Overexpression of *ING3* is associated with attenuation of migration and invasion in breast cancer

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Abstract. Inhibitor of growth 3 (*ING3*) has been identified as a potential cancer drug target, but little is known about its role in breast cancer. Thus, the present study aimed to investigate *ING3* expression in breast cancer, its clinical value, and how *ING3* influences the migration and invasion of breast cancer cells. The Cancer Genome Atlas and UALCAN databases were used to analyze ING3 expression in cancer tissues and normal tissues. Survival analysis was performed using the UALCAN, UCSC Xena and KM-plot databases. In addition, reverse transcription-quantitative PCR and western blot analyses were performed to detect ING3 mRNA and protein expression levels. ING3 was overexpressed via lentiviral vector transfection, while the Transwell and wound healing assays were performed to assess the cell migratory and invasive abilities. Protein interaction and pathway analyses were performed using the GeneMANIA and Kyoto Encyclopedia of Genes and Genomes databases, respectively. The results demonstrated that *ING3* expression was significantly lower in cancer tissues compared with normal tissues (P<0.05). In addition, luminal A and human epidermal growth factor receptor 2 (HER2)-enriched breast cancer tissues expressed lower levels of *ING3* compared with normal breast tissues. Notably, statistically significant differences were observed in long-term survival between patients with luminal A (P=0.04) and HER2-enriched (P=0.008) breast cancer, with high and low expression levels of ING3. The results of the Transwell migration and invasion assays demonstrated that overexpression of *ING3* significantly inhibited the migratory and invasive abilities of MCF7 (P<0.05) and HCC1937 (P<0.05) cells. The results of the wound healing assay demonstrated that the percentage wound closure significantly decreased in cells transfected with LV5-ING3 compared with the negative control group at 12 h (P<0.05) and 24 h (P<0.01). The PI3K/AKT, JAK/STAT, NF-κB and Wnt/β-catenin pathways are the potential pathways regulated by ING3. Notably, overexpression of ING3 inhibited migration and invasion *in vitro*. However, further studies are required to determine whether ING3 regulates the biological behavior of breast cancer via tumor-related pathways.

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Introduction

Breast cancer is the most common malignant tumor in women worldwide; the incidence rate increases by 0.3% per year and it has been estimated that there will be 2.3 million new cases of breast cancer in 2020 and breast cancer has surpassed lung cancer in the number of new cases (1-3). Recurrence and metastasis following systematic therapy are the most common causes of mortality (4). Thus, it is important to identify novel therapeutic targets for breast cancer.

Inhibitor of growth 3 (ING3) is a member of the ING family (5), which consists of five members with different subtypes, according to alternative splicing (6). Their encoded proteins comprise a highly conserved plant homeodomain, a Cys4-His-Cys3 form of zinc finger that directly interacts with histone H3, and a nuclear localization sequence (6,7). ING proteins play significant roles in several biological processes, including apoptosis, DNA repair, cell cycle regulation and histone methylation (8,9). Recent studies have reported that the ING family are closely associated with cancer (10,11). ING family members are tumor suppressors that decrease invasion, migration and proliferation of different types of cancer (12-14).

ING3 is in 7q31 of chromosome seven, and is considered a suppressor in different types of cancer (10,15). Li *et al* (16) suggested that downregulation of *ING3* expression promotes the proliferation of head and neck squamous cell carcinoma cells. Furthermore, Lu *et al* (17) demonstrated that down-regulation of *ING3* expression promotes tumorigenesis in hepatocellular carcinoma. However, *ING3* has been reported to act as an oncogene in prostate cancer, which promotes cell proliferation (18,19). In melanoma, ING3 nuclear expression is downregulated and associated with low disease-specific 5-year survival rates (20), and the nuclear localization sequence of ING3 is critical to its function as a tumor suppressor (21). However, the role of ING3 in breast cancer remains unknown. Previous studies have demonstrated that ING3 is frequently expressed in breast cancer and gynecological cancers; however, ING3 expression has not been detected in the nucleus of breast cancer tissues (7,22).

In most cases, ING3 is considered a tumor suppressor (16,17,23), thus it was hypothesized that ING3 does not play an inhibitory role in breast cancer due to loss of nuclear localization capacity.

To further investigate the effect of ING3 on the biological behavior of breast cancer, *ING3* expression was analyzed in breast cancer tissues and normal tissues to determine its influence on the prognosis of patients with breast cancer. The role of ING3 on the migration and invasion of breast cancer cells was also investigated.

Materials and methods

Patients, tissue samples and follow-up. The present study was approved by the Ethics Committees of the Third Affiliated Hospital of Kunming Medical University, Yunnan Cancer Hospital (Kunming, China; approval no. QT202002), and written informed consent was provided by all patients prior to the study start. The UALCAN database (http://ualcan.path.uab.edu) was used to analyze *ING3* expression in cancer tissues and normal tissues. Follow-up and survival analyses were performed using the UALCAN and KM-plot (http://kmplot.com/analysis) databases. Patient data and *ING3* expression data (FPKM) were downloaded from The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov) (TCGA-BRCA) and UCSC Xena (http://xena.ucsc.edu/public) databases (TCGA.BRCA.sampleMap/BRCA_clinicalMatrix). R (4.0.2) (24) software and Perl (5.28.1; https://www.activestate.com/products/perl/downloads) software were to determine whether the long-term survival of patients with different clinical tumor-node-metastasis (TNM) stages (25) and subtypes (PAM50) were associated with ING3 expression levels. Median *ING3* expression levels between each subgroup was used to distinguish between the high and low expression groups, as follows: Stage I, 2.02357250; stage II, 1.9492; stage III, 1.894254; stage IV, 1.794536; luminal A, 1.803995; luminal B, 1.824826, human epidermal growth factor receptor 2 (HER2)-enriched, 1.497432; basal-like, 2.346218.

Primary cell separation. Normal breast epithelial cells (NBECs) were separated from tissue samples following surgical resection. Tissues were transported on ice in RPMI-1640 medium (Corning, Inc.) supplemented with 1% penicillin/streptomycin (Biological Industries), and used to isolate primary cells within 2 h. The tissues were washed three times with DPBS (Beijing Solarbio Science & Technology Co., Ltd.) and trimmed of excess fat, prior to cutting into 1-2 mm thick sections on ice. Type I collagenase (1.5 mg/ml, Sigma-Aldrich; Merck KGaA) was dissolved in DPBS containing 5% fetal bovine serum (FBS; Corning, Inc.) to digest tissues into cells. Tissues were dissociated by manual agitation for 20-40 min at 37˚C, and digestion was observed under a light microscope (magnification, x100). Cells were washed three times with DPBS containing 0.04% FBS to stop digestion and collected via centrifugation at 4˚C 3,000 x g for 5 min. Red Blood Cell lysis buffer (Invitrogen; Thermo Fisher Scientific, Inc.) was used to lyse erythrocytes on ice. Cells were re-washed three times with DPBS and cultured in RPMI-1640 medium supplemented with 10% FBS (Corning, Inc.), at 37˚C with 95% air and 5% CO_2.

