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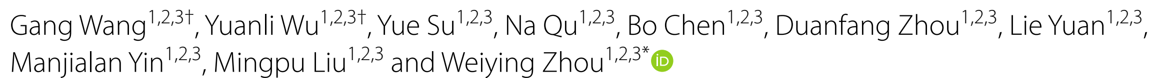

RESEARCH **Open Access**

TCF12-regulated GRB7 facilitates the HER2+ breast cancer progression by activating Notch1 signaling pathway

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Abstract

Background Human epidermal growth factor receptor 2-positive (HER2+) breast cancer (BC), which accounts for approximately one-fifth of all BCs, are highly invasive with a high rate of recurrence and a poor prognosis. Several studies have shown that growth factor receptor-bound protein 7 (GRB7) might be a potential therapeutic target for tumor diagnosis and prognosis. Nevertheless, the role of GRB7 in HER2+ BC and its underlying mechanisms have not been fully elucidated. The aim of this study was to investigate the biological function and regulatory mechanism of GRB7 in HER2+ BC.

Methods Bioinformatics analysis was performed using the TCGA, GEO and CancerSEA databases to evaluate the clinical significance of GRB7. RT quantitative PCR, western blot and immunofluorescence were conducted to assess the expression of GRB7 in BC cell lines and tissues. MTT, EdU, colony formation, wound healing, transwell, and xenograft assays were adopted to explore the biological function of GRB7 in HER2+ BC. RNA sequencing was performed to analyze the signaling pathways associated with GRB7 in SK-BR-3 cells after the cells were transfected with GRB7 siRNA. Chromatin immunoprecipitation analysis (ChIP) and luciferase reporter assay were employed to elucidate the potential molecular regulatory mechanisms of GRB7 in HER2+ BC.

Results GRB7 was markedly upregulated and associated with poor prognosis in BC, especially in HER2+ BC. Overexpression of GRB7 increased the proliferation, migration, invasion, and colony formation of HER2+ BC cells, while depletion of GRB7 had the opposite effects in HER2+ BC cells and inhibited xenograft growth. ChIP-PCR and luciferase reporter assay revealed that TCF12 directly bound to the promoter of the GRB7 gene to promote its transcription. GRB7 facilitated HER2+ BC epithelial-mesenchymal transition (EMT) progression by interacting with Notch1 to activate Wnt/β-catenin pathways and other signaling (i.e., AKT, ERK). Moreover, forced GRB7 overexpression activated Wnt/β-catenin to promote EMT progression, and partially rescued the inhibition of HER2+ BC proliferation, migration and invasion induced by TCF12 silencing.

Conclusions Our work elucidates the oncogenic role of GRB7 in HER2+ BC, which could serve as a prognostic indicator and promising therapeutic target.

Keywords HER2+, Breast cancer, GRB7, TCF12, EMT

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Introduction

Breast cancer (BC) has become the most common malignant tumor worldwide due to the large number of new cases and high mortality rate [1]. BC is mainly divided into four subtypes by characterization of gene mutations and protein expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2): luminal A, luminal B, HER2-positive (HER2+) and triple-negative breast cancer (TNBC) [2]. HER2+ BC accounts for approximately 15% to 20% of all BCs and is characterized by poor differentiation, high aggressiveness and poor prognosis. Current treatment for HER2+ BC is mainly based on chemotherapeutic agents and targeted therapy [3]. However, more than 30% of patients with recurrent metastatic HER2+ BC still present with brain, lung, or bone metastases after early initial treatment [4]. Therefore, exploring the molecular targets and mechanisms associated with metastatic progression could contribute to more comprehensive development of HER2+ BC-targeted therapies and better clinical outcomes.

Growth factor receptor-bound protein 7 (GRB7) is a member of the GRB family, which is an important family of signal transduction proteins involved in cell proliferation, differentiation, survival and migration [5]. GRB7 comprises 554 amino acids and contains an N-terminal Src homology structural domain (SH2) and a C-terminal Src homology structural domain (SH3) [6]. These two structural domains enable GRB7 to interact with a diverse range of signal transduction proteins, thereby regulating cellular signaling pathways. It is known that GRB7 overexpression is associated with the development and drug resistance of several tumors, including gastric, ovarian, cervical and BC, and significantly affects prognosis [7–9]. Mechanistically, GRB7 regulates biological processes such as cell proliferation, migration and transformation by participating in signaling pathways containing EGFR, HER2, and PI3K/AKT in tumor cells [10–12]. It is suggested that GRB7 might be a potential therapeutic target for the diagnosis and prognosis of tumors. However, the malignant process and mechanism of action of GRB7 in HER2+ BC are still unclear.

The precise regulation of gene expression is closely related to the development of organisms and the maintenance of various physiological functions of cells, whereas disorders of gene expression affect many physiological and pathological processes [13, 14]. Transcription factors (TFs), accounting for approximately 8% of all human genes, are proteins that recognize and bind DNA in a sequence-specific manner and regulate transcription to form complex systems that direct genome expression [15]. Transcription factor 12 (TCF12) is the basic helix-loop-helix (bHLH) E-protein family member, which can form homodimers or heterodimers with other family members to regulate cellular development and differentiation in a wide variety of tissues, such as skeletal muscle, neurons, mesenchymal tissues and lymphocytes [16, 17]. Recently, TCF12 has been shown to function as an oncogene in tumor progression. For example, TCF12 promoted tumorigenesis and metastasis in hepatocellular carcinoma (HCC) through upregulation of C-X-C chemokine receptor type 4 (CXCR4) expression [18]. Moreover, TCF12 regulates RNA-binding protein DExH-box helicase 9 (DHX9) signaling to promote glioma growth and tumor-associated macrophage (ATM) infiltration [19, 20]. In addition, TCF12 significantly affects chemoresistance in BC through epithelial-mesenchymal transition (EMT) progression or autophagy injury [21]. However, the action of TCF12 in HER2+ BC and its molecular mechanisms have not been reported until now.

Here, we identified that GRB7 expression is upregulated in HER2+ BC and is inversely associated with the prognosis of the patients. We further revealed that GRB7 is transcriptionally upregulated by TCF12, and promotes the proliferation, migration and invasion of HER2+ BC cells via activating Notch signaling. Our study suggests that GRB7 is a promising target for the development of alternative therapeutic strategies for the treatment of HER2+ BC patients.

Material and methods

Bioinformatics data analysis

GRB7 expression in different cancers and BC subtypes was analyzed by UALCAN (https://ualcan.path.uab.edu/) and TIMER (https://cistrome.shinyapps.io/timer/). We downloaded GRB7 expression data for human HER2+ BC and non-HER2+ BC or normal tissue samples from the Gene Expression Omnibus database (GEO, https://www.ncbi.nlm.nih.gov/geo/) and The Cancer Genome Atlas (TCGA, https://cancergeno.me.nih.gov/). BC patient prognosis was analyzed by Kaplan–Meier Plotter (https://kmplot.com/analysis/). CancerSEA was used to analyze the biological function of GRB7. The cistrome was used to predict potential transcription factors. TIMER was used to analyze the correlation between genes.

Cell lines and culture

All human BC cell lines including ER+ BC cell lines MCF7 and T47D, HER2+ BC cell lines SK-BR-3, MDA-MB-453, and TNBC cell lines MDA-MB-231 and MDA-MB-468 and the immortalized mammary epithelial cell line MCF10A were purchased from the American Type Culture Collection (ATCC) (Manassas, VA, USA). These cell lines were authenticated by short tandem repeat (STR) profiling and tested for mycoplasma contamination. Cells were cultured in DMEM, RPMI-1640, L15 or

Ham's F-12 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin (Procell, Wuhan, China), and streptomycin at 37 °C in a 5% CO_2 humidified atmosphere.

RNA and plasmid transfection

For transient transfection, the indicated small interfering RNAs (siRNAs) (100 pM) or plasmid DNA (5 μg) were introduced into cells in the presence of Lipo8000 (Beyotime, Shanghai, China) and Opti-MEM (Gibco, USA) for 48 h according to the manufacturer's instructions. Plasmids for GRB7 and TCF12 were from Sino Biological Co., Ltd. (Beijing, China). The siRNAs for GRB7 and TCF12 were obtained from Tsingke Co., Ltd. (Beijing, China). The following GRB7 and TCF12 siRNAs were used: siGRB7#1, sense, 5′- CAGAUGUGAACGAGUCCAATT-3′, antisense, 5′- UUGGACUCGUUCACAUCUGTT-3′; siGRB7#2, sense, 5′- GGGUGCAGCUGUACAAGAATT-3′, antisense, 5′- UUCUUGUACAGCUGCACCCTT-3′; siGRB7#3, sense, 5′- GGUUCAGGACGGAAGCUUUTT-3′, antisense, 5′- AAAGCUUCCGUCCUGAACCTT-3′; siTCF12#1, sense, 5′- CAUUCAGUCCUGUCUAGUATT-3′, antisense, 5′- UACUAGGACUGAAUGTT-3′; siTCF12#2, sense, 5′- CCGUGAAUCUCCUAGUUAUTT-3′, antisense, 5′- AUAACUAGGAGAUUCACGGTT-3′. Unless otherwise stated, a cocktail of GRB7 siRNA and TCF12 siRNA was used separately in each of the following experiments.

RT–qPCR

Total RNA was extracted from cells using TRIzol reagent (Invitrogen, Waltham, United States) and subjected to reverse transcription in the presence of PrimeScript RT Master Mix (Takara, Japan). Quantitative PCR was performed using SYBR Green reagent, and β-Actin was used as the internal control. The sequences of primers used are shown in Table 1.

