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The role of HGH1 in breast cancer prognosis: a study on immune response and cell cycle

Hailong Li[1†], Yong Xu[2†], Rong Xu[1*] and Wei Du[1*]

Abstract

Background Breast cancer (BRCA) remains to be among the main causes of cancer-associated mortality in women globally. HGH1 homolog (HGH1) has been reported to be associated with tumor immunity. However, the function of HGH1 in BRCA remains unclear. Therefore, the present study examined the potential role of HGH1 in BRCA.

Methods The Cancer Genome Atlas (TCGA) databases and Gene Expression Omnibus (GEO) were used to obtain RNA-seq data for BRCA. A protein localization of HGH1 was determined by using the Human Protein Atlas (HPA), and immunohistochemistry (IHC) staining revealed an upregulation in the expression of HGH1 in clinical BRCA tissues. Xenograft mice were used to test tumor growth and HGH1 expression in breast cancer cells. The protein interaction information of HGH1 was analyzed using the GeneMANIA website. Based on univariate Cox regression and Kaplan-Meier methods, we evaluated the role of HGH1 in BRCA prognosis. HGH1-related differentially expressed genes were analyzed using GO, KEGG, and GSEA. We also examined the relationship between HGH1 expression, immune checkpoints, and immune infiltration. CCK-8, EdU, and colony formation assays were used to measure cell proliferation, and western blot analysis was used to evaluate HGH1's role in BRCA.

Results IHC results showed that the expression of HGH1 was significantly upregulated in BRCA tissues compared to normal tissues. High levels of HGH1 expression was associated with worse clinical features and a worse prognosis. HGH1 expression was an independent predictor of BRCA outcomes in both univariate and multivariate analyses. Functionally, western blot analysis showed that HGH1 is implicated in cell cycle. As well, knocking down HGH1 significantly reduced BRCA cells' proliferative abilities. Crucially, HGH1 expression levels were positively correlated with Th2 cell infiltration and negatively correlated with Tcm cell infiltration.

Conclusion Biomarkers such as HGH1 can reliably predict prognosis in BRCA patients.

Keywords Breast cancer, Immune infiltration, HGH1 homolog, Prognosis, Cell cycle

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Introduction

Breast cancer (BRCA) is the second most commonly diagnosed cancer worldwide, with approximately ~2.1 million new cases and around ~627,000 BRCA-related deaths annually [1, 2]. In recent years, BRCA has become the most prevalent malignancy among women. As a disease closely linked to aging, the primary treatment strategies for BRCA include surgery and chemotherapy. However, poor prognosis associated with BRCA poses significant challenges for long-term clinical management [3]. Consequently, there is an urgent need to identify novel molecular prognostic markers and therapeutic targets for BRCA.

HGH1 homolog (HGH1), also known as BRP16, BRP16L, FAM203A, FAM203B, C8orf30A, and C8orf30B, is a protein-coding gene linked to maturity-onset diabetes of the young, type 3 [4]. It is located at the chromosomal region 8q24.3. However, the role of HGH1 in cancer remains largely unexplored, with only two studies reporting its involvement in cancer. Specifically, HGH1 was been implicated in the migration and invasion of colorectal cancer cells, suggesting that HGH1 may enhance the malignancy of colorectal cancer [5]. Additionally, a tumor microenvironment characterized by high HGH1 expression has been associated with an immune-depleted environment [6]. However, the expression and clinical significance of HGH1 in BRCA remain unclear. Thus, investigating the function of HGH1 in BRCA could provide valuable clinical insights.

In this study, we the hypothesis that HGH1 may serve as an independent prognostic biomarker in patients with BRCA. We also analyzed the functional networks associated with HGH1 expression, including its role in cell cycle regulation and tumor immunity. The findings of this study may contribute to understanding HGH1 as a potential therapeutic target or predictive biomarker for risk stratification. Moreover, we aim aimed to explore the molecular mechanism underlying BRCA progression and the role of HGH1 in this process.

Materials and methods

Clinical data collection

The Cancer Genome Atlas database (TCGA; https://cancergenome.nih.gov/) [7] is the largest database of cancer genetic information, containing data from 33 types of cancer based on large-scale genome sequencing and various omics techniques, including genomic, transcriptomic, epigenetic, proteomic analyses. The original RNA expression data from 1,083 BRCA cases were downloaded from TCGA (Supplementary Table 1). High throughput sequencing fragments per kilobase of transcript per million values were converted to transcript per million values to identify genes with differential expression among the samples. Corresponding clinical information for patients with BRCA was also retrieved from the TCGA database. To validate the findings from TCGA data, the GSE22820 and GSE42568 datasets were used for further validation [8]. Immunohistochemical (IHC) images of BRCA tissues and normal tissues were obtained from Human Protein Atlas (HPA) database (https://www.proteinatlas.org) [9].

Immunohistochemical (IHC) analysis and immunofluorescence (IF)

60 pair normal breast tissues and breast cancer tissues were collected from the first's people of Changde city (Changde, Chinna). All samples were diagnosed based on the clinical information of patients with BC. All sample collection procedures were approved by the Ethics Committees of the first's people of Changde city (approval number:2024-147-01). Firstly, we collected the breast tissue and breast cancer tissues, which were then fixed it with formalin, dehydrated for 12 h, and then embedded in paraffin to prepare 3 μm thick tissue sections. Primary antibodies were applied and incubated overnight at 4 °C. After adding secondary antibodies, the sections were washed three times with PBS. Positive results were defined as the presence of brown particles in the nucleus and cytoplasm in five random fields under a microscope. For the staining evaluation, we enlisted the assistance of three experienced pathologists [10]. *Immunofluorescence (IF)*: After routine rehydration, the tissue was subjected to antigen retrieval, followed by blocking with 5% BSA. The primary antibody was then added and incubated overnight at 4 °C. The corresponding fluorescent secondary antibody was applied, and the cell nuclei were stained with DAPI. The images were acquired by a fluorescent microscope (Zeiss, Jena, Germany) [11].

Cell culture, plasmids, and western blots

MDA-MB-231and MCF-7 cells (Xiangya School of Medicine, Central South University, China) were cultured. The HGH1 knockdown siRNA sequence was designed and purchased from RIBO Biotechnology (Guangzhou, China). Primary antibodies against HGH1, c-MYC, BCL2, CCNB1 and the loading control GAPDH, as along with HRP-conjugated secondary antibodies were obtained from Abcam (Cambridge, MA).

Cell counting kit-8 (CCK-8)

Cells were transfected with NC and si-HGH1, then transfected to 96-well plates. 24 h later, CCK-8 reagent was added to the culture medium, and the cells were incubated for 1 h at 37 °C. The OD value at 450 nm was measured using a microplate reader.

EdU cell proliferation experiment

2×10^5 cells were seeded in six-well plates. EdU experimental procedures were carried out according to the EdU kit instructions (BeyoClick™), EdU-555, Shanghai, China). Briefly, the cells were incubated with culture medium added EdU solution for 3 h in 37℃. Then fixed with 4% Paraformaldehyde for 30 min at room temperature, Finally, Stain the cell nuclei with DAPI. The images were acquired by a fluorescent microscope (Zeiss, Jena, Germany).

Colony formation assay

In 6-well plates (1000 cells per well), NC and si-HGH1 sequences were transfected into cells. The cells were then cultured for 14 days, with the medium changes every 3 days. To count colonies, the cells were stained with crystal violet and fixed with paraformaldehyde.

Establishment of nude mouse xenograft model

The Beijing Vital River Laboratory Animal Technology Co., Ltd. provided us with four-week-old nude mice. A SPF-grade animal laboratory with a humidity of 60 to 65% and a temperature of 22 to 25 °C was used to house the mice in separate cages. Acclimatization feeding lasted a week before experiments began. Observations were made before the experiment to determine the health status of the mice. Protocols for animal use and experimental procedures were approved by the relevant ethics committee.

To establish the in vivo model, Knockdown and negative control MCF-7 cells (1.0×10^6) were suspended in pre-cooled PBS, and Inject the cells subcutaneously into the mammary fat pad of the nude mice [12]. In the final experiment, we observed the growth of tumors in mice. Once the volume reached about 500 mm^3, the mice were euthanized by cervical dislocation, and their tumor tissue was collected for further study. Tumor volume changes were periodically recorded throughout the experiment using appropriate measuring tools.