Cell lines and culture. Human breast cancer cell lines, HCC1937 and MCF7, were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. Cells were maintained in RPMI-1640 medium supplemented with 10% FBS (both purchased from Corning, Inc.), at 37˚C with 95% air and 5% CO_2.

Lentiviral transfection. The overexpressing ING3 lentivirus (LV5-ING3) and the negative control lentivirus (LV5-NC) were synthesized by Shanghai GenePharma Co., Ltd. The breast cancer cells were transduced with lentivirus (LV5-ING3 or LV5-NC), MCF7 and HCC1937 cells were inoculated into 6-well plates at a density of $5x10^5$/3 ml 24 h prior to transfection. On the day of transfection, $25x10^5$ lentivirus was added to MCF7 cells and $50x10^5$ lentivirus was added to HCC1937 cells, and polybrene (8 μg/ml; Shanghai GenePharma Co., Ltd.) was added to the culture medium. After 72 h of screening with puromycin (1 μg/ml; Beijing Solarbio Science & Technology Co., Ltd.), transfection efficiency was determined via eGFP expression by fluorescence microscopy. Cell viability was determined using the cell counting device (JIMBIO-FIL). Cell suspension (5 μl) was stained with 0.4% trypan blue dye (5 μl) at room temperature and immediately used for cell viability determination; overexpression of *ING3* was detected via reverse transcription-quantitative (RT-q)PCR and western blot analyses.

RT-qPCR. Total RNA was extracted from breast cancer cells and NBECs using the RNAprep Pure cell kit (Tiangen Biotech Co., Ltd.), and reverse transcribed into cDNA using the FastQuant RT kit with gDNase (Tiangen Biotech Co., Ltd.), the RNA was mixed with the genomic DNA removal system and incubated at 42˚C for 3 min, following which, the reverse transcription reaction solution was added and incubated at 42˚C for 15 min and 95˚C for 3 min to synthesize cDNA. qPCR was subsequently performed using SuperReal PreMix Plus (SYBR Green, Tiangen Biotech Co., Ltd.). The following primer sequences were used for qPCR: ING3 forward, 5'-GCTGGATCAGGAACTGGCTAA-3' and reverse, 5'-TCTGTTGTCGTATGGTGAGAAGT-3'; and GAPDH forward, 5'-CAGGAGCGAGATCCCTCCAAAAT-3' and reverse, 5'-AGATGATGACCCTTTTGGCTCCC-3'. The following thermocycling

conditions were used for qPCR: 95˚C for 15 min for 1 cycle and 95˚C for 10 sec following 62˚C for 32 sec for 40 cycles. Relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (18) and normalized to the internal reference gene GAPDH.

Western blotting. Total protein was extracted from the cultured cells using RIPA buffer (Beyotime Institute of Biotechnology) and PMSF (Biosharp Life Sciences) mixed at a 100:1 ratio. Total protein was quantified using BCA protein quantification reagent (Beijing Dingguo Changsheng Biotechnology Co., Ltd.). Proteins (30 μg) were separated via electrophoresis using Spacer on a 12% SDS-PAGE gel. The separated proteins were subsequently transferred onto PVDF membranes (EMD Millipore), washed the PVDF membrane with TBST containing 0.1% Tween-20 and blocked in western blocking fluid (Beyotime Institute of Biotechnology) for 1.5 h at room temperature. The membranes were incubated with primary antibodies against ING3 (1:1,000 dilution; cat. no. GTX102480; GeneTex, Inc.) and GAPDH (1:5,000 dilution; cat. no. GTX100118; GeneTex, Inc.) at overnight 4˚C. Following the primary incubation, membranes were washed three times with TBST (10 min each), and subsequently incubated with secondary antibody (1:5,000 dilution; cat. no. GTX2131110-01; GeneTex, Inc.) at room temperature for 2 h. Protein bands were visualized using the ECL kit (Suzhou Xinsaimei Biotechnology Co., Ltd.) and analyzed using ImageJ software (1.42q; National Institutes of Health).

Migration and invasion assays. Cells were collected and resuspended in culture medium without serum. For the migration assay, $8x10^4$ MCF7 cells and HCC1937 cells transfected with LV5-ING3 and LV5-NC were plated in the upper chambers of Transwell plates without Matrigel, cell culture medium containing 20% FBS (Corning, Inc.) was added to the lower chamber. For the invasion assay, Matrigel was diluted on ice with RPMI-1640 medium (Corning, Inc.) at a ratio of 1:8, and solidified at 37°C for 2 h. Following coating, $8x10^4$ MCF7 cells and HCC1937 cells transfected with LV5-ING3 and LV5-NC were plated in the upper chambers of 8-μm pore size plates coated with 60 μl Matrigel, cell culture medium containing 20% FBS (Corning, Inc.) was added to the lower chamber. Following incubation for 24 h, at 37˚C with 95% air and 5% CO_2, the non-invasive and non-migratory cells were removed. The invasive and migratory cells were fixed with 4% polysorbate 30 min and stained with Giemsa stain 30 min at room temperature. Stained cells were counted for quantification and images captured using a light microscope (magnification, x100) (26).

Wound healing assay. Cells transfected with LV5-ING3 and LV5-NC were suspended and seeded into 12-well plates. Following cell adhesion, sterile 200 μl pipette tips were used to scratch the cell monolayers of each well. Plates were washed with PBS to remove detached cells. Cells were cultured in serum-free medium. Wound closure was observed using a light microscope (magnification, x100) and measured at 0, 12 and 24 h. Image-pro Plus 6.0 software (Media Cybernetics, Inc.) was used to measure the distance of the wound, using the following formula: Percent wound closure = wound closure distance of 12 or 24 h/wound closure distance of 0 h.

GeneMANIA protein interaction and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. Homo ING3 protein and other core protein interactions were analyzed using the GeneMANIA database (http://genemania.org). Pathway analysis was performed using the KEGG database (https://www.kegg.jp). All analyses were completed on March 12, 2020, using the default parameters of the databases.

Statistical analysis. Statistical analysis was performed using SPSS 22.0 software (IBM Corp.) and GraphPad Prism 6.0 software (GraphPad Software, Inc.). Unpaired Student's t-test was used to compare differences between two group, while one-way ANOVA and Bonferroni post hoc test were used to compare differences between multiple groups. Survival analysis was performed using the Kaplan-Meier method and log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

ING3 expression in tissues and prognosis. *ING3* expression in breast cancer tissues and normal tissues was determined using TCGA and UALCAN databases. As presented in Fig. 1A, *ING3* expression was significantly downregulated in cancer tissues compared with normal tissue ($P<0.001$). *ING3* was expressed across different races, TNM stages, subclasses, menopause statuses, ages, sex and histological subtypes, as presented in Fig. 1B-H. No difference in *ING3* mRNA expression among different stages, menopausal states and sex of patient with breast cancer was observed, however, *ING3* mRNA expression was significantly higher in triple negative breast cancer than in luminal and HER2 positive breast cancer ($P<0.001$), *ING3* mRNA expression was also higher in Caucasians than in African Americans ($P<0.01$), the expression of *ING3* was also higher in ILC breast cancer than mixed breast cancer ($P<0.05$).

