Breast Cancer: Targets and Therapy

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ORIGINAL RESEARCH

HGH1 Promotes Breast Cancer Progression Through the PI3K/AKT/NF-κB Signaling Pathway: Potential Role for Prognosis and Targeted Therapy

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Introduction: Previous studies have shown that the HGH1 gene is associated with poor prognosis in a variety of cancers, but its specific function and molecular mechanism in the pathological process of breast cancer remain unclear.

Methods: The relationship between expression of HGH1 and overall survival in BC patients was analyzed. Enrichment analysis of HGH1-related signaling pathways and immune infiltration was performed. BC cell lines with overexpression and knockdown of HGH1 gene were constructed to tested the proliferation, migration, invasion ability and cell apoptosis. Detected the expression of PI3K/AKT pathway in BC cells and treated it with PI3K inhibitor. The effect of HGH1 on breast cancer in vivo was observed by tumor xenograft experiment.

Results: The expression of HGH1 is significantly increased in breast cancer and related to poor prognosis. The high expression of HGH1 is related to the PI3K−Akt signaling pathway, cell cycle, cell senescence, P53 signaling pathway. Overexpression of HGH1 promotes the proliferation, migration, and invasion, and inhibits apoptosis, while its knockdown yields opposite effects. HGH1 promoted the growth of BC cells by activating the PI3K/AKT/NF-κB signaling pathway, and the use of PI3K inhibitors could attenuate the promoting effect. In vivo experiments confirmed that HGH1 promoted breast cancer growth.

Conclusion: HGH1 promotes the growth of BC cells by activating the PI3K/AKT/NF-κB signaling pathway. HGH1 may become a new indicator for evaluating the poor prognosis of BC patients and serve as a potential diagnostic biomarker and therapeutic target for breast cancer.

Keywords: breast cancer, HGH1, PI3K, circadian

Introduction

Breast cancer is one of the most common cancers, ranking first among female malignancies worldwide, and is a significant cause of cancer-related death in women, placing heavy economic pressure on patients and society.[1] Although the continuous optimization of treatment programs has improved the overall survival rate of breast cancer, some patients still suffer from the dilemma of tumor progression or recurrence and metastasis. In the current field of cancer research, finding effective targets for the diagnosis and treatment of breast cancer is still the focus of research.[2,3]

HGH1 is a protein-coding gene that plays a key role in individual growth and development. Studies have shown that *HGH1* gene RNA and secreted protein levels oscillate regularly during the 24-hour cycle, and analysis of the *HGH1* promoter sequence revealed enhancer motifs (E-box) elements that bind circadian transcription mechanisms (*BMAL1* and *CLOCK*).[4–6] In addition, *BMAL1*/*CLOCK* is able to transactivate the *HGH1* promoter, and mutations in this E-box element adversely affect basal activity after gene transfer. Studies have shown that *HGH1* synthesis follows a circadian rhythm and that there is a dynamic association between circadian mechanisms and components of the *HGH1* locus chromosomal architecture that are essential for efficient growth hormone (GH) expression.[6]

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Contemporary research has demonstrated that elevated expression levels of *HGH1* exhibit a significant correlation with adverse prognostic outcomes in a multitude of cancer types, including renal clear cell carcinoma, melanoma, cervical squamous cell carcinoma, hepatocellular carcinoma, breast cancer, and several others.[7] As a form of gene variation, copy number variation is the most common type. It has been observed that *HGH1* not only regulates cell cycle related functions to promote cell proliferation, but also affects autoimmune-related functions within the innate and adaptive immune system, as well as other related immune signaling pathways. These findings highlight the important role of *HGH1* in human cancer, clarifying its involvement in tumorigenesis and cancer immunity.[7]

In our previous bioinformatics analysis, we found that tumor tissues from breast cancer patients with high *HGH1* expression were highly enriched in the PI3K/AKT pathway. PI3K/AKT signaling pathway plays an important role in the proliferation, angiogenesis, metastasis and resistance to radiotherapy and chemotherapy of malignant tumor cells.[8] Based on these findings, we investigated the effects of *HGH1* and its related mechanisms on breast cancer, aiming to establish new targets for breast cancer diagnosis and treatment in the future.

Materials and Methods

Raw Data Preprocessing and Survival Analysis

Breast cancer expression profiles and clinical information were collected from the TCGA Genomic Data Sharing Data Portal and GEO database. The genome expression of *HGH1* was calculated from TCGA database by high throughput sequencing. Kaplan-Meier survival curves were used to assess the association between *HGH1* and overall survival (OS) in patients with breast cancer. *HGH1* expression was divided into high expression group and low expression group, and the relationship between *HGH1* expression and overall survival was determined by confirming the level of *HGH1* expression based on the median.

Immunological Analysis, GO and KEGG Analysis

The CIBERSORT deconvolution procedure was first used to determine the relative composition of immune cells in each sample. An immune differential analysis was then performed to investigate the difference in immune cell content between the two groups. Using R package clusterProfiler (based on the principle of hypergeometric), based on a given gene on the GO, KEGG enrichment analysis.

Patients and Tissue Samples

Tumor and adjacent normal tissues were obtained from 25 female breast cancer patients admitted to the Department of Thyroid and Breast Vascular Surgery, Xijing Hospital, Air Force Medical University. All the enrolled patients underwent radical mastectomy from January 2021 to December 2021, and all patients did not receive neoadjuvant therapy before surgery. The study was approved by the Ethics Committee of Xijing Hospital, Air Force Military Medical University, and informed consent was obtained from all patients. The approval number of the Ethics Committee of Xijing Hospital is No. GKJ-Y-202303-055.

Cell Culture

The breast cancer cell lines used in this study (including T47D, MCF-7, MDA-MB-231, and BT-474) and the non-tumorigenic breast epithelial cell line MCF-10A were procured through iCell (Shanghai) Biotechnology Co., Ltd. from the Shanghai Academy of Sciences Cell Bank. Breast cancer cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 0.01 mg/mL insulin, and maintained at 37°C in a humidified 5% CO_2 atmosphere.

