1 plays an important role in G1 phase regulation of the cell cycle, which is closely related to the growth, deterioration, and metastasis of tumors.[24,26,27] E-cadherin and Snail are key proteins in the epithelial-mesenchymal transition process.[28,29] MMP-2 and MMP-9 are crucial regulators in TPC cell migration.[30,31] Our results showed that SOX12 silencing suppresses the expression of PCNA, Cyclin D1, Snail, MMP-2, and MMP-9 and promotes E-cadherin expression. Accordingly, we deemed that SOX12 promotes the proliferation, migration, and invasion of thyroid cancer cells by regulating the expression of PCNA, Cyclin D1, E-cadherin, Snail, MMP-2, and MMP-9.

Furthermore, through bioinformatic analysis, we found that SOX12 interacts with several members of the POU family (POU2F1, POU2F2, POU3F1, and POU3F2). Multiple studies have demonstrated that POU2F1, POU2F2, and POU3F1 expression is up-regulated in cancer tissues, potentially acting in oncogenic roles.[32-35] Ding, et al.[35] reported that POU2F1 regulates the HOXD10 and HOXD11 expression and facilitates the proliferation and invasion of head and neck cancer cells. Wang, et al.[34] indicated that a POU2F2-oriented network promotes the progression and metastasis of human gastric cancer. Ding, et al.[35] showed that POU3F2 promote tumor cell growth and metastasis in hepatocellular carcinoma, the expression of which appears to be regulated by long non-coding RNA BCYRN1. Notwithstanding, no studies have investigated the expression and role of POU3F2 in thyroid cancer. Our study found that POU3F2 expression in thyroid cancer tissue is not significant and suggests that POU3F2 exerts no obvious effect on the occurrence and development of thyroid cancer. Meanwhile, we found that the expression of POU2F1, POU2F2, and POU3F1 is increased in the cancer tissues of thyroid cancer patients. Moreover, SOX12 expression showed a positive correlation with POU2F1, POU3F1 and POU3F2 expression in thyroid cancer clinical samples. We also found that SOX12 knockdown inhibited the expression of POU2F1, POU2F2, POU3F1, and POU3F2 in thyroid cancer cells. Finally, we proved that overexpression of POU2F1 or POU3F1 effectively reversed the effect of siSOX12 on the proliferation, migration, and invasion of SW579 cells. Altogether, these results indicate that SOX12 promotes the proliferation, migration, and invasion of thyroid cancer cells by regulating the expression of POU2F1 and POU3F1. POU2F1 and POU3F1 might function as oncogenes in the occurrence and progression of thyroid cancer, which needs further research. Due to limitations in the experimental conditions, direct interactions between SOX12 and POU2F1/POU3F1 were not investigated. Further mechanism studies are needed.

In conclusion, we determined that SOX12 is expressed excessively in thyroid cancer samples, promoting tumor cell proliferation, migration and invasion. We noted that SOX12 accelerated the cancerous phenotype of thyroid cancer cells by regulating the expression of POU2F1 and POU3F1. These findings provide new insights into understanding the mechanism of thyroid cancer progression and provide direction for exploring new biomarkers of use in thyroid cancer diagnosis and treatment.

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AUTHOR CONTRIBUTIONS

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