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# Intracellular FGF1 promotes invasion and migration in thyroid carcinoma via HMGA1 independent of FGF receptors

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## Abstract

*Background:* Fibroblast growth factor 1 (FGF1) is extensively amplified in many tumors and accelerates tumor invasion and metastasis. However, the role and precise molecular mechanism by which FGF1 participates in thyroid cancer (TC) are still unclear.
*Methods:* Quantitative real-time polymerase chain reaction- and western blotting were used to detect the mRNA and protein levels of FGF1, high mobility group A (HMGA1), epithelial-to-mesenchymal transition (EMT)-related factors, and FGF receptors in both TC tissues and cell lines. Immunohistochemistry was conducted to examine the expression of FGF1 and HMGA1. Immunofluorescence staining was used to detect the coexpression of FGF1 and HMGA1. Transwell and wound healing assays were conducted to evaluate the effects of FGF1 on the capacity of invasion and migration in cells.
*Results:* FGF1 was upregulated in papillary thyroid carcinoma (PTC) tissues and cell lines and was relatively higher in PTC tissues with cervical lymph node metastasis. Furthermore, FGF1 promotes invasion and metastasis through the EMT pathway. Mechanistically, FGF1 promotes EMT through intracellular function independent of FGF receptors. Interestingly, we demonstrated that FGF1 could upregulate HMGA1 in TC cells, and the correlation of FGF1 and HMGA1 was positive in PTC tissues. FGF1 and HMGA1 had obvious colocalization in the nucleus. We further revealed that FGF1 promotes the invasion and migration of TC cells through the upregulation of HMGA1.
*Conclusion:* Intracellular FGF1 could promote invasion and migration in TC by mediating the expression of HMGA1 independent of FGF receptors, and FGF1 may be an effective therapeutic target in TC.

**Key Words**
- thyroid cancer
- fibroblast growth factor 1
- high mobility group A
- epithelial-to-mesenchymal transition

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## Introduction

Thyroid carcinoma (TC) is one of the most frequent endocrine malignancies, and its incidence has been on the rise for nearly 20 years (1). TC is classified as papillary TC (PTC), follicular TC, poorly differentiated TC, and anaplastic TC (ATC) (2). Among them, PTC is the most common TC, and ATC is one of the most lethal TCs. Although surgical resection remains the most effective treatment for PTC, approximately 30–65% of

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PTC cases feature lymph node metastasis, and <10% of PTC cases have shown distant metastasis in the bones and lungs (3). Additionally, the clinical outcomes of PTC patients with high invasion and proliferation remain poor (4). ATC accounts for less than 2% of all TCs but results in nearly one-third of TC-related deaths because of its high capacity for invasion and metastasis (5). Thus, it remains vital to search for a potential strategy to inhibit the invasion and migration of PTC and ATC. Epithelial-to-mesenchymal transition (EMT) is an important biological process during tumor invasion and migration that affects the initiation and transformation of early-stage tumors into invasive malignancies (6). Evidence has shown that transcription factors, growth factors, signaling pathways, and epigenetic regulation affect thyroid cell migration and the EMT process (6). Fibroblast growth factor 1 (FGF1) was first isolated in bovine brains as a mitotic hormone in 1970 (7). Numerous studies have implied that FGF1 can promote tumor proliferation, migration, and invasion in several types of malignant tumors, such as ovarian cancer (8), squamous cell carcinoma of the oral cavity (9), and colorectal cancer (10). Additionally, an early study demonstrated that the expression of FGF1 was enhanced in differentiated TCs (11). However, the role and precise molecular mechanism by which FGF1 participates in ATC and PTC is still elusive.

High mobility group A (HMGA1), which belongs to the superfamily of nonhistone chromatin binding proteins, has been reported to be associated with high invasion and metastasis of tumors (12, 13). Our previous study demonstrated that HMGA1 could promote TC proliferation and invasion (14). However, the correlation of FGF1 and HMGA1 in TC cells is not yet fully understood. In this study, we found that FGF1 was highly expressed in PTC and ATC tissues and cell lines. Moreover, the expression of FGF1 was higher in TC tissues with lymph node metastasis, and the overexpression of FGF1 could promote the invasion and migration of cancer cells via EMT. Mechanically, although most studies verified that FGF1 functions through FGF receptors (FGFRs) (15), in our study, we found that FGF1 promotes TC cell EMT independent of FGFR. Interestingly, we demonstrated that FGF1 could upregulate the expression of HMGA1 in TC cells, and we further revealed that HMGA1 was the downstream factor of FGF1 that promoted TC progression. Thus, FGF1 may be an effective therapeutic target and even an effective drug response indicator in TC.

## Materials and methods

### Cell lines

The human PTC cell lines (B-CPAP, CTCC-400-0087; TPC-1, CTCC-400-0084), the human thyroid squamous cancer cell line (SW579, CTCC-400-0202), and the human ATC cell line (CAL-62, CTCC-400-0099) were purchased from Meisen (Zhejiang, China) Chinese Tissue Culture Collections. The human ATC cell line (8305C, HTX2750) was purchased from Shenzhen Haodi Huatuo Biotechnology Co., Ltd (Shenzhen, China). The human PTC cell line (K1, IM-H150) was purchased from Xiamen Immocell Biotechnology Co., Ltd (Xiamen, China). The human medullary TC cell line (TT, TCH-C362) was purchased from Suzhou Haixing Biotechnology Co., Ltd (Suzhou, China). Immortalized normal follicular epithelial cell line (Nthy-ori 3-1, BFN680331) was purchased from Qingqi (Shanghai, China) Biotechnology Development Co., Ltd. B-CPAP, TPC1 cells were cultured in RPMI-1640 medium (Gibco) containing 10% fetal bovine serum (HyClone; Thermo Fisher Scientific) and 1% MEMNEAA (Gibco) in 5% $CO_2$ at 37°C. The CAL-62, 8503C, SW579, K1, TT, and Nthy-ori 3-1cell lines were cultured in DMEM (Gibco) containing 10% fetal bovine serum (HyClone; Thermo Fisher Scientific) in 5% $CO_2$ at 37°C.