Relationship between HGH1 expression and clinicopathological features and prognoses

The RNA sequencing data and corresponding clinical information for BRCA were obtained from TCGA-BRCA dataset. The relationship between HGH1 expression and clinicopathological features, as well as prognosis was analyzed using R software (version 4.3.3). The R packages "ggplot2", "ggpubr", "survival", and "survminer" were employed in this process. To the analyze association between HGH1 and clinicopathological parameters, Wilcoxon Rank Sum and Signed Rank Tests were used for pairwise comparisons, while Kruskal-Wallis was applied for multiple sample comparisons. For the overall survival (OS)analysis, cohorts were formed based on HGH1 expression levels using 50% expression levels, with a 50% expression threshold used to categorizing high and low expression groups [13]. The diagnostic value of HGH1 was evaluated using ROC curve analysis. The pROC package was employed for the analysis, and the results were visualized with ggplot2. A nomogram model was constructed based on independent prognostic factors identified through multivariate analysis. The proportional hazards assumption was tested, and Cox regression analysis was performed using the survival package. Nomogram-related models were constructed and visualized with the rms package. Calibration curves were used to assess the difference between predicted probabilities and actual outcomes, with the x-axis representing predicted survival time and the y-axis representing actual survival time. In an ideal predictive model, the predicted survival rates would align with a 45° slope on the curve.

Analysis of differentially expressed genes (DEGs) in low HGH1- and high HGH1-expressing subgroups

Samples were ranked based on HGH1 expression, and the top 25% were classified as the high HGH1 expression subgroup, while the bottom 25% were classified as the low HGH1-expressing subgroup, respectively. Differential expression analysis of the two groups was performed using the 'DESeq2' tool in R, with criteria set at |logFold Change| > 1 and adjusted $P<0.05$. DEGs were identified as the genes with statistically significant differences.

Protein-protein interaction (PPI) and functional enrichment analysis

For PPI network analysis, we utilized the GeneMANIA website [14]. we conducted GO, KEGG, and GSEA analysis to predict biological pathways and molecular functions associated with HGH1. The GSEA analysis was performed using the "h.all.v2023.2.Hs.symbols.gmt" file [15]. TCGA-BRCA data were analyzed by dividing samples into high-expression and low-expression groups, Differential expression analysis was then conducted, and volcano plots were generated to visualize the results. DEGs were further analyzed through enrichment analysis. The R software packages used in the process are "limma", "tibble", "ggplot2, "and "enrichplot". R software GSVA package was used to explore the correlation between HGH1 and signaling pathways. Spearman correlation was used to assess the relationship between gene expression and pathway scores. A p-value of <0.05 was considered statistically significant.

HGH1 and tumor immunity

R software was used to assess immune infiltration, as well as calculate the ImmuneScore, StromalScore, and ESTIMATEScore. Immune invasion data in BRCA were obtained from the TIMER and CIBERSORT [16]

databases. The ssGSEA method, implemented via the GSEA package in R, was used to quantify the infiltration levels of 24 tumor-infiltrating immune cells in BRCA samples. Additionally, the correlation between HGH1 expression and various immune checkpoint genes, as well as tumor mutation burden, was evaluated using R software packages "ggplot2" and "pheatmap".

Statistical analysis

The Wilcoxon rank sum test was used to compare HGH1 expression levels between normal and tumor tissues, while Pairwise t-tests were employed to analyze BRCA samples obtained from hospitals. Prognosis was assessed using both Cox regression and Kaplan-Meier. Additionally, spearman correlation analysis was performed to evaluate the statistical relationship between HGH1 expression and immune cell infiltration levels, immune regulatory genes, and other factors. All calculations were performed using the R package, with $P<0.05$ considered statistically significant.

Results

High HGH1 expression in BRCA

To explore the potential relevance of HGH1 to cancer, we screened for cancers with differentially expressed HGH1 between tumor and healthy tissues using TCGA data (Fig. 1A). TCGA analysis revealed that HGH1 expression was significantly higher in BRCA tissues compared with that in healthy tissues ($P<0.05$), a finding further validated in clinical BRCA tissues and paired normal breast tissues ($P<0.05$; Fig. 1B and C). Additionally, HGH1 expression showed strong discriminatory power with an area under the curve (AUC) value of 0.951, indicating its potential to differentiate BRCA from healthy tissues (Fig. 1D). These results were further validated using the GSE22820 and GSE42568 datasets (Fig. 1E and F). A representative IHC image from the HPA database is displayed (Fig. 1G). Furthermore, Clinical specimens analyzed through IHC experiments confirmed that HGH1 protein levels were significantly upregulated in BRCA samples (Fig. 1H-I).

Association between HGH1 expression and clinical features

Subsequently, we assessed the association between HGH1 expression levels and various clinical features. High HGH1 expression was significantly associated with N stage (Fig. 2A), estrogen receptor (ER) status (Fig. 2B), PMA 50 (Fig. 2C), progesterone receptor (PR) status (Fig. 2D), pathological staging (Fig. 2E), histological type (Fig. 2F), and Race (Fig. 2G) (all $p<0.05$)).

Additionally, logistic regression analysis revealed that elevated HGH1 expression was strongly associated with several clinical features indicative of poor prognosis, including pathological stage [Stages II-IV vs. Stage I; odds ratio (OR), 1.456; 95% CI, 1.055–2.017;$P=0.023$], histological type (Infiltrating Lobular Carcinoma vs. Infiltrating Ductal Carcinoma; OR, 0.390; 95% CI, 0.281–0.538;$P<0.001$), PR status (Positive vs. Negative; OR, 0.691; 95% CI, 0.532–0.897; $P=0.006$) and ER status (Positive vs. Negative; OR, 0.685;95% CI, 0.511–0.915; $P=0.011$; Supplement Table 2).

Prognostic value of HGH1 in BRCA

Univariate and multivariate Cox regression models were used to assess the independent predictive capacity of HGH1 expression. Both analyses revealed that T stage, M stage and HGH1 were significant predictors of overall survival (OS) in BRCA patients (all $p<0.05$) (Fig. 3A and B), indicating that the HGH1 expression is an independent prognostic factor for BRCA. Based on these factors, 1-, 3- and 5-year OS nomograms are constructed (Fig. 3C). Patient prognosis was observed to worsen as the cumulative scores for the four prognostic criteria increased. Additionally, the calibration curve demonstrated that the nomogram accurately predicted the OS at 1, 3 and 5 years (Fig. 3D). These findings suggest that HGH1 could serve as a predictive marker for BRCA patient.

Next, the association between the OS and HGH1 expression in BRCA patients was examined (Fig. 4A). Kaplan-Meier survival curves showed that BRCA with high HGH1 expression were significantly reduced OS compared to those with low HGH1 expression ($P<0.05$). Moreover, HGH1 expression was negatively associated with the OS across various anatomical subdivisions of BRCA patients ($P<0.05$) (Fig. 4B and C).

Detection of differentially expressed genes (DEGs) between the high and low HGH1 expression groups

Data from TCGA were analyzed using the 'DESeq2' tool in R (|logFold Change| > 1); adjusted $P<0.05$), which identified 240 DEGs, including 115 upregulated and 125 downregulated genes between the high and low HGH1 expression groups (Fig. 5A). To further investigate the proteins interacting with HGH1, selected some *DEGs* were used to construct PPI networks, revealing that HGH1 is closely associated with these *DEGs* (Fig. 5B).

Functional annotation and predicted signaling pathways

To comprehensively understand the functional impact of HGH1 in BRCA, we conducted GO, KEGG, and GSEA enrichment analysis based on the *DEGs* associated with HGH1. GO analysis revealed that the most significant biological processes (BP), cellular components (CC), and

