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GNA15 facilitates the malignant development of thyroid carcinoma cells via the BTK-mediated MAPK signaling pathway

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Summary. G protein subunit alpha 15 (GNA15) is recognized as an oncogene for some cancers, however, its role in thyroid carcinoma (TC) is elusive and is investigated in this study. Concretely, bioinformatics was employed to analyze the GNA15 expression profile in TC. The effect of GNA15 on TC cell functions was examined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), colony formation, and Transwell assays. Expressions of extracellular regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 were determined using Western blot. The involvement of Bruton tyrosine kinase (BTK) in the mechanism of GNA15 was investigated by BTK knockdown and rescue assay. GNA15 presented an overexpression pattern in TC samples, which facilitated the viability, proliferation, migration, and invasion of TC cells; GNA15 silencing led to converse results. Ratios of p-ERK/ERK, p-JNK/JNK, and p-p38/p38 were upregulated by GNA15 overexpression. The BTK deficiency weakened the aforementioned behaviors of TC cells and blocked the MAPK signaling pathway, however, these effects were counteracted by GNA15 overexpression. Collectively, GNA15 contributes to the malignant development of TC cells by binding to BTK and thus activating the MAPK signaling pathway.

Key words: Thyroid carcinoma, G protein subunit alpha 15, Bruton tyrosine kinase, MAPK signaling pathway, Malignant phenotype

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Introduction

Thyroid carcinoma (TC) is a prevalent neoplasm of the endocrine system, with an increasing incidence over recent decades (Waguespack et al., 2022). TC is less common in children (0-18 years old) but more prevalent in people aged 15-39 years, who can be categorized as young adults or adolescents, which poses significant risks for morbidity and mortality (Vanden Borre et al., 2017). In addition to surgical resection, postoperative chemotherapy, restoring expressions of tumor suppressor genes, or suppressing the overexpression of oncogenes through molecular intervention is also a promising treatment method for alleviating TC (Shakib et al., 2019). Despite some progress in tumor treatment, patients' quality of life and survival expectancy are still under threat (Lin et al., 2019; Ratajczak et al., 2021). Currently, the pathogenesis of TC is poorly understood but we can confirm that multiple promoter and suppressor genes are involved in TC occurrence and development (Yu et al., 2022). Therefore, exploring the molecular mechanisms affecting tumorigenesis is of great significance for TC management. Profiling of the tyrosine kinome in human TC worldwide has revealed the upregulation of Bruton tyrosine kinase (BTK) in TC tissues (Cho et al., 2012). BTK inhibitors play a role in multiple B-cell malignancies (Pal Singh et al., 2018; Mato et al., 2021). BTK is also associated with the immune escape of tumor cells (Palma et al., 2021; Sharma et al., 2021). On this basis, we can speculate that BTK may be a target gene for TC treatment.

G proteins are a group of signal transmitters, also known as GTP-binding proteins, with about 40 different types, which transmit external signals into cells, thereby activating various downstream signaling pathways (Syrovatkina et al., 2016). Ample evidence indicated that abnormalities in G protein function may be responsible for the development of autoimmune diseases, hypertension, and tumors (O'Hayre et al., 2013; Althoff and Offermanns, 2015; Wu et al., 2020). G protein

subunit alpha 15 (GNA15) is located on human chromosome 19p13.3 and GNA15-encoded protein is closely associated with several cell functions, such as proliferation and apoptosis (Chen et al., 2008). It has been documented that GNA15 could be coupled by G protein-coupled receptors (GPCRs) and modulate downstream signaling pathways, thereby participating in tumorigenesis and metastasis (Zanini et al., 2015). In the study of oncology, *GNA15* has been considered an oncogene in pancreatic ductal adenocarcinoma, hepatocellular carcinoma, and small intestinal neuroendocrine neoplasia (Zanini et al., 2015; Nguyen et al., 2018; Innamorati et al., 2021), yet its biological function in TC is poorly understood.

Mitogen-activated protein kinases (MAPKs) are a class of protein kinases ubiquitously found in eukaryotic cells, which, by linking to receptors on the cell surface, transmit a variety of extracellular physical and chemical stimuli internally, thus regulating cellular gene expression, proliferation, apoptosis, and other physiological processes (Acosta and Kadkol, 2016; Pernice et al., 2016). The MAPK signaling pathway is over-activated in several tumors including TC by receptor tyrosine kinases, GPCRs, or other cytokine receptors, triggering a series of aggressive behaviors in cancer cells (Nucera et al., 2011; Burotto et al., 2014; Masliah-Planchon et al., 2016). Therefore, regulating the MAPK signaling pathway has become a focus of TC research (Al-Jundi et al., 2020). A study of acute myeloid leukemia unveiled that high expression of GNA15 contributes to disease progression by activating the p38 MAPK signaling pathway (Li et al., 2021).

In this study, we probed the biological function of GNA15 in TC and its potential mechanism in the MAPK signaling pathway, hoping to provide new insights for the clinical management of TC.

Materials and methods

Bioinformatics analysis

The GNA15 expression profile in TC (n=505) and normal samples (n=59) was analyzed using UALCAN (http://ualcan.path.uab.edu/) based on the TCGA database. The signal pathways involved in activated BTK were predicted through the Reactome Pathway Database (https://reactome.org/).

Cell culture and transfection

Human TC cell lines TPC-1 (0397, Rio de Janeiro Cell Bank, Brazil) and HTH83 (MZ-2194, Mingzhou Biotech, Ningbo, China) were cultured in RPMI-1640 medium (31870082, ThermoFisher, Waltham, MA, USA) containing 0.1% penicillin-streptomycin (15140148, ThermoFisher, USA) and 10% fetal bovine serum (10099141C, ThermoFisher, USA) under 5% CO_2 at 37°C (He et al., 2021).