Plasmid Construction and RNA Transfection

Three HGH1-interfering plasmids sh-HGH1, namely shRNA-1, shRNA-2 and shRNA-3, and two HGH1-overexpressing plasmids pLenti-HGH1 were designed by Shanghai Shengong Bioengineering Company. Their sequences are shown in the Supplementary Material. $3*10^5$ cells is inoculated to the six-well plate, ensuring that the cell density reaches about

70–80% after 24 hours. Cells were transfected with a 2μg plasmid in serum-free medium using the Lipo8000™ kit (Biyutian Biotechnology, China). Transfected cells were cultured in a medium containing 1μg/mL of purinomycin for 48 hours and further cultured in a medium containing 5μg/mL of purinomycin to generate stable transfected cell lines. The cell clones stably expressing shRNA were screened using flow cytometry, and the selected cells exhibiting high fluorescence intensity were expanded and cultured.

Real-Time Fluorescent Quantitative PCR

Total RNA was extracted from cultured cells using TRIzol reagent, and the PrimeScriptTM II 1st Strand cDNA Synthesis Kit (Vazyme Biotech Co., Ltd) was used to synthesize cDNA. Quantitative real-time PCR was performed using a Bio-Rad C1000 real-time PCR apparatus. The 2^-ΔΔCT method was used to determine the relative expression of target genes. Primer sequence (5' to 3'): F-TGCTGCCCCTTACCCAGTA, R-CAACCACTCGTGATGTCGGT.

Western Blot

Cells were lysed using cell lysis buffer, proteins were separated by 10%SDS-PAGE, and transferred to polyvinylidene difluoride (PVDF, Merck, USA) membrane or nitrocellulose (NC, Merck, USA) membrane by electrophoresis. Proteins were visualized using a Western blot imaging system (MINICHEMI910, Beijing, China) and then quantitatively analyzed using the ImageJ software. The HGH1(20169-1-AP) antibody used in the study was purchased from Proteintech (China). Antibodies to PI3K(#9655), p-PI3K(#4257), AKT(#9272), p-AKT(#9271), and β-Actin(#4967) were purchased from Cell Signaling Technology (USA). IKBα(AF5002), pIKBα(Ser32/36)(AF2002), NF-kB p65(AF5006), and p-NF-kB p65 (AF2006) were purchased from Affinity (China).

Immunohistochemical Staining

The tissues were made into paraffin sections for dewaxing and hydration treatment. After antigen repair, the activity of endogenous peroxidase was blocked and closed, and the primary antibody (ratio 1:100) and secondary antibody (ratio 1:60) were incubated. After Streptomyces antibiotin-peroxidase solution was added for labeling, diaminobenzidine diamine hydrochloride (DAB) solution was added for observing the color reaction. The scan was performed using a digital pathology biopsy scanner. Blind assessment by at least one researcher and one pathologist, with staining scores of 0 for unstained, 2 for weakly staining, 4 for moderate staining, and 6 for strong staining.

Enzyme-Linked Immunosorbent Assay

Phospho-specific antibodies and pan-protein antibodies were immobilized on separate 96-well plates at a concentration of 2 μg/mL in PBS (50 μL per well) overnight at 4°C. Following incubation, the plates were blocked with 5% BSA. Cell lysates, pre-treated either with or without λ-phosphatase as a dephosphorylation control, were then incubated in the wells (100 μL per well) for 2 hours at 25°C. Detection of phosphorylated protein forms was achieved using HRP-conjugated anti-phospho-tyrosine antibody (Abcam ab12345, diluted 1:2,000) with TMB substrate for color development (15 minutes). Total protein levels were quantified using anti-tag antibodies. Absorbance readings at 450 nm were normalized to total protein signals. The specificity of the assay was validated by demonstrating a signal reduction of greater than 95% in phosphatase-treated controls. Data are presented as mean values from triplicate experiments ± SEM.

Cell Proliferation Test

$1*10^{3\sim4}$ breast cancer cells were inoculated in 96-well plates for 0h, 24h, 48h and 72h, then cck-8 reagent (Tontoni Institute of Chemistry, Japan) was added and incubated for 2~4 hours, and the absorbance at 450 nm was measured by enzymoleter.

Colony Formation Experiment

$1*10^{2\sim3}$ breast cancer cells were inoculated in six-well plates and cultured for 2 weeks. After the formation of cell colonies, they were fixed with formaldehyde solution, stained with crystal violet, photographed and counted under a microscope.

Cell Scratch Assay

Breast cancer cells in the logarithmic growth phase were collected and seeded in six-well plates for culture. The cell surface was scratched with the tip of a pipet gun, and the culture was continued by washing with PBS and exchanging fluid. Pictures were taken under microscope at 0h, 6h, 12h and 24 h to observe the difference in the movement distance of the wound edge. Wound healing rate = [(wound width 0 h− wound width 12 or 24 h)/wound width 0 h] × 100%.

Transwell Assay

Matrigel (Corning, USA) was added to the upper chamber of Transwell chamber, cells were suspended in serum-free medium after coagulation, and DMEM medium with 10%FBS was added to the lower chamber, cultured for 24 hours, stained with crystal violet, rinsed and dried. Photographs were taken with a high-power microscope and quantified using ImageJ software.

Flow Cytometry

Apoptosis was detected using Annexin V-FITC/PI (propidium iodide) apoptosis detection kit (Thermo Fisher, China). Transfected cells were harvested, washed with cold PBS, and resuspended in binding buffer before cells were incubated with Annexin V-FITC and PI on ice and analyzed using MoFloAstrios EQ (Beckman, USA).

Xenograft Tumor Construction

BALB/c nude mice of 6–8 weeks were fed in SPF environment and randomly grouped. MDA-MB-231 cells stably transfected with sh-HGH1 and pLenti HGH1 were digested and centrifuged, mixed with PBS, and then fully mixed with an equal amount of matrix glue, and the concentration was adjusted to 5×10^6 cells /100μL which were injected into the upper left mammary fat pad of nude mice. The tumor volume was measured every 4 days, and the mice were euthanized after 5 weeks to weigh the xenograft tumor. Experimental procedures follow ARRIVE guidelines and comply with the NIH guidelines for the care and use of laboratory animals.

Statistical Method

SPSS 26.0 statistical software was used for data analysis. Measurement data were expressed as mean ± standard deviation, and *t*-test and analysis of variance were used to compare whether there were significant differences between the data of each group. $P < 0.05$ was considered statistically significant.