Western blot analysis and immunoprecipitation

The cells were harvested and lysed in RIPA lysis buffer containing protease inhibitor cocktail (Beyotime, Shanghai China). Protein samples (30 μg) were separated by 8%–12% SDS–PAGE and transferred to PVDF membranes for immunoblotting overnight at 4 °C with primary anti-GRB7 (1:1000, Proteintech, 10045-1-Ig), N-Cadherin (1:3000, Proteintech, 22018-1-AP), E-Cadherin (1:10,000, Proteintech, 20874-1-AP), Vimentin (1:5000, Proteintech, 10366-1-AP), Slug (1:1000, Proteintech, 12129-1-AP), Snail (1:500, Proteintech, 13099-1-AP), β-Actin (1:5000, Santa, sc-56459), and anti-TCF12 (#78,), Notch1 (#3608), Jagged1 (#70109), β-catenin (#8480), MMP2 (#40994), p-AKT (#4060), AKT (#4691), ERK1/2 (#9102) antibodies (all from Cell Signaling Technology) with 1:1000 dilution, followed by incubation with secondary antibody (1:5000, ZSBio, ZB-2301 and ZB-5305) for 2 h at room temperature. Protein bands were visualized with a chemiluminescence imaging system (Tanon, Shanghai, China) using ECL substrate. For immunoprecipitation, cell lysates were incubated with S-protein agarose or primary antibodies plus protein A/G agarose (Beyotime, Shanghai, China) and were then analyzed by western blot.

Table 1 Sequences of the primers used in this study

Primer	Direction	Sequences
GRB7	Forward	5′-CGGGACACCCTCCTACC-3′
	Reverse	5′-TGGCGTCTGAGCAAGAGA-3′
E-Cadherin	Forward	5′-GGGGTTAAGCACAACAGCAA-3′
	Reverse	5′-CAAAATCCAAGCCCGTGGTG-3′
N-Cadherin	Forward	5′-ATGGGAAATGGAAACTTGATGGC-3′
	Reverse	5′-CAGTTGCTAAACTTCACTGAAAGG-3′
MMP2	Forward	5′-ATGTTAGGCAAGTGACTTCTCAGT-3′
	Reverse	5′-AGGTGTTCAGGTATTGCATGT-3′
Slug	Forward	5′-CGAACTGGACACACATACAGTGAT-3′
	Reverse	5′-CACACAGTGATGGGGCTGTA-3′
TCF12	Forward	5′-CCAGCAGTTCACCTTACGTTGC-3′
	Reverse	5′-GCCTTTCCAAGTGCATCACCTG-3′
β-Actin	Forward	5′-CCTGGCACCCAGCACAAT-3′
	Reverse	5′-GGGCCGGACTCGTCATAC-3′

GRB7 Growth factor receptor-bound protein 7, *MMP2* Matrix metalloproteinase 2, *TCF12* Transcription factor 12

MTT and EdU assays

3-(4,5)-Dimethylthiahiazo (-z-y1)-3,5-di-phenytetrazoliumromide (MTT) and 5-ethynyl-2′-deoxyuridine (EdU) assays (Beyotime, Shanghai, China) were used to detect cell proliferation. For MTT assay, 2000–3000 cells were inoculated in 96-well plates, and 10 μL MTT solution was added at 24, 48, 72, 96, and 120 h after treatments as indicated. The absorbance was measured at 570 nm by a multiscan spectrophotometer. For EdU assay, 48 h after transfection, SK-BR-3 and MDA-MB-453 cells were incubated in the manufacturer's recommended final concentration of 10 μM EdU solution for 2 h. Next, the cells were reacted with a fluorescent dye mixture, and images were acquired by fluorescence microscopy. Cell colony formation ability was assessed as described previously [22].

Wound healing assay

For the wound healing assay, after cells were inoculated in 6-well plates and grown to 90% confluence, the cell layer was scraped with a 20 μL pipette tip, and a wound was formed. Images were obtained by electron microscopy

at the indicated time intervals, and wound closure rates were assessed using ImageJ software.

Migration and invasion assays

Cell migration and invasive activity were assessed by transwell plates with or without extracellular interstitial gel coating, respectively. Briefly, the cells were designated treated and placed in FBS-free medium in the upper chamber, and fresh medium containing 10% FBS as a chemoattractant was placed in the lower chamber. The cells were incubated under the corresponding culture conditions for 48 h, fixed with 4% paraformaldehyde, and stained with 0.1% crystal violet. The membranes were further washed and scored for cell number after drying.

ChIP assay

ChIP analysis was performed using a chromatin immunoprecipitation kit (Cell Signaling Technology, USA) according to the manufacturer's instructions as previously reported [23]. Briefly, SK-BR-3 cells were soaked in 1% formaldehyde for 15 min and then incubated in glycine for 5 min; cells that had been washed in PBS and incubated with protease inhibitors were collected and sonicated to produce genomic DNA fragments of approximately 200 to 500 bp in length. Chromatin extracts were immunoprecipitated using an anti-TCF12 antibody (1:50, Cell Signaling Technology, #78245) at 4 °C. IgG was used as a negative control.

Luciferase reporter assay

Wild-type and mutant promoter binding region sequences were inserted into the luciferase system. In 96-well plates, the indicated cells were transfected using Lipo8000 with reporter vectors with knockdown or overexpression of GRB7 and TCF12 for 48 h. The luciferase reporter gene assay was performed using the Dual Luciferase Reporter Assay System (Promega Corporation, Madison, WI, USA).

Animal experiments and treatments

Female NOD/scid-IL-2Rgamma(c)(null) (NSG) mice aged 4–5 weeks were obtained from Shanghai Model Organisms Center, Lnc (Shanghai, China) and maintained in a well-ventilated animal transit room with a 12 h light/dark cycle, relative humidity of 60 ± 10%, and a controlled temperature of 22 °C. An orthotopic xenograft model was established in NSG mice by implanting MDA-MB-453 cells (5×10^7 cells/60 μL) mixed with 20 μL Matrigel into the mammary fat pad. Ten days later, the size of the xenograft tumor was measured every 2 days with a caliper. Tumor volume was calculated using the following formula: $\text{length}\times\text{width}^2/2$. After the xenograft tumors grew to an appropriate volume, the nude mice were randomly divided into groups. As previously described [24], in vivo processing with siRNA in a mouse model was used to explore effects of GRB7 knockdown in vivo. Specifically, cholesterol-modified siRNA targeting GRB7 or negative control (Tsingke, 5 nmol/kg) was dissolved in 25 μL sterile physiological saline, mixed with 5 μL in vivo transfection reagent (Engreen, Beijing, China) and then injected into the tumor every 2 days for 2–3 weeks. For in vivo rescue assays, a cholesterol-modified siRNA targeting TCF12 combined with lentivirus containing GRB7 or pCDH was injected into tumor-bearing NSG mice. The lentivirus containing GRB7 with a virus titer of 108/UI was injected into the tumor mass once every 5 days for 3 weeks. Animals were sacrificed with 3% sodium pentobarbital at an appropriate time, and then the orthotopic grafts were exfoliated for follow-up examination. Protocols for animal use were reviewed and approved by the Animal Care and Use Committee of Chongqing Medical University in accordance with Institutional Animal Care and Use Committee guidelines.

Immunofluorescence (IF) and immunohistochemical (IHC) staining

A tissue microarray containing 159 BC pathological samples and normal breast tissue samples was provided by Shanghai Outdo Biotech Co., LTD. Immunofluorescence analysis was performed as described previously [25]. For IHC staining, all xenograft tumor specimens were immersed in formalin. Before staining, tissues were cut to 5 μm thickness and placed on slides. Sections were deparaffinized in xylene and graded alcohols and boiled in sodium citrate buffer (pH 6.0) for antigen recovery, followed by blocking endogenous peroxidase activity with 3% hydrogen peroxide. Treated sections were incubated with anti-GRB7 (1:200, Proteintech, 10045-1-Ig), anti-N-cadherin (1:5000, Proteintech, 22018-1-AP), anti-E-cadherin (1:5000, Proteintech, 20874-1-AP), anti-vimentin (1:5000, Proteintech, 10366-1-AP), anti-slug (1:500, Proteintech, 12129-1-AP), and anti-Ki67 (1:5000, Proteintech, 27309-1-AP) antibodies at 4 °C overnight. Next, the sections were incubated with biotinylated goat anti-rabbit IgG secondary antibody for 30 min at room temperature and visualized with a 3,5-diaminobenzidine (DAB) substrate kit, and microscopy images were produced by light microscopy.

Statistical analysis

All experiments were conducted in triplicate. All of the data were analyzed by GraphPad Prism v.9.0 and are presented as the mean ± standard deviation (SD). Differences between the indicated groups were calculated using Student's t test or ANOVA. $p < 0.05$ was considered statistically significant.

Results

GRB7 expression is upregulated and inversely associated with the prognosis in BC, especially in HER2+ BC

We first compared mRNA levels of GRB7 between different types of tumors and their corresponding normal tissues using the TIMER2 database. The results showed that GRB7 expression was significantly upregulated in some tumors, including BC (Figure S1A). Based on the TCGA database, our analysis revealed that the expression level of GRB7 was significantly higher in BC than in normal breast tissue (Fig. 1A and Figure S1B). Moreover, the results from 33 BC cases paired with normal tissues showed that GRB7 expression was also significantly upregulated in BC tissues compared with the paired normal tissues (Fig. 1B). We further evaluated the association of GRB7 expression with clinicopathologic features and found that GRB7 expression was higher in stage II and III patients, as well as in patients with BC aged 21–80 years (Figure S1C-D). Furthermore, BC patients with high GRB7 expression showed a worse prognosis (Fig. 1C). To comprehend the potential role of GRB7 in the chemotherapy response in BC patients, we retrieved the trend of GRB7 by ROC Plotter. The results showed that the expression of GRB7 was significantly upregulated in non-responders compared with responders to chemotherapy, with a 5-year predictive AUC of 0.562 (Figure S1E-F). These results suggested that GRB7 is closely associated with BC, and the chemotherapy resistance.