The present study investigated whether *ING3* expression influences the prognosis of patients with breast cancer. Survival analysis was performed using the UALCAN, TCGA, UCSC Xena and KM-plot databases. Notably, no significant differences in long-term survival were observed between patients with low and high *ING3* expression, respectively ($P=0.360$ by UALCAN; Fig. 2A and $P=0.078$ by KM-plot; Fig. 2B). However, *ING3* expression was significantly associated with prognosis in the luminal A ($P=0.0039860$; Fig. 2C) and HER2-enriched ($P=0.008149$; Fig. 2E) subtypes. Conversely, ING3 expression was not associated with prognosis in patients with different clinical stages of breast cancer (Fig. 2G-J). In addition, there was no significant difference between luminal B and basal-like breast cancer (Fig. 2D and F).

ING3 expression in cell lines and lentiviral transduction. The results of the present study demonstrated that both *ING3* mRNA and protein expression levels were higher in NBECs compared with MCF7 and HCC1937 cells (Fig. 3C-E). Lentiviral vectors overexpressing *ING3*

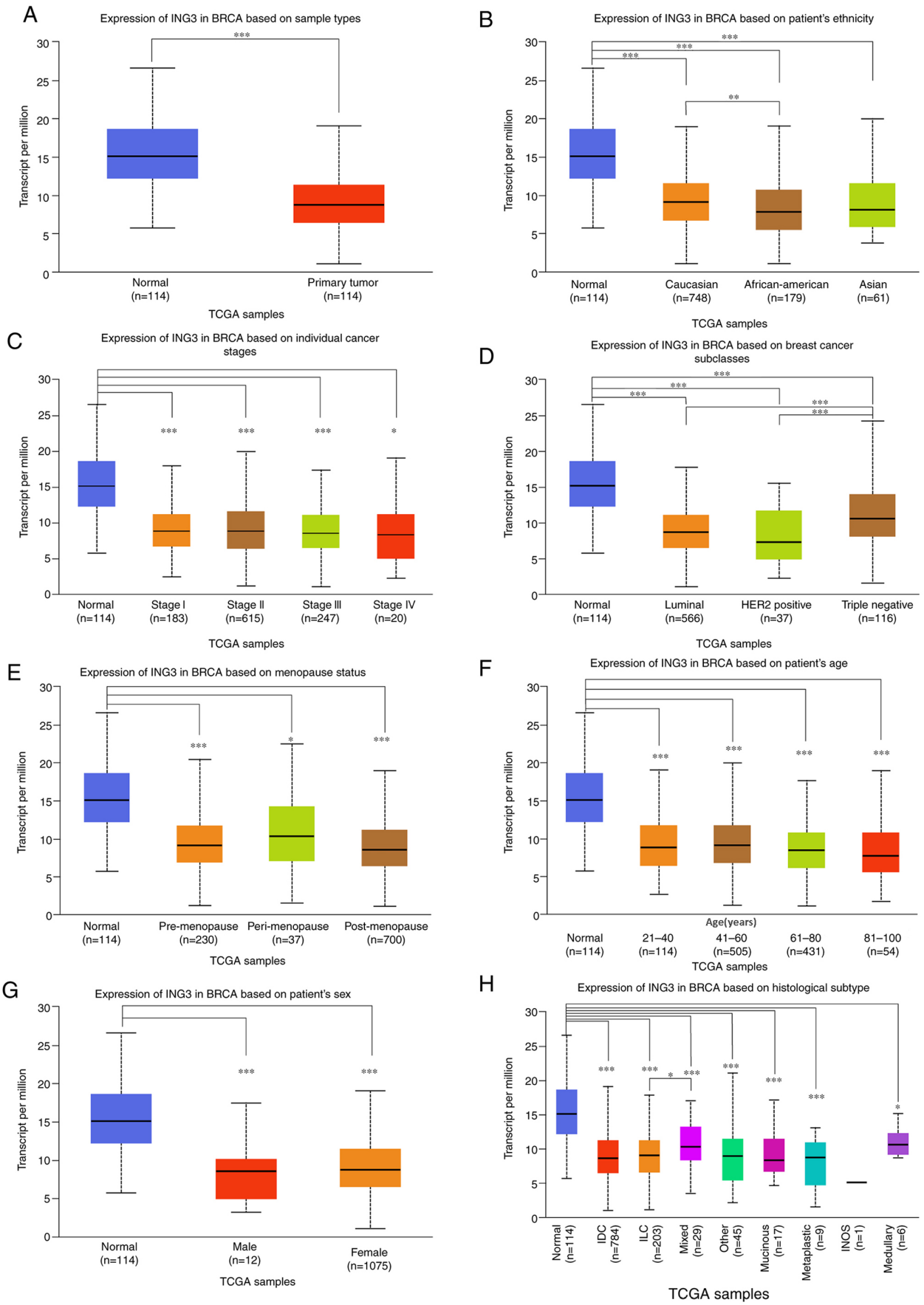

Figure 1. *ING3* expression in breast cancer tissues and normal tissues. (A) *ING3* expression in breast cancer tissues compared with normal tissues. *ING3* expression in normal tissues compared with breast cancer tissues among (B) different races, (C) different tumor-node-metastasis stages, (D) different subclasses, (E) different menopause status, (F) different ages, (G) different sex and (H) different histological subtypes. *P<0.05; **P<0.01; ***P<0.001. ING3, inhibitor of growth 3; TCGA, The Cancer Genome Atlas; HER2, human epidermal growth factor receptor 2; IDC (Infiltrating Ductal Carcinoma), ILC (Infiltrating Lobular Carcinoma), INOS (Infiltrating Carcinoma NOS).