### Tissues of patients

TC and paracancerous tissues were collected from patients who underwent surgery at the First Affiliated Hospital of the University of South China from April 2019 to November 2020. The project was approved by the ethics committee of the First Affiliated Hospital of University of South China and has been registered in the Chinese Clinical Trial Registry (clinical trial registration number: ChiCTR2200065323). Consent was obtained from each patient after full explanation of the purpose and nature of all procedures used. All 38 patient specimens were confirmed as PTC by pathological examination. Patients in the study group were required to meet the following criteria: (1) patients who had undergone thyroid surgery for the first time and had undergone lymph node dissection in the central district or neck dissection at the same time; (2) the diagnosis of TC was confirmed by intraoperative frozen section and postoperative pathology; (3) no history of chemotherapy, radiotherapy, and immunotherapy was found before operation; and (4) age ranged from 18 to 80 years. The following conditions

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were also excluded from the study group: (1) recurrent TC; (2) complicated with other malignant tumors; and (3) pregnant and breastfeeding women. The mean age of the patients was 38.8 years old, including 34 females and 4 males. According to TNM staging, 8 cases were T1N0M0, 3 cases were T2N0M0, 14 cases were T1N1aM0, 5 cases were T2N1aM0, 2 cases were T3N1aM0, 1 case was T1N1bM0, 3 cases were T2N1bM0, and 2 cases were T3N1bM0. The specimens were immediately obtained after surgery and quickly stored in a –80°C refrigerator for further real-time polymerase chain reaction (RT-PCR) and western blot assays. Part of the tissue was embedded in paraffin and sectioned for immunohistochemistry. The carcinoma and adjacent tissues of ATC were obtained from paraffin sections of the First Affiliated Hospital of the University of South China and sectioned for immunohistochemical detection.

## Cell infection and transfection

The knockdown of FGF1 or HMGA1 was conducted by infection of LV-FGF1 shRNA (FGF1 KD) (Shanghai Genechem Co., Ltd, China) and LV-HMGA1 shRNA (B-CPAP KD) (Shanghai Genechem Co., Ltd) into B-CPAP and CAL-62 cells according to the manufacturer's protocol. ShRNA-NC was used as a control. The overexpression of FGF1 was conducted by infection of LV-FGF1 (FGF1 OE) (Shanghai Genechem Co., Ltd) into B-CPAP and CAL-62 cells. The overexpression of HMGA1 (HMGA1 OE) was achieved by transfecting the HMGA1 plasmid into B-CPAP and CAL-62 cells using Lipofectamine 2000 reagent (Life Technologies Corporation) according to the manufacturer's protocol.

## RNA isolation and real-time PCR

Total RNA was extracted by TRIzol® reagent (CWBIO, Beijing, China). The RNA purity was detected through the A260 nm/A280 nm absorption ratio. cDNA synthesis was performed by the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas) using 2 μg of total RNA. Gene expression was determined by an ABI-7500 real-time PCR system with TB Green™ Premix Ex Taq™ II (Takara). The primers were designed with Primer 3 software (Table 1). The primers for the two-step PCR protocol were used according to the manufacturer's instructions. The PCR cycling conditions were 30 s at 95°C followed by 40 cycles at 95°C for 5 s and 60°C for 45 s. The relative gene expression was measured through the $2^{-\triangle\triangle Ct}$ method.

**Table 1** Information for the primers used in this study.

| Genes | Primers | Sequence 5'-3' |
|---|---|---|
| FGF1 | *Forward* | CACAGACACCAAATGAGGAATG |
| | *Reverse* | CATTCTTCTTGAGGCCAACAAA |
| HMGA1 | *Forward* | CCAGTGAAGTGCCAACTCCGAAG |
| | *Reverse* | GCCCTCCTCTTCCTCCTTCTCC |
| FGFR1 | *Forward* | TGGAGTTAATACCACCGACAAA |
| | *Reverse* | GATGATGATCTCCAGGTACAGG |
| FGFR2 | *Forward* | CTAAAGGCAACCTCCGAGAATA |
| | *Reverse* | ACATTTTTGGGAAGCCAAGTAC |
| FGFR3 | *Forward* | GAGGACAACGTGATGAAGATCG |
| | *Reverse* | GGTTGGTCGTCTTCTTGTAGTA |
| FGFR4 | *Forward* | AGTCTAGATCTACCTCTCGACC |
| | *Reverse* | GTTGTCTTTGAGCATCTTGACG |

## Western blotting

The protein of tissues or cells was extracted by the protein cleavage reagent RIPA, including phenylmethylsulfonyl fluoride. The protein concentration was detected using a BCA Assay Kit (CWBIO). The sample extracts were then separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis at 100 V for 2 h and transferred to PVDF membranes (Millipore) at 200 mA for 2 h. Then, the PVDF membranes were blocked with 10% nonfat milk for 2 h at room temperature and incubated with the primary antibodies rabbit anti-FGF1 antibody (1:200, Abcam, WH168080), mouse anti-HMGA1 (1:2000, Santa Cruz Biotechnology, SC-393213), mouse anti-FGFR1 (1:500, Proteintech (Wuhan, China), 60325-1-lg), rabbit anti-FGFR4 (1:500, Proteintech, 11098-1-AP), rabbit anti-phospho-FGFR1 (Y653/Y654) (1:500, Abclonal, AP1317), rabbit anti-phospho-FGFR2 (Tyr463/Tyr466) (1:500, Bioss (Beijing, China), bs-5362R), rabbit anti-phospho-FGFR3 (Y724) (1:500, Abclonal, AP1274), rabbit anti-phospho-FGFR4 (Y642) (1:500, Cusabio (Wuhan, China), CSB-PA008250), mouse anti-E-cadherin (1:500, BD Biosciences (Franklin Lakes, NJ, USA), 610182), rabbit anti-vimentin (1:1000, Cell Signaling, 10366-1-AP), mouse anti-beta-actin (1:1000, ORCIGENE (Rockville, MD, USA), TAB11000), and rabbit anti-slug (1:200, Proteintech, 12129-1-AP) overnight in 5% skim milk solution at 4°C. The membranes were incubated with horseradish peroxidase-conjugated donkey anti-rabbit (1:2000, Millipore) or donkey anti-mouse (1:2000, CWBIO) polyclonal secondary antibodies for 2 h at room temperature the next day. The signals were measured by an enhanced chemiluminescence system (CWBIO).

## Immunohistochemistry

The paraffin sections were soaked in xylene overnight and then dewaxed with anhydrous ethanol, 90% ethanol,

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and 70% ethanol. Endogenous peroxidase was removed from sections with 3% $H_2O_2$. Antigen repair was completed with antigen repair solution at 95°C for 20 min. The sections were then blocked in 5% normal sheep serum with 0.1% Triton for 2 h and incubated with rabbit anti-FGF1 antibody (1:100, Abcam) and mouse anti-HMGA1 (1:500, Santa Cruz Biotechnology) at room temperature for 2 h, followed by incubation at 4°C overnight. Secondary reagents comprised biotinylated goat anti-rabbit or goat anti-mouse IgG (Proteintech). Diaminobenzidine tetrahydrochloride (ZSGB-BIO, Beijing, China) was used as a peroxidase substrate. Washing for all procedures was accomplished in 0.01 M PBS, except for the blocking step. The slices were captured using a digital camera that was attached to the microscope (Olympus). Under the microscope, brown or yellow granules in the cytoplasm or nucleus are positive staining of immunohistochemistry.

