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GNA13 Regulates the β -Catenin Pathway to Promote Esophageal Cancer Cell Invasion while Inhibiting Apoptosis

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ABSTRACT: Background: G protein subunit alpha 13 (GNA13) plays a role in the progression of many malignant tumors, but its expression in esophageal carcinoma (ESCA) cells and impact on their biological behavior are unknown. This research aims to clarify the functions and mechanisms of GNA13 on the malignant phenotype of ESCA cells. **Methods:** GNA13 expression in ESCA tissues and cells was assessed using reverse transcription quantitative polymerase chain reaction (RT-qPCR) and Western blot. Sh-GNA13 was transfected into ESCA cells (TE-1 and KYSE-150) to assess the efficiency of GNA13 silencing. The effects of GNA13 silencing on cell viability, clone formation rate, invasion, and apoptosis of ESCA cells were investigated via Cell Counting Kit-8, clone formation, Transwell, and flow cytometry. The expression of epithelial-mesenchymal transition (EMT) marker proteins, apoptotic proteins, and β -catenin pathway proteins was evaluated by Western blot. **Results:** GNA13 expression in ESCA tissues and cells was significantly higher than in paracancerous tissues and normal human esophageal cells, HET1A ($p < 0.05$). Silencing GNA13 suppressed the malignant phenotypes of ESCA cells: cell viability at 48 h was decreased by 25.8%–42.2% ($p < 0.01$), clone formation rate was reduced by 70%–89% ($p < 0.05$), and invasion capacity was attenuated by 51%–56% ($p < 0.05$). Meanwhile, GNA13 knockdown inhibited the EMT process and increased the apoptotic rate by 3.0–3.2-fold ($p < 0.05$). Silencing GNA13 reduced the expression of β -catenin pathway proteins ($p < 0.05$), while the β -catenin pathway activator SKL2001 reversed the tumor-suppressive effects induced by GNA13 silencing ($p < 0.05$). **Conclusion:** GNA13 increased ESCA cell invasion and prevented apoptosis by stimulating the β -catenin pathway. The findings may lead to new approaches for diagnosing and treating ESCA.

KEYWORDS: Esophageal carcinoma; G protein subunit alpha 13; β -catenin pathway; invasion; apoptosis

1 Introduction

Esophageal carcinoma (ESCA) is among the most aggressive gastrointestinal tumors, accounting for the sixth largest cause of cancer death globally [1,2]. ESCA ranks as one of the most frequent malignant tumors in China, accounting for the fourth highest cause of cancer-related fatalities [3]. The prognosis for patients with ESCA remains poor despite recent advancements in comprehensive treatment options and diagnostic methods, with a survival rate after five years of fewer than 30% and a postoperative recurrence rate of approximately 50% [4,5]. This is primarily owing to the fact that the majority of ESCA patients are diagnosed in the mid- to late phases, and the tumors have metastasized locally and/or exhibited abnormal biological behaviors of the cells [6]. As a result, it is essential to identify and explore the genes and molecular mechanisms associated with ESCA growth and metastasis, as well as to screen for biomarkers with potential application value, which can serve as potential targets for early screening and subsequent therapeutic interventions for ESCA.

The malignant biological activity of ESCA is a complicated process influenced by a variety of variables, phases, signaling pathways, and environmental exposures [7,8]. The progression of ESCA from normal esophageal epithelial cells to mild, moderate, and severe atypical hyperplasia, to carcinoma *in situ*, and finally to invasive and metastatic carcinoma is a long process that is regulated by many related genes, which further affects the signaling pathways critical for the biological functions of cell growth, apoptosis, and metastasis, and induces ESCA [9,10]. Guanine nucleotide-binding protein subunit $\alpha 13$ (G protein subunit $\alpha 13$, GNA13) is found on human chromosome 17 and binds to G protein-coupled receptors (GPCRs) to affect a variety of cellular activities and participates in the regulation of cell proliferation and invasion [11,12]. Accumulating evidence suggests that GNA13 plays an oncogenic role in a variety of tumors [13,14]. For instance, high expression of GNA13 enhances the growth and migration of lung carcinoma with squamous cells [12]. Furthermore, GNA13 induces drug resistance and the tumor-initiating cell (TIC) phenotype in head and neck squamous cell carcinoma [15]. Studies have confirmed that high GNA13 expression is associated with a poor prognosis in ESCA [16], but the mechanism of its action is unknown.

Rho proteins (also known as small G proteins, Rho small GTPases), primarily including RhoA, Rac1, and Cdc42, toggle among active (Rho-GTP-bound) and inactive (Rho-GDP-bound) states modulated by guanine nucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs), acting as molecular switches to regulate a variety of important signaling pathways [17,18]. Notably, overexpression of GNA13 inhibited the activation and protein expression of RhoA and promoted migration and invasion of breast cancer cells [13]. β -catenin is essential for the traditional Wnt signaling pathway, which promotes malignant phenotypes in tumors and regulates biological processes like proliferation, differentiation, and death in human cancer cells [19,20]. The protein stability and nuclear translocation of β -catenin are critical for activating the Wnt pathway. In the resting state, β -catenin is found at low levels in the cytoplasm and binds with E-cadherin at the cell membrane, blocking it from accessing the nucleus. When the Wnt signaling pathway is engaged, β -catenin translocates into the cell nucleus and interacts with TCF/LEF transcription factors to form a functional complex. This complex activates downstream genes and governs cell growth [21,22]. A previous study has shown that down-regulation of RhoA induces the activation of β -catenin signaling and leads to increased intestinal tumorigenesis [23]. The recent discovery of DK419, a novel Wnt inhibitor that effectively blocks β -catenin nuclear translocation and overcomes chemoresistance in glioblastoma, further underscores the therapeutic potential of targeting this pathway in solid tumors [24]. Building on these findings, it is hypothesized that GNA13 regulates the β -catenin pathway, influencing the development of ESCA.

However, the biological role of GNA13 in ESCA and its downstream regulatory mechanisms have not been fully elucidated. This study was designed to characterize GNA13 expression in ESCA clinical tissues and cell lines, evaluate its impact on the malignant phenotypes of ESCA cells, and explore its potential association with the β -catenin pathway. This study aims to deliver new insights into ESCA management and offer experimental evidence for targeted inhibition of GNA13 as a promising therapeutic strategy against ESCA growth and metastasis.

2 Materials and Methods

2.1 Bioinformatics Analysis

GNA13 expression levels in ESCA were examined using the GEPIA platform (<http://gepia.cancer-pku.cn/>). The “Match TCGA normal and GTEx data” option was selected to integrate ESCA tumor samples from The Cancer Genome Atlas (TCGA) with matched normal esophageal tissues from both TCGA and GTEx datasets for pairwise comparison. One-way ANOVA was employed as the built-in statistical method for differential expression testing, with predefined cutoff values set as $|\log_2 \text{ fold change}| > 1$ and $p < 0.01$.