Results

Expression of HGH1 in Breast Cancer Tissues and Prognosis and Related Pathway Analysis

We first compared the expression of HGH1 in multiple malignancies with normal tissues using the TCGA dataset. The results showed that HGH1 (C8ORF30A) expression was elevated in multiple cancer species compared with neighboring non-tumor controls, with HGH1 expression significantly elevated in breast cancer tissues (Figure 1A and B). Kaplan-Meier curves were drawn using TCGA-BRCA dataset (n = 1114) and GSE96058 dataset (n = 3273) to describe the relationship between HGH1 expression and OS in breast cancer patients. It was found that breast cancer patients with high HGH1 expression were significantly correlated with poor OS (TCGA-BC: $p < 0.021$, GSE96058: $p < 0.019$) (Figure 1C). Immunological analysis showed that the high expression of HGH1 was positively correlated with regulatory T cells, macrophage M0, macrophage M1, follicular helper T cells ($P < 0.05$), and negatively correlated with γδ T cells, original B cells, and CD4 memory T cells ($P < 0.05$) (Figure 1D). Subsequently, we performed GO analysis on HGH1, and BP related pathways included regulation of mitotic cell cycle, mitotic cell cycle phase transition, ribonucleoprotein complex biogenesis, and positive regulation of cellular catabolic process. CC terms include mitochondrial inner membrane, mitochondrial matrix, cell-substrate junction, focal adhesion, etc; MF terms include transcription coregulator activity, DNA-binding transcription factor binding, protein serine/threonine kinase activity, etc. (Figure 1E). KEGG

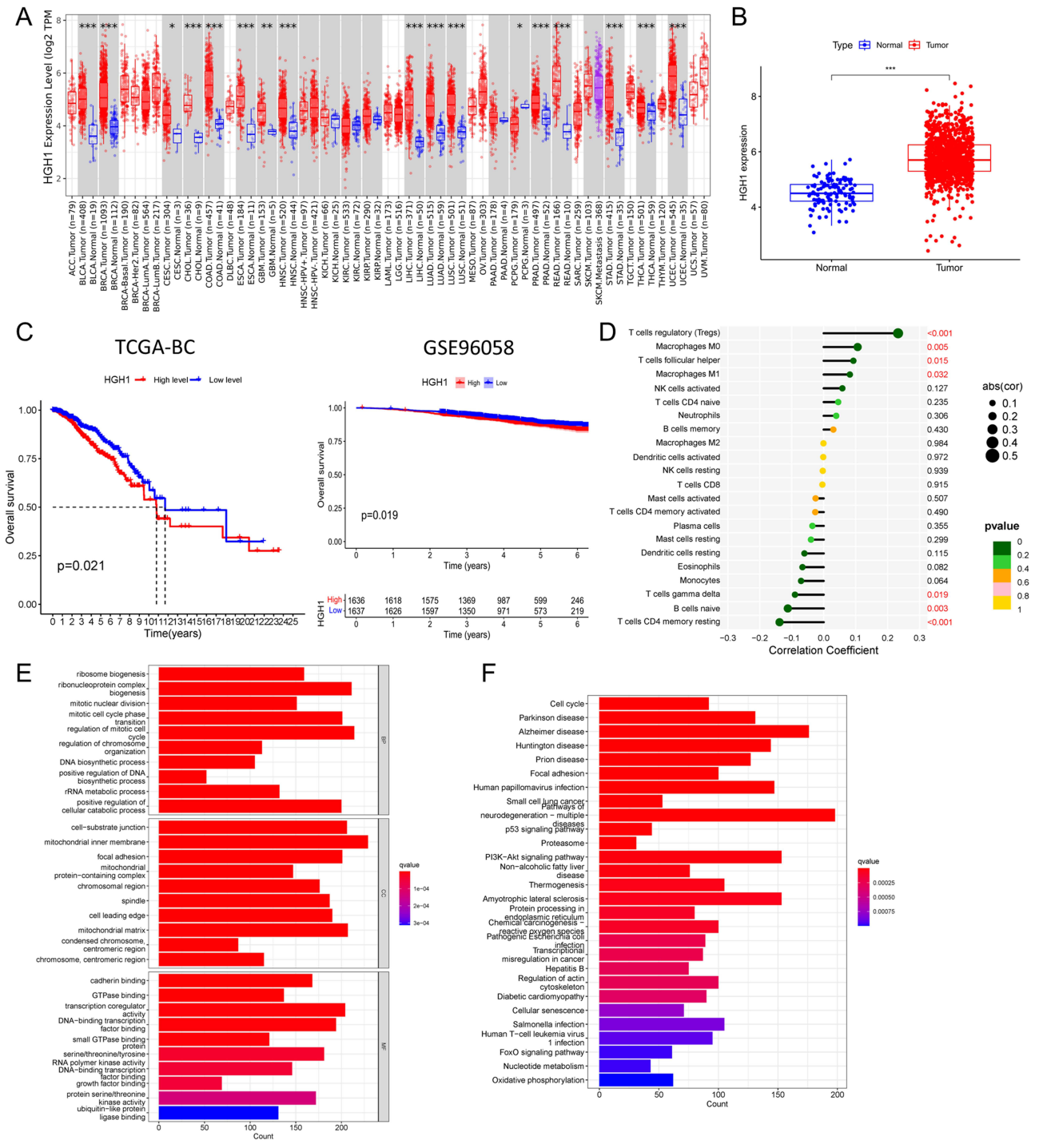

Figure 1 Increased expression of HGH1 is associated with breast cancer prognosis. (**A**) Expression levels of HGH1 in various cancers in the TCGA database. (**B**) HGH1 expression was significantly increased in breast cancer tissues; (**C**) Kaplan-Meier curves describing the association between HGH1 expression and OS of breast cancer patients (TCGA-BC, GSE96058); (**D**) Immunoenrichment analysis of HGH1 high expression; (**E** and **F**) GO and KEGG enrichment analysis of HGH1 high expression. *P<0.05, **P<0.01, ***P<0.001.

analysis showed that high expression of HGH1 was associated with many malignancy-related pathways, including PI3K-AKT signaling pathway, cell cycle, cell aging, P53 signaling pathway, etc. (Figure 1F).