### Immunofluorescence staining

B-CPAP and CAL-62 cells were cultured for 24 h in 6-well plates. Cells were washed three times and fixed in 4% paraformaldehyde at room temperature for 20 min. Paraffin sections were soaked in xylene overnight and then dewaxed with anhydrous ethanol, 90% ethanol, and 70% ethanol. Endogenous peroxidase was removed from sections with 3% $H_2O_2$. Antigen repair was completed with antigen repair solution at 95°C for 20 min. The tissues or cells were blocked with 5% normal sheep serum and 0.1% Triton X-100 at room temperature for 2 h. The tissues or cells were incubated with rabbit anti-FGF1 antibody (1:200, Abcam, WH168080) and mouse anti-HMGA1 (1:500, Santa Cruz Biotechnology, SC-393213) at room temperature for another 2 h, followed by incubation at 4°C overnight. The tissues or cells were incubated for 2 h in the dark at room temperature with Alexa 488-labeled donkey anti-mouse and Cy3-labeled donkey anti-rabbit secondary antibodies (1:250) (Invitrogen). The tissues or cells were then counterstained with Hoechst (Beyotime Institute of Biotechnology, Shanghai, China) at a concentration of 5 μg/mL for 5 min. The results were examined by fluorescence microscopy (EVOS M7000).

### Transwell assay

Migration and invasion of cells were determined using Transwell chambers (Corning Costar, diameter = 8 μm). Cells resuspended in 200 μL serum-free medium were loaded in the apical chambers (with or without matrix glue), while the basolateral chambers were filled with 10% FBS-supplemented medium. After 24 h of incubation at 37°C, the chambers were removed. The cells on the upper surface of the membranes were removed using cotton swabs, and the migrated and invaded cells on the lower surface were fixed in methanol solution, stained with Giemsa dye, and observed under a microscope. The field of view was divided into four quadrants, and the number of cells in the first quadrant was regarded as the number per field.

### Wound healing assay

The intervened cells were seeded in 6-well plates and grown to 60–70% confluence. Then, wounds were created by scratching the cell monolayer with a micropipette tip. Cells were allowed to migrate for 48 h in the serum-reduced medium. The wounds were observed and photographed under a microscope at three timepoints (0, 24, and 48 h) after scratching. ImageJ software (National Institutes of Health) was used to determine the distance between the wound edges.

### Statistical analysis

Statistical analysis was carried out using GraphPad Prism software (version 5.0; GraphPad Prism Software). A paired *t*-test was used for statistical analysis between tumor tissues and adjacent normal tissues. The unpaired *t*-test was used for statistical analysis between two independent groups. Differences among three or more groups were carried out by one-way analysis of variance followed by Bonferroni's *post hoc* test. The difference between the four groups in the rescue experiments was analyzed by two-way ANOVA. Spearman correlation analysis was used to assess the correlation between FGF1 and HMGA1 mRNA levels in PTC tissues. P values < 0.05 were considered statistically significant.

## Results

### Increasing FGF1 in tissues and cells of thyroid carcinoma

To address the role and mechanism of FGF1 in TC, we first detected the level of FGF1 in tissues and cells of TC. Real-time PCR showed that the mRNA level of FGF1 was upregulated in PTC tissues compared with adjacent normal tissues (Nor) ($n = 38$ patients, Fold change (FC) = 2.4, $P < 0.0001$) (Fig. 1A). Moreover, the FGF1 mRNA

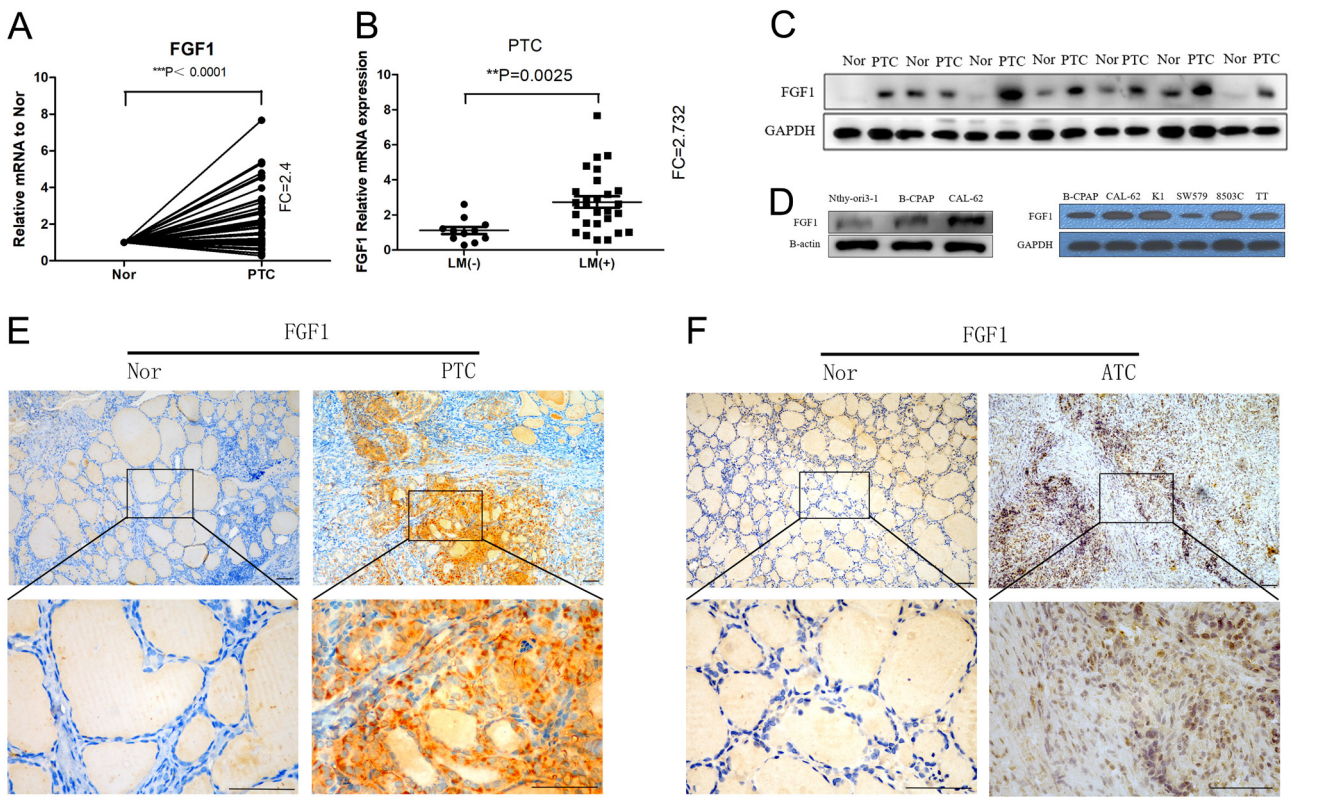


**Figure 1**

The expression of FGF1 in thyroid carcinoma tissues and cells. (A) The mRNA expression level of FGF1 in papillary thyroid carcinoma (PTC) tissues and adjacent normal tissues (Nor). (B) The mRNA level of FGF1 in PTC tissues with (LM(+)) or without ((LM(−)) cervical lymph node metastasis. (C) The protein level of FGF1 in PTC and Nor tissues. (D) The protein level of FGF1 in Nthy-ori3-1 and thyroid cancer cells. (E-F) Representative immunohistochemistry images of FGF1-positive cells in PTC and ATC tissues and their adjacent normal tissues (Nor). Bar = 200 μm. $^{**}P < 0.01$ and $^{***}P < 0.001$.