When cell confluence reached over 80%, cell transfection was conducted. The GNA15 overexpression plasmid (pcDNA-GNA15, HG12687-UT) and pCMV3 vector (CV011) as negative control (NC) were purchased from Sino Biological (Beijing, China). Short hairpin RNA against GNA15 (sh-GNA15, sense: 5'-GAGCAAGACTCATTGTCAA-3' and antisense: 5'-TTGACAATGAGTCTTGCTC-3'), sh-BTK (sense: 5'-GGATAGACGTTGTCAATAA-3' and antisense: 5'-TTATTGACAACGTCTATCC-3'), and shRNA negative control (sh-NC) were synthesized and offered by Genepharma (Shanghai, China). Next, the aforementioned plasmids were independently or jointly transfected into TPC-1 or HTH83 cells using Lipofectamine 3000 reagent (L3000015, ThermoFisher, USA) in accordance with the protocols. After 72 hours (h) of incubation, the transfected cells were harvested and re-suspended for verification of GNA15 expression by quantitative real-time polymerase chain reaction (qRT-PCR).

RNA isolation and qRT-PCR

GNA15 or BTK expression in cells was determined by qRT-PCR, after total RNA isolation with the help of TRIzol reagent (15596026, ThermoFisher, USA). Next, SuperScript IV RT (18090010, ThermoFisher, USA) was applied for cDNA synthesis according to the instructions. Real-Time PCR System (MX3000P, Agilent Stratagene, Santa Clara, CA, USA) equipped with YBR Premix Ex Taq II (RR820A, Takara, Tokyo, Japan) was employed to perform qRT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference of GNA15 or BTK. The relative gene expressions were calculated as per the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The primers used were (5'-3'): GNA15 (GAGGCAGCCAAGAGGTTCAT, CACCCAAGTCCTCAATCCCC), BTK (TAAGTCA GGACTGAGCACACA, CCAGCTTTGCTGGAGTC TCT), and GAPDH (CAATGACCCCTTCATTGACC, TTGATTTTGGAGGGATCTCG).

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The viability of TPC-1 and HTH83 cells with or without transfection of the GNA15 plasmid or/and sh-BTK was measured with the MTT Assay Kit (CC002K, Synthgene, Nanjing, China). 100 µl of cells (5×10^4 cells/ml) were seeded into a 96-well plate and then incubated with 10 µl MTT solution for 4h at 37°C under 5% CO_2. After crystal dissolution by formazan solution, the optical density (OD) value was measured at 24h, 48h, and 72h of transfection using a microplate reader (MR-96A, Mindary, Shenzhen, China) at the wavelength of 490 nm.

Colony formation assay

TPC-1 and HTH83 cells with or without transfection

of the GNA15 plasmid or/and sh-BTK were subjected to colony formation assay. Two ml of resuspended cells were seeded into a 6-well plate and cell density was adjusted to 1×10^3 cells/well. The cells were cultured in an incubator at 37°C with 5% CO_2 for three weeks, followed by fixation using 4% paraformaldehyde (E-IR-R113, Elabscience, Wuhan, China) for 20 minutes (min), and staining with crystal violet (C0121, Beyotime, Suzhou, China) for 30 min. A microscope (CX23, Olympus, Tokyo, Japan) was applied to observe and count colonies.

Transwell assay

Cell migration was determined using Transwell chambers (3422, Corning Life Sciences, Corning, NY, USA), and cell invasion was detected using Matrigel-coated Transwell chambers (354480, Corning Life Sciences, USA). For the migratory capacity test, cells (1×10^5 cells/ml) were cultured in the serum-free medium. Then, 200 µl of cell suspension and 500 µl of medium containing 10% FBS were added into the upper chamber and the basolateral chamber, respectively. Following 48h incubation, the cells were fixed with 4% paraformaldehyde (E-IR-R113, Elabscience, Wuhan, China) for 15 min, and stained with crystal violet (C0121, Beyotime, Suzhou, China) for 20 min. Subsequently, the cells migrating into the basolateral chamber were observed under the CX23 microscope (×250 magnification, Olympus, Tokyo, Japan). For the invasive capacity test, procedures were the same as for the migration assay, except that the cells failing to invade the membrane needed to be wiped off at the end of the incubation.

Western blot

After 48h of cultivation, the transfected TPC-1 and HTH83 cells were harvested for Western blot experiments. First, RIPA buffer (E-BC-R327, Elabscience, Wuhan, China) with the protease inhibitor PMSF (E-EL-SR002, Elabscience, Wuhan, China) was used to lyse TPC-1 or HTH83 cells with or without transfection of the GNA15 plasmid or/and sh-BTK, and the protein in the lysate was quantified with the BCA Protein Concentration Assay Kit (E-BC-K318-M, Elabscience, China). Afterward, electrophoresis was performed in 10% SDS-PAGE gels (P0670, Beyotime, China) to separate proteins, which were then transferred to nitrocellulose membranes (FFN08, Beyotime, China). Subsequently, membranes were treated with 5% BSA Blocking Buffer (SW3015, Solarbio, Beijing, China) at room temperature for 1h, and incubated with primary antibodies against phosphorylated-extracellular regulated protein kinase (p-ERK, #9101, 42 kDa, 44 kDa, 1/1000, Cell Signaling Technology, Beverly, MA, USA), ERK (#4695, 42 kDa, 44 kDa, 1/1000, Cell Signaling Technology, USA), p-c-Jun N-terminal kinase (p-JNK, #9255, 46 kDa, 54 kDa, 1/2000, Cell Signaling Technology, USA), JNK (#9252, 46 kDa, 54 kDa, 1/1000, Cell Signaling Technology, USA), p-p38 (#4511, 43 kDa, 1/1000, Cell Signaling Technology, USA), p38 (#8690, 40 kDa, 1/1000, Cell Signaling Technology, USA), and GAPDH (ab8245, 36 kDa, 1/1000, Abcam, UK) at 4°C overnight in the dark. The next day, secondary antibodies (#14709S, #7074, Cell Signaling Technology, USA) at a dilution of 1:1000 were used to treat the membranes for 2h at room temperature. The primary and secondary antibodies mentioned above were diluted in antibody dilution buffer (E-IR-R121, Elabscience, Wuhan, China). The protein bands were developed in a CL750 iBright Imaging System (Thermo Fisher, USA) with the assistance of ECL Substrates (E-BC-R347, Elabscience, China). GAPDH was used as the housekeeping gene.

