Article

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CCT2 Regulates *ZEB1*-Induced EMT Gene Transcription to Promote the Metastasis and Tumorigenesis of Papillary Thyroid Carcinoma

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Background: Papillary thyroid carcinoma (PTC) is the most common malignant tumor of the thyroid, and its invasiveness and metastatic ability are closely related to patient prognosis. Chaperonin containing TCP1 subunit 2 (CCT2) is an important component of the molecular chaperone protein complex and has been shown to regulate cell proliferation and migration in various tumors. Epithelial-mesenchymal transition (EMT) is a critical process in tumor metastasis, and Zinc Finger E-Box Binding Homeobox 1 (*ZEB1*) is a core transcription factor that regulates EMT. This study aims to explore how *CCT2* induces EMT gene transcription through *ZEB1*, thereby promoting the metastasis and tumorigenesis of PTC.

Methods: CCT2 in PTC tissues was analyzed using quantitative reverse transcription polymerase chain reaction (qRT-PCR) and Western blot. siRNA and overexpression vectors were used to silence and overexpress *CCT2*, respectively, and the effects on PTC cell migration, invasion, proliferation, and apoptosis were observed. Rescue experiments were used to investigate the effect of *CCT2* on *ZEB1* and EMT-related genes. Cell apoptosis was detected by Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) assay. Silencing *ZEB1* was used to verify its effect on the oncogenic activity of *CCT2*.

Results: *CCT2* was found to be highly expressed in PTC tissues ($p < 0.01$). In *in vitro* and *in vivo* experiments, silencing *CCT2* inhibited the migration and invasion of PTC cells and their metastasis, while overexpression of *CCT2* produced the opposite effect. Additionally, *CCT2* promoted PTC cell proliferation and inhibited apoptosis ($p < 0.01$). Mechanistic studies revealed that *CCT2* upregulated *ZEB1* expression ($p < 0.01$), thereby inducing EMT gene transcription ($p < 0.01$). Silencing *ZEB1* reduced the oncogenic effect of *CCT2*.

Conclusion: This study first revealed the high expression of *CCT2* in PTC and its essential role in the migration, invasion, proliferation, and anti-apoptosis of tumor cells. *CCT2* promotes the metastasis and tumorigenesis of PTC by regulating *ZEB1* and EMT-related genes. These findings provide new potential targets for molecular targeted therapy of PTC and explore new directions for future clinical treatment strategies.

Keywords: *CCT2*; thyroid papillary carcinoma; EMT; *ZEB1*; tumor metastasis

Introduction

Thyroid carcinoma is the most common malignant tumor of the endocrine system globally, with papillary thyroid carcinoma (PTC) being the most common subtype, accounting for approximately 80% of all thyroid cancer cases [1–3]. Despite a relatively favorable prognosis, approximately 10–15% of patients experience local recurrence or distant metastasis, reducing survival rates and quality of life [4,5]. Hence, it is crucial to thoroughly comprehend the molecular mechanisms of PTC, specifically the pivotal controlling elements implicated in metastasis and tumorigenesis, to enhance patients' clinical treatment and prognosis [6–8]. Chaperonins, a type of molecular chaperone protein, aid in the accurate folding and formation of additional proteins [9]. Chaperonin containing TCP1 (CCT) is a specific molecular chaperone protein complex composed of 8 different subunits, among which chaperonin containing TCP1 subunit 2 (CCT2) is one [10–12]. Recent studies have shown that CCT2 is essential for the normal physiological functions of cells and significantly impacts the progression of different types of cancer [13,14]. The abnormal manifestation of CCT2 is strongly linked to the growth, movement, and infiltration abilities of cancer cells, although its precise function and mechanism in PTC have yet to be completely understood [15–18].

Epithelial-mesenchymal transition (EMT) refers to a biological phenomenon where epithelial cells transform, losing their adhesion and polarity, gaining the ability to migrate and invade, and adopting a mesenchymal cell pheno-

type [19–22]. EMT is of utmost importance in the development of embryos, the healing of tissues, and the invasion and spread of tumors [23,24]. EMT is considered a crucial process in oncology, facilitating the separation of cancer cells from the main tumor, invasion into nearby tissues, and development of distant metastases [25]. Zinc Finger E-Box Binding Homeobox 1 (ZEB1), a crucial transcription factor for EMT, controls the expression of various EMT-associated genes [26–28]. This includes suppressing epithelial markers like E-cadherin and enhancing mesenchymal markers like vimentin and N-cadherin.

This investigation aims to comprehensively examine the involvement of CCT2 in PTC pathogenesis and its impact on key cellular processes such as migration, invasion, proliferation, and apoptosis. By leveraging quantitative reverse transcription polymerase chain reaction (qRT-PCR) and Western blot analyses, we assessed *CCT2* expression levels in PTC tissues, establishing its correlation with tumor invasiveness and metastatic capacity. Through targeted genetic manipulation involving *CCT2* silencing and overexpression, coupled with functional assays *in vitro* and *in vivo*, we delineated the influence of *CCT2* on PTC cell behavior. Furthermore, we investigated the interplay between *CCT2* and *ZEB1*, shedding light on the molecular mechanisms governing *CCT2*-mediated EMT gene regulation. Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) assays were employed to assess the impact of CCT2 on apoptosis, while *ZEB1* silencing validated its role in mediating the oncogenic effects of *CCT2*. This study elucidates the mechanisms underlying *CCT2*-induced transcriptional regulation of EMT genes via *ZEB1*, thereby fueling PTC's metastatic potential and tumorigenesis.

Materials and Methods

Thyroid Papillary Carcinoma Specimen Collection

Seventeen individuals diagnosed with thyroid papillary carcinoma, consisting of 10 males and 7 females, ranging in age from 31 to 69 years with an average age of 48 years, were chosen for inclusion in the study. The clinical patients involved in this study have all signed informed consent forms. These patients received inpatient care at the hospital's Department of Thyroid and Breast Surgery between January 2022 and July 2022. This study was conducted in accordance with the Declaration of Helsinki. Tumor, Node, Metastasis (TNM) staging was conducted according to the AJCC Cancer Staging Manual criteria. Adjacent non-tumor normal tissues were selected as the control group. All cases had not received radiotherapy, chemotherapy, or hormone therapy before surgery, and their medical records were complete. Around 200 mg of samples were gathered in one go and promptly preserved at –80 °C, while the rest of the tissues were dispatched for regular pathological analysis to verify the findings. Ethical approval: this study has been approved by the Medical Ethics Committee of Taizhou Central Hospital (Taizhou University Hospital) (Approval No.: 2024L-04-06).