The mRNA and protein expression levels of HGH1 in 25 pairs of breast cancer tissues and adjacent normal tissues were analyzed by qRT-PCR and immunohistochemistry, and both were significantly higher in tumor breast tissues than in adjacent normal tissues (Figure 2A–C and Supplementary Figure 1). The following RT-qPCR and Western blot results

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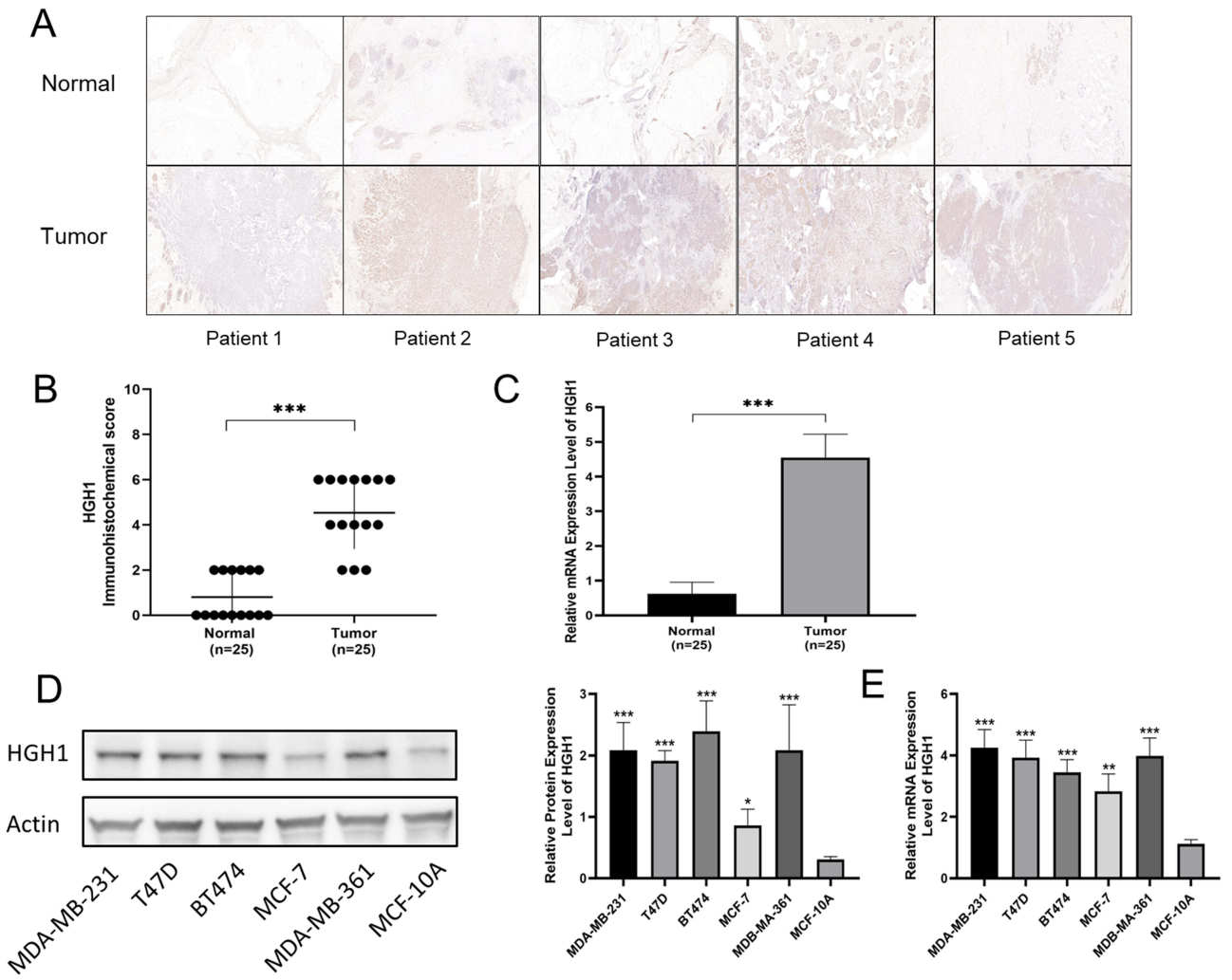

Figure 2 Enrichment of HGH1 in breast cancer tissues and cells. (**A** and **B**) Immunohistochemistry was used to evaluate the expression level of HGH1 in breast cancer tissues; (**C**) RT-qPCR was used to evaluate the expression of HGH1 mRNA in breast cancer tissues; (**D**) HGH1 protein expression in breast cancer cell lines (MCF-7, MDA-MB-231, MDA-MB-361 and T47D) were evaluated using Western blotting; (**E**) RNA expression in breast cancer cell lines. *P<0.05, **P<0.01, ***P<0.001.

showed that the expression of HGH1 in breast cancer cell lines MCF-7, MDA-MB-231, MDA-MB-361 and T47D was significantly higher than that in human normal breast cell line MCF-10A, and the difference was statistically significant (P<0.05, Figure 2D and E). Taken together, these results suggest that HGH1 may play an important role in the development and progression of breast cancer.

HGH1 Promotes Breast Cancer Cell Proliferation, Migration and Invasion, and Inhibits Apoptosis

HGH1 was overexpressed in MCF-7 cells by lentiviral infection to construct a stable breast cancer cell line. Meanwhile, MDA-MB-231 cells with stable HGH1 knockdown were constructed using shRNA transfection and stable protein overexpression and knockdown were verified by Western Blot and RT-qPCR (Figure 3A and B). Cell proliferation activity was detected by cck-8 assay. Cell clonogenesis ability was tested by colony formation assay. Cell invasion ability was detected by Transwell. Cell migration ability was detected by scratch test. Compared with sh-NC group, HGH1 knocking down in sh-HGH1-1 and sh-HGH1-2 groups significantly affected the proliferation activity, colony formation number, migration distance and invasion cell number of MDA-MB-231 cells. Conversely, overexpression of HGH1 promoted the proliferation, migration, and invasion of MCF-7 breast cancer cells (Figure 3C–F). In flow cytometry analysis, knockdown of HGH1 significantly increased the number of late apoptotic cells in MDA-MB-231 cells, while