2.2 Tissue and Cell Specimens

The 28 ESCA tissue samples and paracancerous tissue samples were taken from pathologic biopsy specimens at Medical College of Huanghuai University. This investigation was reviewed and approved by the Ethics Committee of Medical College of Huanghuai University (Approval Number: KY-2025-01-101). All participants were told of the study design and approach, and completed an informed consent form.

Human esophageal normal cells (HET1A; Cat. No.: STM-CL-5534) and human ESCA cell lines KYSE-450 (Cat. No.: STM-CL-5553), KYSE-150 (Cat. No.: STM-CL-5150), TE-1 (Cat. No.: STM-CL-5001), and KYSE30 (Cat. No.: STM-CL-5204) were obtained from Stemcell Biotechnology (Shanghai, China). All the cells have been identified by STR analysis, and there is no mycoplasma contamination. The cells were grown in RPMI-1640 medium with 10% fetal bovine serum (FBS, C0235, Beyotime, Shanghai, China) and 1% penicillin-streptomycin double antibiotic (15140122, Gibco, Big Cabin, OK, USA) in a cell culture incubator at 37°C with 5% CO₂.

2.3 Cell Transfection

Ribobio (Guangzhou, China) offered GNA13 short hairpin RNA (sh-GNA13; TAGCAGTTTACAACCA-GAA) and negative control (sh-NC; TTCTCCGAACGTGTCACGT). KYSE-150 and TE-1 cells in logarithmic growth phase were digested and seeded into 24-well plates: the rapidly growing KYSE-150 cell line was seeded at a density of 5×10^4 cells/well, and the TE-1 cell line was seeded at 1×10^5 cells/well. After 12–16 h of incubation, cells reached 60%–70% confluence and were then subjected to transfection using Lipofectamine 3000 reagent (L3000001, Invitrogen, Carlsbad, CA, USA). At 6 h post-transfection, the culture medium was replaced with fresh complete medium to remove transfection complexes. Cells were harvested at 48 h post-transfection, and transfection efficiency was determined by Western blot. For the sh-GNA13+SKL2001 group, TE-1 and KYSE-150 cells were transfected with sh-GNA13; at 6 h post-transfection, the culture medium was replaced with fresh complete medium. At 24 h post-transfection, the β -catenin pathway activator SKL2001 (40 μ M, HY-101085, MedChemExpress, Monmouth Junction, NJ, USA) was added to the culture medium, and the incubation was continued for another 24 h [25].

2.4 Reverse Transcription Quantitative PCR (RT-qPCR)

The total RNA of ESCA tissue samples and KYSE-150 and TE-1 cells was extracted with the TRIzol kit (DP424, TIANGEN, Beijing, China). Following the instructions of the PrimeScript RT Kit (RR047A, Takara, Tokyo, Japan), the purified total RNA was reverse transcribed into cDNA. RT-qPCR reactions were set up in a 20 μ L reaction system containing 10 μ L of SYBR Green PCR Master Mix (4309155, Applied Biosystems, Foster City, CA, USA), 2 μ L of cDNA template, 0.4 μ L forward and 0.4 μ L reverse primers (both at 10 μ M), and 7.2 μ L of RNase-free water. The amplification was conducted on the RT-qPCR system (PRISM 7300, ABI, Carlsbad, CA, USA). The amplification procedure was set with the following parameters: a pre-denaturation step held at 95°C for 10 min, 40 consecutive cycles of 95°C denaturation for 15 s and 60°C annealing for 60 s, and a final melting curve analysis to confirm the specificity of amplification products. GAPDH was employed as the internal reference gene. The $2^{-\Delta\Delta C_t}$ technique was applied to determine the comparative levels of each gene. The primer sequences (5′–3′) for the examined genes were as follows:

GNA13: F: 5′-AGTGATGGGCGATGGAAAGG-3′, R: 5′-GGCAGGGACTGTCTCACATT-3′.

cytc: F: 5′-CACCCGCAGGAATACCCATT-3′, R: 5′-TTGTTACGTGTCTCGTACTT-3′.

Bax: F: 5′-ACCTACAGAGCGGGGATGAT-3′, R: 5′-CCTAAACAGGCCTCGACGAA-3′.

Bcl-2: F: 5′-GGAACATGGCACACACTGGA-3′, R: 5′-GAACCGGAGGGAGGTTTCAG-3′.

GAPDH: F: 5′-GGGAAACTGTGGCGTGAT-3′, R: 5′-GAGTGGGTGTCGCTGTTGA-3′.

2.5 Western Blot

Pre-chilled RIPA buffer (20101ES60, Yeasen, Shanghai, China) was added to the KYSE-450, KYSE-150, TE-1, and KYSE30 cells and ESCA tissue samples for lysis, and total proteins were extracted. A BCA protein assay kit (P0012, Beyotime) was utilized to measure the protein content of all prepared samples. Equal amounts of total protein (30 µg/lane) were mixed with SDS loading buffer, subjected to 10 min of boiling for denaturation, and then separated by 10% SDS-PAGE. The proteins were transferred to PVDF membranes, which were then blocked with 5% bovine serum albumin for 1 h at 25°C. Membranes were incubated overnight with diluted primary antibodies (Table 1) at 4°C, then washed with TBST, and treated with HRP-labeled goat anti-rabbit IgG (1:2000, ab6721, Abcam) at 25°C for 30 min. Protein bands were developed with an ECL kit (P0018S, Beyotime), and band images were captured using a chemiluminescent image analysis system (5200, Tanon, Shanghai, China). Using GAPDH as the loading control, the gray value of each protein band was analyzed by ImageJ 1.8.0 software (National Institutes of Health, Bethesda, MD, USA). In addition, cytoplasmic and mitochondrial proteins were extracted using the mitochondrial/cytosol separation kit (C3601, Beyotime), respectively, to detect cytc expression in the cytoplasmic fraction.

Table 1: Primary antibodies used in Western blot.