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Total RNA isolation reagent TRIzol (15596026) was purchased from Gibco (Carlsbad, CA, USA). The qRT-PCR kit (4442137) was purchased from Gibco (Carlsbad, California, USA). Primers were synthesized by Beijing Saibaisheng Company (Beijing, China). The tissue extracted total RNA following the manufacturer's instructions for the TRIzol reagent. The extracted RNA was then dissolved in DEPC water and stored at –80 °C. M-MLV reverse transcriptase (K1622, Gibco, Carlsbad, CA, USA) was used, with 3 µL of total RNA from each tissue sample used for reverse transcription. The size of the reaction system was 20 microliters. After adding and combining each reagent as instructed, the mixture was placed in an incubator at 37 °C for 1 hour. Subsequently, the reaction was halted by raising the temperature to 95 °C for 5 minutes. The cDNA obtained was kept at a temperature of –20 °C. To make a reaction system, combine 10× Buffer (2.5 µL), dNTP (2.5 µL), TaqDNA polymerase (0.5 µL), upstream and downstream specific primers (1 µL each), template (1.5 µL), and DEPC-treated ddH_2O to reach a total volume of 25 µL. The components were mixed thoroughly and then centrifuged. The primer sequences used in this study are listed in Table 1. Load the qRT-PCR reaction mixture into the PCR instrument (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) for PCR amplification. During the PCR amplification process, the fluorescently labeled probes will hybridize with the target sequences and emit fluorescent signals. Use the software analysis tools on the qRT-PCR instrument (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) to detect and analyze the fluorescent signals.

Western Blot

Total protein was extracted from transfected cells of each group using RIPA lysis buffer (R0010, Solarbio, Beijing, China). The BCA assay kit (23225, Gibco, Carlsbad, CA, USA) was utilized to measure and modify the protein concentration. The proteins were first separated using SDS-PAGE and transferred onto a PVDF membrane (88518, Gibco, Carlsbad, CA, USA). The membrane was blocked in 5% skim milk TBST buffer for 2 hours. It was then incubated with primary antibodies CCT2 (1:1000 dilution; cat no. ab92746, Abcam, Cambridge, UK), ZEB1 (1:1000 dilution; cat no. Ab203829, Abcam, Cambridge, UK), E-cadherin (1:1000 dilution; cat no. Ab40772, Abcam, Cambridge, UK), Vimentin (1:1000 dilution; cat no. Ab92547, Abcam, Cambridge, UK), Smad3 (1:1000 dilution; cat no. Ab40854, Abcam, Cambridge, UK), phosphorylated Smad3 (p-Smad3) (1:1000 dilution; cat no. Ab52903, Ab-

Table 1. Primer sequences were involved in this study.

Primer name	Primer sequences (5′-3′)
CCT2-F	GCACTACCTCTGTTACCGTTTT
CCT2-E	CTTCTCTCCAACCCGCTATGA
ZEB1-F	GGCATACACCTACTCAACTACGG
ZEB1-R	TGGGCGGTGTAGAATCAGAGTC
Slug-F	CTTCCTGGTCAAGAAGCA
Slug-R	GGGAAATAATCACTGTATGTGTG
Vimentin-F	TGTCCAAATCGATGTGGATGTTTC
Vimentin-R	TTGTACCATTCTTCTGCCTCCTG
E-cadherin-F	CTTCTGCTGATCCTGTCTGATG
E-cadherin-R	TGCTGTGAAGGGAGATGTATTG
FN1-F	GAGAATAAGCTGTACCATCGCAA
FN1-R	CGACCACATAGGAAGTCCCAG
CTNNB1-F	TGATGGAGTTGGACATGGCC
CTNNB1-R	CTCATACAGGACTTGGGAGG
GAPDH-F	CGGAGTCAACGGATTTGGTCGTAT
GAPDH-R	AGCCTTCTCCATGGTGGTGAAGAC

CCT2, chaperonin containing TCP1 subunit 2; ZEB1, Zinc Finger E-Box Binding Homeobox 1; *Slug*, Snail Family Transcriptional Repressor 2; *FN1*, Fibronectin 1; *CTNNB1*, Catenin beta-1; *GAPDH*, Glyceraldehyde 3-phosphate dehydrogenase.

cam, Cambridge, UK) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:1000 dilution; cat no. Ab8245, Abcam, Cambridge, UK) at 4 °C overnight. Following three washes with TBST, the membrane underwent incubation at 37 °C for 1 hour with Horseradish Peroxidase (HRP)-conjugated secondary antibody, specifically goat anti-rabbit IgG (1:1000 dilution; cat no. Ab6728, Abcam, Cambridge, UK). Enhanced Chemiluminescence (ECL) (MT0024, MeilunBio, Dalian, China) was employed, and the grayscale measurements of the protein bands were analyzed utilizing Quantity One software (version 4.6.6, Bio-Rad Laboratories, Inc, Helsingborg, CA, USA).

Cell Culture

Cell lines derived from human thyroid carcinoma (KTC-1 (iCell-h370), B-CPAP (iCell-h022) (Cellverse Bioscience Technology Co. Ltd., Shanghai, China)) were grown in RPMI-1640 medium (11875119, Invitrogen, Carlsbad, CA, USA) containing 100 units/mL penicillin (P4458, Sigma-Aldrich, St. Louis, MO, USA), 0.1 mg/mL streptomycin (P4458, Sigma-Aldrich, St. Louis, MO, USA), and 10% fetal bovine serum (F8067, Sigma-Aldrich, St. Louis, MO, USA). All cell lines were maintained in a humidified atmosphere at 37 °C with 5% CO_2. The cell lines involved in this study have all completed STR identification, and the mycoplasma testing results are negative.

Cell Transfection

CCT2 shRNA-NC (sense: 5′-GCTAAGCTTCGAAGTGTGTT-3′, antisense: 5′-AACACACTTCGAAGCTTAGC-3′), Sh-*CCT2*-1 (sense: 5′-CCTGAAGATGAAGATGAAGA-3′, antisense: 5′-TCTTCATCTTCATCTTCAGG-3′), Sh-*CCT2*-2 (sense: 5′-CCAGAAGGAAGATCAGTTCA-3′, antisense: 5′-TGAACTGATCTTCCTTCTGG-3′), siRNA-NC (Negative control) (sense: 5′-GUACGUACGUACGUACGUACG-3′, antisense: 5′-CGUACGUACGUACGUACGUAC-3′), and siRNA-*ZEB1* (sense: 5′-GAAGGAUCAUCAAGAACUATT-3′, antisense: 5′-UAGUUCUUGAUGAUGCUUCCT-3′) were obtained from Thermo Scientific, Waltham, MA, USA. One day before transfection, cells were seeded in 6-well plates at a density of 1 $\times$ 10^5 cells per well—the transfection efficiency aimed for 50% confluent cells. Lipofectamine 2000 (11668500, Invitrogen, Carlsbad, CA, USA) was used for cell transfection. Confirmation of efficient shRNA knockdown was achieved through qRT-PCR and Western blot analysis. Lipofectamine 2000 (11668500, Invitrogen, Carlsbad, CA, USA) was used to transfect cells with pcDNA3.1-CCT2 (sense: 5′-ATGGAATTCGCCACCATGGCCGCTTCGAGGTGGCGGG-3′, antisense: 5′-ATGCTCGAGTCAGGAACTGCAACGTAG-3′) or the control pcDNA3.1-vector plasmid (sense: 5′-ATGTCTAGATCGGCCGCCACCATGG-3′, antisense: 5′-ATGCTCGAGTCATTTGTAGAGGCT-3′). After 48 hours of transfection, the cells were analyzed using qRT-PCR and Western blot techniques.