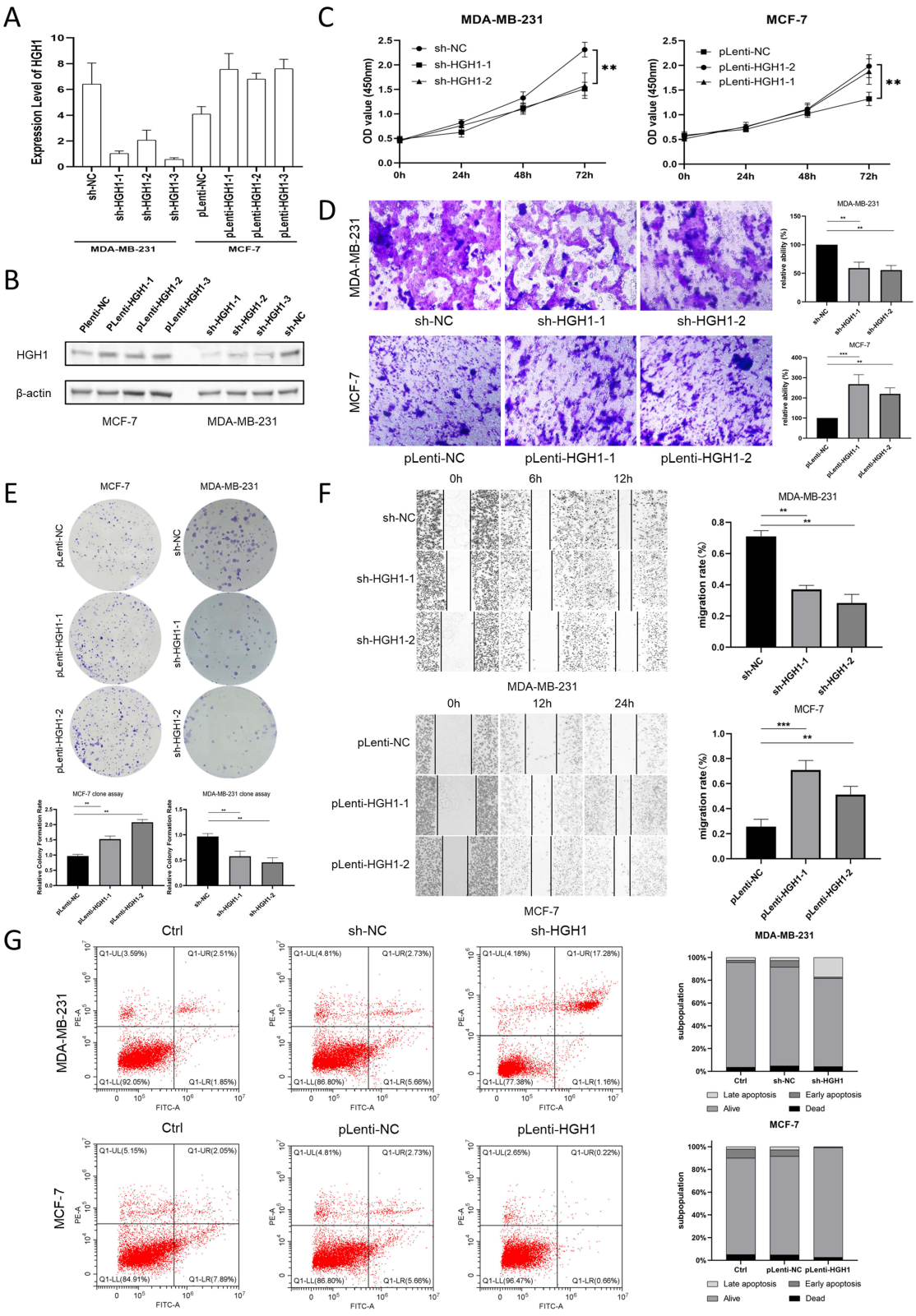

Figure 3 Knockdown or expression of HGH1 in breast cancer cell lines. (**A**) HGH1 mRNA expression after transfection of HGH1 shRNA was assessed using RT-qPCR; (**B**) HGH1 protein expression after transfection with HGH1 shRNA was assessed using Western blotting; (**C**) The proliferation ability of breast cancer cell lines (MDA-MB-231, MCF-7) after overexpression or knockdown of HGH1 was detected by CCK-8 method; (**D**) The invasive ability was assessed by Transwell method; (**E**) The proliferation ability was detected by colony formation experiment; (**F**) Cell migration ability was assessed by scratch test; (**G**) Flow cytometry analysis of apoptosis of MDA-MB-231 and MCF-7 cells after shHGH1 and pLenti-HGH1 transfection. *P<0.05, **P<0.01, ***P<0.001.

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overexpression of HGH1 significantly decreased the early apoptotic rate in MCF-7 cells. These in vitro results revealed that abnormal expression of HGH1 affects the proliferation, migration and invasion ability of breast cancer cells and can regulate the occurrence of apoptosis of breast cancer cells (Figure 3G).

HGH1 Activates NF-κB Signaling in the PI3K/AKT Pathway to Promote Breast Cancer Progression

According to the above KEGG analysis results, the high expression of HGH1 is associated with many malignancy-related pathways, including the PI3K-Akt signaling pathway. Next, we investigated whether HGH1 regulates the PI3K/AKT signaling pathway in breast cancer cells. Inhibition of HGH1 decreased the levels of proteins phosphorylated PI3K (p-PI3K) and phosphorylated AKT (p-AKT), while overexpression of HGH1 increased the levels of p-PI3K and p-AKT (Figure 4).

Previous studies have shown that the upstream PI3K/AKT signaling pathway regulates the inhibitory IKB protein, thereby affecting the expression of NF-κB.[9] It has been confirmed that the signaling mechanism of NF-κB plays a crucial role in the growth and progression of tumor cells.[10] To verify whether activation of the PI3K/AKT signaling pathway promotes the up-regulation of NF-κB p65 protein expression, we used LY294002, an inhibitor of PI3K, to block this signaling pathway. We treated pLenti-HGH1/MDA-MB-231 cells with 30 μM and 50 μM PI3K inhibitors for 48 hours, respectively. As expected, the levels of p-PI3K and p-AKT in the cells were significantly reduced, and at the same time, LY294002 inhibited the expression levels of p-IKBα and, NF-κB p65, and p-NF-κB p65 in pLenti-HGH1/MDA-MB-231 cells (Figure 5). These results were re-validated in ELISA analysis (Figure 5). The findings observed in breast cancer cells suggest that the PI3K/AKT/NF-κB signaling pathway is involved in HGH1-induced pathologic progression.