Statistical analysis

GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA) was applied for analyzing data. Measurement data were described as mean ± standard deviation. The independent samples t-test and one-way analysis of variance were applied for comparison between two groups and among multiple groups, respectively. $p<0.05$ was considered statistically significant.

Results

GNA15 presented high expression in TC samples

The analysis of UALCAN results showed that GNA15 was highly expressed in patients with TC compared with normal samples (Fig. 1A, p=7.544E-07).

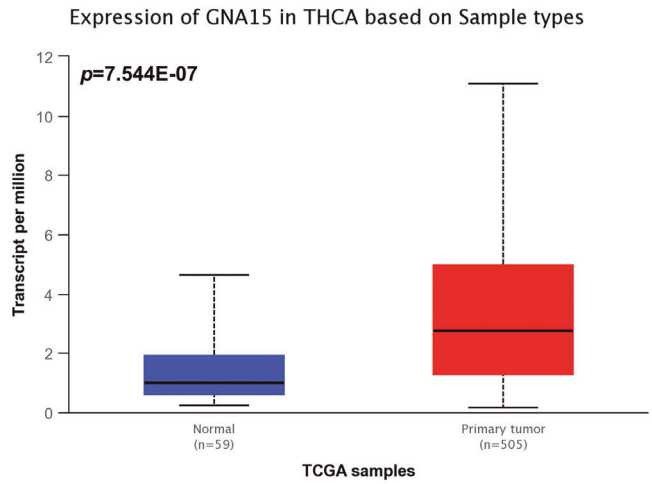

Fig. 1. The expression of GNA15 in TC samples. UALCAN (http://ualcan.path.uab.edu/) was employed to analyze GNA15 expression in thyroid carcinoma (TC) (n=505) and normal samples (n=59).

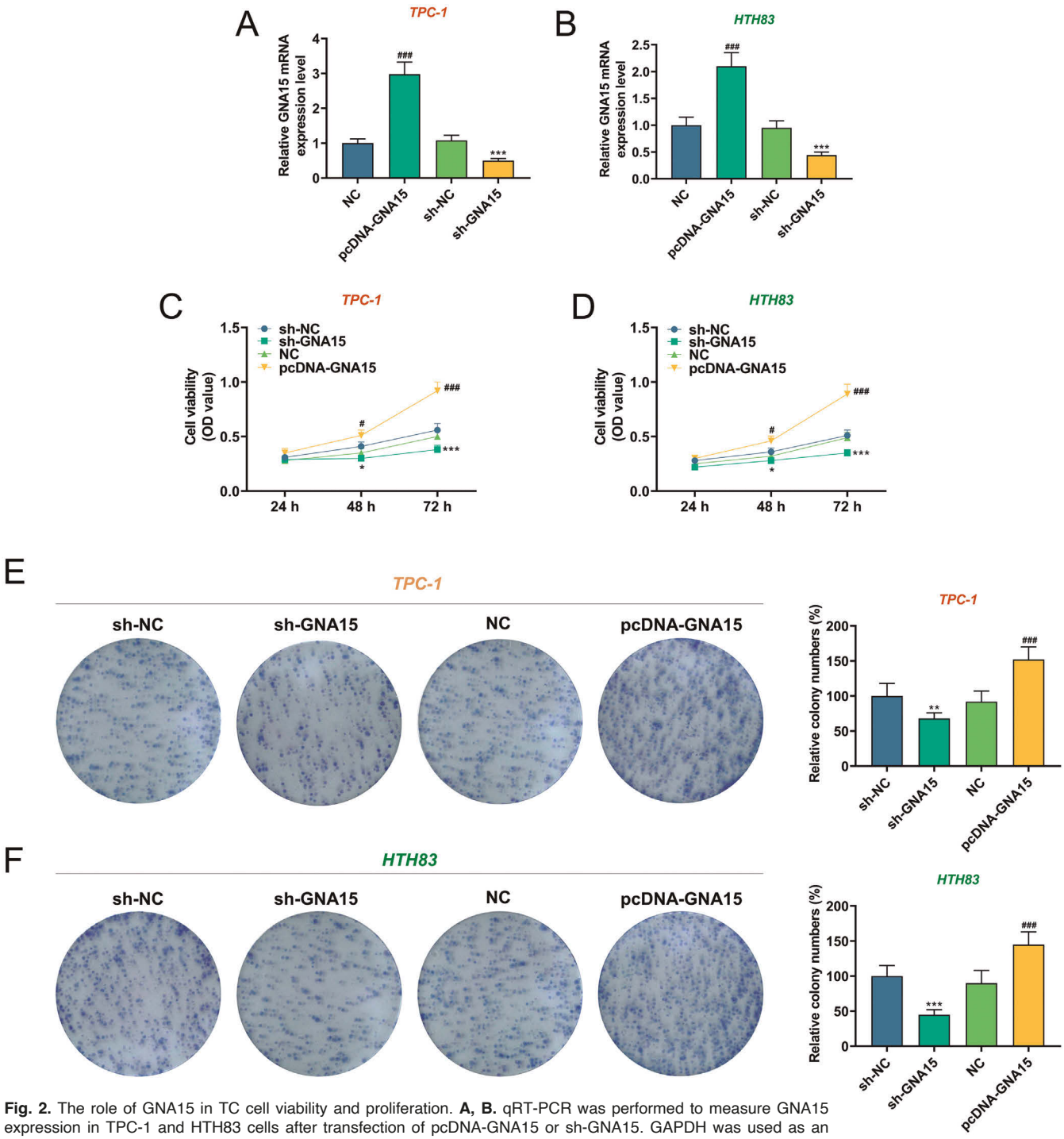

Fig. 2. The role of GNA15 in TC cell viability and proliferation. **A, B.** qRT-PCR was performed to measure GNA15 expression in TPC-1 and HTH83 cells after transfection of pcDNA-GNA15 or sh-GNA15. GAPDH was used as an internal control. **C, D.** MTT assay was used to measure optical density (OD) values in transfected cells. **E, F.** Colony formation assay was carried out for proliferative capacity measurement in transfected cells. *p<0.05, **p<0.01, ***p<0.001 vs. sh-NC; $^{\#}p$<0.05, $^{\#\#\#}p$<0.001 vs. NC. TC, thyroid carcinoma; GNA15, G protein subunit alpha 15; qRT-PCR, quantitative real-time polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; sh-GNA15, short hairpin RNA against GNA15; NC, negative control.