Transwell Assay for Cell Migration and Invasion

Migration assay: 2 $\times$ 10^4 cells were seeded in the upper chamber of Transwell (3381, Corning Corporation, Corning, NY, USA). For the invasion experiment, a total of 100,000 cells were placed in the top compartment of a Transwell, which had been coated with matrix gel. The lower chamber was filled with 800 µL of RPMI-1640 medium containing 10% FBS (with double antibodies). The cells were cultured for 2 days, treated with 70% ethanol at low temperature for 60 minutes, and then subjected to staining using 0.5% crystal violet for 20 minutes. The process of cell movement and infiltration was observed using a microscope (CX83, Olympus, Tokyo, Japan), and the total count of cells that migrated and invaded was recorded in 5 different fields of view.

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL)

The cells were treated with 4% paraformaldehyde to be fixed. Following permeabilization with 0.1% Triton X-100 in PBS on ice for 2 minutes, the cells were exposed to TUNEL solution (T2195, Solarbio, Beijing, China) at 37 °C in a lightless environment for 60 minutes. Subsequently, cells were sealed with an anti-fluorescence quencher. The observation was conducted under a fluorescence microscope (IX83, Olympus, Tokyo, Japan).

EDU Cell Proliferation Assay

The cells were placed in a 96-well plate with 5 $\times$ 10^3 cells per well. Following 6 hours, the cells were subjected

to an incubation period of 2 hours with 100 uL of EDU solution (CA1174, Solarbio, Beijing, China). After fixing and incubating with 2 mg/mL glycine for 5 minutes, the cells were treated with 100 uL of permeabilization solution for 10 minutes. Then, 100 uL of 1× Apollo staining reaction solution was added and incubated for 30 minutes. The wells were washed with 100 uL of permeabilization solution for 3 minutes and then with 100 µL of methanol for 5 minutes. Subsequently, 100 µL of 1× DAPI reaction solution was added to the wells and incubated in the dark on a shaker for 30 minutes. The cells were then sealed with an anti-fluorescence quencher. Finally, cell images were captured under a fluorescence microscope (IX83, Olympus, Tokyo, Japan), and the cell count was recorded. Cells with red-stained nuclei were considered positive.

Cell Proliferation Assessed through CCK-8 Assay and Plate Clone Formation Experiment

The cells from every group were cultured at 37 degrees Celsius in 5% carbon dioxide. 10 uL of Cell Counting Kit-8 (CCK-8) solution (CA1210, Solarbio, Beijing, China) was introduced into every well, and the microplate reader (SpectraMax iD3, Molecular Devices, San Jose, CA, USA) was utilized to measure the absorbance value (OD) at a wavelength of 450 nm for each well. Cells from each group were uniformly seeded in culture dishes at a density of 200 cells per well and cultured in a 37 °C, 5% CO_2 incubator for 2 weeks. After the appearance of clones, crystal violet staining was performed for 30 minutes, and counting and photography were conducted.

Statistical Analysis

Statistical analysis was performed using SPSS version 21.0 software (IBM, Armonk, NY, USA). The data were reported as the average plus the standard deviation. Between-group comparisons were made using *t*-tests, and comparisons among multiple groups were made using one-way analysis of variance (ANOVA). The Tukey test is conducted for post hoc analysis. A *p*-value below 0.05 was deemed to be statistically significant.

Results

The Expression of CCT2 is Upregulated in PTC

Initially, *CCT2* mRNA in 17 adjacent normal and primary tumor tissue pairs were analyzed. The results showed a high expression of *CCT2* in PTC (Fig. 1A, $p < 0.01$). This indicates an upregulation of *CCT2* mRNA levels in cancer tissues, which may be associated with the occurrence and development of cancer. The detection of CCT2 protein in normal tissues and primary tumor tissues was performed using Western blot analysis. Fig. 1B showed that tumor tissues exhibited increased expression of CCT2 compared to adjacent normal tissues. These findings are consistent with the mRNA level data, providing additional confirmation of the elevated expression of *CCT2* in PTC ($p < 0.01$).

Silencing of CCT2 Inhibits the Migration and Invasion of PTC Cells in Vitro

B-CPAP cells were transfected with two different *CCT2* silencing plasmids to silence the *CCT2* gene. qRT-PCR and Western blot were used to assess transfection efficiency, revealing the highest expression of *CCT2* in the shNC group and relatively lower expression in the sh*CCT2*-1 and sh*CCT2*-2 groups ($p < 0.05$). This indicates successful silencing of the *CCT2* gene (Fig. 2A,B). Transwell assay results showed that cell migration and invasion were highest in the shNC group while relatively lower in the sh*CCT2*-1 and sh*CCT2*-2 groups ($p < 0.01$). This suggests that silencing of *CCT2* inhibited PTC cells' migration and invasion ability (Fig. 2C,D). In summary, the experimental results demonstrate that silencing of *CCT2* inhibits the occurrence of PTC metastasis by suppressing cell migration, invasion, and the formation of lung metastatic foci.

Overexpression of CCT2 Promotes the Migration and Invasion of PTC Cells in Vitro

KTC-1 cells were transfected with *CCT2* overexpression plasmids for the overexpression of *CCT2* gene. Transfection efficiency was evaluated using qRT-PCR and Western blot, revealing that *CCT2* expression was higher in the OE-*CCT2* group compared to the OE-NC group ($p < 0.01$). This indicates successful overexpression of the *CCT2* gene (Fig. 3A,B). The transwell assay results showed increased cell migration and invasion in the OE-*CCT2* group compared to the OE-NC group ($p < 0.01$). These findings suggest that the upregulation of *CCT2* enhances PTC cells' migratory and invasive potential, as shown in Fig. 3C,D. The experimental results indicate that the overexpression of CCT2 promotes cell migration and invasion.