level in PTC tissues with cervical lymph node metastasis (LM(+)) was higher than that in tissues without cervical lymph node metastasis (LM(−)) ($n = 11$ LM(−) patients and 27 LM(+) patients, FC = 2.732, $P = 0.0025$) (Fig. 1B). In order to further determine the protein levels of FGF1, we randomly selected seven pairs of samples which with higher mRNA levels of FGF1 for western blot testing, and the results showed that the FGF1 protein was increased significantly in PTC tissues compared with adjacent normal tissues, which was consistent with the level of FGF1 mRNA (Fig. 1C). Because of the low incidence rate of ATC in the clinic, cell lines were also used to estimate the protein level of FGF1. We found that when compared with the Nthy-ori3-1 cells (representing normal thyroid cells), the protein level of FGF1 in B-CPAP (representing PTC) and CAL-62 cells (representing ATC) increased significantly; moreover, the FGF1 protein level in CAL-62 cells was higher than that in B-CPAP cells. We also examined the protein of FGF1 in a variety of TC cells and confirmed that the expression of FGF1 was still relatively high in B-CPAP and CLA-62 (Fig. 1D). Furthermore, we investigated the expression of FGF1 in both PTC and ATC tissues by immunohistochemistry, and representative images of FGF1 are shown in Fig. 1E and F. The results showed that FGF1 was mainly expressed in the cytoplasm and nucleus of TC cells, and FGF1 was highly expressed in five out of eight cases in PTC and three out of four cases in ATC. These results indicated that FGF1 was upregulated in TC tissues and cells and might play an important role in the invasion and metastasis of TC.

## FGF1 promotes the invasion and metastasis of thyroid carcinoma cells through the EMT pathway

In the present study, the wound healing assay showed that when compared to those of the control and the NC group, the migration capability of B-CPAP and CAL-62 cells in the FGF1 overexpression (FGF1 OE) group increased significantly ($P < 0.05$), while FGF1 knockdown (FGF1 KD) produced the opposite functions ($P < 0.05$) (Fig. 2A and B). Additionally, transwell assays revealed that FGF1 OE enhanced the invasive and migration capabilities of

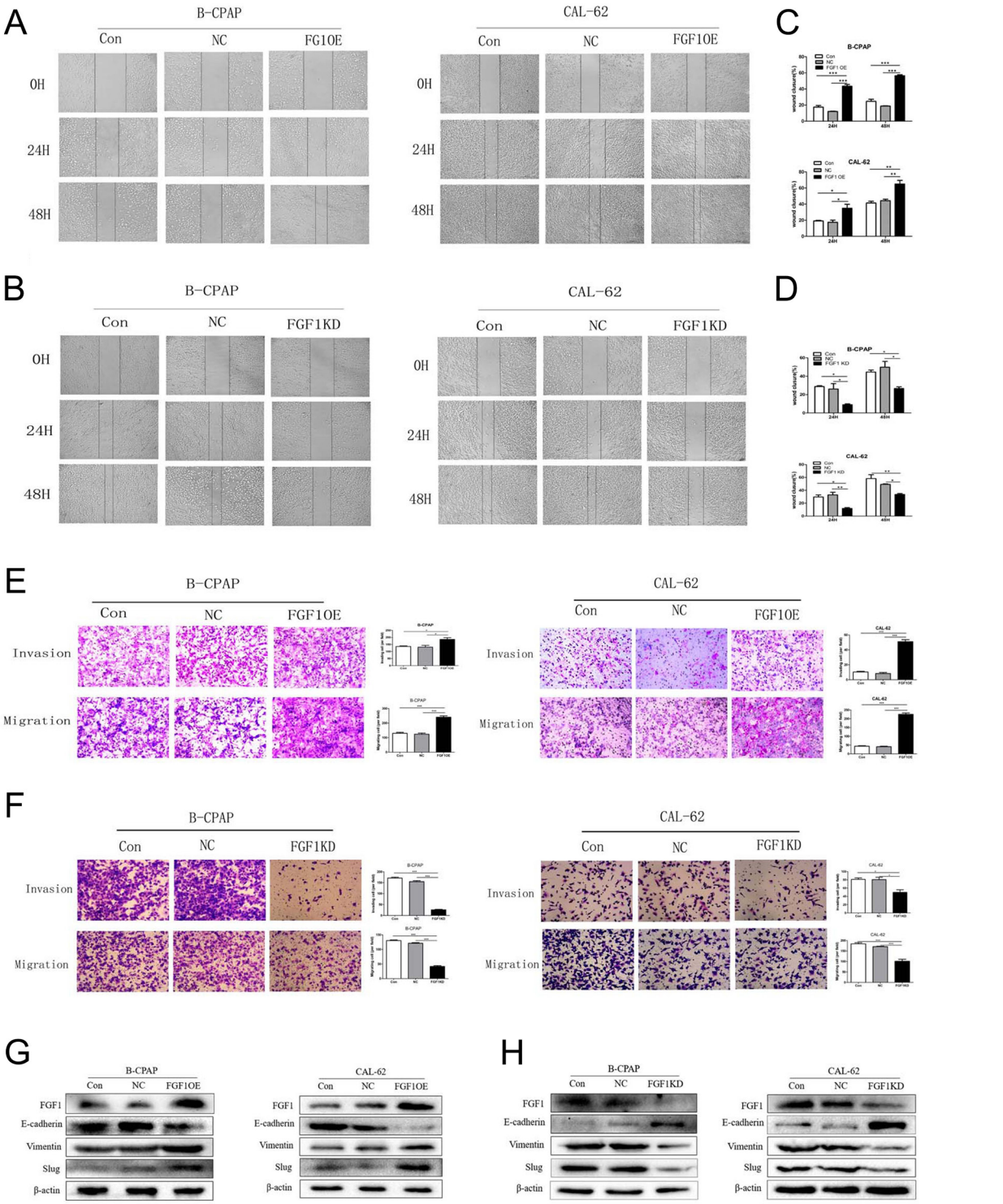


**Figure 2**

The role of FGF1 in the invasion and metastasis of thyroid carcinoma. (A) The effects of FGF1 overexpression (OE) or (B) knockdown (KD) on the migration capability of B-CPAP and CAL-62 cells with a wound healing assay. (C) The effects of FGF1 OE or (D) KD on the invasive and migration capabilities in B-CPAP and CAL-62 cells with transwell assays. (E) The influences of FGF1 OE or (F) KD on the protein levels of E-cadherin, Vimentin, and Slug in B-CPAP and CAL-62 cells. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$.