BC is a highly heterogeneous disease in terms of histological, epidemiological and molecular characteristics. Therefore, we analyzed GRB7 expression levels according to the molecular type of BC. The results showed that both mRNA and protein levels of GRB7 were significantly increased in HER2+ BC tissues compared with normal tissues, luminal A/B BC and TNBC subtypes (Fig. 1D-E). Notably, GRB7 was among the top 25 overexpressed genes in HER2+ BC (Fig. 1F). Then, we explored HER2+ highly expressed genes using data published in GEO, which contained GRB7 (Fig. 1G). The high expression level of GRB7 was confirmed by analyzing 9 other microarray datasets (Fig. 1H and Figure S2), all of which confirmed the high expression level of GRB7. Meanwhile, immunofluorescence revealed higher levels of GRB7 protein expression in HER2+ BC than in normal human breast tissue (Fig. 1I). Kaplan–Meier survival curves showed that high GRB7 expression was associated with significant reductions in overall survival (OS) and disease-free survival (DFS) (Fig. 1J). Next, we detected the expression of GRB7 in different BC cell lines and normal human BC cell lines by RT–qPCR and western blot. Compared with other cell lines, GRB7 mRNA and protein expression levels were higher in HER2+ cell line SK-BR3 and MDA-MB-453 cells than other types of BC cell lines (Fig. 1K).

GRB7 is critical for the viability of HER2+ BC cells

To explore the GRB7 biological function in HER2+ BC, we first analyzed the genes co-expressed with GRB7 by KEGG pathway enrichment, and found that these co-expressed genes were widely enriched in signaling for cell growth and adhesion (Figure S3A). In addition, we found that GRB7 is associated with the cell cycle, EMT, and stemness in BC by single-cell databases (Figure S3B), as confirmed by several BC datasets (Figure S3C). Further analysis by GSEA showed that GRB7 expression was significantly correlated with the cell cycle and apoptosis (Figure S3D). We evaluated the in vitro and in vivo effects of GRB7 knockdown or overexpression in HER2+ BC cells. To this end, GRB7 siRNA or negative control (NC) siRNA, or GRB7 plasmids or vector control were transfected into SK-BR-3 and MDA-MB-453 cells, and the transfection efficiency was validated by RT–qPCR and western blot analysis (Fig. 2A, B). MTT assays showed that knockdown of GRB7 in SK-BR-3 and MDA-MB-453 cells significantly decreased cell viability, while GRB7 overexpression promoted cell proliferation (Fig. 2C). Knockdown of GRB7 resulted in a significant decrease in cell colony forming ability, while GRB7 overexpression enhanced colony formation (Fig. 2D). EdU assay further confirmed that depletion of GRB7 inhibited HER2+ BC cell proliferation, while overexpression of GRB7 promoted cell proliferation (Fig. 2E). Correspondingly, we constructed an orthotopic xenograft model of MDA-MB-453 cells to probe GRB7 effect in HER2+ BC. As shown in Fig. 2F-H, the tumor volume and weight were significantly lower in the mice with GRB7 knockdown than those in the control group of mice. Furthermore, by IHC staining, the number of Ki67-positive cells was significantly reduced in tumor tissue sections after knockdown of GRB7 in xenograft tumors (Fig. 2I). Consistent results were also obtained with western blot analysis (Fig. 2J). These results suggest that the GRB7 is critical for viability of HER2+ BC cells.

GRB7 potentiates the migration and invasion of HER2+ BC cells

We then investigated the role of GRB7 in regulation of the migration and invasion of HER2+ BC cells. The wound healing assay results showed that knockdown of GRB7 expression significantly suppressed the cell migration, while overexpression of GRB7 enhanced the cell migration (Fig. 3A, B). In transwell assays, the number of migrating and invading cells was reduced after knockdown of GRB7 in SK-BR-3 and MDA-MB-453, whereas

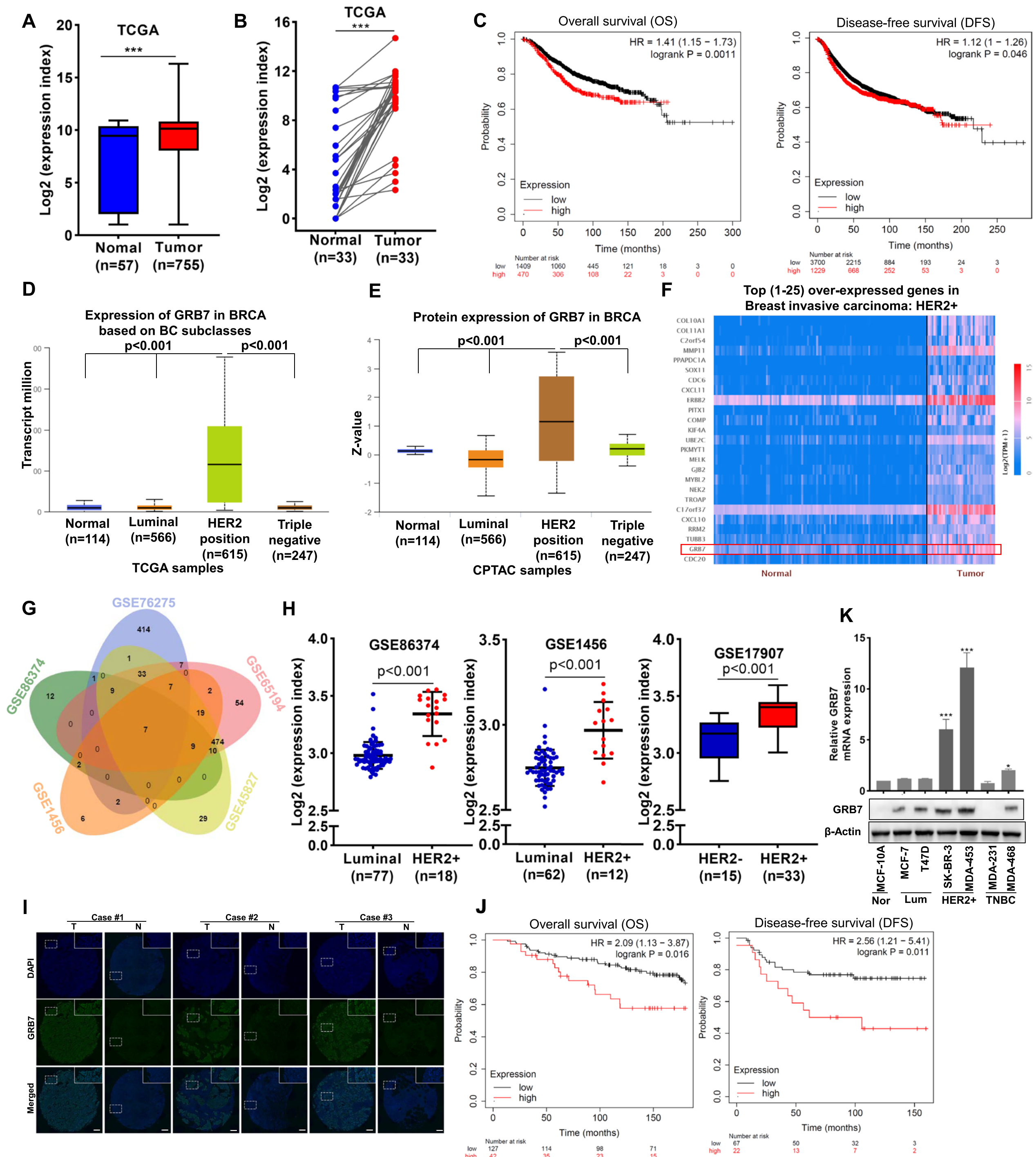


Fig. 1 GRB7 is overexpressed in HER2+ BC and associated with poor prognosis. **A** Expression of GRB7 in BC and normal breast tissue. **B** Expression of GRB7 in 33 pairs of BC tissues and normal adjacent tissues. **C** Kaplan–Meier analysis of OS and DFS based on GRB7 expression in BC from the online Kaplan–Meier plotter database. **D**, **E** GRB7 mRNA and protein expression in different subtypes of BC based on TCGA and CPTAC databases. **F** The top 25 differentially expressed genes between HER2+ BC and normal tissues were analyzed from the UALCAN database. **G** The Venn diagram shows the upregulated genes in HER2+ BC. **H** Expression of GRB7 in HER2+ samples from public microarray datasets (GSE86374, GSE1456 and GSE17907). **I** The results of immunofluorescence staining against GRB7 in clinical HER2+ BC samples. GRB7 protein is shown in green, and nuclei are shown in blue (stained with DAPI) (4× and 40×). **J** Kaplan–Meier OS and DFS curves stratified by GRB7 expression in 120 patients with HER2+ BC. **K** Protein and mRNA expression of GRB7 in BC cell lines and MCF-10A cells. The relative quantification was calculated by the $2^{-\Delta\Delta Ct}$ method and normalized based on β-actin. Nor, normal; Lum, luminal. Data are shown as the mean ± SD, N ≥ 3. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

the opposite was true for overexpression of GRB7 (Fig. 3C, D). These results indicate that GRB7 potentiates the migration and invasion of HER2+ BC cells.