GNA15 positively regulated malignant phenotypes of TC cells

After TC cells were transfected with pcDNA-GNA15 or sh-GNA15, GNA15 expression was quantified by qRT-PCR. As shown in Figure 2A,B, pcDNA-GNA15 remarkably elevated (p<0.001), while sh-GNA15 strikingly reduced the expression of GNA15 in both TPC-1 and HTH83 cells (p<0.001). The OD value indicated that the viability of TC cells was enhanced after GNA15 overexpression (Fig. 2C,D, p<0.05), and suppressed after GNA15 silencing (Fig. 2C,D, p<0.05). The following colony formation assay results proved that TC cell colony number declined after

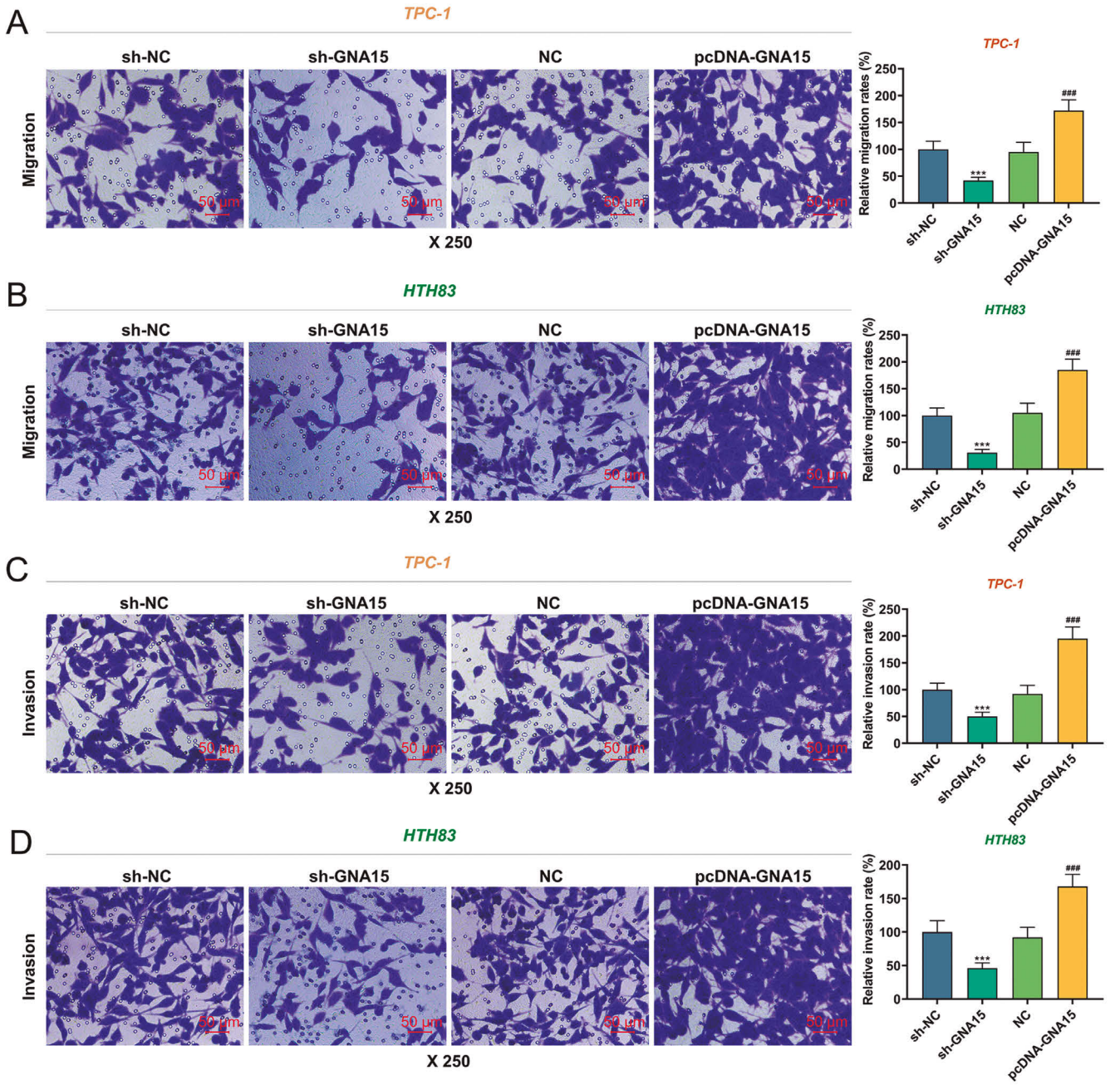

Fig. 3. The role of GNA15 in TC cell migration and invasion. **A-D.** After transfection with pcDNA-GNA15 or sh-GNA15, the migratory and invasive capacities of TPC-1 and HTH83 cells were detected by Transwell assay. A microscope was used to observe the results. ***p<0.001 vs. sh-NC; ###p<0.001 vs. NC. TC, thyroid carcinoma; GNA15, G protein subunit alpha 15; sh-GNA15, short hairpin RNA against GNA15; NC, negative control.

GNA15 silencing (Fig. 2E,F, $p<0.01$) but was elevated after GNA15 overexpression (Fig. 2E,F, $p<0.001$). As illustrated in Figure 3A-D, overexpression of GNA15 not only strengthened the migratory capacity of TPC-1 and HTH83 cells but also potentiated their invasive capacity ($p<0.001$), while silencing of GNA15 generated opposite results ($p<0.001$).

The MAPK signaling pathway was activated by GNA15 overexpression in TC cells

According to Figure 4A,B, Western blot results indicated that the ratios of p-ERK/ERK, p-JNK/JNK, and p-p38/p38 were upregulated by GNA15 overexpression in TPC-1 and HTH83 cells ($p<0.01$).