HGH1 Promotes the Development of Breast Xenografts in vivo

sh-HGH1 and Lenti-HGH1 stably transfected MDA-MB-231 cells were implanted into the subcutaneous fat pad of nude mice to establish a xenograft model to study the role of HGH1 in the occurrence of breast cancer in vivo. Compared with the control group, the sh-HGH1/MDA-MB-231 group showed a significant reduction in tumor volume after 5 weeks of tumor implantation. In contrast, the pLenti-HGH1/MDA-MB-231 group had a significant increase in tumor volume as compared with the control group (Figure 6A and B). The expression level of HGH1 in tumor tissues of sh-HGH1/MDA-

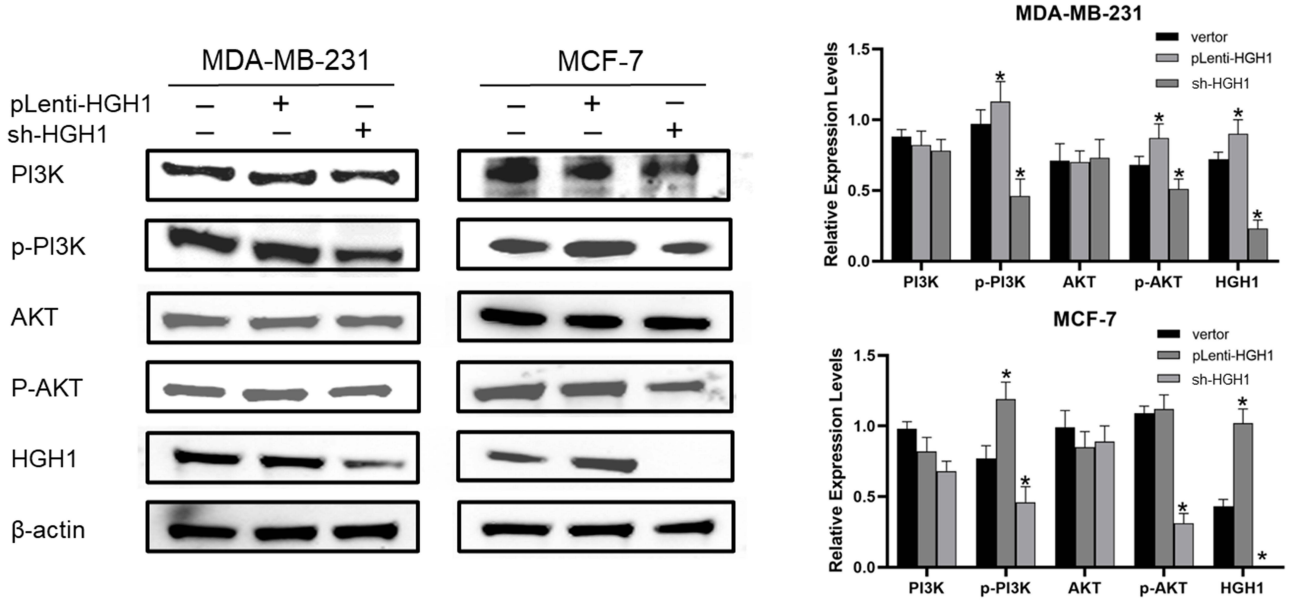

Figure 4 HGH1 promotes the expression of the PI3K/AKT pathway in breast cancer cells. Western blot analysis showed that the protein levels of phospho-PI3K and phospho-Akt were increased in Lenti-HGH1 cells and decreased in shHGH1 cells. The experiment was repeated three times. Representative results are presented. *P < 0.05.

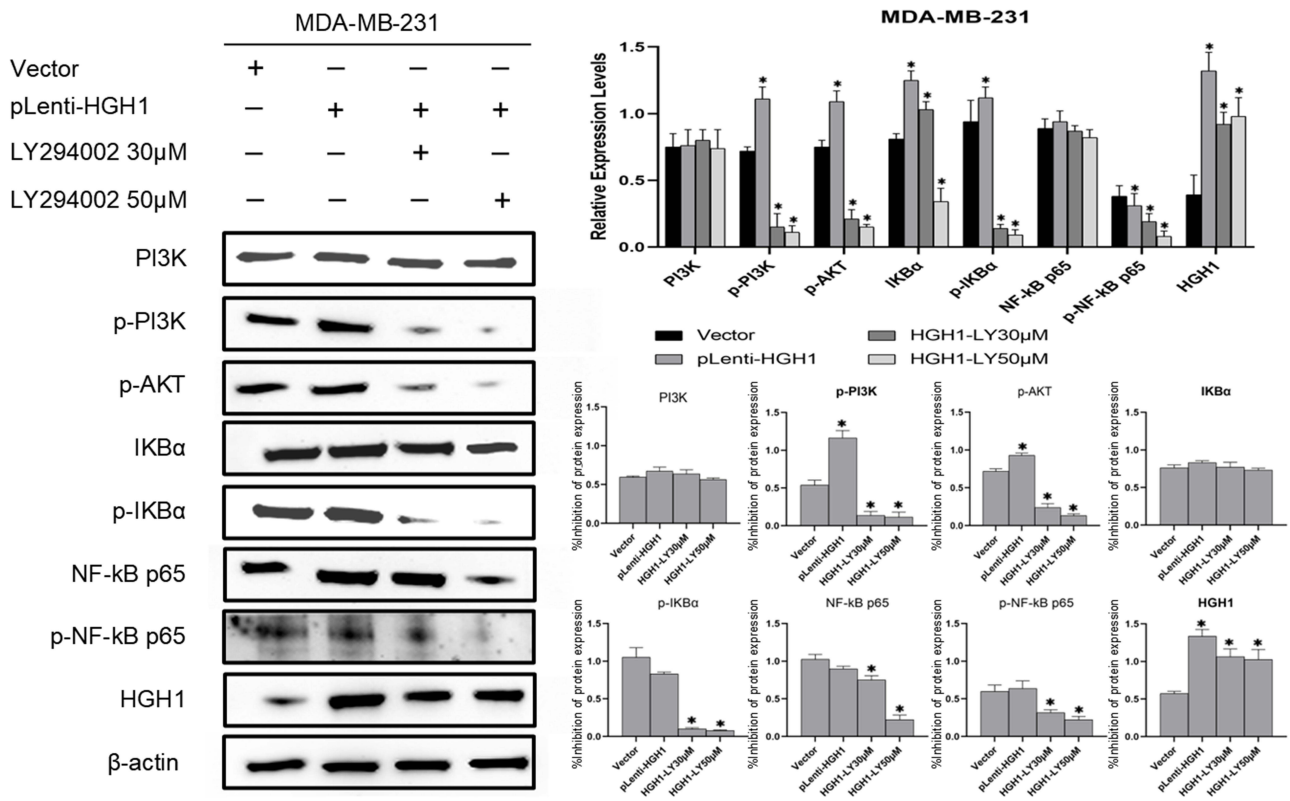

Figure 5 Phosphorylation-Nf-κb p65 is regulated by HGH1. Western blot analysis showed that treatment of MDA-MB-231 cells with LY294002 (30 μM, 50 μM) attenuated hGH1-induced phospho-PI3K, phospho-Akt, phospho-IKBα, and phospho-p-Nf-Kb p65. The experiment was repeated three times. Representative results are presented. ELISA analysis revealed the differences in the mRNA expression of phospho-PI3K, phospho-Akt, phospho-IKBα, p-NF-kB p65 and phospho-p-Nf-Kb p65 in MDA-MB-231 cells. *P < 0.05.