Antibody	Cat. No.	Dilution	Manufacturer
GNA13	ab128900	1:2000	Abcam
E-cadherin	ab40772	1:1000	Abcam
cytc	ab133504	1:5000	Abcam
N-cadherin	ab76011	1:5000	Abcam
Bcl-2	ab182858	1:2000	Abcam
Vimentin	ab92547	1:1000	Abcam
Bax	ab32503	1:1000	Abcam
DVL-1	ab233003	1:1000	Abcam
p-GSK-3β	ab75814	1:10,000	Abcam
GSK-3β	ab32391	1:5000	Abcam
β-catenin	ab6302	1:4000	Abcam
GAPDH	ab9485	1:2500	Abcam

2.6 CCK-8 Detection

Post-transfection, TE-1 and KYSE-150 cells were digested with 0.25% trypsin (25200114, Gibco), centrifuged at 4°C for 5 min (500× g) using a centrifuge (Pro2415R, Everfuge Biotech Instrument, Beijing, China), and then inoculated into 96-well plates (1 × 10⁴/well). Each well received 200 µL of culture solution and was placed in a 37°C, 5% CO₂ incubator for 24, 48, 72, or 96 h. 20 µL of CCK-8 solution (HY-K0301, MedChemExpress) was introduced into each well, which was thoroughly mixed and incubated for 2 h. The optical density (OD) of each well at 450 nm was then assessed by a SpectraMax Mini microplate reader (Molecular Devices, San Jose, CA, USA). The cell viability ((OD value of the treated group/OD value of the sh-NC group) × 100%) was then computed [26].

2.7 Clone Formation Experiments

Following transfection, TE-1 and KYSE-150 cells were inoculated in 12-well cell culture plates (inner diameter of well is 21.1 mm, CCP-12H, Servicebio, Wuhan, Hubei, China) at a density of 1 × 10³ cells/well and grown in a 37°C incubator for 2 weeks. The medium was replaced every 2~3 days, and the assay was halted when distinct colonies emerged in the wells. The wells were rinsed twice with PBS (0.01 M, pH 7.2), followed by fixation in 100% methanol for 15 min. After removal of the supernatant, the cultures were

treated for 30 min with 0.5% crystal violet (C0121, Beyotime) at 25°C. After washing off the staining solution with running water and drying in air, the clones (>50 cells as 1 clone) were counted, and the relative clone formation rate = colonies in the experimental group/colonies in the control group.

2.8 Cell Invasion Test

Transwell chambers were inserted into 24-well plates. Matrigel (354234, Corning, Corning, NY, USA) was diluted with serum-free RPMI-1640 medium at a ratio of 1:8, and 50 µL of the diluted Matrigel was added to the upper chamber. The plates were incubated at 37°C for 2 h to allow the Matrigel to fully polymerize into gel. The upper chamber received 100 µL of TE-1 or KYSE-150 cell suspension (1×10^5 /mL in serum-free medium), and the lower chamber received 500 µL of RPMI-1640 medium with 15% FBS. Following a 48-h culture period, the plates were removed, and the non-invaded cells on the chambers were carefully removed with a sterile cotton swab. The lower surface-attached invasive cells were subjected to fixation with 4% paraformaldehyde (60536ES, Yeasen, Shanghai, China) for 30 min before being stained with 0.1% crystal violet for 30 min, rinsed twice with PBS, and air-dried. Five random non-overlapping fields of view per chamber were selected and photographed under microscopic photography (DM IL LED, Leica Microsystems, Wetzlar, Germany), and the relative number of invading cells was counted.

2.9 Flow Cytometry

At 48 h post-transfection, both floating apoptotic KYSE-150 and TE-1 cells in the culture supernatant and adherent cells digested with EDTA-free trypsin were combined into one tube, washed twice with PBS (0.01 M, pH 7.2), and gathered by centrifugation (500× g, 4°C, 5 min). Cell pellets were resuspended in 100 µL of binding buffer provided in the Annexin V-FITC/PI kit (40302ES50, Yeasen), mixed well, and then double-stained with 5 µL of Annexin V-FITC and 10 µL of PI, respectively. The mixture was incubated for 15 min in a light-protected condition at 25°C. Unstained blank control, Annexin V-FITC single-stained control, and PI single-stained control were prepared in parallel with test samples to calibrate flow cytometry fluorescence compensation and accurately define the quadrant gates. Following the addition of 400 µL binding buffer, all samples were maintained on ice and detected with a flow cytometer (2010284AA, Agilent, Santa Clara, CA, USA) within 1 h to determine the apoptotic rate.

2.10 Data Analysis

All data processing and statistical analyses were performed using SPSS 26.0 software (IBM Corp., Armonk, NY, USA). Data were presented as mean ± standard deviation ($\bar{x} \pm SD$). The normality of data and homogeneity of variance were verified for all datasets. Statistical differences between two groups were evaluated using Student's *t*-test. One-way ANOVA coupled with Tukey's post hoc test was applied for multiple-group comparisons among three or more experimental groups. $p < 0.05$ indicates statistically significant differences. The figures were drawn with GraphPad Prism 9.0 (GraphPad Inc., La Jolla, CA, USA).

3 Results

3.1 GNA13 Is Abundantly Expressed in ESCA

To investigate GNA13 expression in human ESCA, we performed a GEPIA database search, which revealed that GNA13 was considerably overexpressed in ESCA samples (Fig. 1A). We obtained fresh tumor tissues and corresponding paracancerous tissues from 28 groups of ESCA patients from the Medical College of Huanghuai University, and examined GNA13 expression levels via RT-qPCR and Western blot. GNA13 expression was considerably higher in ESCA tumor specimens compared with paracancerous tissues

(Fig. 1B–D), which was consistent with the results of the GEPIA search. Subsequently, we examined GNA13 expression in the cells and discovered that GNA13 expression was significantly higher in ESCA cell lines (KYSE-450, KYSE-150, TE-1, and KYSE30), particularly in TE-1 and KYSE-150 cells, than in human esophageal normal cells, HET1A (Fig. 1E–G), implying that GNA13 may act as a pro-carcinogenic factor to drive ESCA development. We chose TE-1 and KYSE-150 cells for subsequent cell biological behavior studies.

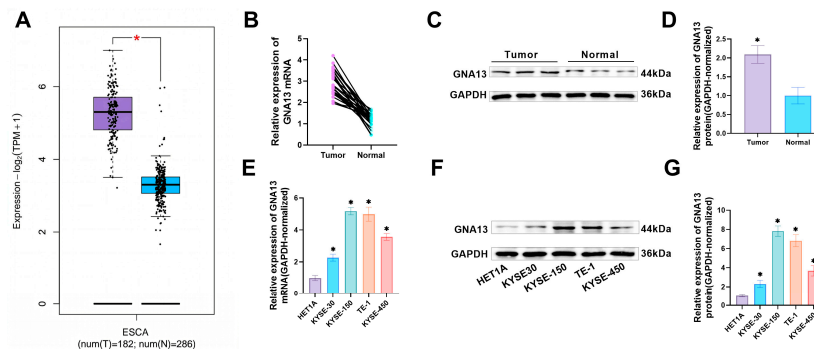


Figure 1: GNA13 is abundantly expressed in ESCA. (A) The GEPIA database was utilized to obtain GNA13 expression discrepancies between normal and ESCA samples. (B) The mRNA levels of GNA13 in 28 ESCA and paracancerous tissues were examined using RT-qPCR (n = 28). (C,D) GNA13 protein levels were assessed in 28 ESCA and paracancerous tissues through Western blot. (E) GNA13 mRNA levels in human esophagus normal cells HET1A and ESCA cell lines (KYSE-450, KYSE-150, TE-1, and KYSE30). (F,G) GNA13 protein expression levels in human esophagus normal cells HET1A and ESCA cell lines. n = 3, *p < 0.05 vs. Normal/HET1A group.