The depletion of BTK reversed the effects of GNA15 overexpression on malignant phenotypes of TC cells

Based on our previous findings that BTK could be activated through binding with GNA15, we then investigated whether BTK is implicated in the mechanism of GNA15 in TC progression. After transfection with sh-BTK, we observed an evident decrease in BTK expression in TPC-1 and HTH83 cells (Fig. 5A,B, $p<0.001$). Notably, sh-BTK suppressed the viability (Fig. 5C,D, $p<0.05$), proliferation (Figure 5E,F, $p<0.001$), migration and invasion (Fig. 6A-F, $p<0.01$) of TC cells. Moreover, the enhancement of cell viability, proliferation, migration, and invasion by GNA15 overexpression was abrogated by BTK silencing (Figs. 5C-F, 6A-F, $p<0.01$). Similarly, the suppressing effect of sh-BTK on malignant behaviors of TC cells was reversed by GNA15 overexpression (Figs. 5C-F, 6A-F, $p<0.05$).

The effect of GNA15 on the MAPK signaling pathway in TC cells was mediated by BTK

As demonstrated in Figure 7A-D, significant downregulation of p-ERK/ERK, p-JNK/JNK, and p-p38/p38 ratios was observed in TPC-1 and HTH83 cells transfected with sh-BTK ($p<0.05$), which was abrogated by GNA15 overexpression ($p<0.05$). Elevation of p-ERK/ERK, p-JNK/JNK, and p-p38/p38 ratios by GNA15 overexpression in TC cells was reversed by sh-

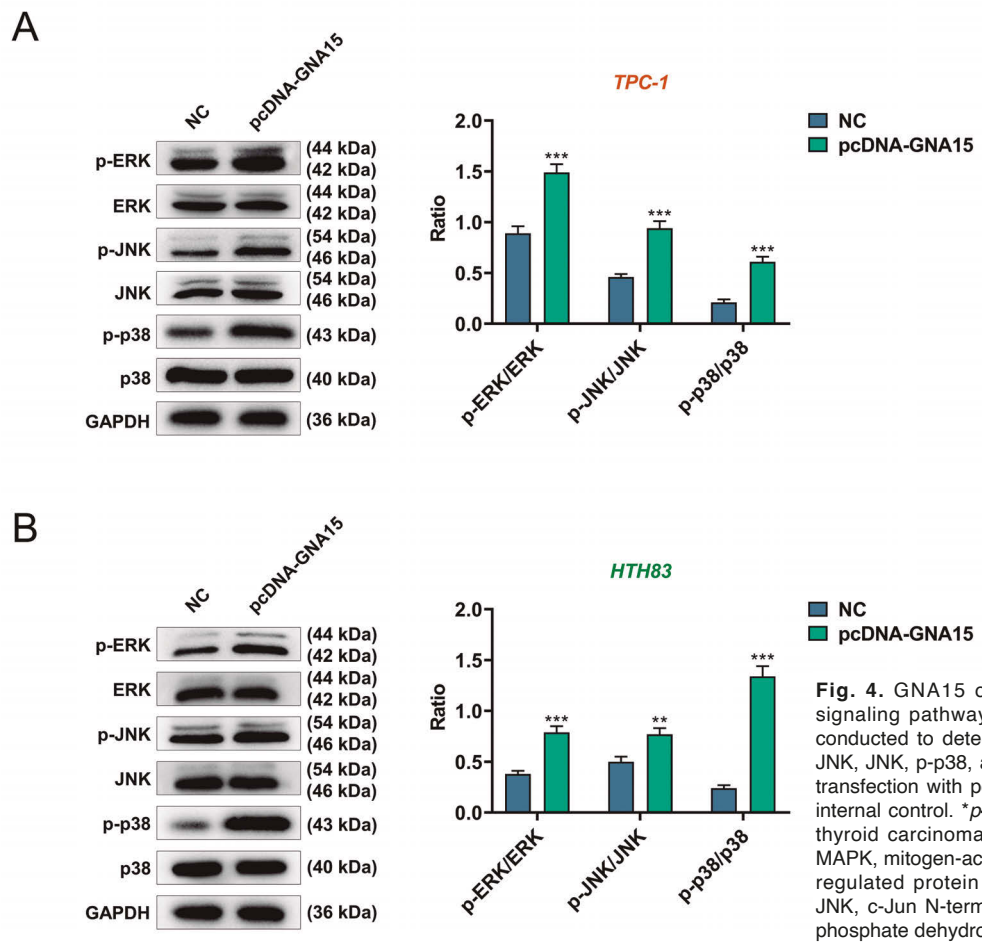

Fig. 4. GNA15 overexpression activated the MAPK signaling pathway in TC cells. **A, B.** Western blot was conducted to detect the protein levels of p-ERK, ERK, p-JNK, JNK, p-p38, and p38 in TPC-1 and HTH83 cells after transfection with pcDNA-GNA15. GAPDH was used as an internal control. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. NC. TC, thyroid carcinoma; GNA15, G protein subunit alpha 15; MAPK, mitogen-activated protein kinase; ERK, extracellular regulated protein kinase; p-ERK, phosphorylated ERK; JNK, c-Jun N-terminal kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NC, negative control.

BTK (Fig. 7A-D, $p<0.001$).

Discussion

The present study produced the first evidence that GNA15 is implicated in TC tumorigenesis. Loss/gain-of-function experiment results demonstrated that the increment in tumor cells was attributed to the upregulation of GNA15 in TC cells. Furthermore, we found that GNA15 could activate the MAPK signaling pathway by binding to BTK in TC cells. These findings implied that GNA15 can act as an oncogene for TC development by triggering the MAPK signaling pathway.