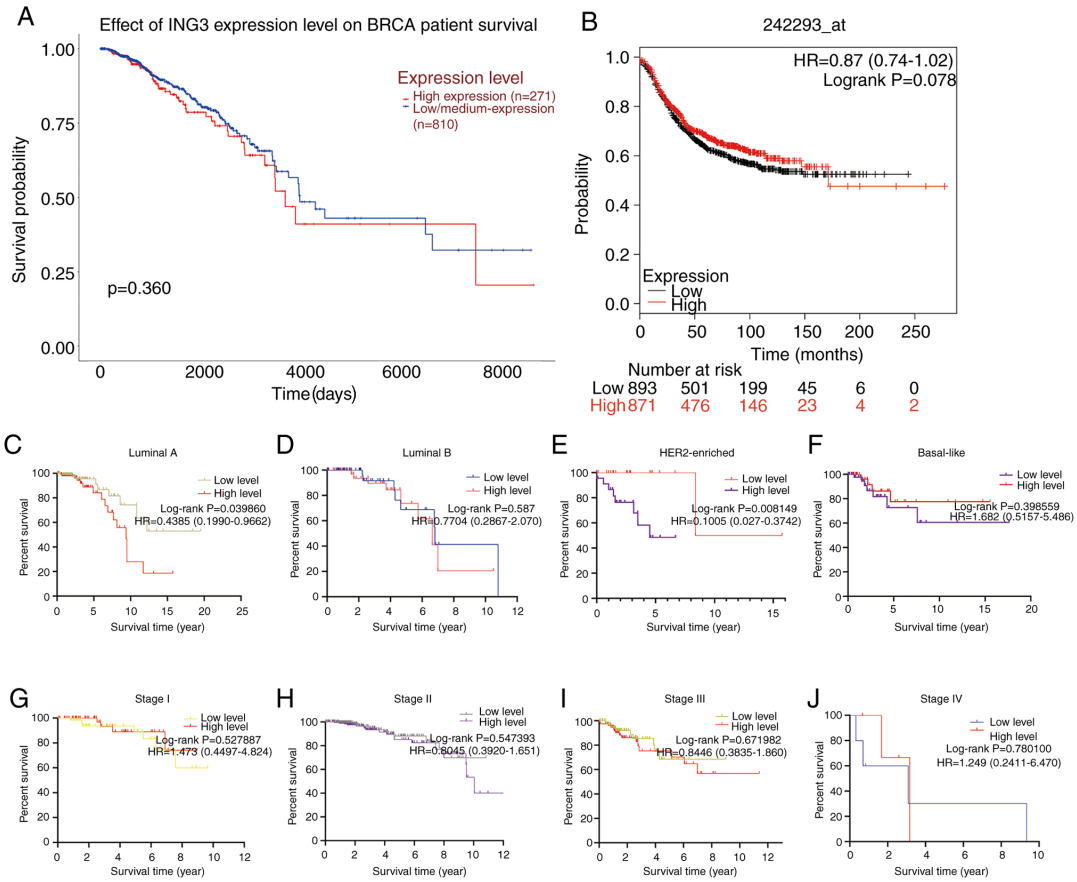

Figure 2. Survival analysis of patients with breast cancer with different *ING3* expression levels. (A) No significant differences were observed in long-term survival between patients with low and high *ING3* expression levels, according to (A) UALCAN and (B) KM-plot. (C-F) Long-term survival analysis in different subclasses of patients with low and high *ING3* expression levels. (G-J) Long-term survival analysis in different tumor-node-metastasis stages of patients with low and high *ING3* expression levels. ING3, inhibitor of growth 3; HER2, human epidermal growth factor receptor 2; HR, hazard ratio.

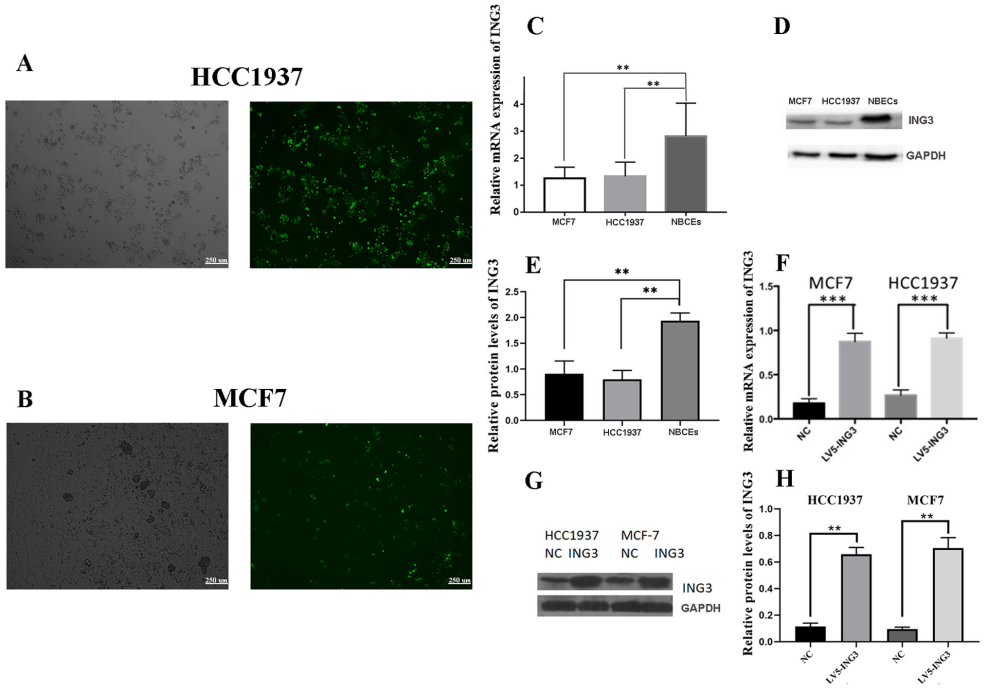

Figure 3. *ING3* expression in cell lines and following transfection. Transfection efficiency of (A) HCC1937 and (B) MCF7 cells was detected via eGFP expression by fluorescence microscopy. (C) *ING3* mRNA expression was higher in NBECs compared with MCF7 and HCC1937 cells. (D and E) ING3 protein expression was higher in NBECs compared with MCF7 and HCC1937 cells. (F) *ING3* mRNA expression was higher in MCF7 and HCC1937 cells transfected with LV5-ING3 compared with the LV5-NC group. (G and H) ING3 protein expression was higher in MCF7 and HCC1937 cells transfected with LV5-ING3 compared with the LV5-NC group. **P<0.01; ***P<0.001. ING3, inhibitor of growth 3; NBECs, normal breast epithelial cells; NC, negative control.

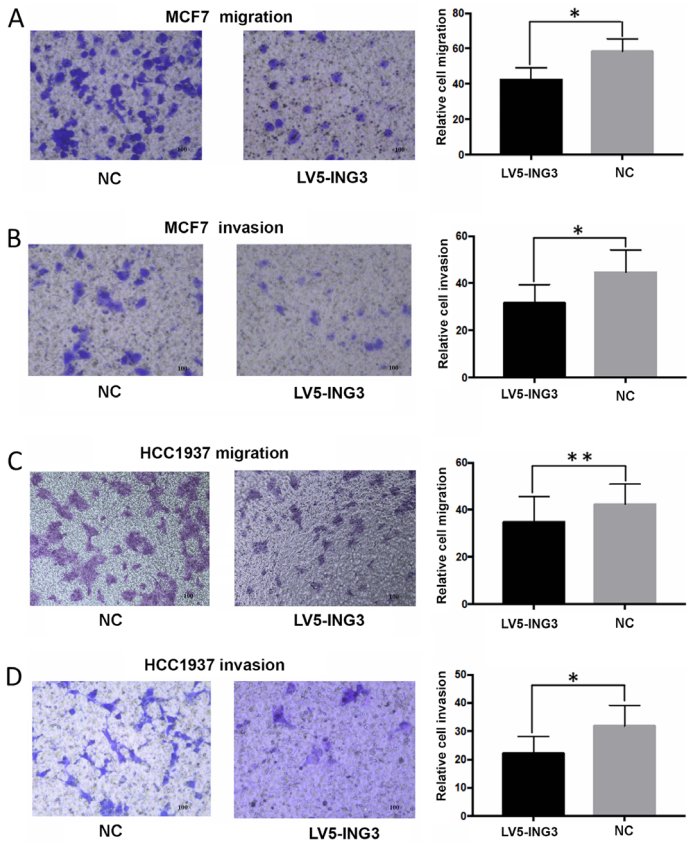

Figure 4. Overexpression of *ING3* inhibits migration and invasion. Overexpression of *ING3* inhibited the (A) migratory and (B) invasive abilities of MCF7 cells. Overexpression of *ING3* inhibited the (C) migratory and (D) invasive abilities of HCC1937 cells. *P<0.05; **P<0.01. ING3, inhibitor of growth 3; NC, negative control.