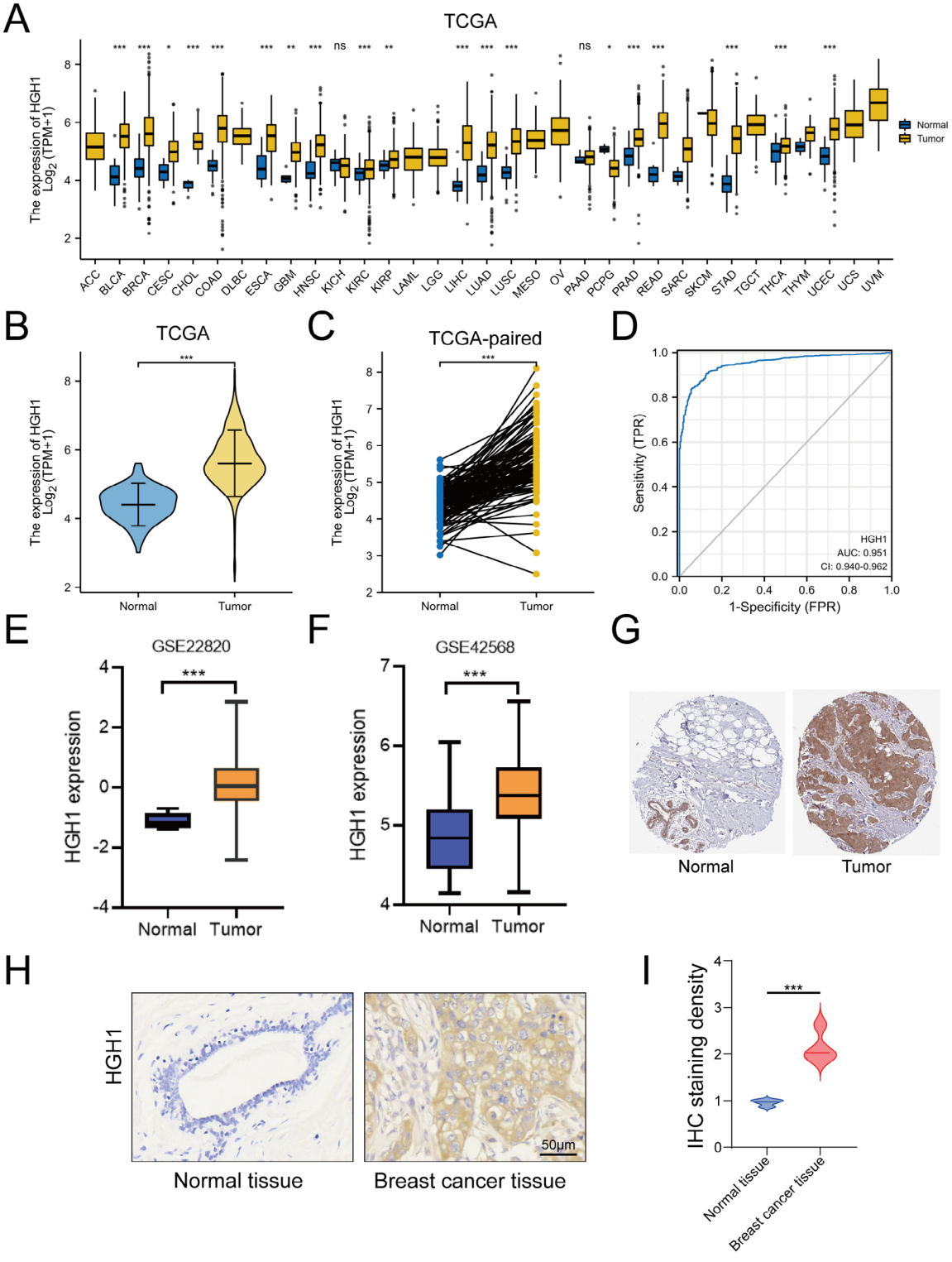


Fig. 1 HGH1 expression levels and diagnostic value. (**A**) Expression patterns of HGH1 in pan-cancer and corresponding healthy tissues. (**B**) Expression patterns of HGH1 in cancerous compared to unmatched healthy tissues. (**C**) HGH1 expression levels in BRCA and corresponding paired non-cancerous tissues. (**D**) Receiver operating analysis showing the potential of HGH1 to distinguish between tumor and healthy tissues. (**E**-**F**) The expression data of HGH1 from GSE22820 and GSE42568 datasets. (**G**) Representative IHC photomicrographs of HGH1 in breast tissue samples and BRCA from the HPA database (**H**-**I**) Representative IHC micrographs of HGH1 in collected clinical samples of breast samples and BC. All data are derived from a minimum of three independent experiments and are expressed as the mean ± SD

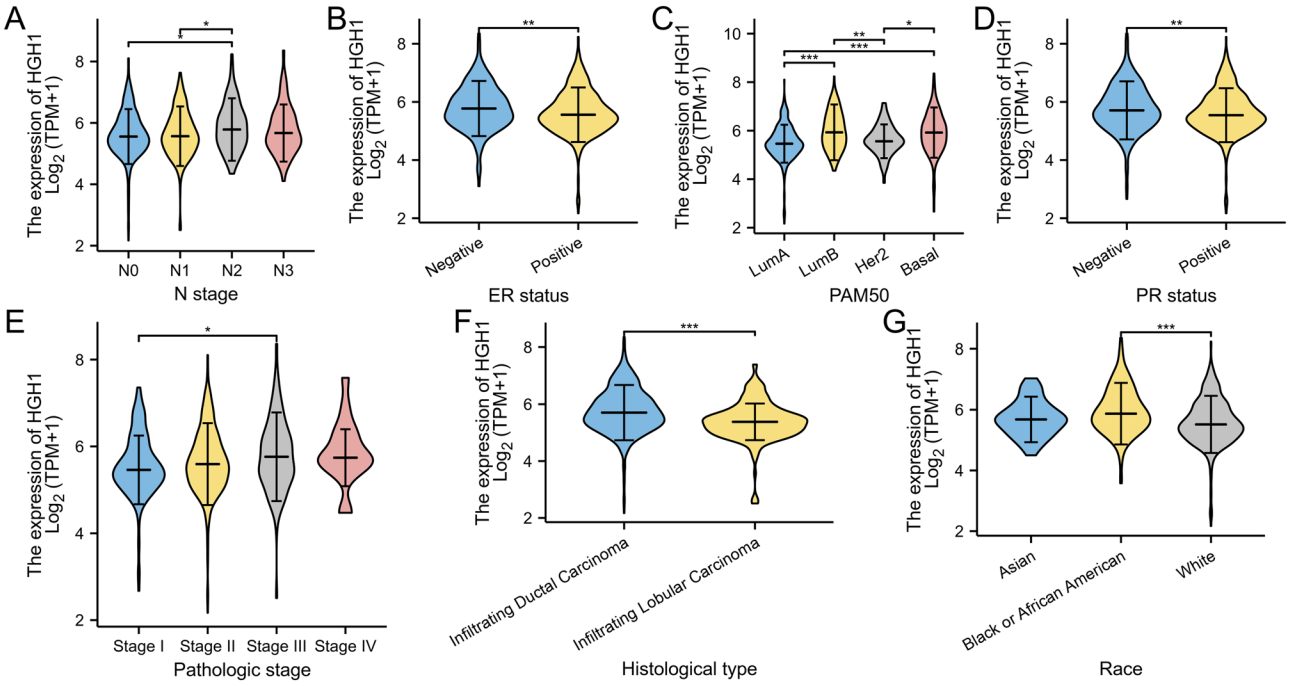


Fig. 2 HGH1 expression in breast cancer patients according to the different clinical characteristics. (**A**) N stage, (**B**) ER status, (**C**) PMA 50, (**D**) PR status, (**E**) pathological stage (**F**), histological type and (**G**) race. ER, estrogen receptor; PR, progesterone receptor

molecular functions (MF) include the mitotic cell cycle, cell division, extracellular space, extracellular region, extracellular matrix structural constituent, and heparin binding (Fig. 6A). KEGG enrichment analysis identified significant enrichment in the cell cycle and PPAR signaling pathway (Fig. 6B). Furthermore, GSEA analysis indicated that the high HGH1 expression is primarily associated with the activation of MYC_TARGETS, E2F_TARGETS and G2M_CHECKPOINT. The intersecting genes involved in these three activated signaling pathways include MYC, CDC20, CDK4, and MCM2. In summary, our findings demonstrate a significant enhancement in cell cycle, suggesting that HGH1 plays a role in regulating this process. (Fig. 6C-D).

HGH1 promoted the proliferation, colony formation, and cell cycle of BRCA cells

To validate the impact of HGH1 on the phenotype of breast cancer cells, Western blotting (WB) was used to analyze the efficiency of HGH1 knockdown. The results showed that siRNA-2 sequences specifically targeting HGH1 significantly suppressed HGH1 expression levels (Fig. S1). The EdU and CCK-8 assay was employed to evaluate the role of HGH1 in the growth of BRCA cells. Compared to the control group cells, the proliferation of MDA-MB-231 and MCF-7 cells with HGH1 knockdown was significantly inhibited (Fig. 7A-C). A colony formation assay further evaluated the impact of HGH1 on the colony formation ability of BRCA cells. The results demonstrated that MDA-MB-231 and MCF-7 cells with HGH1 knockdown exhibited a reduced colony formation capacity compared to the control group (Fig. 7D-E). Additionally, to further investigate the mechanism of HGH1, we utilized bioinformatics approaches and found that HGH1 is closely associated with the cell cycle and MYC pathway (Fig. 7F-H). Subsequently, WB assay was conducted to examine the protein expression levels of c-MYC [17], CCNB1 [18], and BCL2 [19] in MDA-MB-231 and MCF-7 cells with HGH1 knockdown. The results demonstrate that the expression levels of c-MYC, CCNB1, and BCL2 were lower in the HGH1 knockdown cells compared to the control group (Fig. 7I-K). These findings suggest that HGH1 promotes the proliferation, colony formation, and cell cycle progression of BRCA cells in vitro.