The Proliferation of PTC Cells is Enhanced, and Apoptosis is Suppressed by CCT2

CCK-8 assay was used to evaluate the proliferation ability of cells. In this experiment, B-CPAP cells were divided into shNC, sh*CCT2*-1, and sh*CCT2*-2. According to the findings, the cells in the shNC group exhibited the highest proliferation capacity, whereas the sh*CCT2*-1 ($p < 0.05$) and sh*CCT2*-2 groups ($p < 0.01$) displayed comparatively lower proliferation abilities (Fig. 4A). This suggests that the suppression of *CCT2* hampers the capacity of PTC cells to proliferate. EdU assay was also used to evaluate the proliferation ability of cells. The findings indicated that the cell's capacity to significantly decreased in the sh*CCT2*-1 and sh*CCT2*-2 groups compared to the shNC group ($p < 0.001$). Fig. 4B,B-1 provides additional evidence for the suppressive impact of *CCT2* knockdown on the growth potential of PTC cells. Clonogenic assay was used to evaluate the proliferation and growth potential of cells. According to the findings, the shNC group had the highest number of cell clones, whereas the sh*CCT2*-1 and sh*CCT2*-2 groups had comparatively fewer ($p < 0.01$). This also indicates

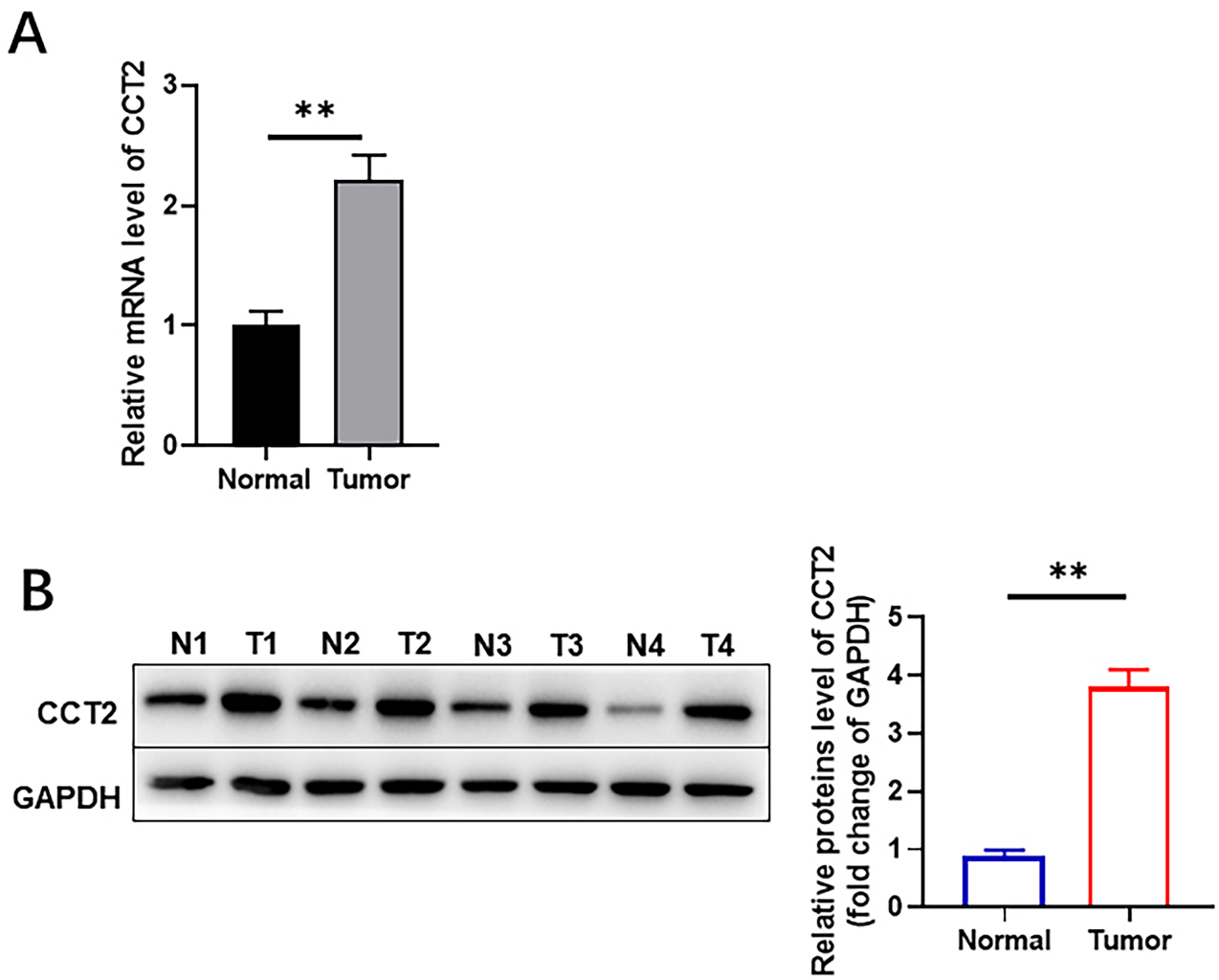

Fig. 1. Expression analysis of *CCT2* in thyroid papillary carcinoma, showing high expression of *CCT2* in thyroid papillary carcinoma. (A) *CCT2* mRNA in 17 pairs of normal tissues and primary tumor tissues. (B) Analysis of CCT2 expression levels in normal and primary tumor tissues using Western blot. $^{**}p < 0.01$. n = 17.

that silencing of *CCT2* inhibits PTC cells' proliferation and growth potential (Fig. 4C). TUNEL assay was used to evaluate the apoptosis rate of cells. The results showed an increase in the apoptosis rate of cells in the sh*CCT2*-1 and sh*CCT2*-2 groups compared to the NC group ($p < 0.01$). This indicates that silencing or inhibition of *CCT2* induces apoptosis of PTC cells (Fig. 4D). The findings from the experiment suggest that suppressing *CCT2* hinders the growth potential of PTC cells and triggers apoptosis.

CCT2 Regulates EMT-Related Genes in PTC

Experimental results showed that after knocking down the *CCT2* gene, *ZEB1* was highest in the shNC group and lower in the sh*CCT2*-1 and sh*CCT2*-2 groups ($p < 0.01$) (Fig. 5A). Following *CCT2* gene knockdown, Snail Family Transcriptional Repressor 2 (*Slug)* was highest in the shNC group and lower in the sh*CCT2*-1 and sh*CCT2*-2 groups ($p < 0.05$) (Fig. 5B). During the EMT process, cells express more Vimentin. Experimental results showed that after *CCT2* gene knockdown, Vimentin was highest in the shNC group and lower in the sh*CCT2*-1 ($p < 0.01$) and sh*CCT2*-2 groups ($p < 0.05$) (Fig. 5C). The experimental findings indicated that following the suppression of the *CCT2* gene, the levels of E-cadherin were the lowest in the shNC group. At the same time, they were comparatively higher in the sh*CCT2*-1 and sh*CCT2*-2 groups ($p < 0.01$) (Fig. 5D). This suggests that *CCT2* knockdown may promote E-cadherin, affecting the EMT process. Following *CCT2* gene knockdown, Fibronectin 1 (*FN1)*, and Catenin beta-1 (*CTNNB1)* were highest in the shNC group and lower in the sh*CCT2*-1 ($p < 0.05$) and sh*CCT2*-2 groups ($p < 0.01$). This indicates that *CCT2* knockdown may inhibit *FN1* and *CTNNB1*, affecting the EMT process (Fig. 5E,F).