3.2 Silencing the GNA13 Gene Decreases ESCA Cell Proliferation, Migration, and Invasion

To explore the role of GNA13 in the malignant biological behavior of ESCA cells, we transfected sh-GNA13 or sh-NC into TE-1 and KYSE-150 cells. After transfection with sh-GNA13, the GNA13 protein level was notably decreased compared with the sh-NC group (Fig. 2A,B). The impacts of GNA13 gene silencing on ESCA cell growth and clone formation were investigated using the CCK-8 and cell cloning assays. After transfection with sh-GNA13 for 24, 48, 72, and 96 h, the proliferation viability of both cell lines was markedly suppressed when compared to the control group (Fig. 2C,D). Additionally, the number of clones formed by TE-1 and KYSE-150 cells was significantly reduced by the naked eye, and the rate of clone formation was decreased to less than 50% in the sh-GNA13 group (Fig. 2E,F). Protein levels of EMT-associated markers were examined after GNA13 silencing; E-cadherin was notably elevated, while N-cadherin and Vimentin were both markedly reduced (Fig. 2G–J), showing that the cellular epithelial-mesenchymal transition pathway was blocked. As demonstrated in Fig. 2K,L, after transfection with sh-GNA13, the number of TE-1 and KYSE-150 cells invading the bottom chamber was much lower than in the control group. GNA13 gene suppression was revealed to decrease both the malignant phenotype of ESCA cells and the EMT process, implying that GNA13 may be a crucial protein in producing the malignant phenotype of ESCA.

3.3 GNA13 Gene Silencing Promotes Apoptosis in ESCA Cells

GNA13's influence on ESCA cell death was investigated further using flow cytometry. The findings demonstrated that the apoptotic rate of TE-1 and KYSE-150 cells silenced with GNA13 was much higher than that of the cells in the sh-NC group (Fig. 3A,B). We examined the specific alterations in apoptosis-related proteins in ESCA cells after silencing GNA13. Fig. 3C–E showed that GNA13 silencing caused marked upregulation of pro-apoptotic cytc and Bax mRNA levels, and concurrent downregulation of anti-apoptotic Bcl-2 mRNA expression in both cell lines. Additionally, following GNA13 down-regulation, cytc and Bax

protein levels were markedly elevated, whereas Bcl-2 protein was prominently decreased in TE-1 and KYSE-150 cells (Fig. 3F–I).

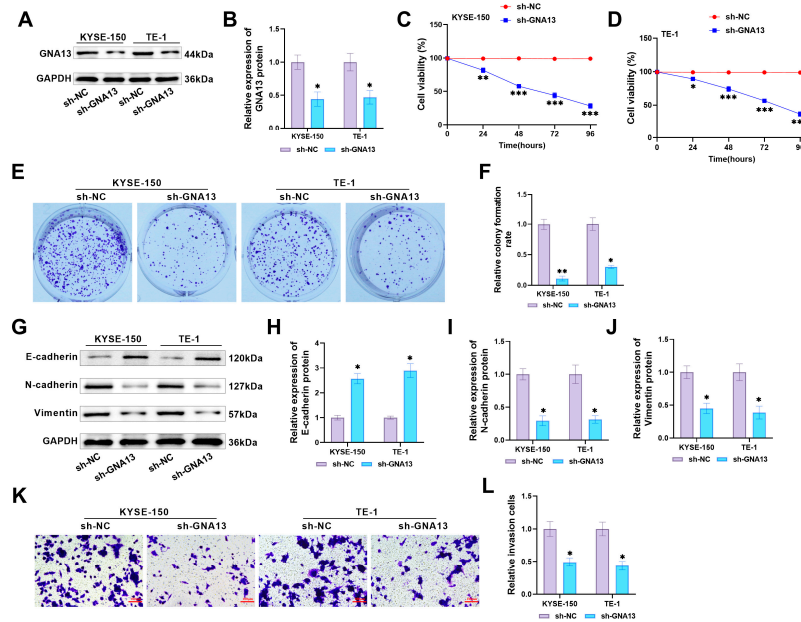


Figure 2: GNA13 gene silencing reduces ESCA cell proliferation, migration, and invasion. (A,B) sh-GNA13 was transfected into TE-1 and KYSE-150 cells, and the effectiveness of GNA13 silencing was assessed by Western blot. (C,D) CCK-8 was employed to assess the impact of GNA13 silencing on the proliferation of ESCA cells. (E,F) GNA13 silencing impaired clone formation in ESCA cells. (G–J) The effect of GNA13 silencing on EMT in ESCA cells was assessed by measuring N-cadherin, E-cadherin, and Vimentin protein expression. (K,L) Transwell assays were used to calculate changes in the number of ESCA cells that invaded the lower chamber following GNA13 silencing (20×; bar = 100 μ m). n = 3, * p < 0.05, ** p < 0.01, *** p < 0.001 vs. sh-NC group.

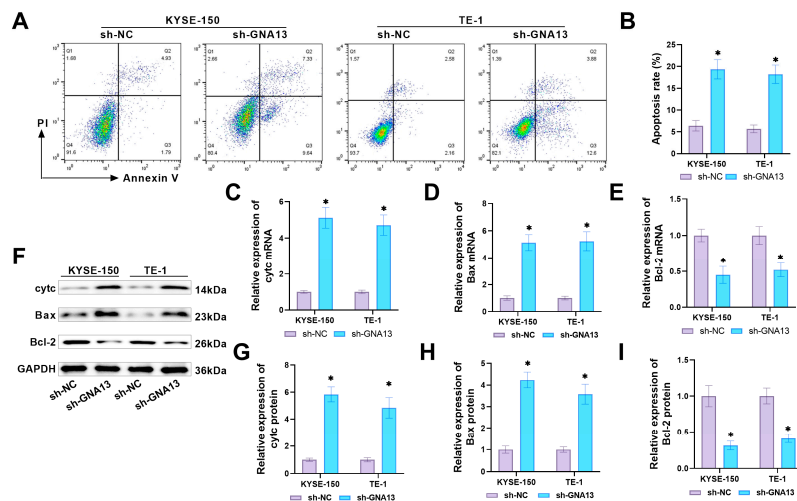


Figure 3: GNA13 gene silencing promotes apoptosis in ESCA cells. (A,B) Flow cytometry analysis of apoptotic levels in TE-1 and KYSE-150 cells following GNA13 gene silencing. (C–E) The impact of GNA13 silencing on cytc, Bax, and Bcl-2 mRNA levels. (F–I) The impact of GNA13 silencing on cytc, Bax, and Bcl-2 protein levels. n = 3, * p < 0.05 vs. sh-NC group.