Impact of HGH1 expression on the growth of BRCA cell xenografts

An in vivo study was conducted to investigate the potential regulatory mechanisms of HGH1 on BRCA cell proliferation and its role in BRCA progression. By subcutaneously inoculating MCF-7 cells with inhibited

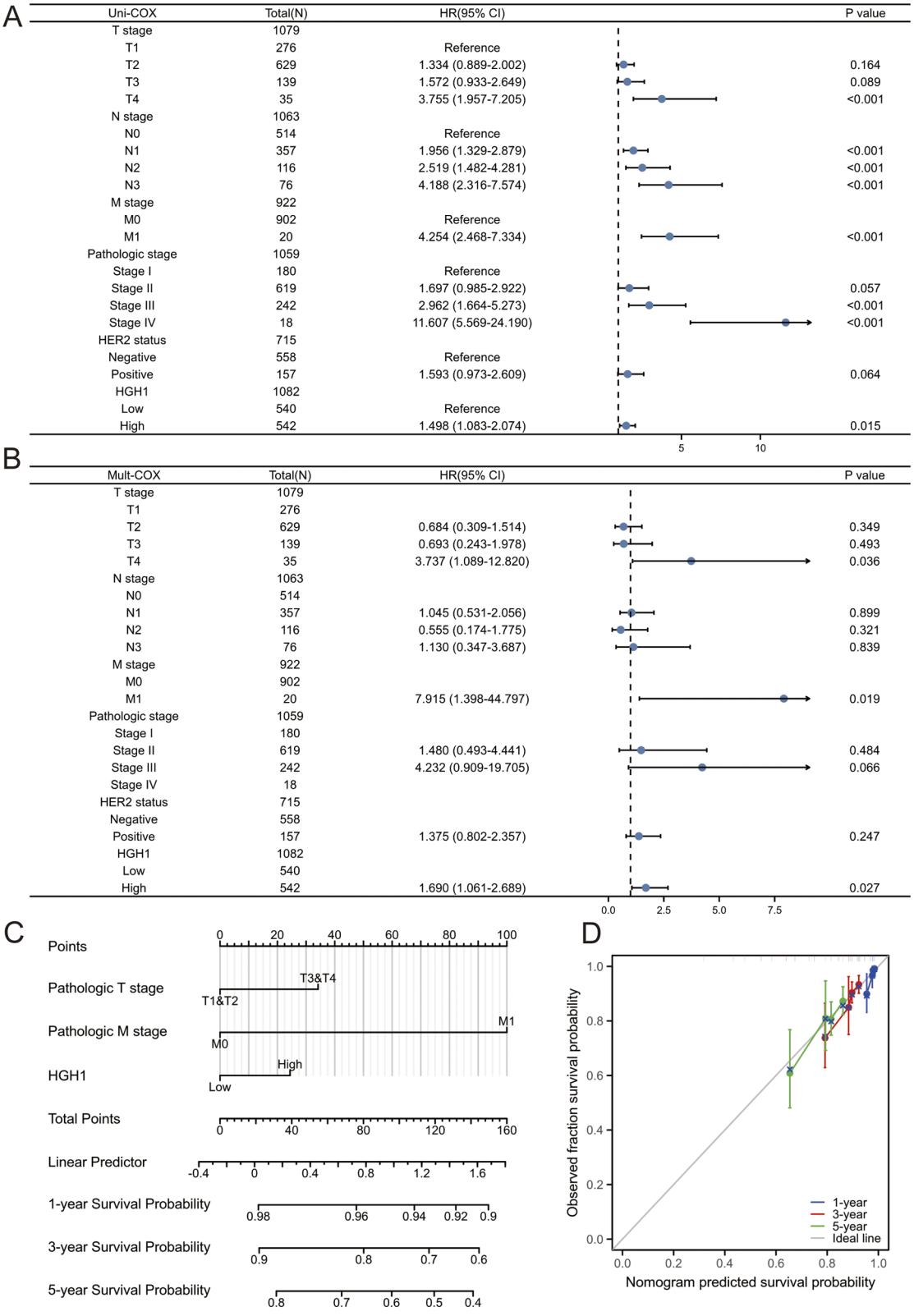


Fig. 3 Predictive value of HGH1 levels in patients with BRCA. Forest plots were used to depict the findings of (**A**) univariate and (**B**) multivariate Cox regression analyses. (**C**) Nomogram for forecasting clinical prognosis after the inclusion of HGH1 expression in patients with BRCA. (**D**) Validation of calibration graphs for 1-, 3- and 5-year clinical prognoses for patients with BRCA

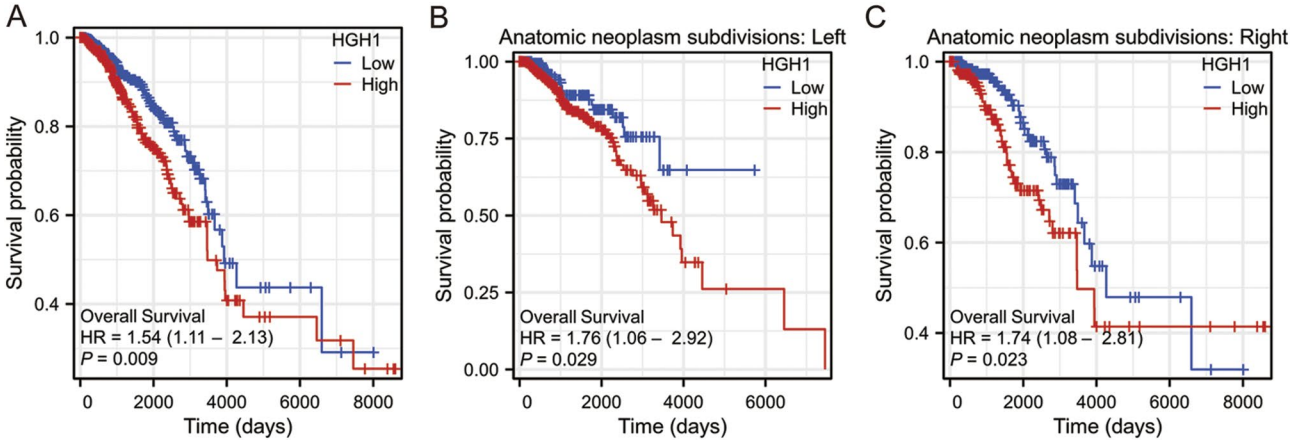


Fig. 4 Association between HGH1expression and patient prognosis. (**A**) Association of HGH1 expression with OS according to The Cancer Genome Atlas database. (**B**-**C**) Association of HGH1 expression with OS in Anatomic neoplasm subdivisions patients. OS, overall survival; HR, hazard ratio

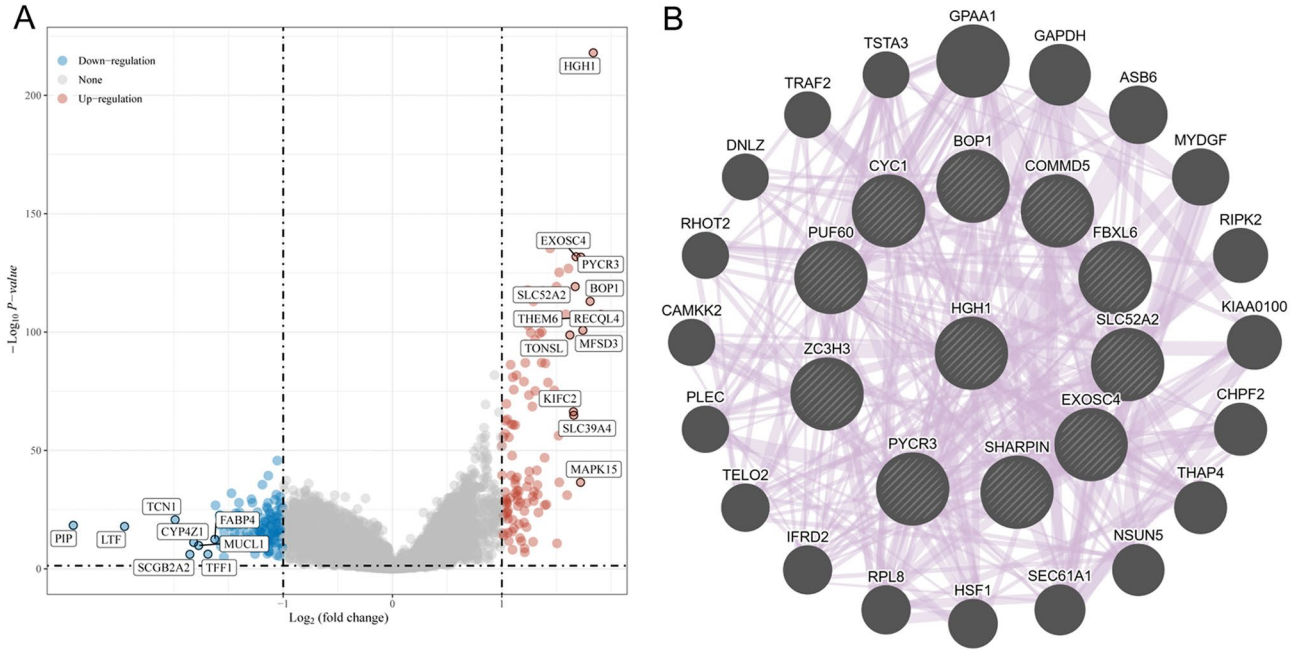


Fig. 5 DEGs between patients with high and low HGH1 and PPI networks. (**A**) A volcano plot of DEGs in groups with high and low HGH1 expression. (**B**) PPI networks. DEGs, differentially expressed genes

HGH1, we successfully established a xenograft mouse model of BRCA. The growth trends of tumors and the expression of HGH1 were continuously monitored and recorded throughout the experiment. Results indicated that the downregulation of HGH1 significantly reduced the growth of tumors, as evidenced by reductions tumor volume and weight ($P<0.01$) (Fig. 8A–C). Immunofluorescence experiment showed that Ki-67 positive cells was observed alongside reduced HGH1 expression ($P<0.01$) (Fig. 8D). Additionally, IHC data showed that compared to the NC group, the expression of HGH1 protein in the si-HGH1 group was significantly reduced ($P<0.01$) (Fig. 8E). These findings indicate that HGH1 plays a crucial role in promoting MCF-7 cell proliferation.