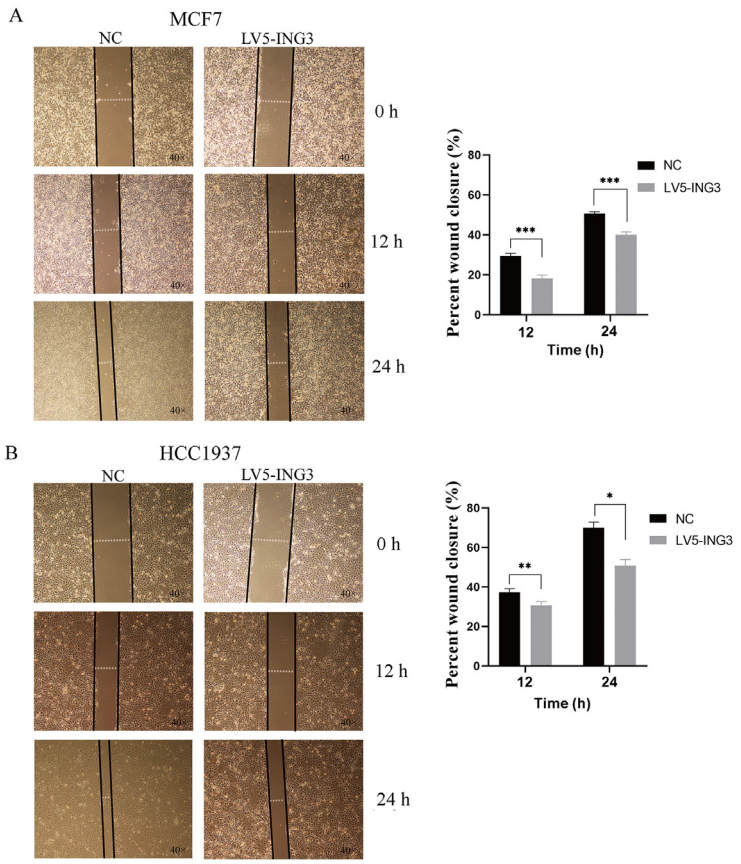

Figure 5. Overexpression of *ING3* attenuates the percentage wound closure. Overexpression of *ING3* attenuated the percentage wound closure in (A) MCF7 and (B) HCC1937 cells after 12 and 24 h. *P<0.05; **P<0.01; ***P<0.001. ING3, inhibitor of growth 3; NC, negative control.

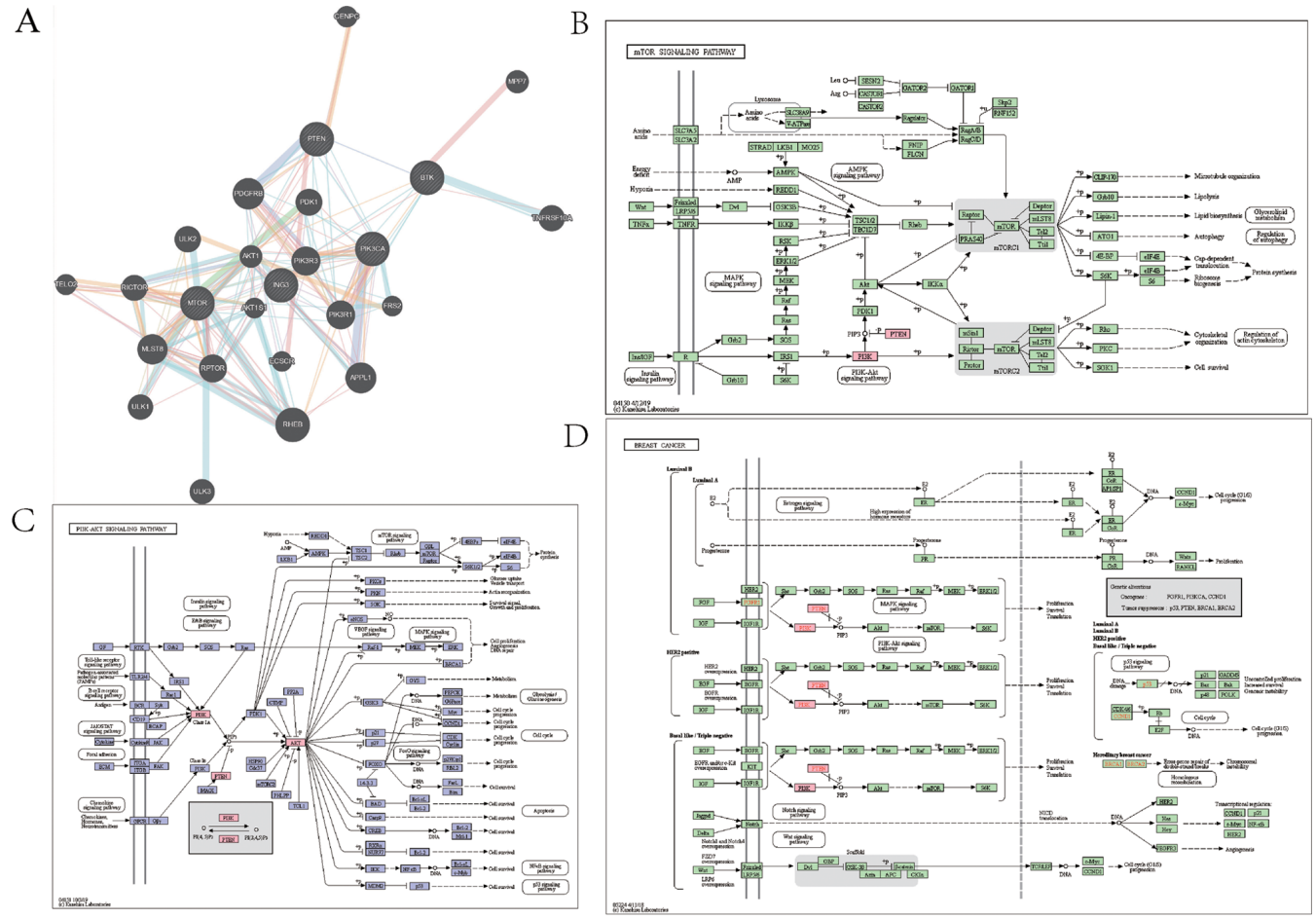

Figure 6. Potential pathway regulated by ING3. (A) GeneMANIA analysis revealed interaction between ING3 and a core protein of the PI3K/AKT pathway, while Kyoto Encyclopedia of Genes and Genomes pathway analysis demonstrated that ING3 may regulate the PI3K/AKT pathway. (B) PI3K/Akt Pathway was involved in mTOR signaling. (C) PI3K/Akt Pathway. (D) PI3K/ Akt pathway involved in breast cancer. ING3, inhibitor of growth 3.