GRB7 promotes EMT of HER2+ BC cells

To explore the molecular mechanisms by which GRB7 regulates HER2+ BC malignant progression, RNA-seq was used to probe potential targets and related signaling pathways of GRB7 (Figure S4A). There was a total of 862 differentially expressed genes (DEGs) in the GRB7 knockdown group compared with the NC group, including 285 upregulated genes ($P < 0.05$ and $FC > 1$) and 577 downregulated genes (Figure S4B). These DEGs were further subjected to signal enrichment by GO, KEGG and protein network analysis (Figure S4C-E). Notably, the Wnt pathway was highly correlated in KEGG analysis (Fig. 4A). The Wnt signaling pathway plays an important role in embryonic development, tumorigenesis and metastasis, confirming that Wnt signaling can directly or indirectly induce the phenomenon of EMT in the early stage of tumor metastasis [26, 27]. we first confirmed the correlation of GRB7 with EMT-related proteins such as N-cadherin (CDH2), MMP2 and Slug (SNAI2) in HER2+ BC (Fig. 4B). We further examined the expression of EMT-related markers by western blot and RT–qPCR after altering GRB7 expression. The results showed that depletion of GRB7 significantly decreased protein levels of N-cadherin, Vimentin, Snail, and Slug and increased E-cadherin expression levels in SK-BR-3 and MDA-MB-453 cells, while overexpression of GRB7 had the opposite effect on those proteins (Fig. 4C and E), suggesting that GRB7 regulates β-catenin/MMP2 signaling. The effects of knockdown or overexpression of GRB7 on mRNA levels of those molecules related to β-catenin/MMP2 signaling were verified by RT-qPCR, and the results were consistent with the alterations in protein levels (Fig. 4D and F). Furthermore, EMT-associated proteins were stained by IHC in orthotopic xenograft tumors of MDA-MB-453, and the results were consistent with those of western blot analysis (Fig. 4G). Together, these data suggest that GRB7 promotes EMT of HER2+ BC cells, thereby facilitating the migration and invasive capacity of tumor cells.

GRB7 promotes HER2+ BC via the Notch and other signaling

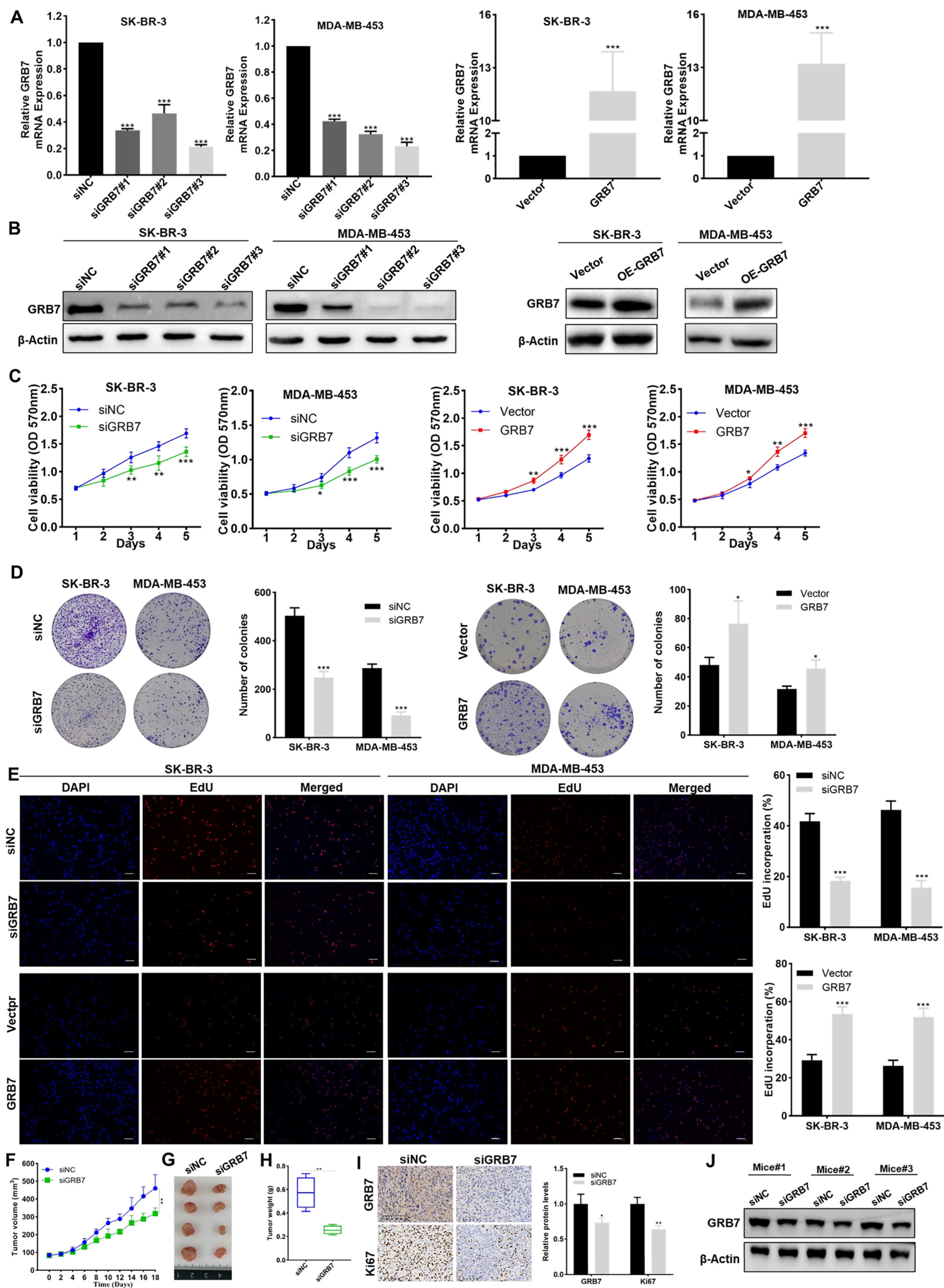
Notch signaling can lead to dysregulation and loss of control of cellular processes in proliferation, cell cycle inhibition, differentiation, and apoptosis, which results in transformation and malignancy, ultimately leading to the development of malignant tumors [28, 29]. Previous studies have shown that Notch signaling plays a crucial role in HER2+ BC [30, 31]. In the results of RNA-seq gene set enrichment analysis (GSEA) of SK-BR-3 cells with GRB7 knockdown, the altered genes were associated with gene sets of several signaling pathways (Figure S5A), especially the Notch signaling pathway (Fig. 5A). Notably, the expression level of GRB7 was strongly correlated with that of Notch1 (Fig. 5B). GRB7 and Notch1 were found to interact by immunoprecipitation (IP) assay (Fig. 5C). In addition, knockdown of GRB7 in SKBR-3 and MDA-MB-453 cells significantly downregulated the levels of Jagged1, p-AKT and ERK1/2 proteins (Fig. 5D). Conversely, overexpression of GRB7 increased the expression levels of these proteins (Fig. 5E). In addition, the expression levels of the related proteins in xenografts of MDA-MB-453 cells were verified (Fig. 5F), and the results were consistent with the in vitro results. To clarify the role of Notch1 in GRB7-induced HER2+ BC progression, we applied a selective inhibitor of Notch signaling, FLI-06 (TargetMol, T3075, MA, USA), which prevents the early secretion of Notch signaling. Consistent with the previous studies [32], FLI-06 downregulated Notch1 expression (Fig. 5G, H). As expected, FLI-06 significantly impaired the proliferation, migration and invasion induced by GRB7 overexpression in HER2+ BC cells (Fig. 5I–K).

TCF12 directly regulates GRB7 expression

TFs are involved in a great amount of human diseases, and dysregulation of TFs can lead to the development of cancer cells and tumor progression[33, 34]. We analyzed TFs from the Cistrome DB database that might regulate GRB7 expression (Fig. 6A). Based on the predicted results, the correlation between the top 10 TFs and GRB7 expression in HER2+ BC was further analyzed in the TIMER database (Figure S6). The results showed

(See figure on next page.)

Fig. 2 GRB7 facilitates HER2+ cell survival and proliferation in vitro and in vivo. **A** RT−qPCR was used to verify the efficiency of GRB7 knockdown and overexpression in SK-BR-3 and MDA-MB-453 cells. **B** western blot was adopted to validate the efficiency of GRB7 knockdown and overexpression in SK-BR-3 and MDA-MB-453 cells. The cell survival or proliferation of SK-BR-3 and MDA-MB-453 cells transfected with GRB7 siRNAs or GRB7 plasmid was detected by **C** MTT assay, **D** colony-forming ability, and **E** EdU assays. Data are shown as the mean ± SD, N ≥ 3. **F** siGRB7 was administered for 18 days, and tumor volume was measured with a sliding caliper every 2 days (n = 4). **G** Images of dissected orthotopic xenografted tumors (n = 4). **H** Tumor weights were measured after mouse sacrifice (n = 4). **I** Representative images of GRB7 and Ki67 expression evaluated by IHC in xenografted tumor tissues. **J** Western blot was used to detect the protein expression level of GRB7 in xenografts. Three different visual fields were randomly selected for each slice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$