Correlation analysis of immune response

To understand the impact of HGH1 expression on the tumor microenvironment (TME), single-sample GSEA analysis of immune infiltration was conducted. Spearman's correlation was used to examine the relationship

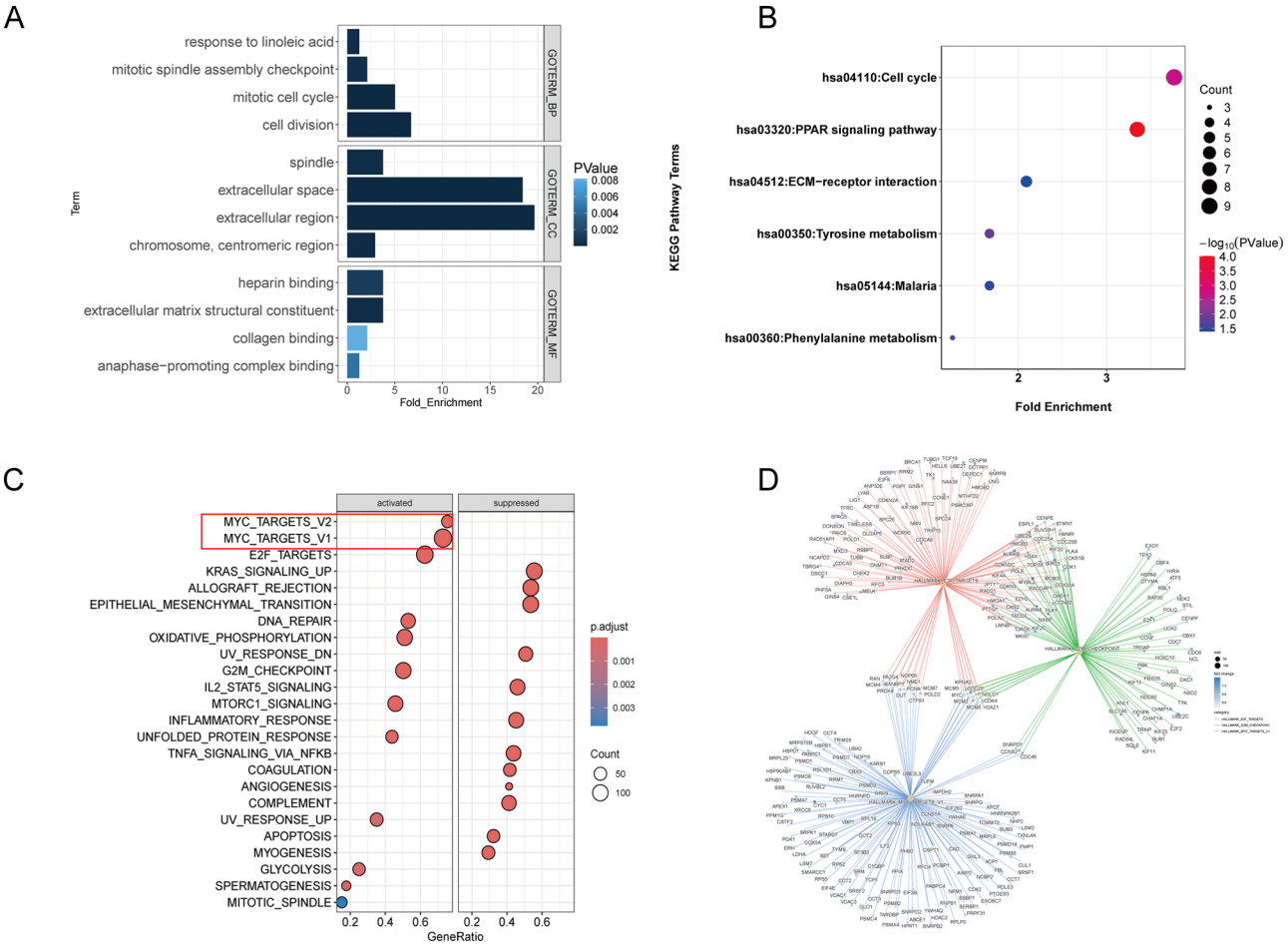


Fig. 6 Functional enrichment analysis. (**A**) GO (**B**) KEGG enrichment analyses. (**C**) GSEA analysis. (**D**) The intersection genes involved in MYC_TARGETS, E2F_TARGETS, and G2M_CHECKPOINT activated signaling pathways. BP, biological process; CC, cellular component; MF, molecular function

between immunity cell enrichments in BRCA tissues and HGH1 expression patterns. The results revealed a negative correlation between HGH1 expression and the infiltration levels of several immune cells, such as Tcm and T helper cells($P<0.05$) (Fig. 9A). Moreover, compared to the low HGH1 expression group, the high HGH1 expression group exhibited lower StromalScore, ImmuneScore and ESTIMATScore (Fig. 9B). Additionally, a negative correlation was found between HGH1 expression levels and immune checkpoint-related genes (Fig. 9C). Lastly, we assessed the relationship between tumor mutation burden and HGH1 expression, discovering a positive correlation between HGH1 expression and tumor mutation burden($P<0.05$) (Fig. 9D).

Discussion

BRCA currently has the highest rate of incidence rate among all malignancies in women [20]. This study explored the clinical value and function of HGH1 using bioinformatics databases and found that HGH1 is a potential prognostic molecular marker in clinical practice (Fig. Scheme 1). Identifying novel prognostic factors can aid in assessing treatment efficacy and predicting disease progression. For instance, Cytokine-induced apoptosis inhibitor 1 (CIAPIN1) is significantly associated with tumor immune cells infiltration and poor prognosis in invasive breast cancer, making it a potential prognostic marker for this condition [21].

Data from TCGA database revealed that HGH1 is expressed at higher levels in tumor tissues compared with normal tissues, which was validated in the HPA dataset and GEO database. Additionally, IHC assay demonstrated the HGH1 protein expression is significantly increased in BRCA tissues compared to normal tissues. ROC curve suggested that HGH1 gene expression may serve as a future diagnostic marker to distinguish between BRCA and healthy individuals. To determine whether the expression level of HGH1 is associated with