(LV5-ING3) and LV5-NC were transfected into MCF7 and HCC1937 cells. No significant differences were observed in cell viability following transfection with the corresponding lentivirus vectors (Fig. S1). The transfection efficiency was detected via eGFP expression by fluorescence microscopy (Fig. 3A and B). The mRNA and protein expression levels of *ING3* increased in MCF7 and HCC1937 cells following transfection with LV5-ING3 compared with the LV5-NC group (Fig. 3F-H).

Overexpression of ING3 inhibits migration and invasion. Overexpression of *ING3* inhibited the migratory and invasive abilities of MCF7 cells. The average migration cell counts were 41±8 for the LV5-ING3 group compared with 58±6 for the NC group (P<0.05; Fig. 4A). The average invasion cell counts were 33±7 for the LV5-ING3 group compared with 42±8 for the NC group (P<0.05; Fig. 4B). Furthermore, overexpression of *ING3* inhibited the migratory and invasive abilities of HCC1937 cells. The average migration cell counts were 37±7 for the LV5-ING3 group compared with 41±6 for the NC group (P<0.01; Fig. 4C). The average invasion cell counts were 23±6 for the LV5-ING3 group compared with 32±7 for the NC group (P<0.05; Fig. 4D).

The results of the wound healing assay suggested a similar phenomenon. In MCF7 cells, the percentage wound closure was 18.16±1.76 in the LV5-ING3 group vs. 29.40±1.37 in the NC group in 12 h (P<0.001; Fig. 5A). Furthermore, the percentage wound closure was 40.03±1.48 in the LV5-ING3 group vs. 50.63±0.95 in the NC group in 24 h (P<0.001; Fig. 5A). In HCC1937 cells, the percentage wound closure was 30.69±1.85 in the LV5-ING3 group vs. 37.35±1.78 in the NC group at 12 h (P<0.01; Fig. 5B). Furthermore, the percentage wound closure was 50.76±3.22 in the LV5-ING3 group vs. 70.03±2.89 in the NC group at 24 h (P<0.05; Fig. 5B).

Potential pathways regulated by ING3. The present study aimed to investigate the potential regulating mechanism of ING3. The PI3K/AKT, JAK/STAT, NF-κB and Wnt/β-catenin pathways are closely associated with different types of cancer, including breast cancer (27-33). In the present study, GeneMANIA analysis exhibited interaction between ING3 and the core protein of the PI3K/AKT pathway, while KEGG pathway analysis demonstrated that ING3 may regulate the PI3K/AKT pathway (Fig. 6). Similar results were observed for the JAK/STAT (Fig. 7), NF-κB (Fig. 8) and Wnt/β-catenin (Fig. 9) pathways.

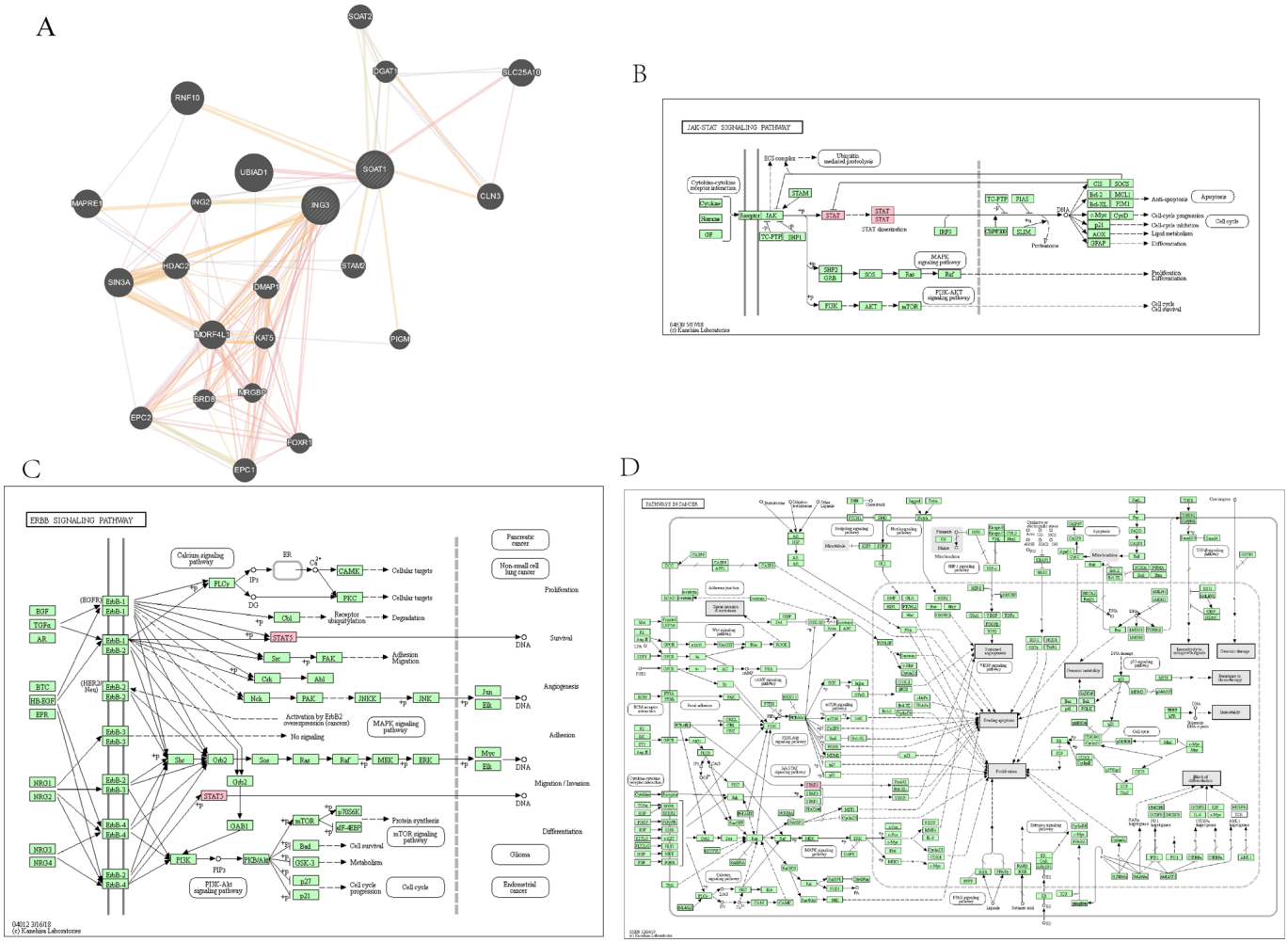

Figure 7. Potential pathway regulated by ING3. (A) GeneMANIA analysis revealed interaction between ING3 and a core protein of the JAK/STAT pathway, while Kyoto Encyclopedia of Genes and Genomes pathway analysis demonstrated that ING3 may regulate the JAK/STAT pathway. (B) The JAK/STAT pathway. (C) The JAK/STAT pathway is involved in ERBB signaling. (D) The JAK/STAT pathway is involved in breast cancer. ING3, inhibitor of growth 3.