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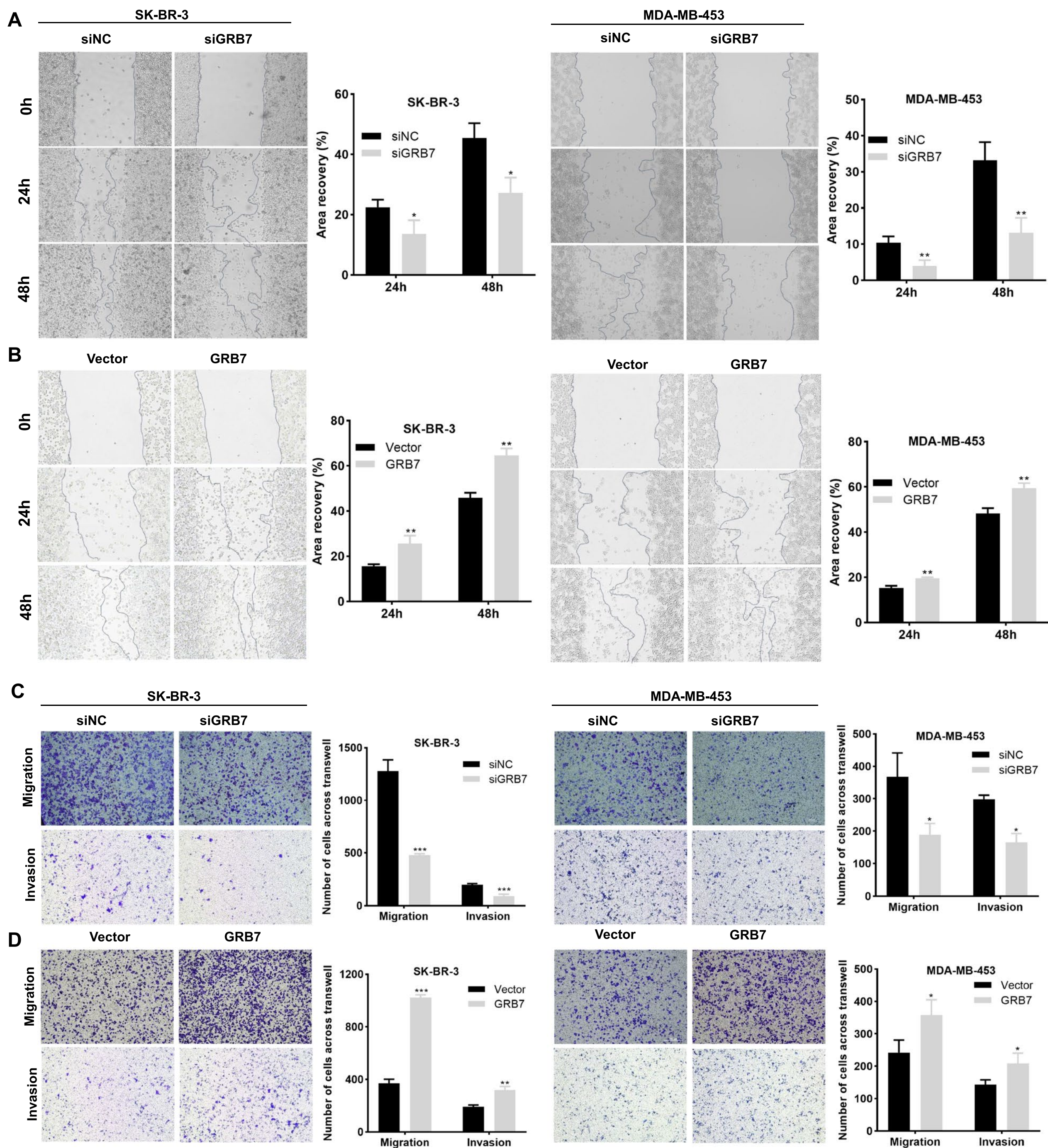


Fig. 3 GRB7 promotes HER2+ cell migration and invasion in vitro. **A**, **B** The migration of SK-BR-3 and MDA-MB-453 cells was assessed by wound healing assays at 0 h, 24 h and 48 h after knockdown or overexpression of GRB7. **C**, **D** Representative images of migration and invasion of GRB7 knockdown or overexpression SK-BR-3 and MDA-MB-453 cells. Data are shown as the mean ± SD, N ≥ 3. *p < 0.05, **p < 0.01, ***p < 0.001

that TCF12 was most correlated with GRB7 expression (Fig. 6B). We then performed bioinformatics to analyze the TCF12-binding motifs and found that the GRB7 promoter contains four binding motifs in a relatively concentrated and overlapping region (Fig. 6C). To confirm TCF12 binding to the GRB7 promoter region, we performed ChIP-PCR in SK-BR-3 cells. The result showed that the fragment 1 (nt-304 to nt-294) and fragment 6 (nt-1211 to nt-1201) of the GRB7 promoter was enriched by anti-TCF12 (Fig. 6D-E), indicating that TCF12 bound

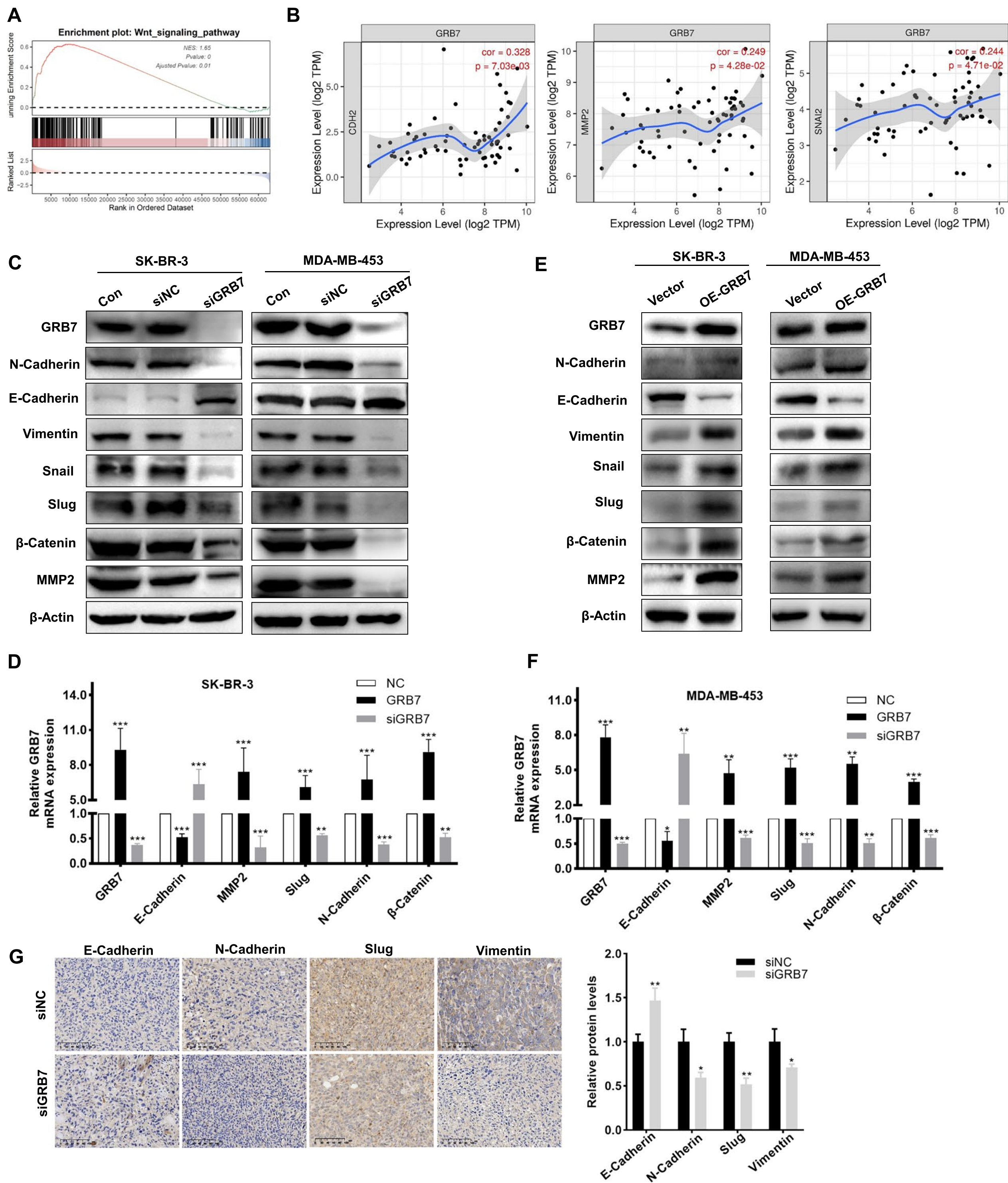


Fig. 4 GRB7 enhances EMT progression and promotes related protein expression. **A** GSEA enrichment analysis after knockdown of GRB7. **B** Correlation analysis of GRB7 with N-cadherin (CDH2), MMP2 and Slug (SNAI2) in HER2+ BC from the TIMER database. **C**, **D** Western blot and RT–qPCR were used to detect the protein and mRNA levels of EMT biomarkers in SK-BR-3 and MDA-MB-453 cells following GRB7 knockdown. **E**, **F** Western blot and RT–qPCR were used to detect the protein and mRNA levels of EMT biomarkers in SK-BR-3 and MDA-MB-453 cells following GRB7 overexpression. **G** Representative images of EMT biomarker expression evaluated by IHC in xenograft tumor tissues. Data are shown as the mean ± SD, N ≥ 3. *p < 0.05, **p < 0.01, ***p < 0.001

to those regions. In addition, the luciferase reporter gene assay showed that transfection of TCF12 expression vector increased the luciferase activity of the GRB7 reporter gene (Fig. 6F). Next, the expression levels of GRB7 in HER2+ cells with TCF12 silencing or overexpression were measured by RT–qPCR and western blot. The results showed that knockdown of TCF12 significantly decreased GRB7 expression levels (Fig. 6G), while overexpression of TCF12 increased GRB7 expression levels (Fig. 6H). Taken together, our results demonstrated that TCF12 could bind to the GRB7 promoter region to enhance GRB7 expression.