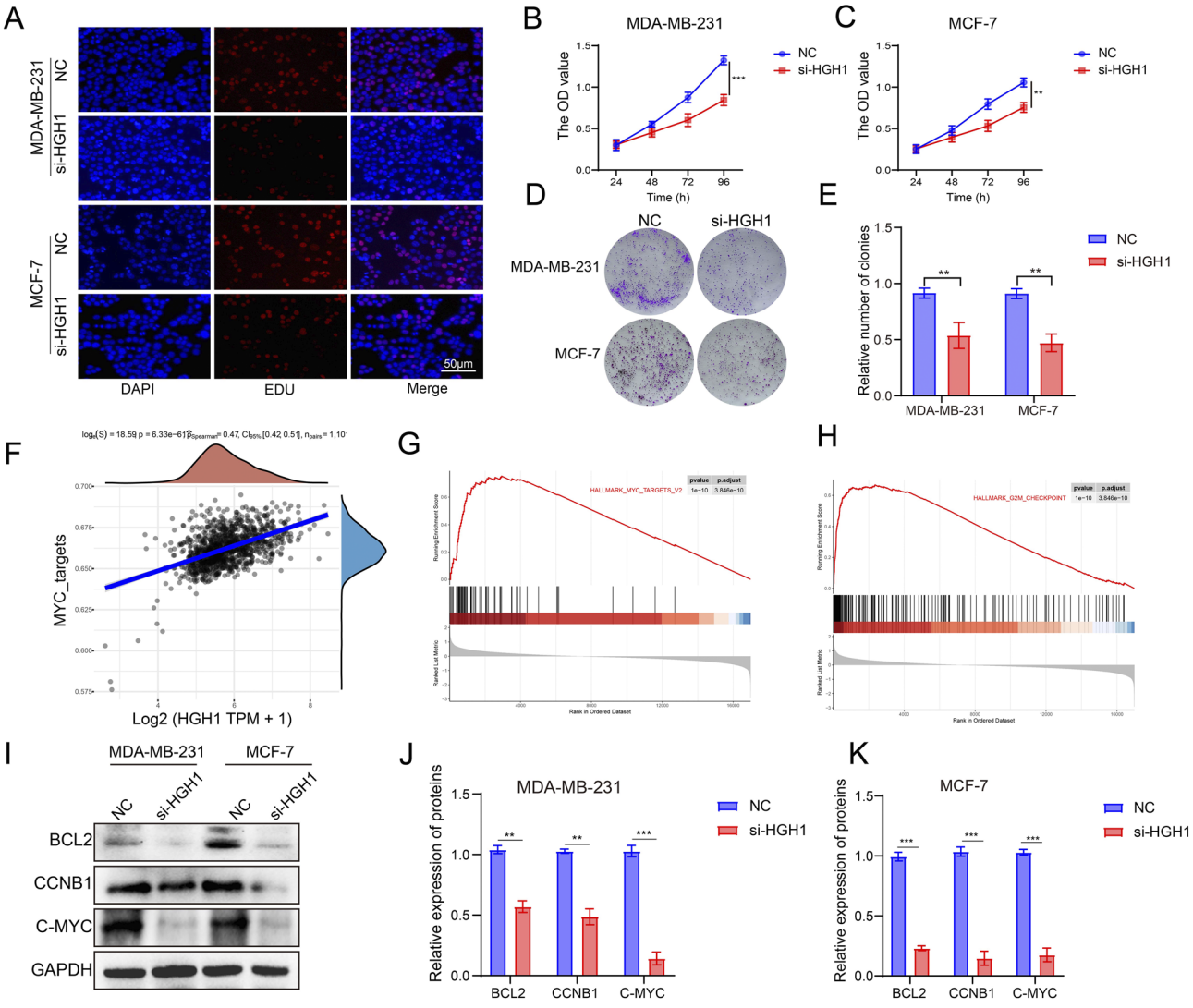


Fig. 7 HGH1 promote cell proliferation of breast cancer cells. (**A**) EdU assay was used to detect cell proliferation. (**B**, **C**) CCK-8 assay was used to detect the proliferation of MDA-MB-231 and MCF-7 cells. (**D**-**E**) Colony formation assay results for MDA-MB-231 and MCF-7 cells transfected with NC or si-HGH1. (**F**-**H**) Correlation analysis and enrichment analysis. (**I**-**K**) Western Blot validation of c-MYC, CCNB1, and BCL2 protein expression changes in MDA-MB-231 and MCF-7 cell xenografts after HGH1 silencing. Data are presented as mean ± SD, based on at least three independent experiments

the clinical characteristics, one-way ANOVA was conducted using clinical data from TCGA database. It was found that BRCA patients who are PR- and ER-negative had worse prognose [22]. Our study showed that HGH1 expression was significantly higher in PR- and ER- negative patients compared to PR- and ER-positive patients in BRCA. Moreover, invasive ductal carcinoma tissues exhibited higher HGH1 expression than lobular adenocarcinoma tissues. Breast cancer metastasis is the leading cause of cancer-related deaths worldwide. Early diagnosis, identification of therapeutic targets, and prognostic markers are crucial for improving the management of metastatic breast cancer. Tubulin α-1b chain (TUBA1B), a subtype of α-tubulin, has the potential to serve as both a diagnostic marker and a promising therapeutic target for breast cancer treatment [23]. F-actin, known for its sensitivity to mechanical stimuli, plays critical roles in cell attachment, migration, and cancer metastasis. Moreover, interfering with F-actin using a low-frequency rotating magnetic field has been shown to effectively inhibit breast cancer metastasis. This approach suggests a new, non-invasive, and highly penetrative physical therapy

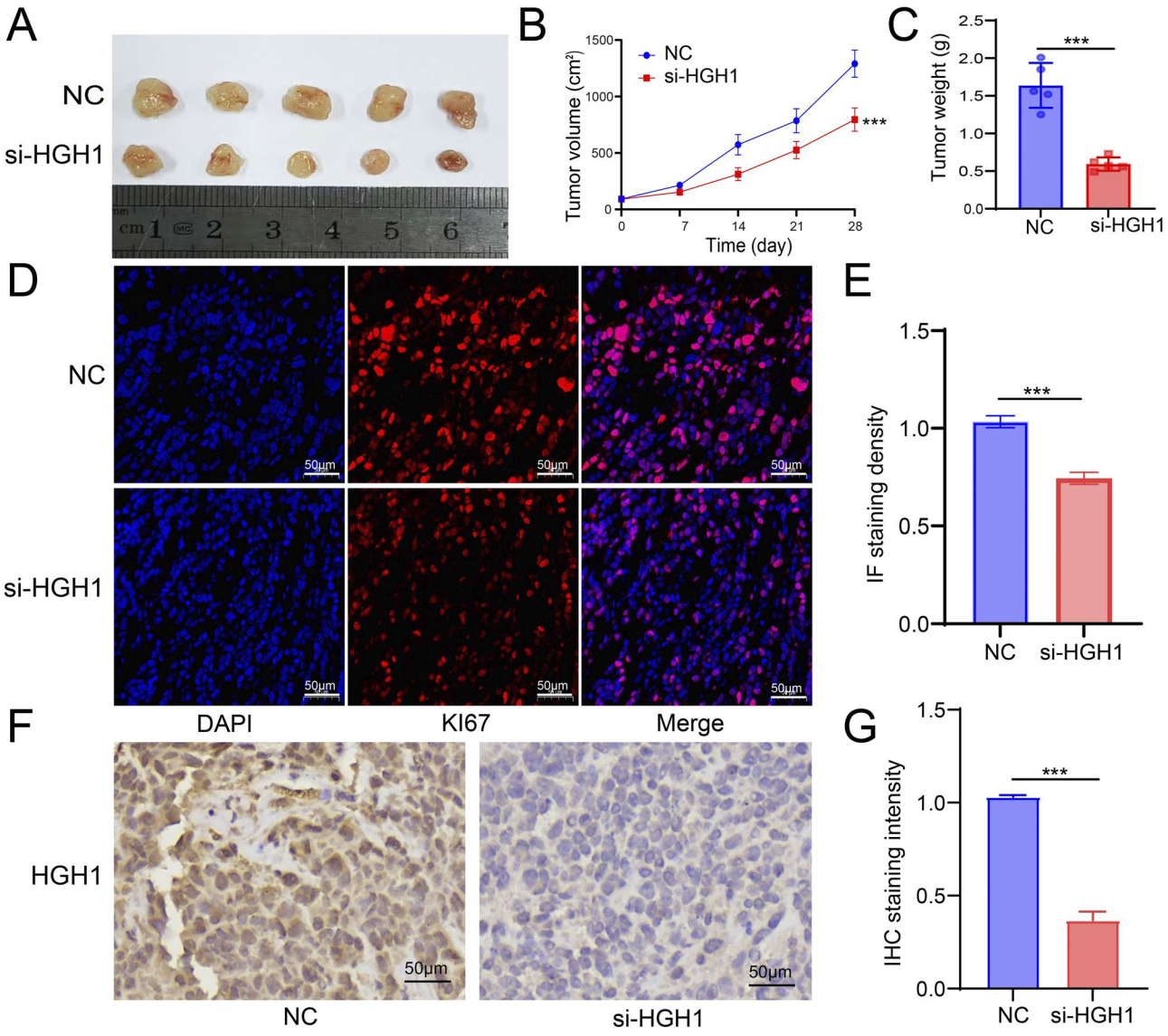


Fig. 8 Effects of HGH1 on the growth of MCF-7 cell xenograft in BRCA. (**A**) Images of MCF-7 xenografts. (**B**) Impact of HGH1 silencing on MCF-7 cell xenograft volume. (**C**) Effect of HGH1 silencing on the weight of MCF-7 xenografts, with quantitative analysis. (**D**) Immunofluorescence detection changes in Ki-67 expression. (**E**) IHC assay for detecting HGH1 expression. All data are derived from a minimum of three independent experiments and are expressed as the mean ± SD.**Indicates $P < 0.01$

strategy for controlling metastatic cancer [24]. Herein, the prognostic value of HGH1 in BRCA patients was analyzed. Univariate and multivariate analysis revealed that HGH1 is an independent prognostic marker. A prognostic model incorporating HGH1, T stage, N stage, M stage and Histological stage was developed to predict the clinical treatment efficacy of BRCA. Kaplan-Meier survival curve analysis showed that that BRCA patients with high HGH1 expression levels had a significantly lower overall survival (OS) probability compared to those with low HGH1 expression. Additionally, the expression of HGH1 was negatively associated with OS across various BRCA anatomical subtypes of. Further exploration of potential marker genes in BRCA is necessary to determine their clinical significance in treatment and drug development [25]. T This study screened differentially expressed genes (DEGs) associated with HGH1 and performed functional enrichment analysis on these genes, revealing that HGH1 may be involved in cell proliferation and the cell cycle. Targeting cell cycle-related proteins, such as MYC [26] and CCNB1 [27], could offer a solution to current issues with BRCA drug resistance [28].