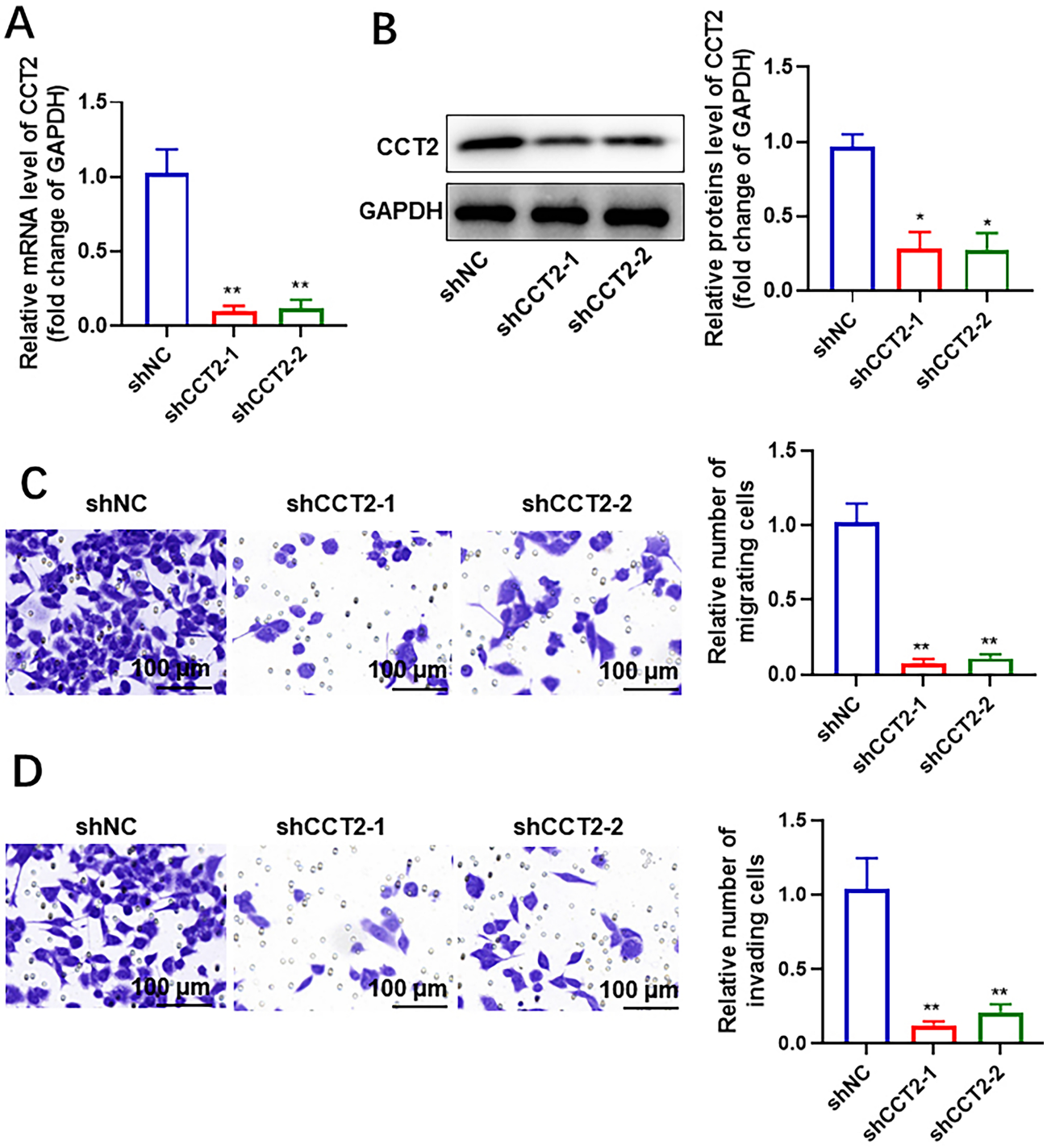

Fig. 2. Silencing of *CCT2* inhibits the migration and invasion of papillary thyroid carcinoma (PTC) cells *in vitro*. (A,B) B-CPAP cells were transfected with two different *CCT2* silencing plasmids. qRT-PCR (A) and Western blot (B) to detect transfection efficiency. (C,D) Representative images of migration (C) and invasion (D). *$p < 0.05$, **$p < 0.01$. n = 6. NC, Negative control.

Silencing ZEB1 Reduces the Oncogenic Effect of CCT2

Transwell experiments showed that silencing *ZEB1* reversed the increased migration and invasion abilities of KTC-1 cells expressing *CCT2*. In KTC-1 cells expressing *CCT2*, migration and invasion abilities were increased compared to the control group (OE-NC) ($p < 0.01$). However, when *ZEB1* was silenced, the migration and invasion abilities were reversed ($p < 0.01$) (Fig. 6A–D). This indicates that silencing *ZEB1* inhibits the *CCT2*-induced cell migration and invasion abilities. Western blot analysis was used to detect ZEB1, Smad3, E-cadherin, Vimentin, and phosphorylated Smad3. The results showed that in KTC-1 cells