3.4 Silencing the GNA13 Gene Reduces Activation of the β -Catenin Pathway

The foregoing investigations have proven that GNA13 gene silencing inhibits the malignant development of ESCA cells, although the mechanism and downstream signaling pathways have yet to be investigated. Aberrant activation of the β -catenin pathway has been reported in ESCA [27]. We therefore hypothesized that the β -catenin pathway may be implicated in GNA13-mediated malignant development of ESCA cells. To confirm the speculation, we divided the experiment into three groups: control (sh-NC), GNA13-silencing (sh-GNA13), and GNA13-silencing + β -catenin pathway activator (sh-GNA13+SKL2001). We examined the β -catenin signaling pathway by Western blot to detect changes in protein and phosphorylated protein levels of DVL-1, GSK3 β , and β -catenin in the pathway. The findings demonstrated that GNA13 silencing dramatically lowered the levels of DVL-1, p-GSK3 β /GSK3 β , and β -catenin, which was effectively reversed by the addition of SKL2001 (Fig. 4A–H). This suggests that the suppression of GNA13 silencing on the malignant biological phenotypes of ESCA cells may be associated with inhibition of the β -catenin pathway.

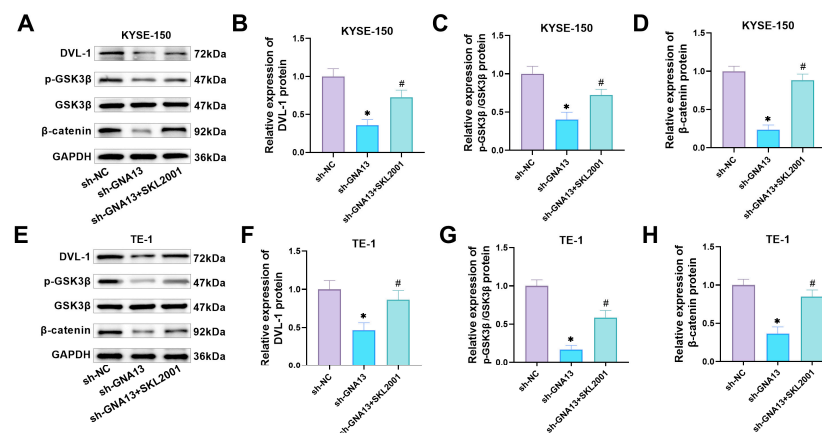


Figure 4: GNA13 silencing blocks the β -catenin pathway. (A–D) GNA13 silencing impaired β -catenin pathway-associated protein levels, including DVL-1, p-GSK-3 β /GSK-3 β , and β -catenin in KYSE-150 cells. (E–H) GNA13 silencing suppressed β -catenin pathway-associated protein levels, including DVL-1, p-GSK-3 β /GSK-3 β , and β -catenin in TE-1 cells. n = 3, * p < 0.05 vs. sh-NC group; # p < 0.05 vs. sh-GNA13 group.

3.5 Silencing GNA13 Suppresses ESCA Cellular Malignant Phenotype via Inhibiting the β -Catenin Pathway

We investigated whether GNA13 influences the malignant phenotype of ESCA cells via the β -catenin pathway. The findings revealed that SKL2001 treatment reversed the inhibitory effects of GNA13 silencing on ESCA cells, resulting in increased cell proliferation viability and relative colony formation rate (Fig. 5A–C), down-regulation of the epithelial marker E-cadherin, and up-regulation of the mesenchymal markers N-cadherin and Vimentin (Fig. 5D–G), and an increase in the number of invasive cells (Fig. 5H,I). These results demonstrated that GNA13 regulates ESCA cells by mediating the β -catenin pathway.

3.6 Silencing GNA13 Promotes Apoptosis in ESCA Cells via Inhibiting the β -Catenin Pathway

Flow cytometry results revealed that SKL2001 treatment dramatically reduced apoptosis rates in TE-1 and KYSE-150 cells when compared to the sh-GNA13 group (Fig. 6A,B). Silencing GNA13 markedly upregulated the mRNA levels of cytc and Bax, while downregulating Bcl-2; this regulatory effect was mitigated by SKL2001 treatment (Fig. 6C–E). Additionally, both cytc and Bax protein levels were dramatically

raised, whereas Bcl-2 was significantly lowered after silencing GNA13; these changes were also partially reversed by SKL2001 (Fig. 6F–I). Collectively, these findings indicate that silencing GNA13 promotes apoptosis of ESCA cells through hindering the β -catenin pathway.

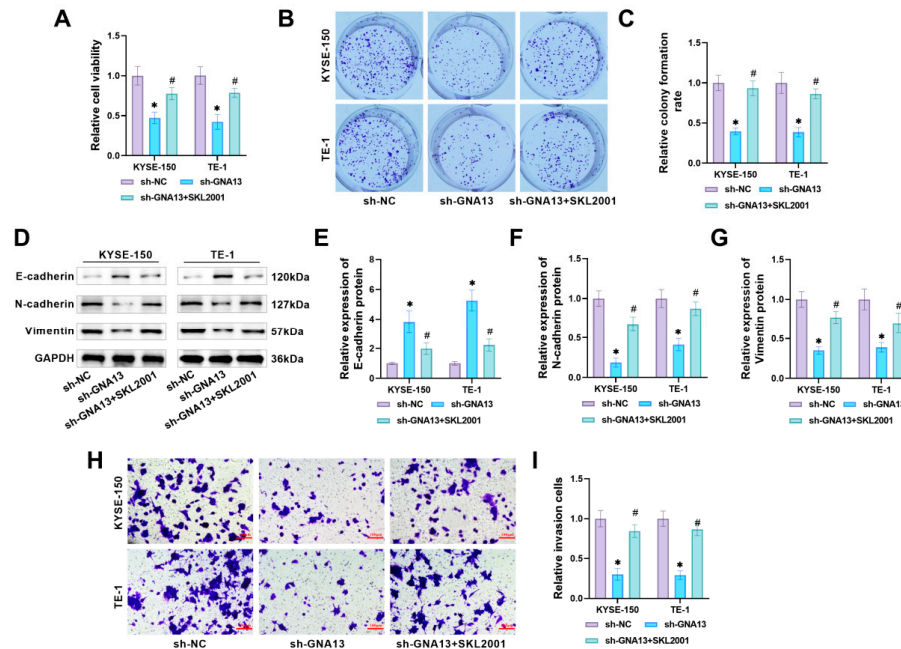


Figure 5: Silencing GNA13 suppresses ESCA cellular malignant phenotype via inhibiting the β -catenin pathway. (A) CCK-8 was used to measure the impact of SKL2001 on the proliferative viability of GNA13-silenced cells. (B,C) SKL2001's effect on silencing GNA13 inhibits clone creation rate in ESCA cells. (D–G) SKL2001's effect on suppressing GNA13 to block EMT in both ESCA cell lines. (H,I) SKL2001's effect on GNA13 silencing to prevent cell penetration into the lower compartment (20 $\times$; bar = 100 μ m). n = 3, * p < 0.05 vs. sh-NC group; # p < 0.05 vs. sh-GNA13 group.