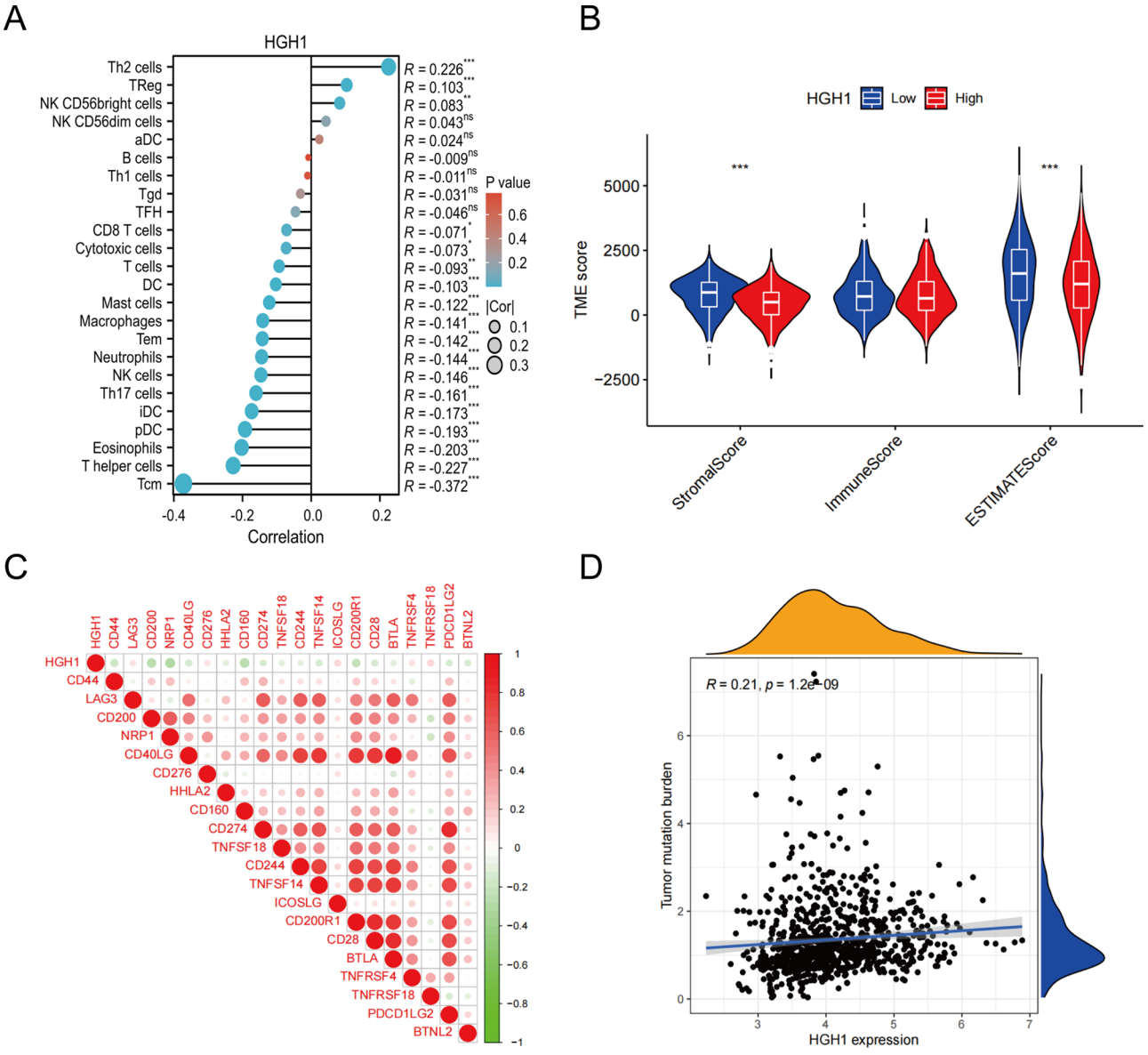
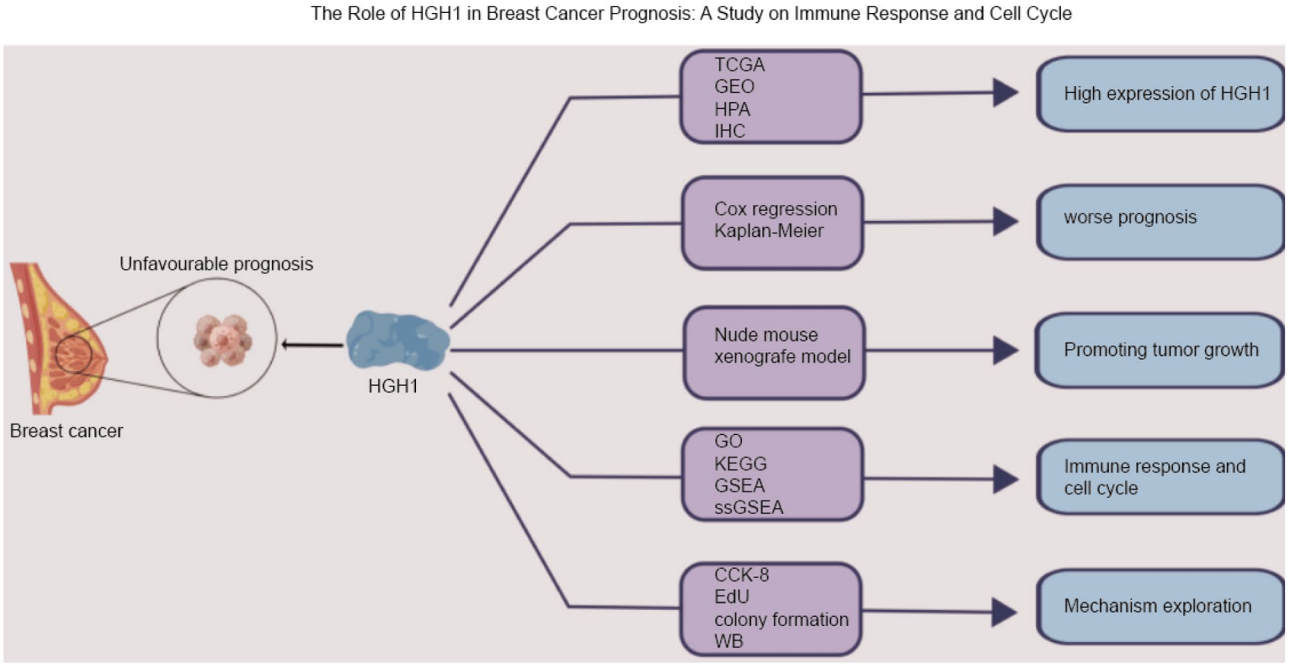


Fig. 9 *Correlation analysis of immune response.* (**A**) Correlation between HGH1 expression and infiltration by 24 immune cell types. (**B**) Analysis of StromalScore, ImmuneScore and ESTIMATScore. (**C**) Correlations between HGH1 expression and the key immune checkpoint genes. (**D**) Correlation between tumor mutation burden correlation with HGH1 expression

Immunotherapy is currently a powerful approach in cancer treatment [29]. Studies have found that Gelsolin (GSN) has significant diagnostic value in breast cancer and is involved in immune-related pathways, making it a potential predictive, diagnostic, and immune marker in pan-cancer [30]. Additionally, TIGIT has been identified as a promising target for immunotherapy in invasive breast cancer, providing a solid foundation for new treatment strategies [31]. In this study, the relationship between the expression level of HGH1 and tumor immune cell infiltration was analyzed. Results indicated that HGH1 expression is positively correlated with Th2 cell infiltration and negatively correlated with Tcm cell infiltration. Additionally, HGH1 expression is closely associated with the tumor microenvironment (TME) score, immune checkpoint genes, and tumor mutation burden. Therefore, from an immunotherapy perspective, HGH1 may become an effective target for BRCA immunotherapy. However, this study has certain imitations, It relied on open-access databases, which may introduce biases in the BRCA data. Moreover, the study focused solely on gene expression data without considering other



Scheme 1 The role of HGH1 in breast cancer prognosis and immune response

factors that could influence tumor occurrence and development. To enhance our understanding of tumors, future research should incorporate more clinical and epidemiological data. Rigorous experiments are also needed to explore how HGH1 affects the tumor immune response. Despite these limitations, this study is the first to investigate the relationship between HGH1 and BRCA and its prognostic effects, laying the foundation for further research. Targeting HGH1 could potentially improve BRCA treatment and survival rates.