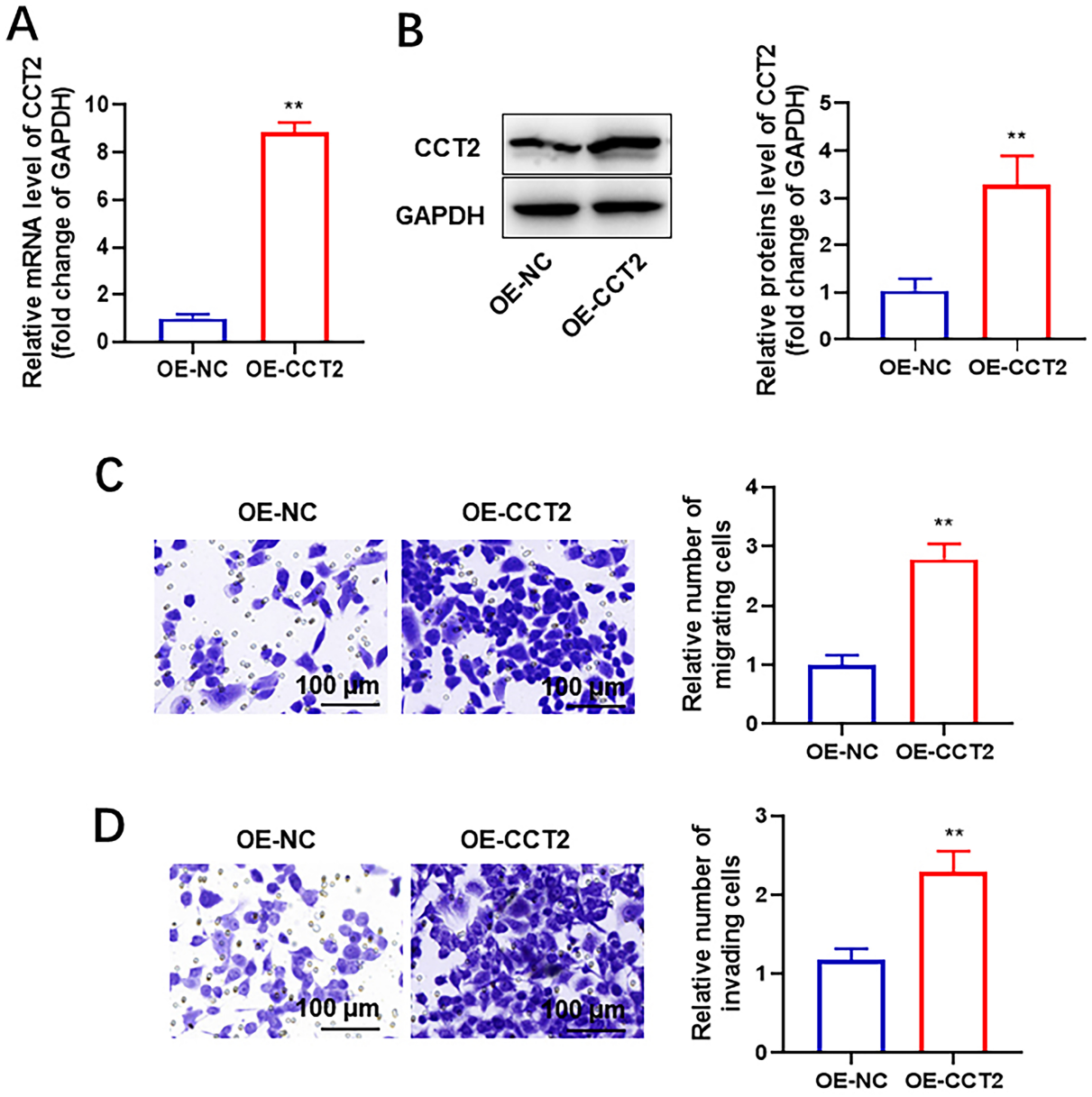

Fig. 3. Overexpression of *CCT2* promotes the migration and invasion of thyroid papillary carcinoma cells *in vitro*. (A) KTC-1 cells transfected with *CCT2* overexpression plasmid. qRT-PCR to detect transfection efficiency. (B) KTC-1 cells transfected with *CCT2* overexpression plasmid. Western blot to detect transfection efficiency. (C,D) Representative pictures illustrating the migration (C) and invasion (D) of KTC-1 cells. $^{**}p < 0.01$. n = 6.

overexpressing *CCT2*, *ZEB1*, *Vimentin* and *p-Smad3* were high ($p < 0.01$), while *E-cadherin* was low ($p < 0.01$). Conversely, ZEB1 silencing resulted in reduced ZEB1 and Vimentin levels, increased E-cadherin ($p < 0.01$), and a decrease in p-Smad3 ($p < 0.01$) (Fig. 6E–I).

Discussion

Many studies have indicated that the overexpression of *CCT2* is associated with tumor invasion and metastasis in various cancers [14,17,29,30]. In this study, we found that *CCT2* induces the transcription of EMT genes through the regulation of *ZEB1*, promoting the metastasis and tumorigenesis of PTC. This discovery provides new targets and strategies for the treatment of PTC.

Our research results demonstrate that the high expression of *CCT2* in PTC is more pronounced than in normal thyroid tissue, consistent with previous studies that revealed *CCT2* overexpressed in various cancer types and associated with poor clinical prognosis [16,31]. We further revealed the impact of *CCT2* on PTC cell migration and invasion. Silencing *CCT2* inhibited the migration and invasion capabilities of PTC cells, while overexpression of *CCT2* pro-

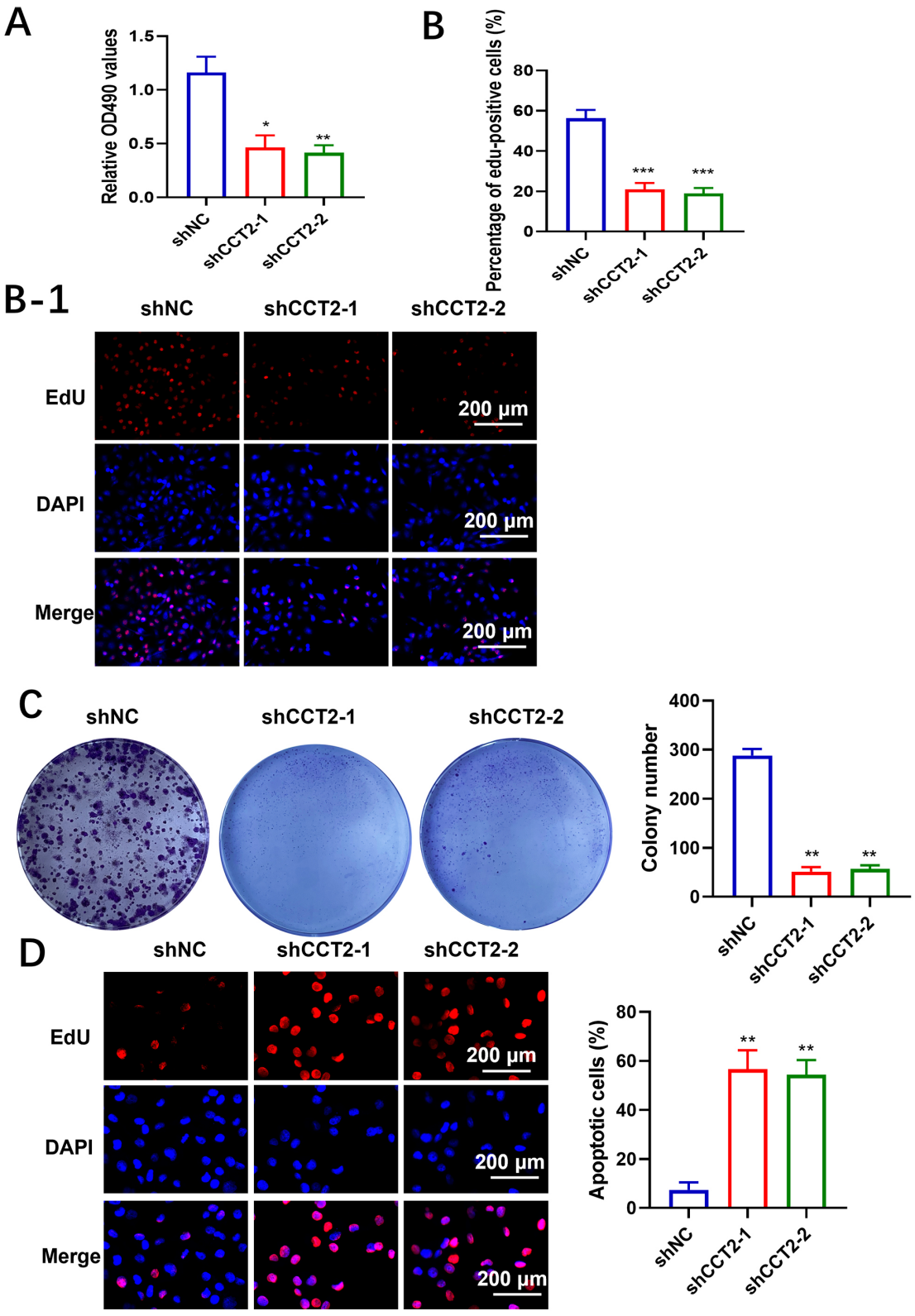

Fig. 4. *CCT2* promotes proliferation and inhibits apoptosis of thyroid papillary carcinoma cells. (A) Cell Counting Kit-8 (CCK-8) assay in B-CPAP cells. (B&B-1) EdU assay in B-CPAP cells. (C) Clone formation assay showing that *CCT2* silencing inhibits the proliferation of B-CPAP cells. (D) Terminal deoxynucleotidyl transferase dUTP Nick End Labeling (TUNEL) results indicate that *CCT2* silencing or inhibition induces apoptosis in B-CPAP cells. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. n = 6.