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B-CPAP and CAL-62 cells compared with the control and NC groups ($P < 0.05$) (Fig. 2E), while the invasive and migration capabilities of B-CPAP and CAL-62 cells were inhibited by FGF1 KD ($P < 0.05$) (Fig. 2F). Furthermore, we investigated whether the EMT pathway is involved in these effects. Western blotting was used to examine the protein levels of the epithelial marker E-cadherin, the interstitial marker Vimentin, and the transcription factor Slug. The results showed that FGF1 OE decreased the protein level of E-cadherin but increased the expression of Vimentin and Slug in both B-CPAP and CAL-62 cells (Fig. 2G). Conversely, FGF1 KD increased the protein level of E-cadherin but decreased the expression of Vimentin and Slug in the two cell lines (Fig. 2H). Thus, these results indicated that FGF1 could promote invasion and migration through the EMT pathway in TC.

## FGF1 promotes EMT in thyroid carcinoma through intracellular function independent of FGF receptors

Next, we demonstrated the mRNA levels of FGFR1–4 in PTC tissues. Interestingly, the mRNA levels of FGFR1 (FC = 0.613, $P = 0.0001$), FGFR2 (FC = 0.654, $P = 0.002$), and FGFR4 (FC = 0.752, $P = 0.032$) were significantly decreased in PTC tissues compared with adjacent normal tissues, while FGFR3 was not significantly different between the two groups (FC = 1.22, $P = 0.157$) (Fig. 3A). The mRNA levels of FGFR1–4 were not affected by FGF1 OE in B-CPAP or CAL-62 cells (Fig. 3B and C). Moreover, the phosphorylation of proteins of FGFR1–4 was not affected by FGF1 OE in B-CPAP or CAL-62 cells (Fig. 3D). Western blotting further demonstrated that the protein levels of FGFR1 and FGFR4 were not significantly different after FGF1 OE in the two cell lines (Fig. 3E). To explore whether the effects of FGF1 on EMT depend on FGFRs, the highly specific FGFR1 inhibitor SU5402 or FGFR4 inhibitor H3B-6527 was used to block the FGFR-dependent signaling pathway, and the results showed that the promotional effect of FGF1 on EMT was not influenced by SU5402 and H3B-6527 in either B-CAPA or CAL-62 cells (Fig. 3F and G). All these results indicated that FGF1 may promote EMT in TC through intracellular function, which is independent of FGF receptors.

## FGF1 regulates the expression of HMGA1 in thyroid cancer

Our earlier study confirmed that classical extracellular function-dependent FGFRs were not essential for FGF1 to promote EMT in TC, so we speculate that FGF1-promoted EMT may occur through intracellular function. Our previous studies have demonstrated that high mobility group AT-hook 1 (HMGA1) plays a pivotal role in the progression of TC (14, 16). Here, we also investigated the regulation of FGF1 on the expression of HMGA1. Western blot analysis revealed that FGF1 OE increased the protein level of HMGA1 (Fig. 4A), while FGF1 KD exhibited the opposite effect (Fig. 4B) in B-CPAP and CAL-62 cells. Moreover, the mRNA levels of FGF1 and HMGA1 were positively correlated (R = 0.5916, $P < 0.0001$) in PTC tissues (Fig. 4C). Not only in PTC tissues but also in B-CPAP and CAL-62 cells, FGF1 was largely expressed in the cytoplasm and nucleus, while HMGA1 was only largely expressed in the nucleus. FGF1 and HMGA1 had obvious colocalization in the nucleus of PTC tissues, B-CAP, and CAL-62 cells (Fig. 4D and E). Interestingly, we found that HMGA1 was also highly expressed in PTC tissues, B-CAP, and CAL-62 cells with enhanced FGF1 expression (Fig. 4D and E). These results indicate that FGF1 could mediate the expression of HMGA1 in TCs. Then, we detected whether FGFRs were essential in the process and found that SU5402 or H3B-6527 intervention could not reverse the protein level of HMGA1 induced by FGF1 OE in the cell lines (Fig. 4F and G). Thus, FGF1 promoted the expression of HMGA1 independent of FGFRs. Additionally, the mRNA level of HMGA1 in TC tissues was also evaluated (FC = 2.083, $P = 0.0018$) (Fig. 4H). We first selected all samples with increased expression of FGF1 mRNA in cancer tissue compared to adjacent tissues, and randomly selected seven pairs of samples to verify the level of FGF1 protein by western blot in papillary thyroid cancer and adjacent tissues; the results showed that HMGA1 protein increased significantly in PTC tissues compared with adjacent normal tissues, which was consistent with the level of HMGA1 mRNA (Fig. 4J) HMGA1 was also enhanced in B-CPAP and CAL-62 compared with Nthy-ori3-1 and other TC lines (Fig. 4K). Moreover, the HMGA1 mRNA level in PTC tissues with cervical lymph node metastasis (LM(+)) was higher than that in tissues without cervical lymph node metastasis (LM(–)) ($n = 11$ patients for LM(–) and 27 patients for LM(+), FC = 2.538, $P = 0.0349$) (Fig. 4I). Immunohistochemistry further revealed that HMGA1 was mainly expressed in the nucleus of TC cells, and the expression of HMGA1 in PTC and ATC tissues was increased significantly compared with that in adjacent normal tissues. HMGA1 was highly expressed in six out of eight cases in PTC and three out of four cases in ATC (Fig. 4L and M). These results suggest that HMGA1 is regulated by FGF1 through intracellular function in TC.