Abbreviations

BRCA	Breast cancer
TCGA	The Cancer Genome Atlas
OS	Overall survival
GSEA	Gene Set Enrichment Analysis
TPM	Transcripts per million
ROC	Receiver operating characteristic

Supplementary Information

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Supplementary Material 1

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Author contributions

LHL organized the manuscript and all the bioinformatics analyses. XR contributed to the statistical analysis and completed in vivo and in vitro experiments. DW designed the figures and provided many ideas. XY provides important data and corrections to the article. All authors reviewed and accepted the paper for publication.

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Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

The animal experiments in the study was approved by the Ethics Committee of the Central South University. The patients/participants provided their written informed consent to participate in this study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Katayama A, Aoki Y, Watanabe Y, Horiguchi J, Rakha EA, Oyama T. Current status and prospects of artificial intelligence in breast cancer pathology: convolutional neural networks to prospective Vision transformers. Int J Clin Oncol, (2024).
2. Veronesi U, Boyle P, Goldhirsch A, Orecchia R, Viale G. Breast cancer. Lancet (London England). 2005;365:1727–41.
3. Khan MM, Yalamarty SSK, Rajmalani BA, Filipczak N, Torchilin VP. Recent strategies to overcome breast cancer resistance. Crit Rev Oncol/Hematol. 2024;197:104351.

4. Mönkemeyer L, Klaips CL, Balchin D, Körner R, Hartl FU, Bracher A. Chaperone function of Hgh1 in the Biogenesis of Eukaryotic Elongation Factor 2, molecular cell, 74 (2019) 88–e100109.
5. Zhang C, Yu JEE. LncRNA CASC21 induces HGH1 to mediate colorectal cancer cell proliferation, migration, EMT and stemness. RNA Biol. 2021;18:369–81.
6. Wu G, Dong Y, Hu Q, Ma H, Xu Q, Xu K, Chen H, Yang Z, He M. HGH1 and the immune landscape: a novel prognostic marker for immune-desert tumor microenvironment identification and immunotherapy outcome prediction in human cancers, cell cycle (Georgetown, Tex.), 22 (2023) 1969–85.
7. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge, contemporary oncology. (Poznan Poland). 2015;19:A68–77.
8. Wang M, Liu H, Wu W, Zhao J, Song G, Chen X, Wang R, Shao C, Li J, Wang H, Wang Q, Feng X. Identification of differentially expressed plasma lncRNAs as potential biomarkers for breast Cancer. Clin Breast Cancer. 2022;22:e135–41.
9. Thul PJ, Lindskog C. The human protein atlas: a spatial map of the human proteome. Protein Science: Publication Protein Soc. 2018;27:233–44.
10. Guo Z, Zhang X, Zhu H, Zhong N, Luo X, Zhang Y, Tu F, Zhong J, Wang X, He J, Huang L. TELO2 induced progression of colorectal cancer by binding with RICTOR through mTORC2. Oncol Rep. 2021;45:523–34.
11. Tan WCC, Nerurkar SN, Cai HY, Ng HHM, Wu D, Wee YTF, Lim JCT, Yeong J, Lim TKH. Overview of multiplex immunohistochemistry/immunofluorescence techniques in the era of cancer immunotherapy. Cancer Commun (London England). 2020;40:135–53.
12. Jiang B, Wu S, Zeng L, Tang Y, Luo L, Ouyang L, Feng W, Tan Y, Li Y. Impact of NDUFAF6 on breast cancer prognosis: linking mitochondrial regulation to immune response and PD-L1 expression. Cancer Cell Int. 2024;24:99.
13. Shao J, Fu Q, Wang M. Multivariate and Univariate Prognostic Analysis of postoperative patients with gastric Cancer. Am Surg. 2019;85:e201–3.
14. Franz M, Rodriguez H, Lopes C, Zuberi K, Montojo J, Bader G.D., Morris Q. GeneMANIA update 2018. Nucleic Acids Res. 2018;46:W60–4.
15. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular signatures database (MSigDB) hallmark gene set collection. Cell Syst. 2015;1:417–25.
16. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods. 2015;12:453–7.
17. Yao Y, Liu C, Wang B, Guan X, Fang L, Zhan F, Sun H, Li H, Lou C, Yan F, Lu X, Cui L, Liao Y, Han S, Yao Y, Zhang Y. HOXB9 blocks cell cycle progression to inhibit pancreatic cancer cell proliferation through the DNMT1/RBL2/c-Myc axis. Cancer Lett. 2022;533:215595.
18. Xiao X, Rui B, Rui H, Ju M, Hongtao L. MEOX1 suppresses the progression of lung cancer cells by inhibiting the cell-cycle checkpoint gene CCNB1. Environ Toxicol. 2022;37:504–13.
19. Barillé-Nion S, Bah N, Véquaud E, Juin P. Regulation of cancer cell survival by BCL2 family members upon prolonged mitotic arrest: opportunities for anticancer therapy. Anticancer Res. 2012;32:4225–33.
20. Hsu R, Al-Zubeidy B, Flores D, Nazarian A, Baugh A, Gonzalez E, Castanon S, Xiu J, Kang I, Spicer D, Lenz HJ, Dara L, Ademuyiwa FO, Korn WM, Irshad S, Chan IS. E.T. Roussos Torres, evaluation of markers of immunity in different metastatic immune microenvironments suggests more suppression within breast to liver metastases in breast cancer. Breast cancer research and treatment; 2024.
21. Luo Z, Wang Y, Bi X, Ismtula D, Wang H, Guo C. Cytokine-induced apoptosis inhibitor 1: a comprehensive analysis of potential diagnostic, prognosis, and immune biomarkers in invasive breast cancer. Translational cancer Res. 2023;12:1765–86.
22. Chen H, Gui X, Zhou Z, Su F, Gong C, Li S, Wu W, Rao N, Liu Q, Yao H. Distinct ER and PR expression patterns significantly affect the clinical outcomes of early HER2-positive breast cancer: a real-world analysis of 871 patients treated with neoadjuvant therapy. Breast (Edinburgh Scotland). 2024;75:103733.
23. Wang Y, Li Y, Jing Y, Yang Y, Wang H, Ismtula D, Guo C. Tubulin alpha-1b chain was identified as a prognosis and immune biomarker in pan-cancer combing with experimental validation in breast cancer. Sci Rep. 2024;14:8201.
24. Ji X, Tian X, Feng S, Zhang L, Wang J, Guo R, Zhu Y, Yu X, Zhang Y, Du H, Zablotskii V, Zhang X. Intermittent F-actin perturbations by magnetic fields inhibit breast Cancer metastasis. Res (Washington D C). 2023;6:0080.
25. Karampuri A, Kundur S, Perugu S. Exploratory drug discovery in breast cancer patients: a multimodal deep learning approach to identify novel drug candidates targeting RTK signaling. Comput Biol Med. 2024;174:108433.
26. Dang CV. MYC on the path to cancer. Cell. 2012;149:22–35.
27. Chen EB, Qin X, Peng K, Li Q, Tang C, Wei YC, Yu S, Gan L, Liu TS. HnRNPR-CCNB1/CENPF axis contributes to gastric cancer proliferation and metastasis. Volume 11. Aging; 2019. pp. 7473–91.
28. Li CX, Wang JS, Wang WN, Xu DK, Zhou YT, Sun FZ, Li YQ, Guo FZ, Ma JL, Zhang XY, Chang MJ, Xu BH, Ma F, Qian HL. Expression dynamics of periodic transcripts during cancer cell cycle progression and their correlation with anticancer drug sensitivity. Military Med Res. 2022;9:71.
29. Luke JJ, Davar D, Andtbacka RH, Bhardwaj N, Brody JD, Chesney J, Coffin R, de Baere T, de Gruijl TD, Fury M, Goldmacher G, Harrington KJ, Kaufman H, Kelly CM, Khilnani AD, Liu K, Loi S, Long GV, Melero I, Middleton M, Neyns B, Pinato DJ, Sheth RA, Solomon SB, Szapary P, Marabelle A. Society for Immunotherapy of Cancer (SITC) recommendations on intratumoral immunotherapy clinical trials (IICT): from premalignant to metastatic disease. J Immunother Cancer, 12 (2024).
30. Wang Y, Bi X, Luo Z, Wang H, Ismtula D, Guo C. Gelsolin: a comprehensive pan-cancer analysis of potential prognosis, diagnostic, and immune biomarkers. Front Genet. 2023;14:1093163.
31. Guo C, Luo Z, Ismtula D, Bi X, Kong H, Wang Y, Yang Z, Mao X. TIGIT as a novel prognostic marker for Immune infiltration in invasive breast Cancer. Comb Chem High Throughput Screen. 2023;26:639–51.

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