Forced GRB7 overexpression partially reverses the inhibition of HER2+ BC progression caused by TCF12 knockdown

Several studies have demonstrated that TCF12 is involved in regulation of cell development and differentiation, thus promoting malignant tumor progression [18, 19]. To test whether TCF12 is involved in the function of GRB7 in HER2+ BC, we investigated the role of TCF12 on proliferation, migration and invasion in SK-BR-3 and MDA-MB-453 cells by inhibiting TCF12 expression through siRNA. The results showed that viability of SK-BR-3 and MDA-MB-453 cells was significantly inhibited after TCF12 knockdown (Fig. 7A). Colony formation and EdU assays also showed that knockdown of TCF12 significantly inhibited cell proliferation (Fig. 7B, C), but forced GRB7 overexpression could partially reverse the reduction in cell viability and proliferation caused by TCF12 knockdown (Fig. 7D and Figure S7A). Furthermore, forced GRB7 overexpression partially rescued the reduction in migration and invasion caused by TCF12 knockdown (Fig. 7E, F and Figure S7B-C). These results suggested that GRB7 was a key target of TCF12 to regulate HER2+ BC progression.

To further confirm the role of TCF12 in the regulation of GRB7 in vivo, MDA-MB-453 cells were orthotopically inoculated into NSG mice to form the xenograft. As shown in Fig. 8A–C the tumor size and weight in the cholesterol-modified siTCF12 group were significantly lower than those in the control group. Moreover, lentivirus-mediated GRB7 overexpression significantly attenuated the inhibitory effect of TCF12 knockdown on tumor growth. We further verified the expression of Ki67 and EMT markers by immunohistochemistry (Fig. 8D, E), further supporting that TCF12 promotes HER2+ BC progression via GRB7 in vivo.

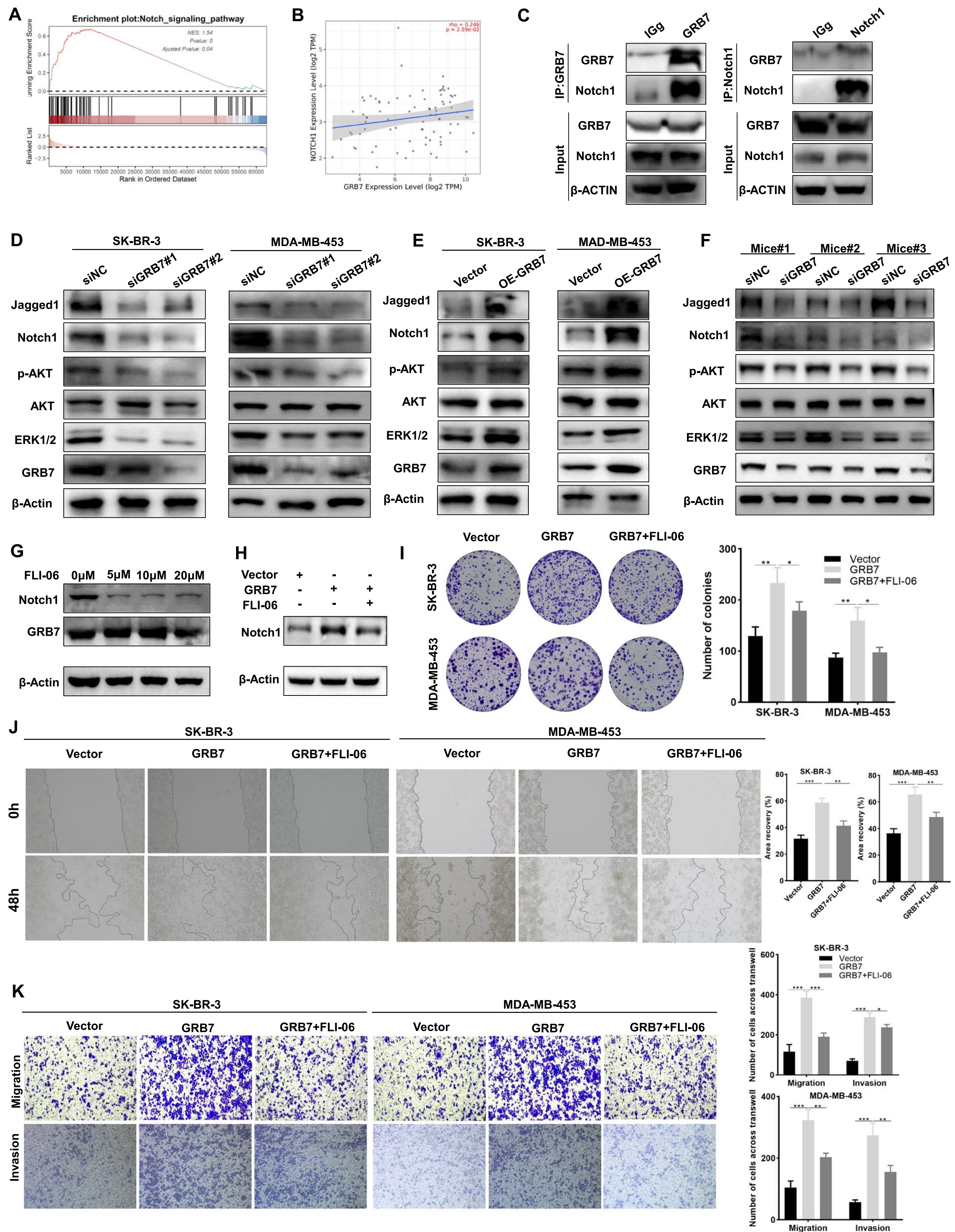
Discussion

HER2+ BC is an aggressive malignancy more prone to recurrence than luminal A and luminal B BC subtypes. BC patients with HER2 overexpression show worse prognosis and shorter OS [35, 36]. HER2 overexpression is involved in maintaining cell proliferation, avoiding apoptosis, promoting cell spreading and metastasis formation [37, 38]. HER2+ BC patients frequently develop brain, liver and lung metastases [39]. The prognosis of patients with the HER2+ subtype of BC remains unsatisfactory despite standard methods of treatment. Because the biological behavior of HER2+ BC is extremely complex, the molecular mechanisms and signaling pathways remain largely unknown despite extensive research. Therefore, there is a need to identify new molecules and explore different molecular mechanisms for guiding the diagnosis, prognosis estimation, and targeted therapies for patients with HER2+ BC. In this study, based on our results and TCGA data, we consistently verified that GRB7 expression was upregulated in HER2+ BC and correlated with poor prognosis. In addition, we observed increased expression of GRB7 in chemotherapy non-responders, indicating a feasible role for GRB7 in chemotherapy resistance. Functional studies showed that GRB7 promoted the proliferation, migration and invasion of HER2+ BC, consistent with the previous findings [40, 41]. These results suggest that GRB7 plays an oncogenic role in HER2+ BC and further motivate us to deeply explore the molecular mechanism of GRB7 action.

Increasing evidence suggests that GRB7 causes tumor progression through cascade signaling or signal transduction. For example, GRB7 could regulate mitogen-activated protein kinases (MAPKs), such as JNK or ERK signaling, to promote cancer cell motility, and conversely, knockdown or inhibition of GRB7 expression or

(See figure on next page.)

Fig. 5 GRB7 binds to Notch1 and activates the Jagged1/AKT/ERK1/2 signaling pathway to promote HER2+ BC progression. **A** GSEA of differentially expressed genes after GRB7 knockdown. **B** Correlation analysis between GRB7 and Notch1. **C** The interaction between GRB7 and Notch1 was detected by immunoprecipitation (IP). **D**, **E** After GRB7 knockdown and overexpression treatment, whole cell lysates were prepared and analyzed by immunoblotting. **F** Western blot was used to detect the protein expression levels of pathway-related genes after in vivo knockdown of GRB7. **G** The effect of SK-BR-3 cells treated with FLI-06 (5, 10, and 20 μM) for 24 h on Notch1 and GRB7 was verified by western blot. **H** The effect of SK-BR-3 cells on Notch1 after transfection with GRB7 plasmid for 48 h or FLI-06 (10 μM) treatment for 24 h was detected by western blot. β-Actin was used as a loading control. **I** Colony-forming ability was detected after transfected with GRB7 or GRB7 plasmid and FLI-06 (10 μM). The migration and invasion of SK-BR-3 and MDA-MB-453 cells was assessed by **J** wound healing assays, **K** transwell assays after transfected with GRB7 or GRB7 plasmid and FLI-06 (10 μM). Data are shown as the mean ± SD, N ≥ 3. *p < 0.05, **p < 0.01, ***p < 0.001