MB-231 group was significantly decreased compared with the control group, while pLenti-HGH1/MDA-MB-231 group was significantly higher than that of control group. Meanwhile, the expression patterns of PI3K, p-PI3K, p-AKT, iKB-α, p-IKB-α, NF-κB p65, and p-NF-κB p65 in vivo were consistent with our in vitro measurements (Figure 6C). These findings suggest that HGH1 may promote breast tumor growth in vivo by enhancing the expression of genes associated with the PI3K/AKT/NF-κB signaling pathway.

Discussion

Breast cancer remains the leading cause of cancer-related death in women and is the most common cancer among women.[11] Breast cancer is characterized by heterogeneity in pathology, genomic alterations, gene expression, and the tumor microenvironment (TME), which collectively influence its clinical behavior and response to therapy. However, the classical parameters to guide treatment decisions–including histopathology, tumor size and grade, nodal involvement, and marker expression–are imperfect, especially for advanced cancers which eventually develop drug resistance. Therefore, it is necessary to continuously explore therapeutic targets to improve and optimize breast cancer treatment. The integration of modern genomic and transcriptomic data at an unprecedented scale has revealed diverse breast cancer subtypes, key molecular drivers, clonal evolution trajectories, and prognostic features, providing a deeper understanding of the biology of breast cancer and potential opportunities for the discovery of new therapeutic targets and predictive markers.[12,13]

Growth hormone (GH), a major metabolic homeostasis factor, is secreted in a circadian pattern, and studies have shown that RNA and secreted protein levels of the human (H) GH gene (HGH1) oscillate over a 24-hour cycle. BMAL1/ClOCK can transactivate the HGH1 promoter, and the mutation of this E-box element adversely affects the basal activity after transgene. The ability of BMAL1 to bind to the HGH1 promoter region containing the E-box element has been demonstrated in situ in the pituitary gland of HGH1 transgenic mice. This experiment demonstrates that the circadian

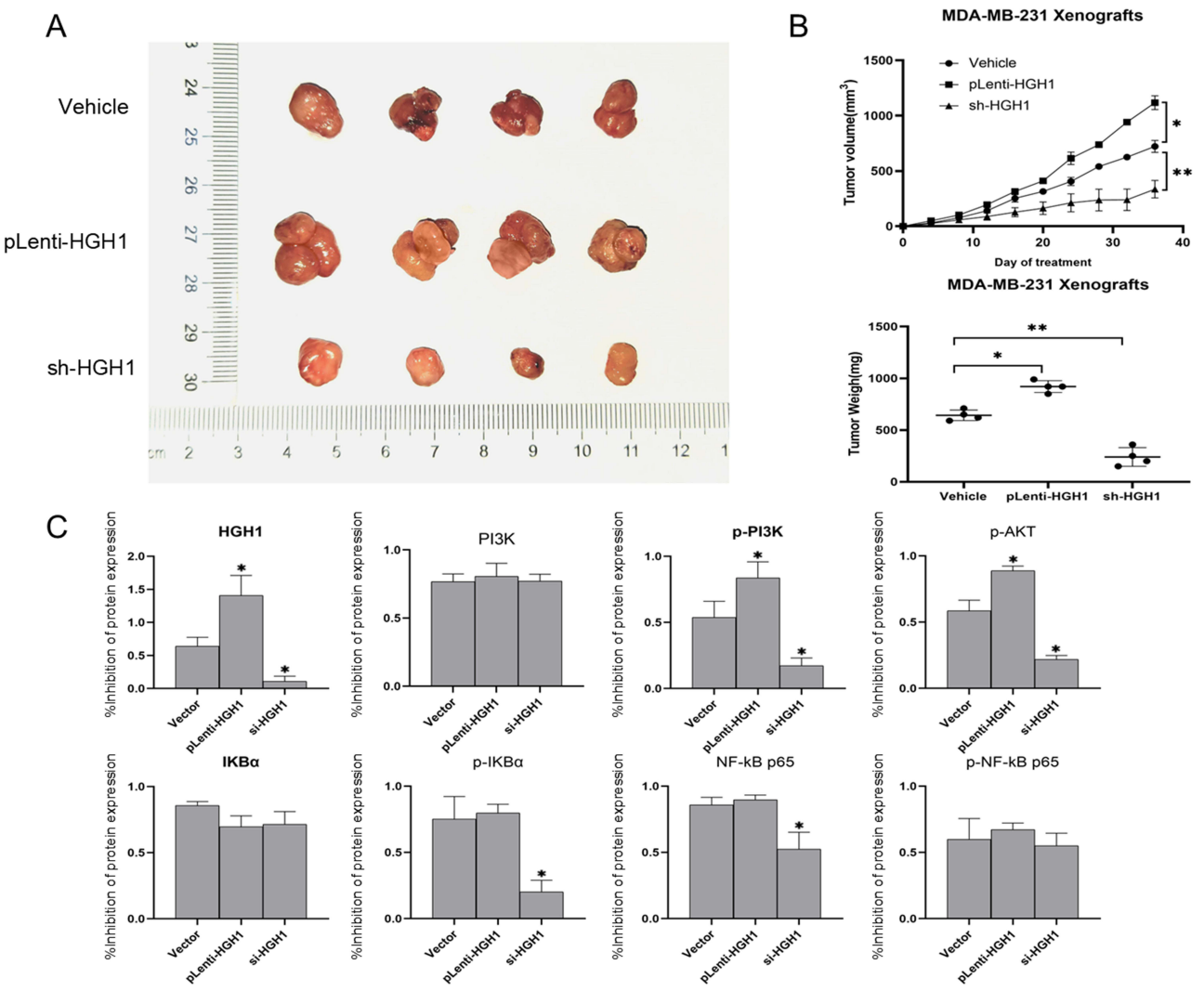

Figure 6 HGH1 promotes the growth of breast cancer xenografts in mice. (**A** and **B**) Compared with the vector control injected cells, the tumor volume and weight of Lenti-hGH1 injected cells were significantly increased, while those of sh-HGH1 injected cells were significantly reduced; (**C**) HGH1 induced PI3K/AKT signaling in vivo as observed by ELISA analysis. *P < 0.05, **P<0.01.