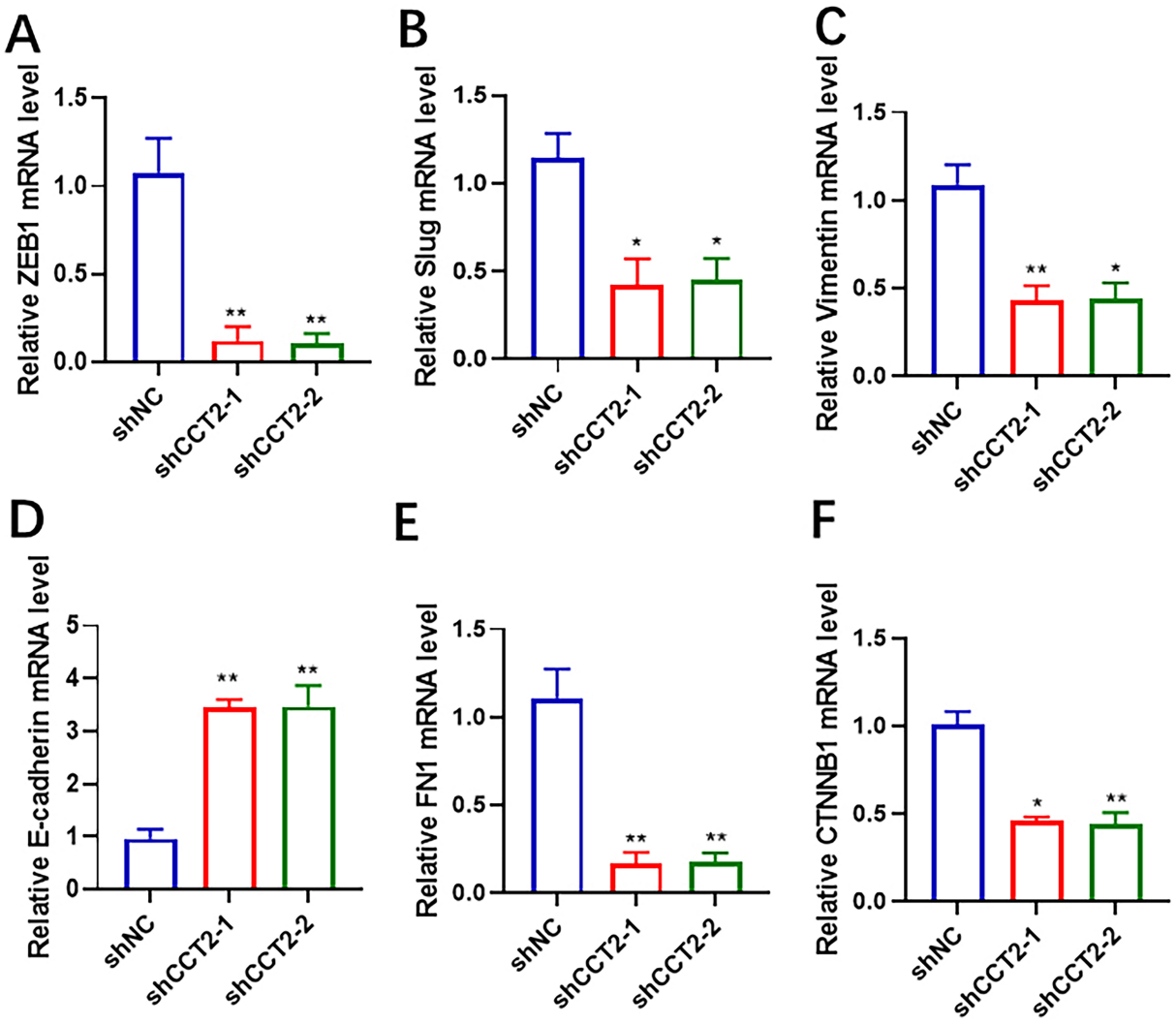

Fig. 5. *CCT2* regulates *EMT*-related genes in thyroid papillary carcinoma. (A–F) Quantitative analysis of *ZEB1* (A), *Slug* (B), *Vimentin* (C), *E-cadherin* (D), *FN1* (E), and *CTNNB1* (F) levels. *$p < 0.05$, **$p < 0.01$. n = 6.

moted these processes. These findings suggest that *CCT2* may serve as an essential regulatory factor in the progression of PTC. Additionally, *CCT2* can encourage the proliferation of PTC cells and inhibit apoptosis, further indicating its pro-carcinogenic role in PTC development. This is consistent with the role of *CCT2* in other cancers, such as breast cancer and colorectal cancer, where its overexpression is associated with increased proliferation and survival of cancer cells [14,17,32].

Further mechanistic studies revealed that *CCT2* can regulate multiple *EMT*-related genes, implying that *CCT2* may promote PTC metastasis through the EMT mechanism. EMT is the process by which cancer cells undergo a transition from epithelial to mesenchymal cells, enhancing their migration and invasion capabilities and being associated with chemotherapy resistance [15,33]. Silencing *ZEB1* can attenuate the pro-carcinogenic effect of *CCT2*, indicating the crucial role of *ZEB1* in the *CCT2*-mediated EMT process. *ZEB1* is a known EMT transcription factor that promotes EMT by suppressing epithelial markers and activating mesenchymal markers [34]. Our data support a model in which *CCT2* regulates the transcription of *EMT*-related genes through *ZEB1*, thereby promoting the migration and invasion of PTC.

Based on our research findings, we observed a significant increase in phosphorylated Smad3 levels in *CCT2*-expressing KTC-1 cells, whereas silencing *ZEB1* resulted in a notable decrease in phosphorylated Smad3 levels. This suggests that *CCT2* may promote tumor cell migration and invasion by activating the Smad3 signaling pathway. Smad3 is a crucial member of the TGF-β/Smad signaling pathway, and its phosphorylation status is often considered a key indicator of pathway activity. Under normal circumstances, TGF-β signaling regulates cell growth, proliferation, and differentiation by inducing Smad3 phosphorylation. However, in cancer, aberrant activation of the TGF-β/Smad signaling pathway is frequently associated with tumor progression and metastasis.