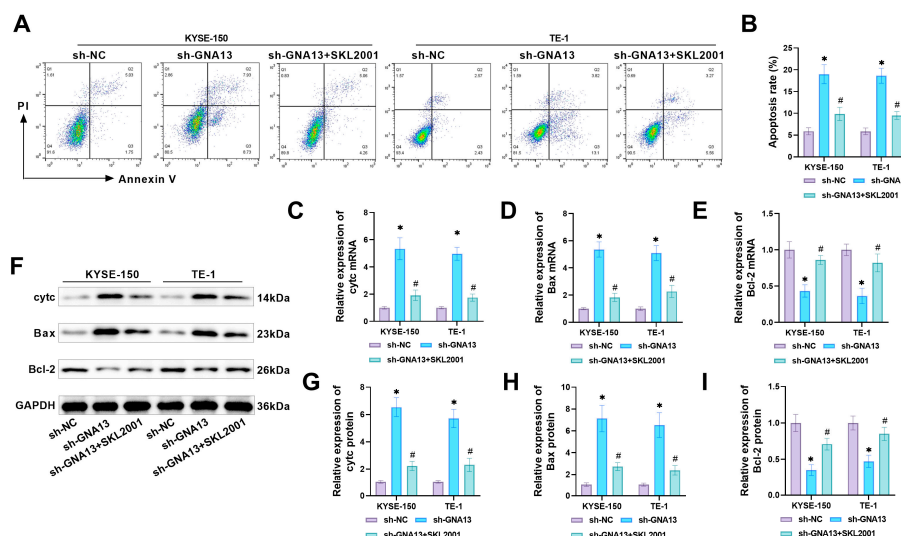


Figure 6: Silencing GNA13 promotes apoptosis in ESCA cells by inhibiting the β -catenin pathway. (A,B) Flow cytometry to assess the impact of SKL2001 on GNA13 silencing-mediated promotion of apoptosis in ESCA cells.

(C–E) SKL2001 reversed GNA13 silencing-mediated upregulation of *cytc* and *Bax* mRNA and downregulation of *Bcl-2* mRNA. (F–I) SKL2001 reversed GNA13 silencing-induced changes in *cytc*, *Bax*, and *Bcl-2* protein expression. $n = 3$, * $p < 0.05$ vs. sh-NC group; # $p < 0.05$ vs. sh-GNA13 group.

4 Discussion

Because of ESCA's strong proliferative and invasive capacity, most patients have already established tumor metastases or have progressed to advanced stages when they are first diagnosed, resulting in a poor prognosis [9]. To enhance the clinical prognosis of ESCA patients, additional biomarkers must be identified to aid the clinic in the early prediction of their prognosis. Using bioinformatics and *in vitro* cellular tests, we discovered GNA13 as a pro-carcinogenic component that induces the malignant phenotype of ESCA cells. We validated the effect of β -catenin pathway activation on GNA13's participation in ESCA, indicating its probable mechanism.

GPCRs are the biggest protein superfamily in the human body, situated on the cell surface. They work by coupling to heterotrimeric G proteins, which include $G\alpha$, $G\beta$, and $G\gamma$ subunits. $G\alpha$ has four subfamilies: G_s , G_q , G_i , and G_{12} [28]. Cancer is driven by a number of GPCRs that maintain abnormal signaling networks; factors such as compensatory mechanisms make it difficult to treat by targeting GPCRs [29]. The G_{12} family, comprised of $G\alpha_{12/13}$ (the product of the *GNA12/13* gene), is one of the most important families in cancer, and the majority of GPCRs signal through the G_{12} protein [30]. As a result, targeting G_{12} or its encoding genes, *GNA12/13*, could be an effective technique for treating malignant development in solid tumors [15]. Most prior investigations on the role of *GNA12/13* in cancer focused on *GNA12*, yet in the last few years, it has been revealed that *GNA13* is a significant predictor marker for lifespan and poor migration in a variety of solid cancers (head and neck, ovarian, lung, and gastric) [28]. *GNA13* causes phenotypic alteration in tumor cells and governs cell motility, invasion, and metastasis via activating Rho GTPases or modifying signal transduction pathways [31,32]. Additionally, activated RhoA phosphorylates and inhibits $GSK3\beta$ through its downstream effector kinase ROCK, which stabilizes β -catenin protein and amplifies Wnt/ β -catenin pathway activation [33]. In non-small cell lung cancer, elevated *GNA13* expression enhances invasion and metastasis through activating the WNT/ β -catenin axis [34]. Deletion of miR-182 in prostate cancer cells led to increased production of *GNA13*, which regulates the Wnt/ β -catenin pathway to promote malignant phenotypes of cancer cells and suppresses apoptosis [35,36]. In our study, *GNA13* was notably upregulated in both ESCA tissues and cell lines. Silencing *GNA13* caused a raised level of E-cadherin, along with reduced levels of N-cadherin and Vimentin, a notable decline in the number of cells proliferating and invading, an increase in apoptotic proteins *cytc* and *Bax*, and a decline in *Bcl-2* in ESCA cells. *GNA13* was shown to induce EMT and invasion while inhibiting apoptosis in ESCA cells, which aligns with previous findings in other malignancies.