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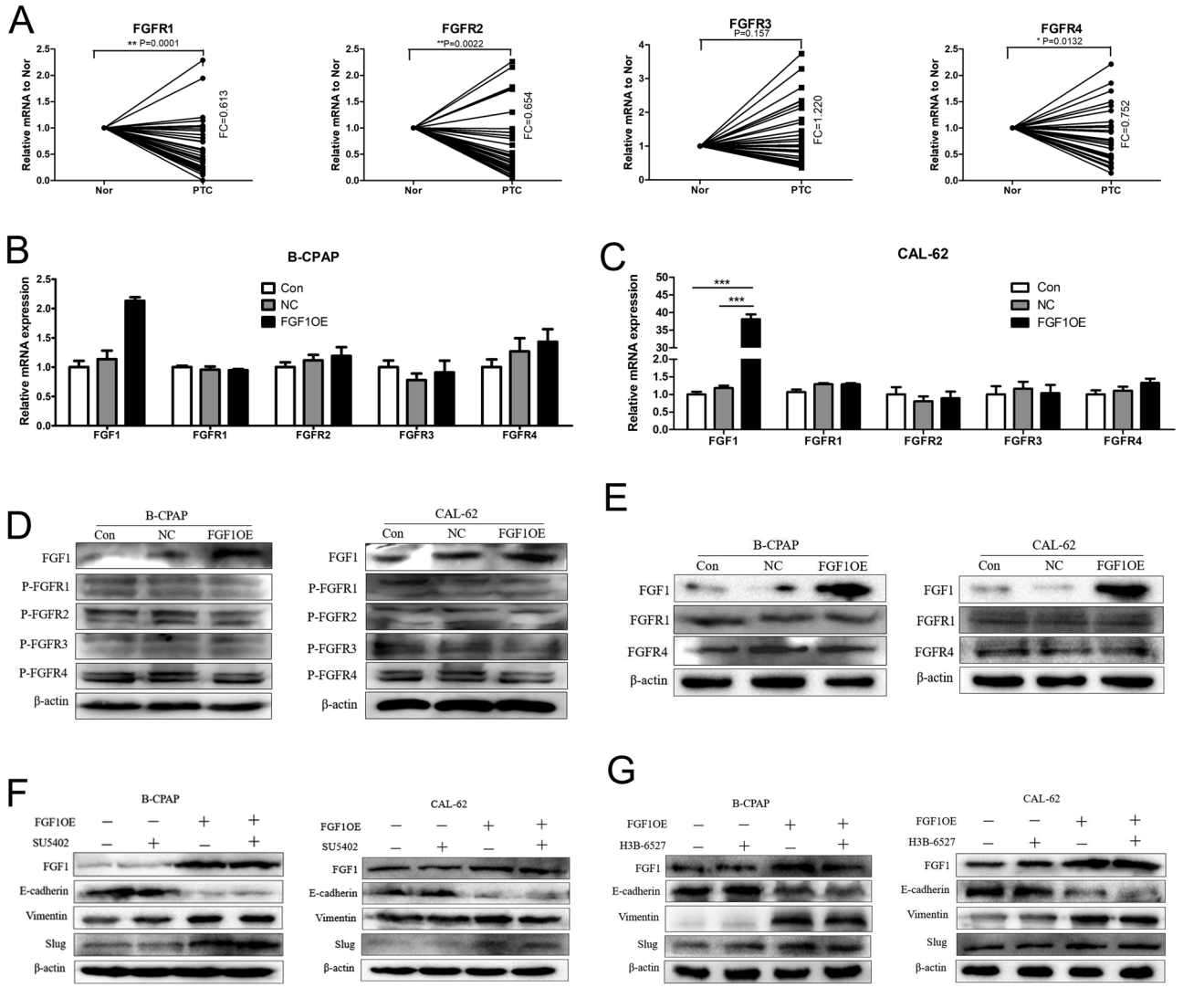


**Figure 3**

FGF receptors are not essential for FGF1 to promote EMT in thyroid carcinoma. (A) The mRNA expression levels of FGFR1, FGFR2, FGFR3, and FGFR4 in PTC tissues and Nor tissues. (B–C) The influences of FGF1 OE on the mRNA levels of FGFR1, FGFR2, FGFR3, and FGFR4 in B-CPAP and CAL-62 cells. (D) The influences of FGF1 OE on the phosphorylation of proteins of FGFR1, FGFR2, FGFR3, and FGFR4 in B-CPAP and CAL-62 cells. (E) The effects of FGF1 OE on the protein levels of FGFR1 and FGFR4 in B-CPAP and CAL-62 cells. (F) The effects of FGF1 OE on the protein levels of E-cadherin, Vimentin, and Slug in the presence of the FGFR1 inhibitor SU5402 in B-CPAP and CAL-62 cells. (G) The influences of FGF1 OE on the protein levels of E-cadherin, Vimentin, and Slug in the presence of the FGFR4 inhibitor H3B-6527 in B-CPAP and CAL-62 cells. *$P$ < 0.05, **$P$ < 0.01, and ***$P$ < 0.001.

## FGF1 promotes invasion and migration via HMGA1 in thyroid cancer

Next, we conducted a series of rescue experiments to further investigate whether FGF1 promoted invasion, migration, and EMT via HMGA1 in TC. Wound healing assays showed that HMGA1 KD inhibited the effect of FGF1 OE on the migration capability of B-CPAP and CAL-62 cells ($P$ < 0.05) (Fig. 5A and B), while the inhibitory effect of FGF1 KD on the migration capability was partially rescued by HMGA1 OE in the two cell lines ($P$ < 0.05) (Fig. 5C and D). Further transwell assays also revealed that HMGA1 KD could suppress the invasive and migration capabilities induced by FGF1 OE; conversely, HMGA1 OE could partially rescue the invasive and migration capabilities after FGF1 KD in both B-CPAP and CAL-62 cells ($P$ < 0.05) (Fig. 5E and F). We also found that the reduction in E-cadherin and the elevation of Vimentin and Slug induced by FGF1 OE could be partially reversed when HMGA1 was knocked down in B-CPAP and CAL-62 cells (Fig. 5G). Conversely, overexpression of HMGA1 eliminated the influence of FGF1 KD on the protein

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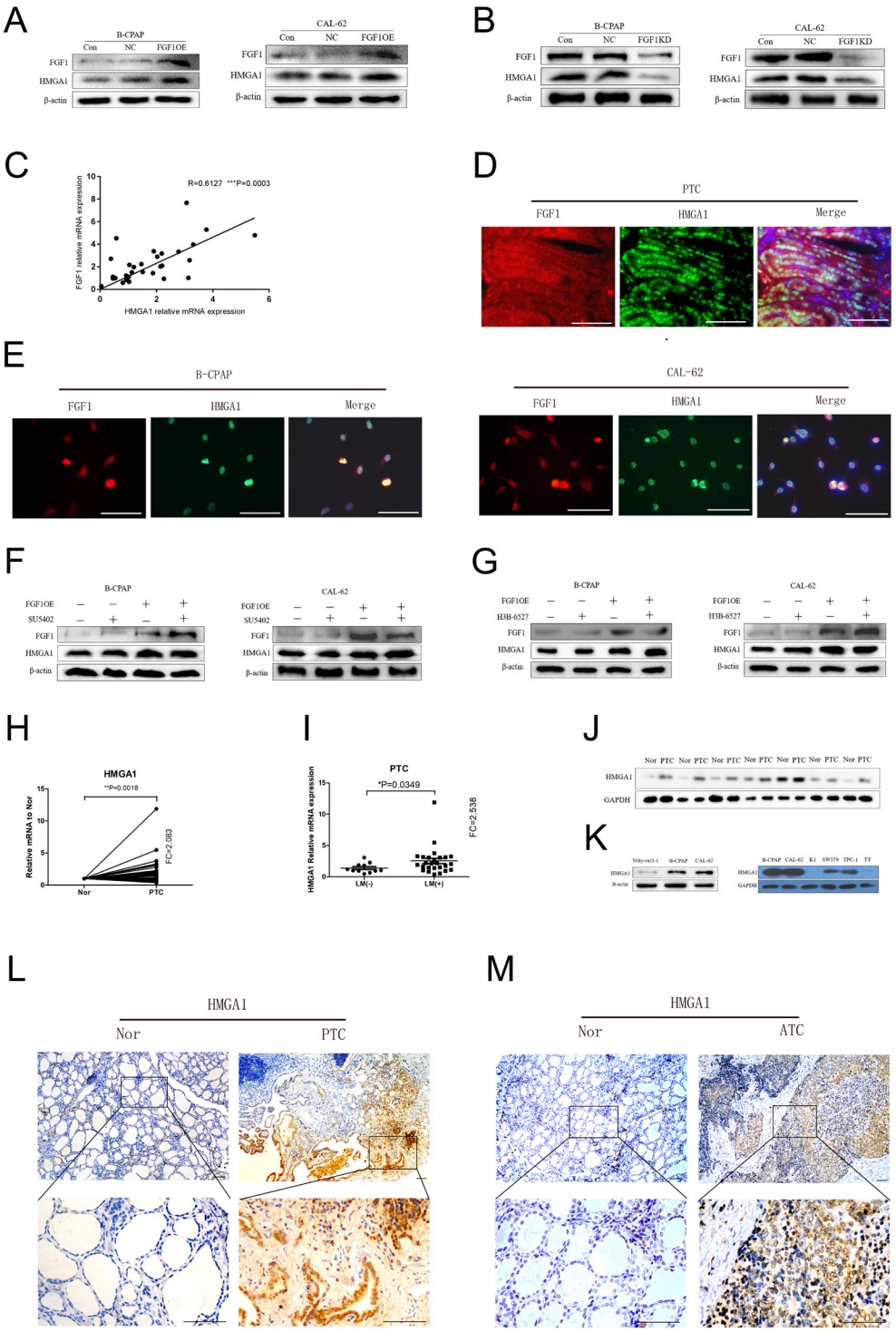