Discussion

Data from TCGA and UALCAN databases confirmed that ING3 expression is downregulated in breast cancer tissues compared with normal breast tissues, and similar results were observed in breast cancer cell lines and NBECs. In the present study, ING3 had prognostic significance in certain types of breast cancer, such as luminal A and HER2-enriched breast cancer. To the best of the authors' knowledge, the present study was the first to demonstrate that overexpression of ING3 inhibits the migratory and invasive abilities of breast cancer cells. The results demonstrated that the PI3K/AKT, JAK/STAT, NF-κB and Wnt/β-catenin pathways are potential pathways regulated by ING3.

Studies investigating the association between ING3 and cancer are gaining significant interest (18,34-36). Increasing evidence suggest that ING3 is a key protein in cell apoptosis (37), cell proliferation and renewal (36), and tumor biological behaviors (18). Yang *et al* (35) reported that ING3 expression is downregulated in gastric cancer. However, Nabbi *et al* (19) demonstrated that ING3 expression is upregulated in prostate cancer. The role of ING3 in breast cancer remains largely unknown. The present study compared *ING3* expression across different races, TNM stages, subclasses, menopause status, ages, sex and histological subtypes. Notably, no significant differences were observed in the respective comparisons. To the best of our knowledge, the present study is the first to reveal that ING3 may act as a tumor suppressor gene in breast cancer. The results of the present study differ from previous findings on prostate cancer (18,19), but are similar to studies on head and neck cancer (16,23).

The results of the present study suggest that *ING3* may be a prognostic biomarker for patients with breast cancer. In the present study, high ING3 expression predicted poor prognosis in patients with luminal A and HER2-enriched breast cancer. However, ING3 expression was not associated with prognosis in patients with breast cancer without classification, and no significant association was observed between ING3 expression and prognosis in patients with different clinical stages.

Metastasis is a key feature of malignancies (38,39). Migration and invasion initiate metastasis *in vitro* (40-42). ING3 is considered a tumor suppressor in hepatocellular carcinoma, which attenuates proliferation, migration and invasion (17). However, it is considered a tumorigenesis promoter

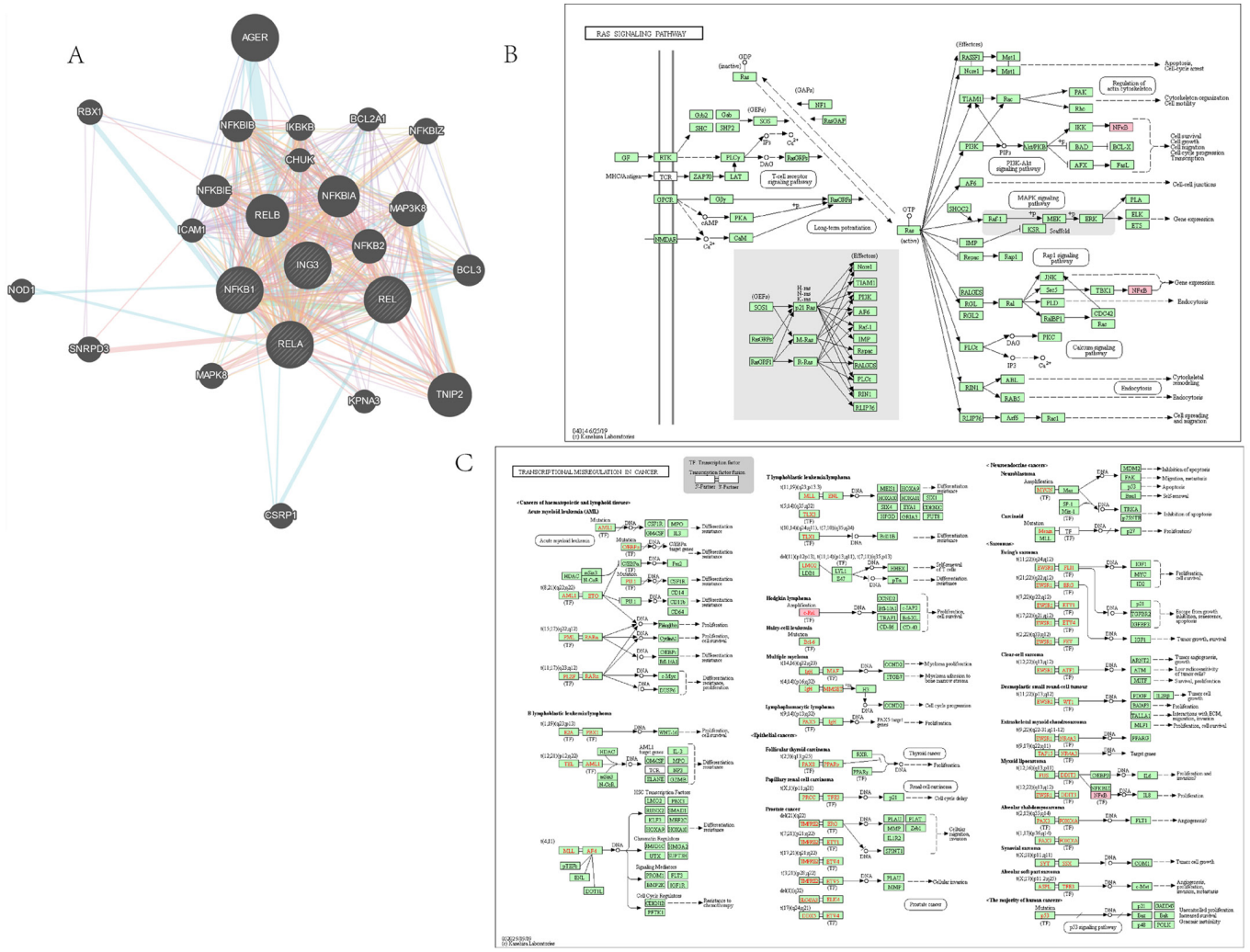

Figure 8. Potential pathway regulated by ING3. (A) GeneMANIA analysis revealed interaction between ING3 and a core protein of the NF-κB pathway, while Kyoto Encyclopedia of Genes and Genomes pathway analysis demonstrated that ING3 may regulate the NF-κB pathway. (B) The NF-κB pathway is involved in the RAS signaling pathway. (C) The NF-κB pathway is involved in transcriptional misregulation in cancer. ING3, inhibitor of growth 3.

in prostate cancer (18,19). The results of the present study demonstrated that ING3 mRNA and protein expression levels were higher in NBECs compared with MCF7 and HCC1937 cells. Similar results were observed between breast cancer tissues and normal tissues. The results of the Transwell migration and invasion, and wound healing assays demonstrated that overexpression of *ING3* inhibited the migratory and invasive abilities of MCF7 cells. Collectively, these results suggest that ING3 acts as a tumor suppressor in breast cancer, influencing biological behaviors, particularly attenuating migration and invasion.