Most patients with TC can be treated satisfactorily with surgery, radioactive iodine therapy, locoregional therapy, systemic therapy, and personalized medicine, however, for some patients with distant metastasis, tumor recurrence, or resistance, there is still no effective therapeutic strategy (Filetti et al., 2019). Tumor invasion and metastasis are major causes of death in patients with TC. A recent study identified that a GNA15 mutation is involved in the development of congenital vascular tumors, and makes cells independent of growth factors via MAPK activation (Lim et al., 2016). In hepatocellular carcinoma, Wu et al. demonstrated that high expression of GNA15 is involved in the aggressive proliferation of tumor cells, which could be inhibited

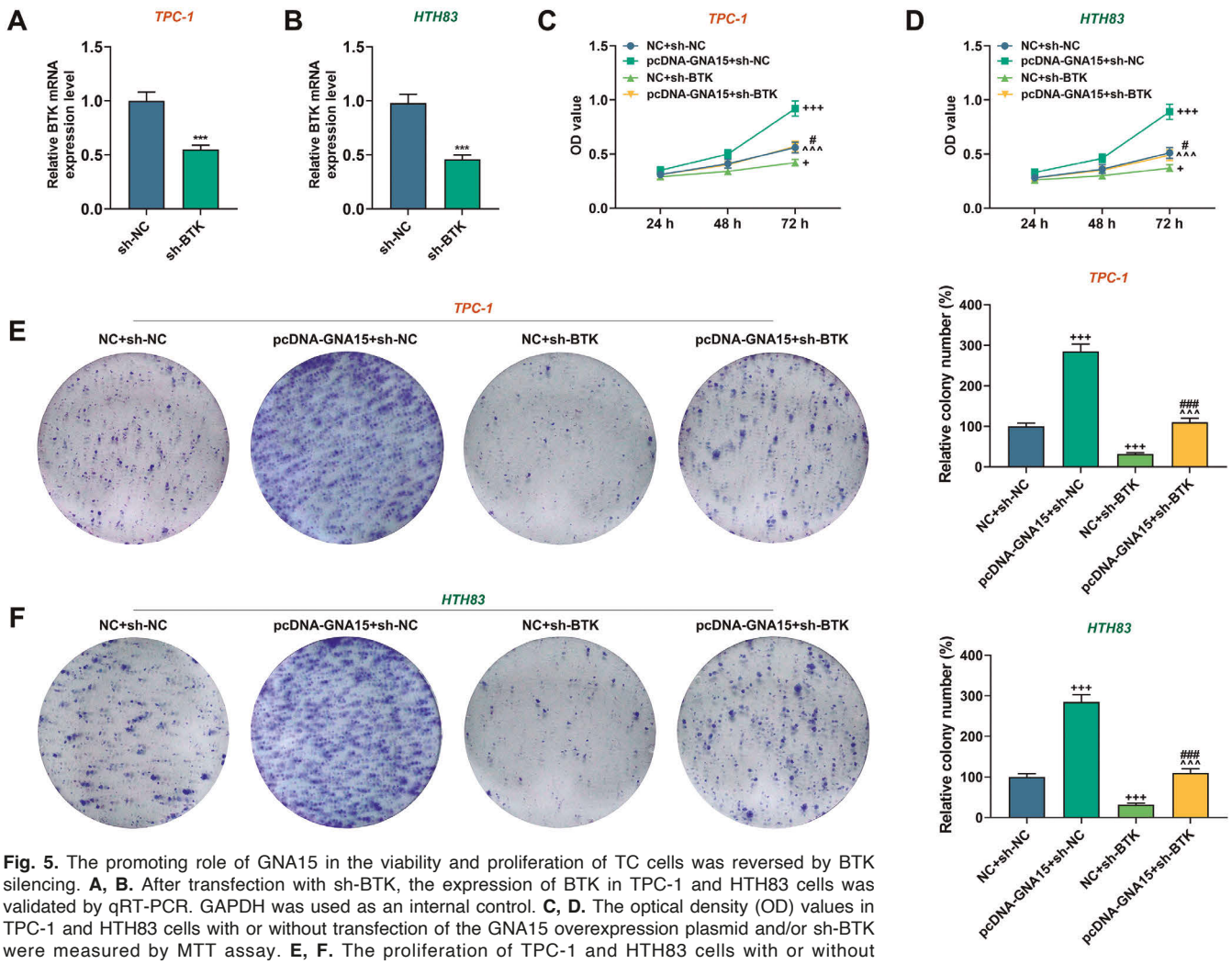

Fig. 5. The promoting role of GNA15 in the viability and proliferation of TC cells was reversed by BTK silencing. **A, B.** After transfection with sh-BTK, the expression of BTK in TPC-1 and HTH83 cells was validated by qRT-PCR. GAPDH was used as an internal control. **C, D.** The optical density (OD) values in TPC-1 and HTH83 cells with or without transfection of the GNA15 overexpression plasmid and/or sh-BTK were measured by MTT assay. **E, F.** The proliferation of TPC-1 and HTH83 cells with or without transfection of GNA15 overexpression plasmid and/or sh-BTK was determined by the colony formation assay. $^{***}p<0.001$ vs. sh-NC; $^{+}p<0.05$, $^{+++}p<0.001$ vs. NC + sh-NC; $^{\wedge\wedge\wedge}p<0.001$ vs. pcDNA-GNA15 + sh-NC; $^{\#}p<0.05$, $^{\#\#\#}p<0.001$ vs. NC + sh-BTK. TC, thyroid carcinoma; BTK, Bruton tyrosine kinase; GNA15, G protein subunit alpha 15; qRT-PCR, quantitative real-time polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; sh-BTK, short hairpin RNA against BTK; NC, negative control.