mechanism is dynamically associated with components of the HGH1 locus chromosomal architecture that are essential for efficient expression.[6] Hgh1, a highly conserved armadillos repeat protein, binds primarily to the structural dynamic domain III of eEF2 and appears to prevent aberrant intramolecular interactions that would otherwise slow folding of flanking domains. HGH1 recruits the chaperone TRiC to the C-terminal eEF2 module. In the absence of HGH1, much of eEF2 misfolds, leading to aggregation or degradation.[14] Another study found that the co-chaperone Cns1 and the recruitment protein Hgh1 could link Hsp90 to translation elongation via chaperone elongation factor 2.[15]

Studies have shown an association between circadian disruptors and breast cancer, such as night shift work, nocturnal family light exposure, sleep timing, and circadian gene polymorphisms.[16] Animal experiments showed that the circadian rhythm disorders and its recently caused by abnormal circadian rhythm related gene expression can contribute to the occurrence and development of breast cancer in mice and distant metastasis.[17,18] As a circadian rhythm-related gene, the abnormal expression of HGH1 may play a role in promoting malignant tumors, which was confirmed in this study.

Disruption of the circadian rhythm leads to the loss or reversal of the daily pattern and cytokine levels of M0 and M2 macrophages, which exhibit immunosuppression and homeostasis in favor of tumor cell proliferation.[19,20] In our immunological analysis, we found that the level of M0 macrophages in the group with high expression of HGH1 was significantly higher than low expression, while the level of T cell CD4 memory activation was significantly reduced. As Kaplan-Meier

survival analysis showed that the survival time of HGH1 high expression risk group was shorter, immune infiltration was an important factor affecting the prognosis of breast cancer patients. In the GO gene enrichment pathway analysis, it was found that the enrichment of ribosome pathway, ribosome biogenesis is a common marker of cell growth and proliferation, because ribosome is necessary for protein production, and the upregulation of ribosome biogenesis during G1/S stagnation can promote tumor metastasis.[21,22] In the analysis of KEGG gene enrichment pathway, the high expression of HGH1 was associated with many malignancy related pathways, including PI3K-AKT signaling pathway, cell cycle, cell aging, P53 signaling pathway, etc. Phosphorylation of AKT in the PI3K/AKT signaling pathway can activate and regulate important cellular activities, such as body growth, cell proliferation, tissue differentiation, carbohydrate metabolism, and tumorigenesis,[23–26] and is presumed to lead to resistance to systemic therapies, including chemotherapy and HER2-targeted therapy.[27] In this study, we found that HGH1 was involved in the development of breast cancer through the PI3K/AKT signaling pathway. The transcription factor p53 is an important regulator of a variety of cellular processes and is involved in the control of the cell cycle, DNA repair, and the induction of senescence and apoptosis by activating or inhibiting its target genes.[28,29] Mutations of p53 protein showed a variety of carcinogenic properties, these features give it regulates cancer cell proliferation, escape the apoptosis mechanism of the immune system, and promote the ability of invasion and metastasis.[30,31] These enriched pathways are consistent with the conclusion that patients in the HGH1 low expression group have a longer survival time.

In this study, we observed a significant increase in HGH1 expression in breast cancer tissues. In vitro experiments showed that the expression of HGH1 can affect the malignant behavior of breast cancer cells, and knocking down HGH1 can inhibit the proliferation, migration and invasion of breast cancer cells, and promote the occurrence of apoptosis. Conversely, overexpression of HGH1 promotes the proliferation and migration of breast cancer cells and helps to reduce apoptosis. In subsequent mechanism analysis, it was found that down-regulation of HGH1 inhibited the growth of breast cancer cells in vitro and in vivo by inhibiting the activation of PI3K/AKT/NF-κB signaling, and induced apoptosis of BC cells by regulating the PI3K/AKT pathway. Further studies will explore how HGH1 specifically interacts with or regulates these signaling pathways at the molecular level, involving the discovery of upstream regulators or downstream effectors of HGH1 within these pathways, which will gradually reveal the potential of the HGH1/PI3K/AKT regulatory network for the clinical treatment of breast cancer.

The circadian gene HGH1 emerged as a potential oncogenic mediator in this study. While its role in ribosome biogenesis via eEF2 folding and TRiC recruitment is well-documented,[14,15] our findings extend its function to breast cancer progression through PI3K/AKT signaling—a pathway central to therapy resistance.[23–27] However, the causal link between HGH1 dysregulation and PI3K/AKT activation remains speculative. Mechanistically, HGH1 may act indirectly via circadian disruption-induced metabolic reprogramming or direct interactions with kinase cascades. Furthermore, the reliance on transcriptional data (eg, TCGA) limits insight into post-translational modifications or non-cell-autonomous effects of HGH1 within the TME.

In conclusion, these data indicate that HGH1 plays a cancer-promoting role in breast cancer, and further reveals the HGH1 / PI3K/AKT regulation network potential impact on the progress of breast cancer. HGH1 is expected to be a new biomarker for the diagnosis of breast cancer, and also provides a potential therapeutic target for the treatment of breast cancer.

Data Sharing Statement

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

Ethics Statement

All animal procedures were approved by Welfare and Ethics Committee of the Experimental Animal center of the Air Force Military Medical University. The procedures conformed to the guidelines of NC3Rs ARRIVE. This study followed the Guidelines for Ethical Review of Laboratory Animal Welfare in China. The study complies with the Declaration of Helsinki.

Scope Statement

Our study is aimed at novel target development for breast cancer diagnosis and treatment and is closely related to the content of the Frontiers in Oncology journal. Meanwhile, we investigated the role of circadian HGH1 in cancer development and explored the downstream PI3K-AKT signaling pathway. We believe that our study is suitable for publication in the sections of breast Cancer, Cancer Genetics, and Cancer Molecular Targets and Therapeutics.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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