In the present study, high ING3 mRNA expression was associated with poor prognosis, while overexpression of ING3 inhibited the metastasis of breast cancer cells. However, gene expression is a complex biological process, and further studies are required to validate gene function at the mRNA level, as only a weak association was observed between mRNA and protein expression (43,44). Previous studies have demonstrated that ING3 protein can be rapidly degraded by the SCF^{skp2}-mediated ubiquitin-protease system (45,46). Thus, prospective studies will focus on investigating the association between ING3 protein expression and prognosis.

Studying the mechanisms of metastasis is important in identifying novel anti-cancer drugs and developing cancer therapy (47-49). The activation of cancer-related pathways as a promotor of tumorigenesis, proliferation, migration and invasion in several types of cancer is generally accepted (50,51). ING3 regulates cell proliferation, apoptosis and cell cycle in gastric cancer via the PI3K/AKT pathway (23). However, the mechanisms by which ING3 regulates pathways in breast cancer remain unclear. The results of the present study revealed interactions between ING3 and core proteins of the PI3K/AKT, JAK/STAT, NF-κB and Wnt/β-catenin pathways. In addition, KEGG pathway analysis indicated that ING3 potentially regulates the PI3K/AKT, JAK/STAT, NF-κB and Wnt/β-catenin pathways. Although these assumptions were not proven in the present study, they remain valid hypotheses and will be the focus of prospective studies.

In conclusion, the results of the present study suggest that ING3 plays a key role in breast cancer. ING3 expression

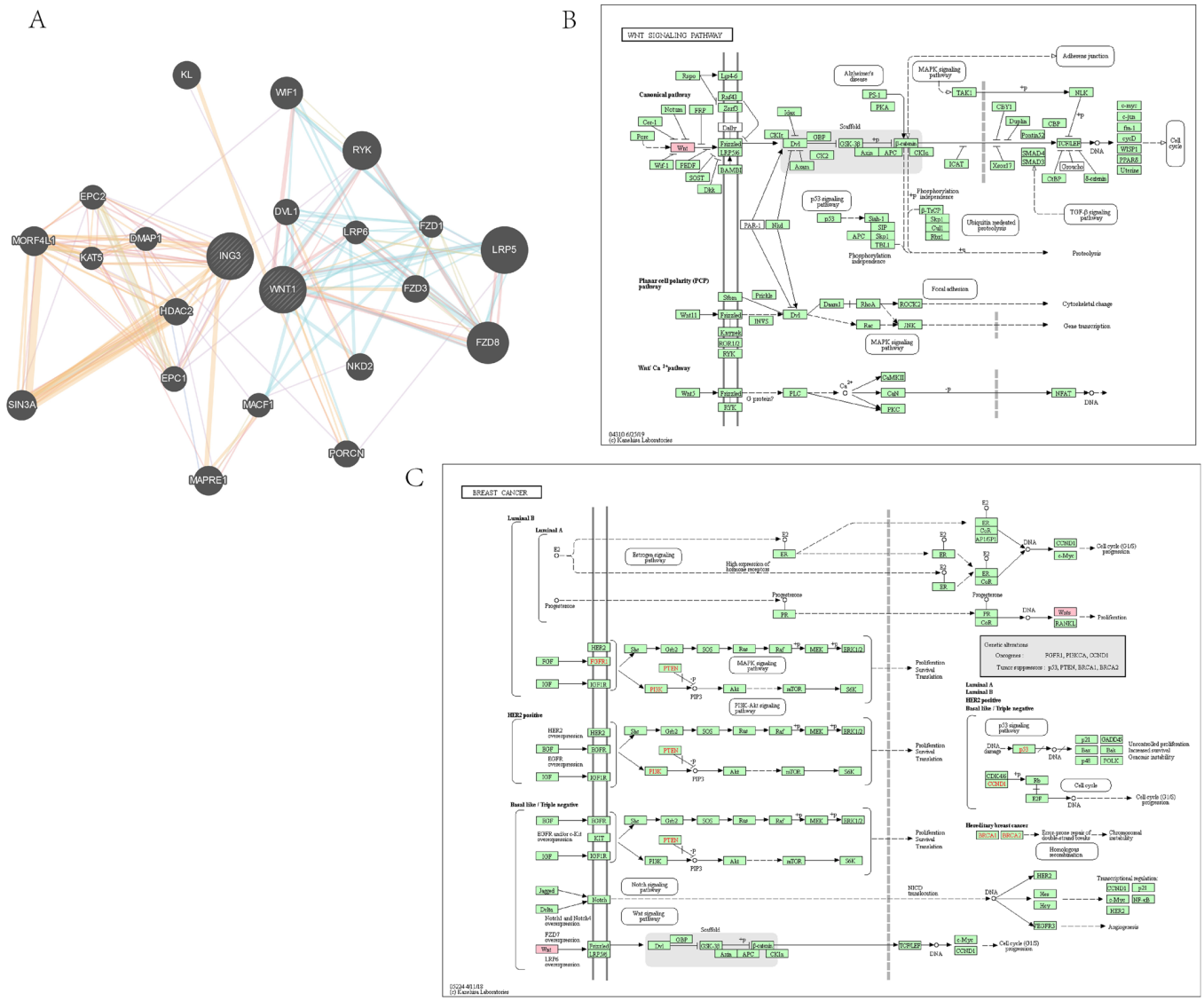

Figure 9. Potential pathway regulated by ING3. (A) GeneMANIA analysis revealed interaction between ING3 and a core protein of the Wnt/β-catenin pathway, while Kyoto Encyclopedia of Genes and Genomes pathway analysis demonstrated that ING3 may regulate the Wnt/β-catenin pathway. (B) Wnt signaling pathway. (C) Wnt signaling pathway involved in breast cancer. ING3, inhibitor of growth 3.

was downregulated in breast cancer tissues compared with normal tissues. In addition, ING3 expression influenced the prognosis of patients with different molecular subtypes. Notably, overexpression of ING3 inhibited migration and invasion *in vitro*. Thus, ING3 may be used to regulate the biological behavior of breast cancer via tumor-related pathways.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

The study was designed by ST, RL, DL and HZ. DL, MW, YT, KZ and WC carried out the funding obtain and manuscript review. HL (first author), RG, DL, RL downloaded the gene expression and clinical data from the TCGA database.ST, HL (first author), XT in charge of bioinformatics analysis. HZ, YT and WC is responsible for cell transfection, molecular biology

experiments and cell function experiments. The data were statistically analyzed by MW, KZ, HL, MT and KW. HL(first author) and ST write the manuscript, and all authors participate in the revision. ST and HZ confirm the authenticity of all the raw data. All authors reviewed and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committees of the Third Affiliated Hospital of Kunming Medical University, Yunnan Cancer Hospital, Kunming, China; approval no. QT202002, and written informed consent was provided by all patients prior to the beginning of the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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