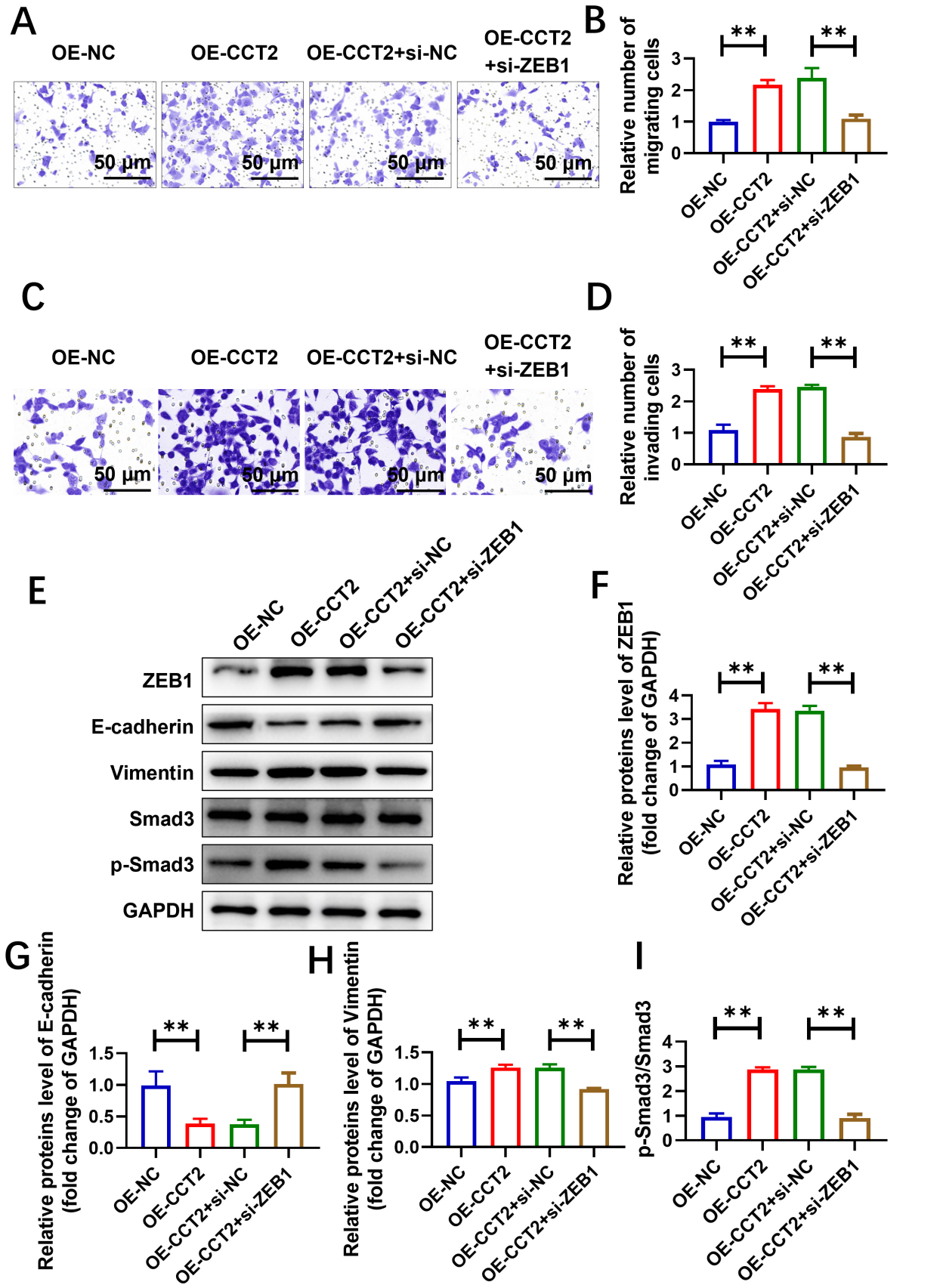

Fig. 6. Silencing of ZEB1 reduces the oncogenic effect of CCT2. (A–D) The transwell experiment showing that the knockdown of ZEB1 reverses the increased migration (A,B) and invasion (C,D) ability of KTC-1 cells to express CCT2. (E–I) Western blot analysis of ZEB1(F) expression levels and levels of E-cadherin (G), Vimentin (H), and phosphorylated Smad3/Smad (I). $^{**}p < 0.01$. n = 6.

EMT is an important cellular biological process associated with tumor invasion and metastasis [35]. Our findings indicate that *CCT2* can promote the metastasis and tumorigenesis of PTC through the EMT pathway mediated by *ZEB1*. Therefore, our study provides important clues for further exploration of the mechanisms of *CCT2* and *ZEB1* in PTC. This discovery suggests that *CCT2* may be a potential prognostic factor, aiding clinicians in better-assessing patient prognosis and developing personalized treatment plans.

This study elucidates for the first time, the mechanistic role of *CCT2* in the occurrence and metastasis of PTC, revealing a novel pathway through which it promotes the transcription of EMT genes by regulating *ZEB1*. This finding offers a new perspective for understanding the molecular mechanisms of PTC and may have profound implications for the diagnosis and treatment of thyroid cancer. Additionally, we confirmed the potential therapeutic effects of *CCT2* inhibitors in inhibiting the migration and invasion of PTC cells in *in vitro* models, providing a theoretical basis for developing novel PTC treatment drugs.

Despite the findings of this study, there are limitations. Our research primarily focuses on *in vitro* cell experiments, *in vivo* animal metastasis model experiments, and several clinical samples. Future studies are needed to validate the function and mechanism of *CCT2* in more extensive clinical samples. Furthermore, further research is required to investigate whether other molecules regulate the interaction between *CCT2* and *ZEB1* and whether this interaction applies to different tumors beyond PTC. Future studies should also focus on exploring the safety and efficacy of *CCT2* inhibitors in clinical applications and how to integrate them with existing treatment strategies to improve the therapeutic outcomes for PTC patients.

Conclusion

In conclusion, our study reveals the mechanism by which *CCT2* induces the transcription of *EMT* genes through the regulation of *ZEB1*, promoting the metastasis and tumorigenesis of PTC. This provides new targets and strategies for the treatment of PTC. Our findings offer important clues for further exploration of the mechanisms of *CCT2* and *ZEB1* in PTC and the development of related treatment strategies, potentially bringing breakthroughs in treating PTC.

Availability of Data and Materials

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Author Contributions

WPL: conception, design, materials, data collection, analysis, literature review, writing. RZL: design, supervision, materials, analysis, literature review, writing. CMZ: supervision, data collection, analysis, literature review, writing. YXC: design, materials, analysis, literature review, writing. QGG: supervision, materials, data collection, analysis, writing. JJZ: materials, data collection, analysis, writing, critical review. All the listed authors in the study carried out the experiments, participated in the design of the study and performed the statistical analysis, conceived of the study, and helped draft the manuscript. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study has been approved by the Medical Ethics Committee of Taizhou Central Hospital (Taizhou University Hospital) (Approval No.: 2024L-04-06). This study was conducted in accordance with the Declaration of Helsinki. The clinical patients involved in this study have all signed informed consent forms.

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Conflict of Interest

The authors declare no conflict of interest.

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