**Figure 4**

The effects of FGF1 on the expression of HMGA1 in thyroid carcinoma. (A) The influences of FGF1 OE or (B) KD on the protein level of HMGA1 in B-CPAP and CAL-62 cells. (C) The correlation between FGF1 and HMGA1 mRNA levels in PTC tissues. (D) Immunofluorescence colocalization of FGF1 and HMGA1 in PTC tissues. (E) Immunofluorescence colocalization of FGF1 and HMGA1 in B-CPAP and CAL-62 cells. (F) The effects of FGF1 OE on the protein level of HMGA1 in the presence of the FGFR1 inhibitor SU5402 in B-CPAP and CAL-62 cells. (G) The influence of FGF1 OE on the protein level of HMGA1 in the presence of the FGFR4 inhibitor H3B-6527 in B-CPAP and CAL-62 cells. (H) The mRNA level of HMGA1 in PTC tissues and Nor tissues. (I) The mRNA level of HMGA1 in PTC tissues with (LM(+)) or without (LM(−)) cervical lymph node metastasis. (J) The protein level of HMGA1 in PTC and Nor tissues. (K) The protein level of HMGA1 in Nthy-ori 3-1 and thyroid cancer cells. (L–M) Representative immunohistochemistry images of HMGA1-positive cells in PTC and ATC tissues and their adjacent normal tissues (Nor). Bar = 200 μm. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

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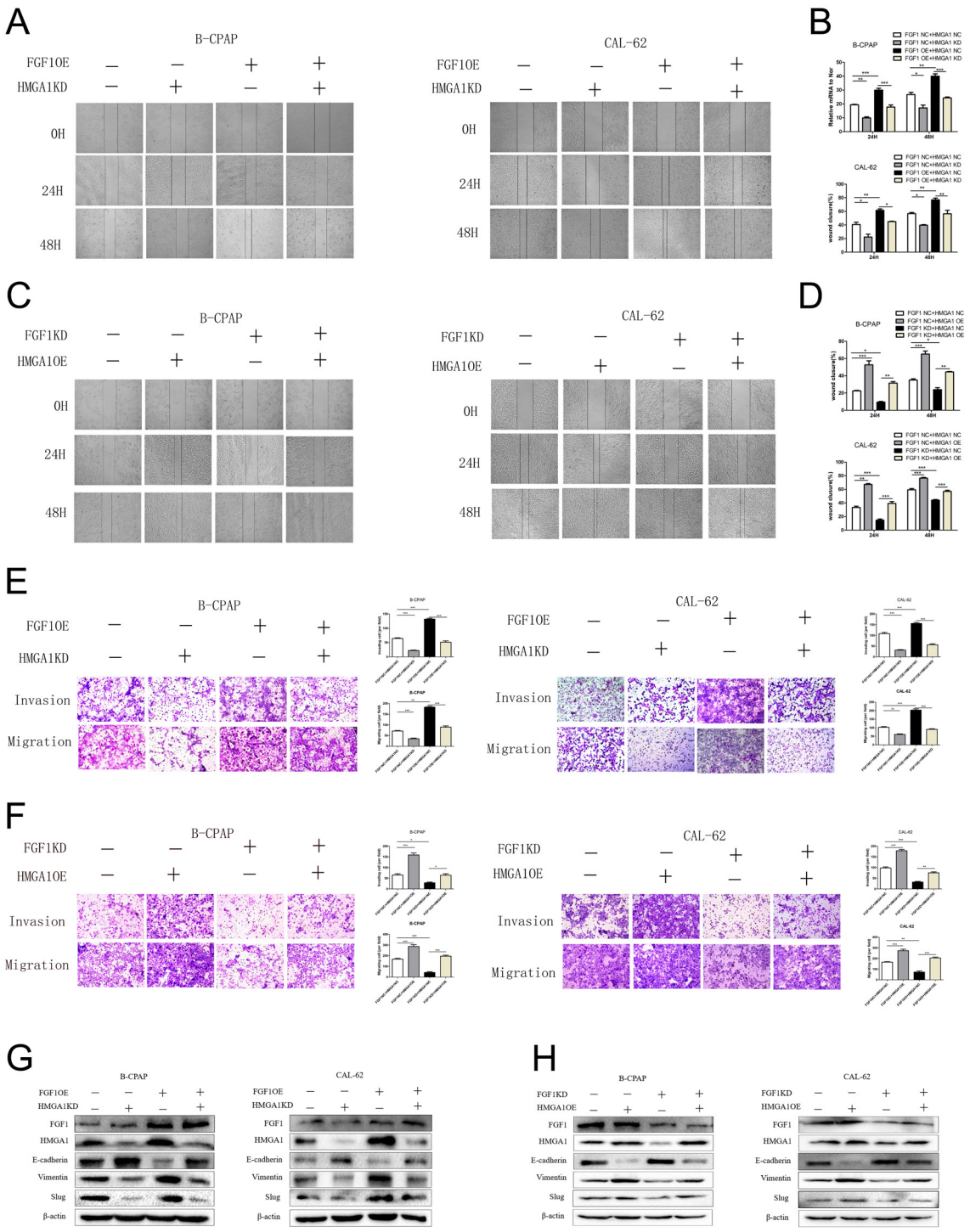


**Figure 5**

FGF1 promotes invasion and migration via HMGA1 in thyroid carcinoma. (A–B) The effects of FGF1 OE and HMGA1 KD or (C–D) FGF1 KD and HMGA1 OE on the migration capability of B-CPAP and CAL-62 cells in a wound healing assay. (E) The effects of FGF1 OE and HMGA1 KD or (F) FGF1 KD and HMGA1 OE on the invasive and migration capabilities in B-CPAP and CAL-62 cells with transwell assays. (G) The protein levels of E-cadherin, Vimentin, and Slug under FGF1 OE and HMGA1 KD conditions or (H) under FGF1 KD and HMGA1 OE conditions in B-CPAP and CAL-62 cells. $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

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levels of E-cadherin, Vimentin, and Slug (Fig. 5H). These results fully demonstrated that FGF1 promoted invasion, migration and EMT through HMGA1 in TC, and the FGF1-HMGA1 axis might play an important role in TC.