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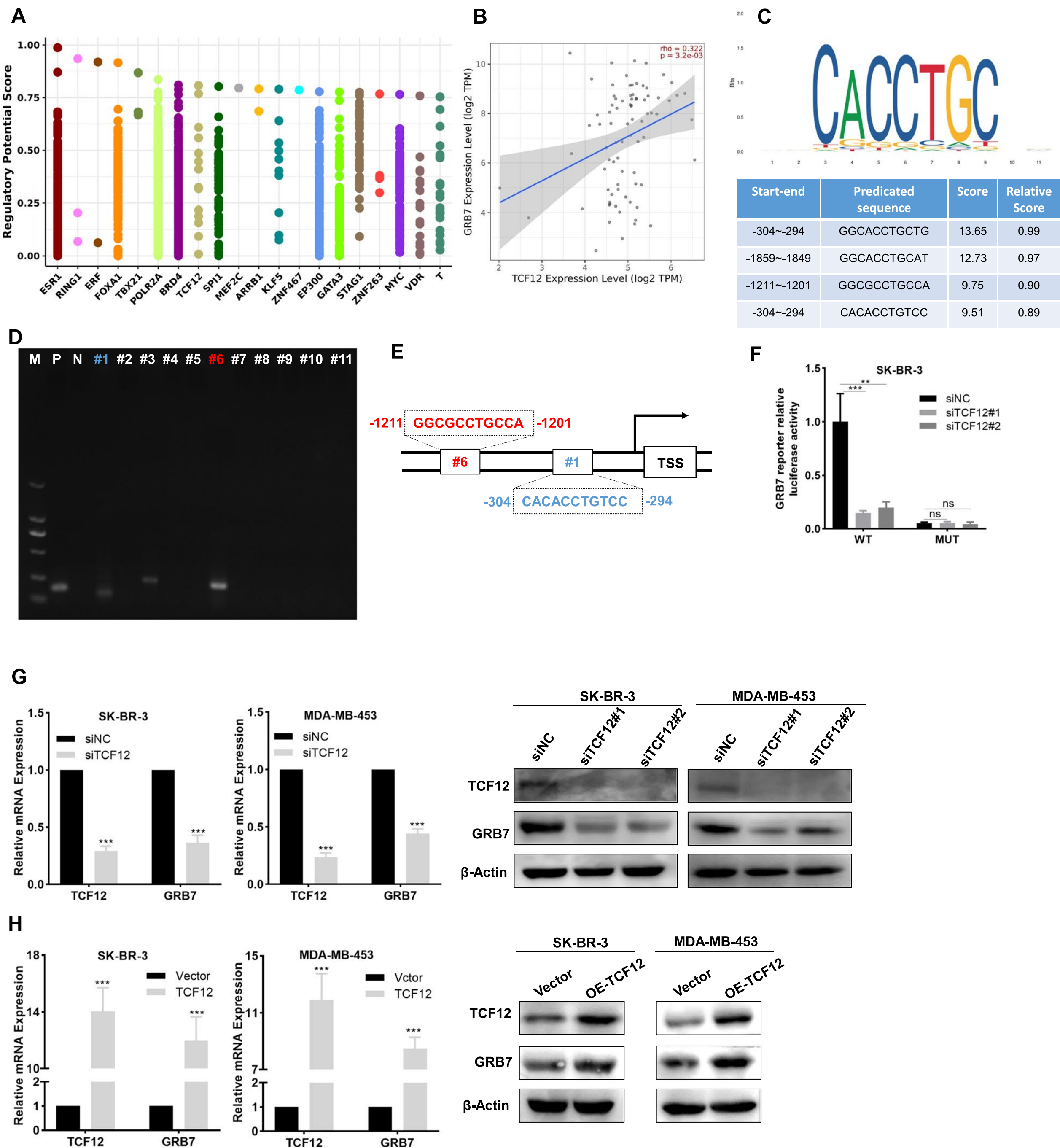

Start-end	Predicated sequence	Score	Relative Score
-304~-294	GGCACCTGCTG	13.65	0.99
-1859~-1849	GGCACCTGCAT	12.73	0.97
-1211~-1201	GGCGCCTGCCA	9.75	0.90
-304~-294	CACACCTGTCC	9.51	0.89

Fig. 6 TCF12 binds to the GRB7 promoter and promotes GRB7 expression. **A** Prediction of TFs that may regulate GRB7 expression by the Cistrome DB database. **B** The correlation between the expression of TCF12 and GRB7. **C** The potential binding sites of GRB7 were analyzed by JASPAR. **D** Agarose electrophoresis was used for chromatin immunoprecipitation (ChIP) analysis of TCF12 binding to the GRB7 promoter in SK-BR-3 cells. **E** Detailed fragments of TCF12 bound to the GRB7 promoter in SK-BR-3 cells were analyzed by ChIP. **F** Dual-luciferase reporter assays of the corresponding luciferase activities in SK-BR-3 cells. **G** Western blot and RT−qPCR were used to analyze the mRNA and protein levels of TCF12 and GRB7 in SK-BR-3 and MDA-MB-453 cells after TCF12 knockdown. **H** Western blot and RT-qPCR were used to analyze the mRNA and protein levels of TCF12 and GRB7 in SK-BR-3 and MDA-MB-453 cells after TCF12 overexpression. Data are shown as the mean ± SD, N ≥ 3. *p < 0.05, **p < 0.01, ***p < 0.001. *ns* no significant difference

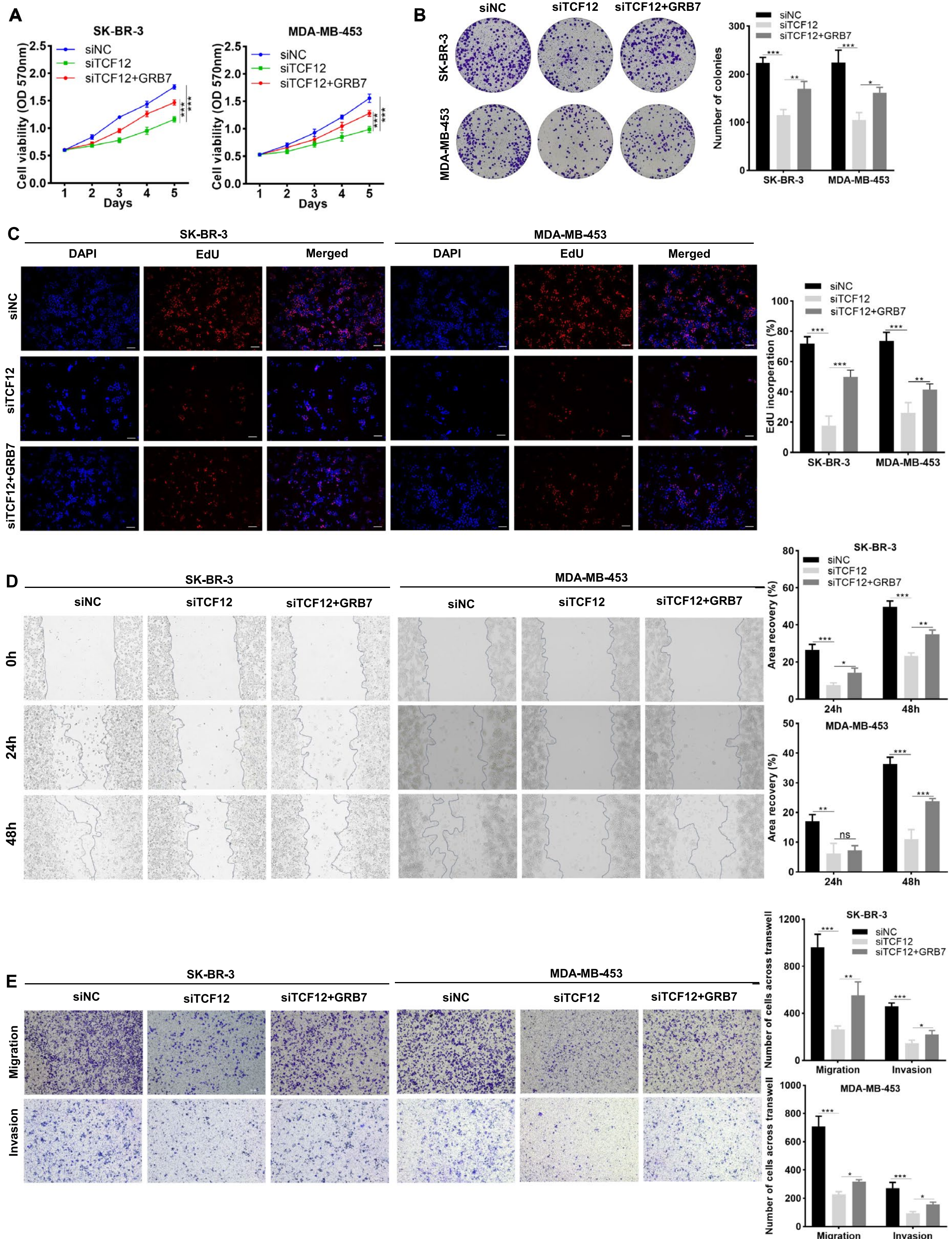

Fig. 7 GRB7 partially rescues the effect of downregulation of TCF12 in HER2+ cells. **A** MTT assay, **B** colony formation assay and **C** EdU assay showed that GRB7 could partially reverse the inhibitory effect of TCF12 knockdown on cell proliferation. **D** Wound healing assays and **E** Transwell assays indicated that overexpression of GRB7 partially restored the inhibitory effect of TCF12 downregulation on cell migration and invasion. Data are shown as the mean ± SD, N ≥ 3. *p < 0.05, **p < 0.01, ***p < 0.001

GRB7-mediated downstream signaling by specific kinase activity abrogates GRB7-mediated cancer migration [42]. Accordingly, our results indicated that GRB7 promoted HER2+ BC progression by activating AKT and increasing ERK levels. GRB7 is one of the most important mediators in oncogenic ERBB family-mediated signaling and can promote anchored anchorage-independent growth of tumor cells through interaction with phosphotyrosine-related signaling molecules [43, 44]. Notably, we first demonstrated that GRB7 was able to bind to Notch1, subsequently increasing the enrichment of its ligand Jagged1, further activating the Wnt/β-catenin signaling pathway, and promoting the progression of EMT in HER2+ BC. Our results showed that GRB7 bound to Notch1 to activate Notch signaling and other signaling (i.e. AK and ERK) therefore promoting HER2+ BC cell proliferation and metastasis. Our study suggests that GRB7 might be a potential therapeutic target for HER2+ BC. For this reason, the development of targeted inhibitors for GRB7 and natural or synthetic bioactives is particularly important. Studies have shown that the cell-penetrating non-phosphorylated peptide (G7-18NATE) binds to the SH2 domain of GRB7 and inhibits its binding to several different tyrosine kinases, including ERBB family members and FAK [45, 46], thereby inhibiting the ability of TNBC cells to migrate, invade, form colonies in 3D cultures[46], and significantly inhibit pancreatic cancer metastasis [47]. Moreover, this highly specific peptide does not interfere with other closely related SH2-containing adaptor proteins, including GRB2, GRB10, and GRB14 [45, 47]. So far, studies on biologically active inhibitors against GRB7 in HER2+ BC have not yet been reported, which opens up more potential possibilities for future development of targeted drugs against GRB7.