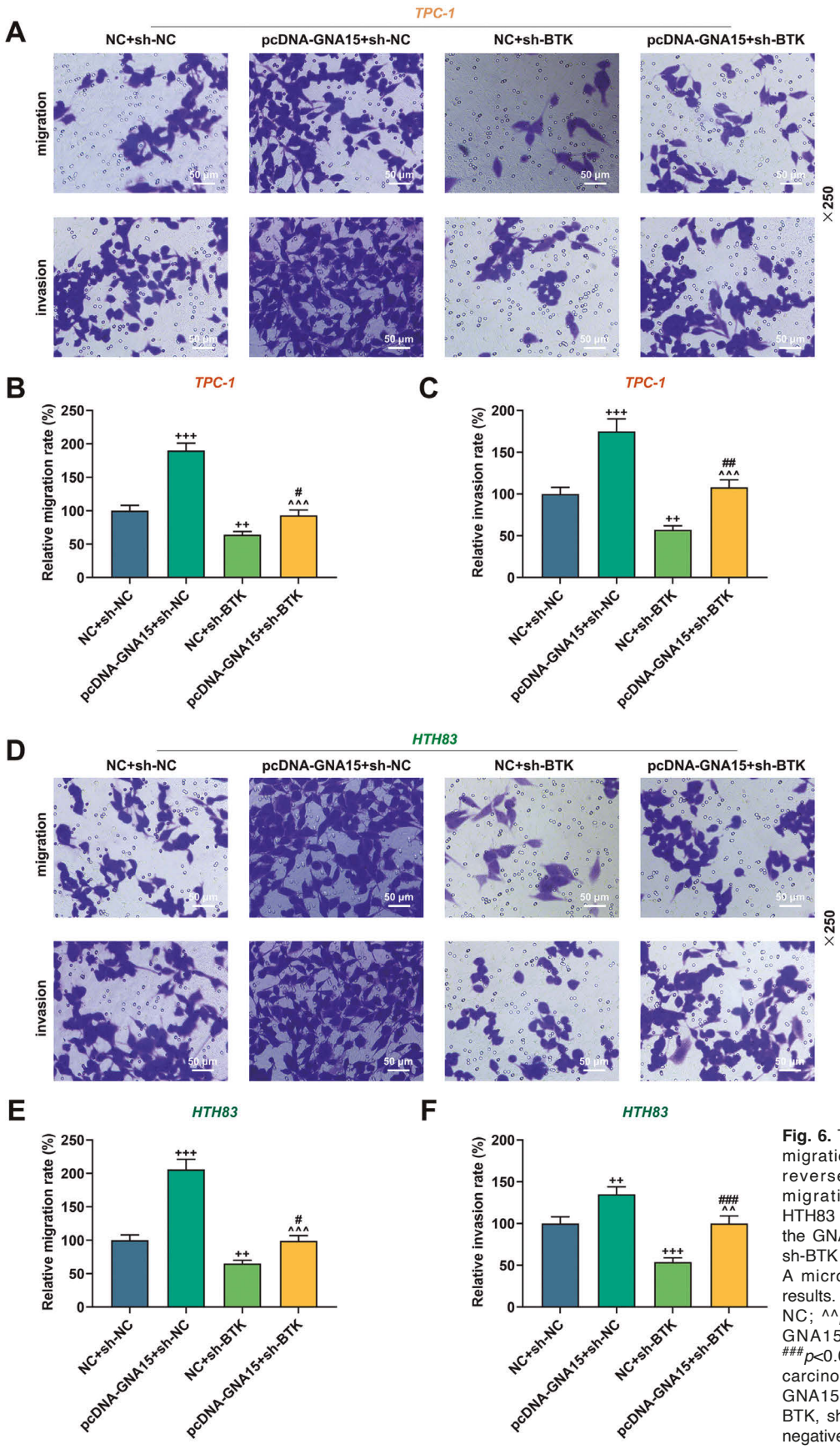

Fig. 6. The promoting role of GNA15 in the migration and invasion of TC cells was reversed by BTK silencing. **A-F.** The migration and invasion of TPC-1 and HTH83 cells with or without transfection of the GNA15 overexpression plasmid and/or sh-BTK were detected by Transwell assay. A microscope was used to observe the results. $^{++}p<0.01$, $^{+++}p<0.001$ vs. NC + sh-NC; $^{\wedge\wedge}p<0.01$, $^{\wedge\wedge\wedge}p<0.001$ vs. pcDNA-GNA15 + sh-NC; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. NC + sh-BTK. TC, thyroid carcinoma; BTK, Bruton tyrosine kinase; GNA15, G protein subunit alpha 15; sh-BTK, short hairpin RNA against BTK; NC, negative control.

when GNA15 expression is downregulated by the monoclonal antibody AC10364 (Wu et al., 2019). In pancreatic ductal adenocarcinoma, Innamorati et al. revealed that GNA15 expression is positively related to the infiltration of neoplastic cells, and the demethylation of the 5' GNA15 promoter region promotes the diffusion of transformed cells (Innamorati et al., 2021). In this study, we first confirmed that GNA15 overexpression enhanced TC cell proliferation, migration, and invasion *in vitro*, suggesting that GNA15 contributed to the malignant development of TC.

Studies have reported that some oncogenes, such as VEGF and certain calcium-binding proteins, can promote tumor infiltration and metastasis by reducing intercellular adhesion through phosphorylation of p38 (Kwon et al., 2013; Lu et al., 2020). It has been evidenced that TC cells were killed by a certain concentration of vitamin C by inhibiting the MAPK/ERK pathway, prompting downregulation of epidermal growth factor (EGF) and thus reducing the number of proliferating cells (Su et al., 2019). Besides, ERK is also involved in tumor invasion and metastasis, abnormal activation of the ERK/MAPK signaling pathway facilitates the proliferation of breast cancer cells (Chen et al., 2017). The infiltration and metastasis of tumors require epithelial-mesenchymal transition (EMT), and the development of EMT involves the transduction of multiple signaling pathways. JNK is an important branch of the MAPK pathway that is involved in a variety of physiological and pathological cell processes, and JNK has been verified to modulate EMT (Guan et al., 2017). Based on the above findings, we measured ERK, JNK, and p38 levels as well as their phosphorylated levels in TC cells with GNA15 overexpression, and found that p-ERK/ERK, p-JNK/JNK, and p-p38/p38 ratios were strikingly increased by GNA15 overexpression, indicating that the promoting role of GNA15 in TC progression was realized by activating the MAPK signaling pathway.