## Discussion

FGF1 is a well-characterized growth factor among the 22 members of the FGF superfamily in humans. Mounting evidence has shown that FGF1 plays an important role in tumor progression. FGF1 has oncogenic activity and has been found to modulate numerous cellular processes, including proliferation, invasion, and survival (7). The level of FGF1 is elevated in a variety of tumors, and the gene is implicated in malignant progression (17). In the present study, we identified that FGF1 is closely related to invasion and migration in both PTC and ATC, as indicated by EMT-related factors and transwell and wound healing assays. Moreover, our current study also demonstrated that FGFRs were not mediated by FGF1 in TC, but HMGA1 was positively correlated with the expression of FGF1. In addition, the role of FGF1 was abolished by the knockdown of HMGA1 in both B-CPAP and CAL-62 cells. Thus, the FGF1-HMGA1 axis may promote invasion and migration in both PTC and ATC, and FGF1 might be an ideal biomarker for the prediction or drug response of TC. Inhibiting the expression of FGF1 may halt disease progression.

FGF1 is involved in the regulation of diverse physiological processes, such as development (18), angiogenesis (19), wound healing (20), adipogenesis (21), and neurogenesis (22), while dysfunction of FGF1 is associated with various diseases, including tumor progression. For example, FGF1 could promote invasion and migration in squamous cell carcinoma of the oral cavity (9), ovarian cancer (8), angiosarcoma (23), and tongue squamous cell carcinoma (24). However, whether or how FGF1 participates in TC remains unclear. Importantly, our study demonstrated that the level of FGF1 was upregulated in both PTC and ATC tissues and cell lines, which was consistent with a previous study (11). It is well established that FGF1 promotes the invasion and metastasis of various tumors (24). However, the effects of FGF1 on invasion and metastasis are not yet completely understood in TC. The expression of FGF1 was higher in TC tissues with lymph node metastasis than in tissues without lymph node metastasis, indicating that FGF1 may participate in invasion and migration. Furthermore, we demonstrated that overexpression of FGF1 could enhance the capacity of invasion and migration in B-CPAP and CAL-62 cells through EMT, as indicated by the reduction of E-cadherin and increase of Vimentin and Slug, while FGF1 KD could produce the opposite effects. These data demonstrated that FGF1 promotes TC metastasis through the EMT pathway, which was also verified in tongue squamous cell carcinoma (24).

Numerous studies have demonstrated that FGF1 can bind to all four known surface FGF receptors (FGFR1–4) to activate intracellular signaling and regulate a plethora of functions, including cell growth, proliferation, migration, differentiation, and survival, in different cell types (25). Studies have shown that FGF1 participates in bladder tumor progression through the activation of FGFR1 (26); FGF1 promotes ovarian proliferation, migration, and invasion through the activation of FGFR4 signaling (8). In addition to classical extracellular activity, among all growth factors, FGF1 has a specific N-terminal nuclear localization sequence, which is essential for the efficient transport of FGF1 to cross the cell membrane and to translocate into the cytosol and nucleus (27, 28); thus, FGFRs are not essential for FGF1 to function. A previous study revealed that FGF1 antiapoptotic activity is independent of FGFR activation (29). Another study also demonstrated that intracellular FGF1 protected the cell against stress conditions independent of FGFR signaling cascades (30). In our current study, we illustrated that the expression of FGFRs was inconsistent with that of FGF1 in PTC tissues, and overexpression of FGF1 had no significant influence on the expression and phosphorylation of FGFRs either B-CPAP or CAL-62 cells. Because the specific sequence in the C-terminal tail of FGFR1 and FGFR4 is necessary for FGF1 translocation (31), our study also explored the role of SU5402 (an inhibitor of FGFR1) and H3B-6527 (an inhibitor of FGFR4) in the EMT of B-CPAP and CAL-62 cells, and the results showed that neither SU5402 nor H3B-6527 could reverse the role of FGF1 in EMT, thereby suggesting that FGF1 promotes TC metastasis through intracellular function independent of FGFRs.

HMGA1 has been reported to play an important role in tumor invasion and migration, including hepatocellular carcinoma (32), bladder cancer (33), colorectal cancer (34), and osteosarcoma (35). Additionally, our previous studies have demonstrated that HMGA1 could mediate TC proliferation and invasion (14, 16). Furthermore, HMGA1 exerts important roles in EMT, and knockdown of HMGA1 could decrease the expression of N-cadherin and vimentin while E-cadherin was increased (36). However, the regulatory mechanism of HMGA1 in TC

remains elusive. Herein, we found that the expression of HMGA1 could be mediated by FGF1 in cells. FGF1 and HMGA1 had obvious colocalization in the nucleus of TC cells. Interestingly, we found that HMGA1 was also highly expressed in cells with strong FGF1 expression. Moreover, the expression of FGF1 and HMGA1 in TC tissues exhibited a significant positive correlation, indicating that FGF1 may promote TC metastasis via HMGA1. To further prove our hypothesis, we determined the expression of HMGA1 in TC and found that it is highly expressed in TC tissues and cell lines. Critically, the role of FGF1 in mediating the expression of EMT-related factors and invasion and migration functions could be abolished by HMGA1 intervention. Thus, we can conclude that FGF1 could modulate TC invasion and migration through HMGA1. In our future study, we will deeply explore the regulatory mechanism by which FGF1 mediates HMGA1.

## Conclusions

Taken together, the present work is the first study to report that intracellular FGF1 may promote the process of invasion and migration in TC through HMGA1, and FGF1 may be an effective drug response indicator and even a potential treatment target in TC.

### Declaration of interest

The authors have declared that no competing interest exists.

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### Author contribution statement

Zuyao Chen and Xiaolin Zhong performed PCR and immunohistochemistry; Zuyao Chen and Weiqiang Tang performed western blot tests; Zuyao Chen, Min Xia and Chang Liu performed cell culture, cell infection and transfection; Zuyao Chen, Yinping Guo and Yan Yi performed wound healing and Transwell assays; Qingshan Jiang, Shaoxin Gong and Shanji Fan collected thyroid cancer tissues; Zuyao Chen, Xiaolin Zhong and Jing Zhong performed statistical analysis and drafted the manuscript. Xiaolin Zhong and Jing Zhong performed the research design and manuscript revision.

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