Wnt/ β -catenin signaling system has been linked to tumor cell proliferation, apoptosis, migration, and invasion, making it a viable therapeutic target [37]. When Wnt signaling is engaged, the Wnt ligand binds to its receptor Frizzled (FZD) and forms co-receptor complexes, triggering the classical Wnt signaling axis [21]. Dishevelled (DVL) proteins in the cytoplasm are activated, and the scaffolding protein Axin is recruited to the cell membrane. The protein complex of Axin/APC/ $CK1\alpha$ / $GSK3\beta$ is disrupted, and cytoplasmic β -catenin escapes from being degraded by phosphorylation, thus stabilizing and accumulating in the cytoplasm and nucleus, further inducing oncogenic properties, promoting cancer cell proliferation and survival [38], and facilitating tumor progression by inhibiting T-cell responses [39]. Our experimental results showed that *GNA13* silencing resulted in significant reductions in DVL-1, p- $GSK3\beta$ / $GSK3\beta$, and β -catenin protein levels, indicating suppression of the Wnt/ β -catenin pathway. The addition of SKL2001 substantially reversed this

tendency and greatly reduced sh-GNA13's inhibitory effect on proliferation, clone formation, invasion, and EMT in KYSE-150 and TE1 cells. Thus, GNA13 may regulate ESCA cells' malignant behavior by activating the β -catenin pathway. Notably, we observed that SKL2001 not only restored β -catenin levels but also reversed the downregulation of upstream DVL-1 and the decreased p-GSK3 β /GSK3 β ratio induced by GNA13 silencing. This phenomenon can be partially explained by the conserved transcriptional positive feedback loop of the Wnt pathway: the nuclear β -catenin/TCF complex binds to the promoter region of DVL-1 and promotes its transcription, and accumulated DVL-1 further inhibits GSK3 β kinase activity, forming a positive regulatory loop to amplify pathway activation. Additionally, potential off-target effects of SKL2001 or indirect feedback regulation within the signaling network cannot be entirely excluded. Future studies employing genetic approaches, such as β -catenin-specific knockdown, are warranted to further explore the causal relationship between GNA13, β -catenin, and the observed phenotypic changes.

Some limitations of this work should be acknowledged. A primary limitation of this research is the modest sample size ($n = 28$), and all specimens were collected from a single center; larger multicenter cohorts are needed to confirm our findings. Additionally, the present study was conducted exclusively *in vitro*; *in vivo* animal models are required to further validate the tumor-promoting role of GNA13 and the therapeutic potential of targeting this gene in ESCA.

In summary, we discovered that GNA13 is a pro-carcinogenic gene in ESCA cells, and that silencing GNA13 decreased ESCA cell proliferation, clone formation, invasion, and EMT. The β -catenin pathway, which is regulated by GNA13, was inhibited after silencing GNA13. However, adding SKL2001, a pathway activator, reversed the suppression of GNA13 silencing on the malignant phenotypes of ESCA cells.

5 Conclusion

ESCA is an aggressive tumor of the digestive tract that poses a major threat to human health, and elucidating its pathogenesis and searching for effective therapeutic targets are essential issues that must be addressed today. This study found that GNA13 increases ESCA cell proliferation and metastasis via activating the β -catenin pathway. The findings are extremely important for discovering new and effective prognostic markers and intervention targets for ESCA, optimizing ESCA therapeutic modalities, advancing precision therapy for ESCA, and improving the prognosis of ESCA patients. However, the development of ESCA involves complicated control in many domains, and the regulation of the β -catenin pathway by GNA13 is only one of them. The exact molecular mechanism has to be supplemented and improved. Furthermore, it is required to develop GNA13 medications for human usage, improve and optimize the GNA13 dose through preclinical basic research and animal testing, and then provide feedback to the clinic to formulate optimized therapy regimens to improve the prognosis of ESCA patients.

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References

1. Yang H, Wang F, Hallemeier CL, Lerut T, Fu J. Oesophageal cancer. *Lancet*. 2024;404(10466):1991–2005. [\[CrossRef\]](#).
2. Sung J, Lin D, Fitzgerald R, Sharma P, Law S, Ilson D. Carcinoma of esophagus. *J Gastroenterol Hepatol*. 2025;40(8):1861–75. [\[CrossRef\]](#).
3. Zhao YX, Zhao HP, Zhao MY, Yu Y, Qi X, Wang JH, et al. Latest insights into the global epidemiological features, screening, early diagnosis and prognosis prediction of esophageal squamous cell carcinoma. *World J Gastroenterol*. 2024;30(20):2638–56. [\[CrossRef\]](#).
4. Boerner T, Carr RA, Hsu M, Michel A, Tan KS, Vos E, et al. Incidence and management of esophageal cancer recurrence to regional lymph nodes after curative esophagectomy. *Int J Cancer*. 2023;152(10):2109–22. [\[CrossRef\]](#).
5. Zhang S, Shen Y, Zhu L, Lai Y, Zhu L, Xiao X, et al. Esophageal cancer: From pathogenesis to precision therapies. *Sig Transduct Target Ther*. 2026;11(1):134. [\[CrossRef\]](#).
6. Lagergren J, Smyth E, Cunningham D, Lagergren P. Oesophageal cancer. *Lancet*. 2017;390(10110):2383–96. [\[CrossRef\]](#).
7. Abnet CC, Arnold M, Wei WQ. Epidemiology of esophageal squamous cell carcinoma. *Gastroenterology*. 2018;154(2):360–73. [\[CrossRef\]](#).
8. Chen Q, Zhang W, Wu X, Chen S. Expression of circRNA-0036474 in esophageal cancer and its clinical significance. *Am J Cancer Res*. 2024;14(5):2228–39. [\[CrossRef\]](#).
9. Smyth EC, Lagergren J, Fitzgerald RC, Lordick F, Shah MA, Lagergren P, et al. Oesophageal cancer. *Nat Rev Dis Primers*. 2017;3:17048. [\[CrossRef\]](#).
10. Talukdar FR, di Pietro M, Secrier M, Moehler M, Goepfert K, Lima SSC, et al. Molecular landscape of esophageal cancer: Implications for early detection and personalized therapy. *Ann New York Acad Sci*. 2018;1434(1):342–59. [\[CrossRef\]](#).
11. Subramanyan LV, Rasheed SAK, Wang L, Ghosh S, Ong MSN, Lakshmanan M, et al. GNA13 suppresses proliferation of ER+ breast cancer cells via ER α dependent upregulation of the MYC oncogene. *Breast Cancer Res*. 2024;26(1):113. [\[CrossRef\]](#).
12. Na J, Zhou W, Yin M, Hu Y, Ma X. GNA13 promotes the proliferation and migration of lung squamous cell carcinoma cells through regulating the PI3K/AKT signaling pathway. *Tissue Cell*. 2022;76:101795. [\[CrossRef\]](#).
13. Teo CR, Casey PJ, Rasheed SA. The GNA13-RhoA signaling axis suppresses expression of tumor protective Kallikreins. *Cell Signal*. 2016;28(10):1479–88. [\[CrossRef\]](#).
14. Xu Y, Rong J, Duan S, Chen C, Li Y, Peng B, et al. High expression of GNA13 is associated with poor prognosis in hepatocellular carcinoma. *Sci Rep*. 2016;6:35948. [\[CrossRef\]](#).
15. Rasheed SAK, Leong HS, Lakshmanan M, Raju A, Dadlani D, Chong FT, et al. GNA13 expression promotes drug resistance and tumor-initiating phenotypes in squamous cell cancers. *Oncogene*. 2018;37(10):1340–53. [\[CrossRef\]](#).
16. Pan Z, Zheng Z, Ye W, Chen C, Ye S. Overexpression of GNA13 correlates with poor prognosis in esophageal squamous cell carcinoma after esophagectomy. *Int J Biol Markers*. 2022;37(3):289–95. [\[CrossRef\]](#).
17. Crosas-Molist E, Samain R, Kohlhammer L, Orgaz JL, George SL, Maiques O, et al. Rho GTPase signaling in cancer progression and dissemination. *Physiol Rev*. 2022;102(1):455–510. [\[CrossRef\]](#).
18. Svensmark JH, Brakebusch C. Rho GTPases in cancer: Friend or foe? *Oncogene*. 2019;38(50):7447–56. [\[CrossRef\]](#).
19. Perugorria MJ, Olaizola P, Labiano I, Esparza-Baquer A, Marziani M, Marin JJG, et al. Wnt- β -catenin signalling in liver development, health and disease. *Nat Rev Gastroenterol Hepatol*. 2019;16(2):121–36. [\[CrossRef\]](#).
20. Zhang Y, Wang X. Targeting the Wnt/ β -catenin signaling pathway in cancer. *J Hematol Oncol*. 2020;13(1):165. [\[CrossRef\]](#).
21. Liu J, Xiao Q, Xiao J, Niu C, Li Y, Zhang X, et al. Wnt/ β -catenin signalling: Function, biological mechanisms, and therapeutic opportunities. *Sig Transduct Target Ther*. 2022;7:3. [\[CrossRef\]](#).