Previous evidence has shown that BTK presents a high expression pattern in thyroid tumors, signifying that

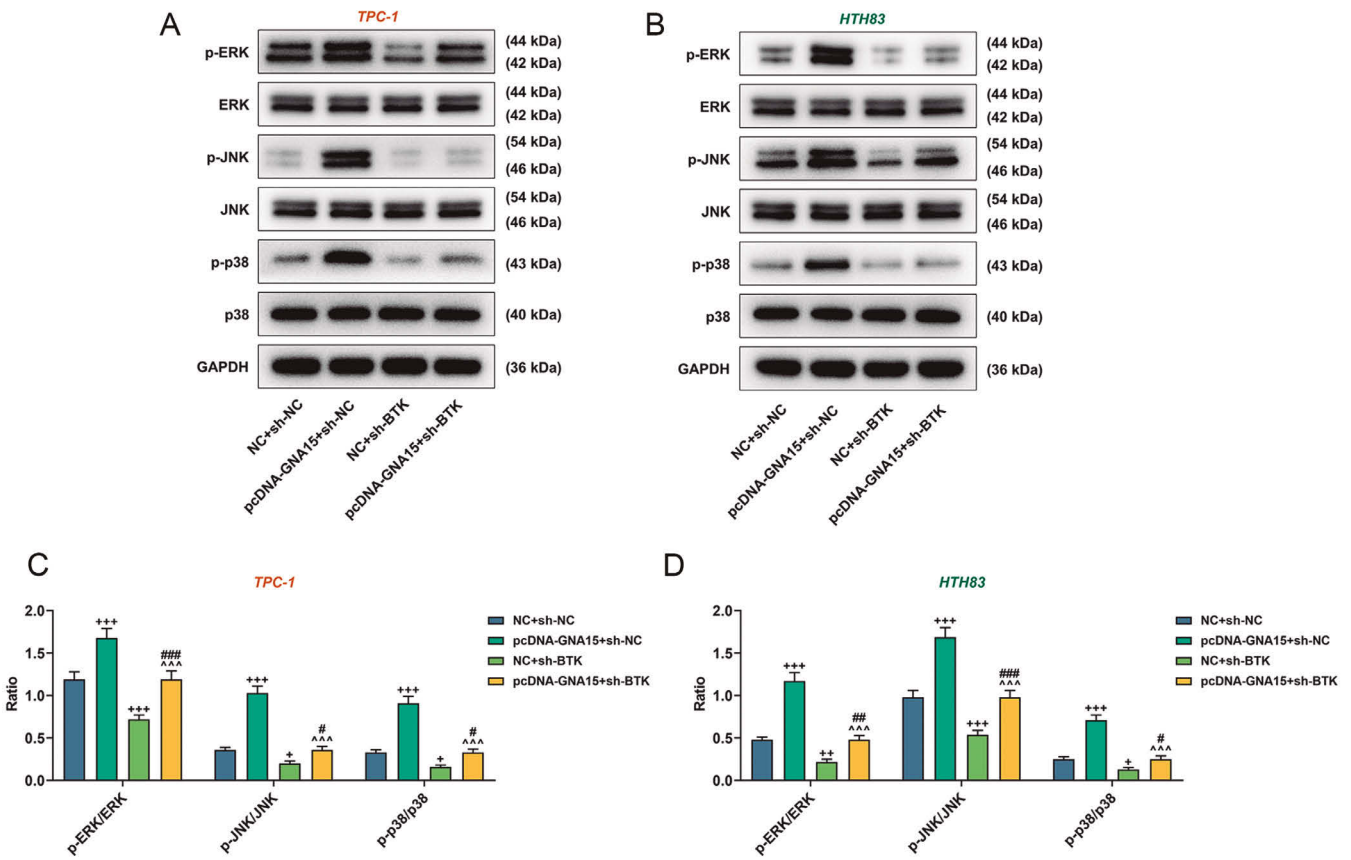

Fig. 7. The activation of the MAPK signaling pathway in TC cells by GNA15 overexpression was counteracted by BTK silencing. **A-D.** After transfection with the GNA15 overexpression plasmid and/or sh-BTK in TPC-1 and HTH83 cells, protein levels of p-ERK, ERK, p-JNK, JNK, p-p38, and p38 were measured by Western blot. GAPDH was used as an internal control. $^{+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$ vs. NC + sh-NC; $^{\wedge\wedge\wedge}p<0.001$ vs. pcDNA-GNA15 + sh-NC; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. NC + sh-BTK. TC, thyroid carcinoma; BTK, Bruton tyrosine kinase; GNA15, G protein subunit alpha 15; MAPK, mitogen-activated protein kinase; ERK, extracellular regulated protein kinase; p-ERK, phosphorylated ERK; JNK, c-Jun N-terminal kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; sh-BTK, short hairpin RNA against BTK; NC, negative control.

BTK is a promising target for the treatment of advanced TC (Cho et al., 2012). BTK belongs to the TEC family of non-receptor tyrosine kinases and plays a pivotal role in the B-cell receptor signaling pathway (Herman et al., 2017). Excessive activation of BTK induces abnormal B-cell growth, which is an important factor in the development of many B-cell-related malignant tumors with high recurrence rates, poor prognosis, and unsatisfactory clinical outcomes. Besides, BTK could be stimulated by GNAs in a binding way (Bence et al., 1997; Jiang et al., 1998). Accordingly, we reasonably inferred that BTK might be involved in the development of TC by binding with GNA15. After BTK silencing, we observed that the proliferative, migratory, and invasive abilities of TC cells were weakened, and the promoting effect of GNA15 overexpression on these cell behaviors was offset, which confirmed our inference. Through the Reactome Pathway Database, we also found that the high expression of BTK could activate the downstream p38 MAPK signaling pathway. In the study of B cell lymphoma, ibrutinib, an inhibitor of BTK, blocks the MAPK signaling pathway and thereby suppresses the proliferation of tumor cells *in vivo* (He et al., 2019). As expected, the ratios of p-ERK/ERK, p-JNK/JNK, and p-p38/p38 were significantly reduced in response to BTK silencing, which was reversed with the upregulation of GNA15, confirming that the regulatory effect of GNA15 on the MAPK signaling pathway was mediated by BTK in TC cells.

Taken together, our study revealed, for the first time, a new pathogenesis of TC, in which GNA15 contributes to the malignant development of TC cells by binding to BTK and then triggering the MAPK signaling pathway. Although this study lacks further *in vivo* validation, the current findings may lay novel theoretical foundations for developing diagnostic and therapeutic strategies for TC.

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Data Availability Statement. The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions. Substantial contributions to conception and design: Yihan Sun; Data acquisition, data analysis, and interpretation: Yifan Han; Drafting the article or critically revising it for important intellectual content: Yihan Sun; Final approval of the version to be published: All authors; Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved: Yihan Sun and Yifan Han.

Declaration of conflicting interest. The authors declare that there are no conflicts of interest.

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