22. Yu F, Yu C, Li F, Zuo Y, Wang Y, Yao L, et al. Wnt/ β -catenin signaling in cancers and targeted therapies. *Sig Transduct Target Ther*. 2021;6:307. [[CrossRef](#)].
23. Dopeso H, Rodrigues P, Cartón-García F, Macaya I, Bilic J, Anguita E, et al. RhoA downregulation in the murine intestinal epithelium results in chronic Wnt activation and increased tumorigenesis. *iScience*. 2024;27(4):109400. [[CrossRef](#)].
24. Bai P, Wang P, Ren T, Tang Q, Zhang N, Zhao L, et al. Discovery of a novel Wnt inhibitor DK419: Reversing temozolomide resistance in glioblastoma by switching off Wnt/ β -catenin signaling pathway to inhibit MGMT expression. *Eur J Med Chem*. 2025;288:117411. [[CrossRef](#)].
25. Liao S, Chen H, Liu M, Gan L, Li C, Zhang W, et al. Aquaporin 9 inhibits growth and metastasis of hepatocellular carcinoma cells via Wnt/ β -catenin pathway. *Aging*. 2020;12(2):1527–44. [[CrossRef](#)].
26. Zhao R, Zhao D, Zhu X, Li F, Xiong P, Li S, et al. The influence of miR-3149 on the malignancy progression of gastric cancer by negatively regulating CEACAM5. *J Cancer Biomol Ther*. 2024;1(1):1–10. [[CrossRef](#)].
27. Hu X, Wu D, He X, Zhao H, He Z, Lin J, et al. circGSK3 β promotes metastasis in esophageal squamous cell carcinoma by augmenting β -catenin signaling. *Mol Cancer*. 2019;18(1):160. [[CrossRef](#)].
28. Rasheed SA, Teo CR, Beillard EJ, Voorhoeve PM, Zhou W, Ghosh S, et al. MicroRNA-31 controls G protein alpha-13 (GNA13) expression and cell invasion in breast cancer cells. *Mol Cancer*. 2015;14:67. [[CrossRef](#)].
29. Ghosh P. Heterotrimeric G proteins as emerging targets for network based therapy in cancer: End of a long futile campaign striking heads of a Hydra. *Aging*. 2015;7(7):469–74. [[CrossRef](#)].
30. Rasheed SAK, Subramanyan LV, Lim WK, Udayappan UK, Wang M, Casey PJ. The emerging roles of G α 12/13 proteins on the hallmarks of cancer in solid tumors. *Oncogene*. 2022;41(2):147–58. [[CrossRef](#)].
31. Lim WK, Chai X, Ghosh S, Ray D, Wang M, Rasheed SAK, et al. G α -13 induces CXC motif chemokine ligand 5 expression in prostate cancer cells by transactivating NF- κ B. *J Biol Chem*. 2019;294(48):18192–206. [[CrossRef](#)].
32. Yi Q, Huang M, Zhang X, Xu Z, Sun J, Wang S, et al. GNA13 inhibits glioblastoma metastasis via the ERKs/FOXO3 signaling pathway. *Cell Signal*. 2023;109:110789. [[CrossRef](#)].
33. Shan S, Su M, Li Y, Wang Z, Liu D, Zhou Y, et al. Mechanism of RhoA regulating benign prostatic hyperplasia: RhoA-ROCK- β -catenin signaling axis and static & dynamic dual roles. *Mol Med*. 2023;29(1):139. [[CrossRef](#)].
34. Wu JQ, Zou HP, Fan RY, Cai J, Wei P, He MF, et al. *GNA13* promotes brain metastasis of non-small cell lung cancer and EMT through the WNT/ β catenin signaling pathway. *Front Cell Dev Biol*. 2025;13:1652200. [[CrossRef](#)].
35. Rasheed SAK, Teo CR, Beillard EJ, Voorhoeve PM, Casey PJ. MicroRNA-182 and microRNA-200a control G-protein subunit α -13 (GNA13) expression and cell invasion synergistically in prostate cancer cells. *J Biol Chem*. 2013;288(11):7986–95. [[CrossRef](#)].
36. Wang D, Lu G, Shao Y, Xu D. MiR-182 promotes prostate cancer progression through activating Wnt/ β -catenin signal pathway. *Biomed Pharmacother*. 2018;99:334–9. [[CrossRef](#)].
37. Pai SG, Carneiro BA, Mota JM, Costa R, Leite CA, Barroso-Sousa R, et al. Wnt/beta-catenin pathway: Modulating anticancer immune response. *J Hematol Oncol*. 2017;10(1):101. [[CrossRef](#)].
38. Valkenburg KC, Graveel CR, Zylstra-Diegel CR, Zhong Z, Williams BO. Wnt/ β -catenin signaling in normal and cancer stem cells. *Cancers*. 2011;3(2):2050–79. [[CrossRef](#)].
39. Hong Y, Manoharan I, Suryawanshi A, Majumdar T, Angus-Hill ML, Koni PA, et al. β -catenin promotes regulatory T-cell responses in tumors by inducing vitamin A metabolism in dendritic cells. *Cancer Res*. 2015;75(4):656–65. [[CrossRef](#)].