TCF12 is a key transcription factor that finely regulates a wide range of effector proteins and thus plays a crucial role in different types of cancer, including melanoma, glioma and oral squamous cell carcinoma [19, 48, 49]. In addition, the high expression of TCF12 in ovarian cancer is associated with its histologic grading and metastasis and significantly promotes the growth, migration, and invasion of ovarian cancer cells, which may provide a new strategy for targeting ovarian cancer [50, 51]. In the present study, we identified in the public ChIP-Seq dataset that TCF12 might be recruited and bound to the promoter region of GRB7 and that the expression levels of both showed a significant positive correlation in HER2+ BC tissues. Moreover, we found that knocking down TCF12 expression significantly inhibited the proliferation, migration and invasion of HER2+ BC cells in vitro as well as tumor growth in vivo, while overexpression of GRB7 partially mitigated the effects of TCF12 inhibition. Furthermore, depletion of TCF12 inhibited the Wnt/β-catenin signaling pathway and blocked HER2+ BC EMT progression, which is consistent with previous studies confirming that TCF12 is a key regulator in promoting the EMT process [52]. Accordingly, TCF12 could expedite HER2+ BC progression by regulating GRB7 transcriptional activity. Nonetheless, GRB7 may not be the only mediator of TCF12 transcriptional regulation. Previous studies have shown that TCF12 directly binds to the CXCR4 promoter to regulate its expression, promoting tumorigenesis and progression of HCC by activating the MAPK/ERK and PI3K/AKT signaling pathways [18]. Furthermore, TCF12 directly binds to the promoter region of CSF1 to regulate and regulate TAMs infiltration, thereby promoting glioma progression [19]. In addition, TGFB2 was identified as a direct downstream target of TCF12, and depletion of TCF12 confers melanoma sensitive to BRAF inhibition in vitro and in vivo, making it a potential therapeutic target [53]. Overall, we first reported that TCF12 could bind to the promoter region of GRB7 and increase its expression, thereby promoting HER2+ BC progression, providing a theoretical basis for further targeting the TCF12/GRB7 axis for the treatment of HER2+ BC.

EMT is a crucial biological phenomenon involved in embryonic development, exhibiting loss of cell polarity or intercellular adhesion by epithelial cells, which then acquire migratory and invasive features to become mesenchymal cells [54, 55]. EMT is characterized by cellular and molecular changes, including decreased epithelial markers, such as E-cadherin, and increased mesenchymal markers, such as vimentin and N-cadherin [56]. In recent years, EMT has been considered a pivotal link in the invasion and metastasis of several cancers [57]. Several studies have demonstrated that EMT is associated with HER2+ BC progression [58, 59]. Our results showed that GRB7 significantly increased N-cadherin/Vimentin/β-Catenin expression levels and decreased E-cadherin

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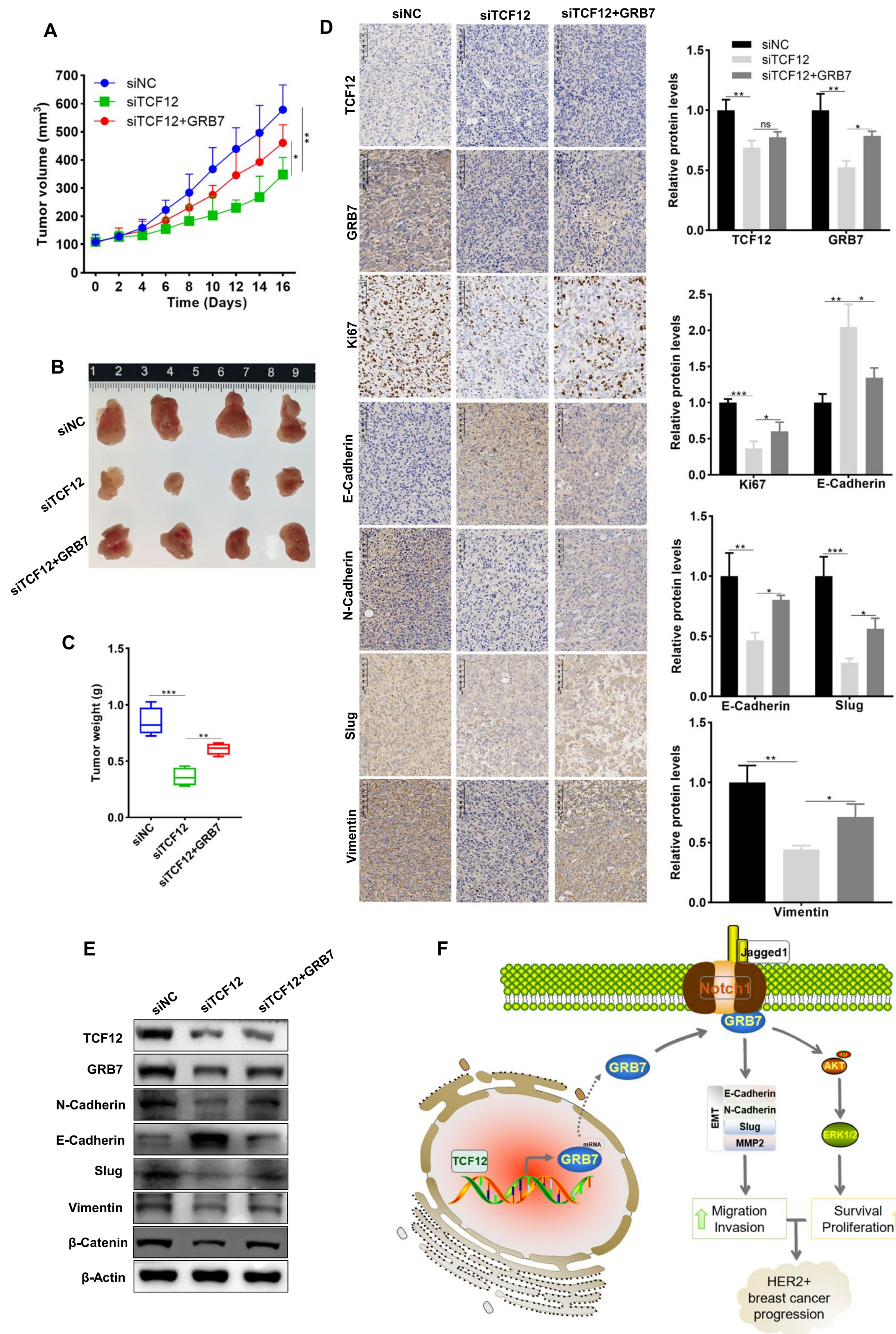
Fig. 8 GRB7 promotes HER2 progression in vivo by increasing TCF12-induced EMT reduction. **A** Tumor growth curve of xenograft tumors from the indicated groups. **B** Representative images of xenograft tumors from the indicated groups. **C** Tumor weights from the indicated groups. **D**, **E** EMT-related protein expression was analyzed by IHC and western blot in tumor samples from the indicated groups. Data are shown as the mean ± SD, *p < 0.05, **p < 0.01, ***p < 0.001. **F** Schematic of the molecular mechanism by which GRB7 is transcriptionally regulated by TCF12 to regulate HER2+ BC progression through the Notch/AKT/ERK axis



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levels, suggesting an enhanced HER2+ BC cell mesenchymal cell-like feature. Conversely, knockdown of GRB7 or its upstream transcription factor TCF12 reduced the proliferative capacity of HER2+ BC cells in vitro and in vivo. Moreover, overexpression of GRB7 partially rescued the reduction in proliferation, migration and invasion ability of HER2+ BC cells induced by TCF12 inhibition. Thus, targeting the TCF12/GRB7 might be a promising strategy for developing antimetastatic drugs against HER2+ BC.

Conclusions

In summary, we illustrated that GRB7 is highly expressed in HER2+ BC and is introvertedly corelated with the patient's prognosis. Functionally, GRB7 facilitates HER2+ BC cell proliferation, migration and invasion. Mechanistically, GRB7 is transcriptionally regulated by TCF12, interacts with Notch1, and promotes EMT progression and proliferation by activating Wnt/β-catenin pathways and other signaling (i.e., AKT, ERK) in HER2+ BC cells (Fig. 8F). Taken together, our findings elucidate the oncogenic role of GRB7 in HER2+ BC, providing a novel molecular mechanism and therapeutic target for the development of HER2+ BC./>

Abbreviations

BC Breast cancer
ER Estrogen receptor
PR Progesterone receptor
HER2 Human epidermal growth factor receptor 2
TNBC Triple-negative breast cancer
GRB7 Growth factor receptor-bound protein 7
TCF12 Transcription factor 12
EMT Epithelial-mesenchymal transition
CXCR4 C-X-C chemokine receptor type 4
DHX9 DExH-box helicase 9
RT–qPCR Real-time quantitative polymerase chain reaction
ChIP Chromatin immunoprecipitation
IF Immunofluorescence
IHC Immunohistochemical

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-024-05536-6.

Supplementary Material 1. Figure S1. GRB7 is highly expressed in BC and affects prognosis. Pan-cancer expression profile of GRB7 from the TIMER database.Expression of GRB7 in BC and normal breast tissue. Expression level of GRB7 in different stages and ages of BC patients from the TCGA database.Box plot showing increased GRB7 levels in non-responderscompared to responders in BC.AUC curve of GRB7 based on the 5-year RFS of BC after any chemotherapy. All data were from http://www.rocplot.org. Data are shown as the mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Figure S2. Expression of GRB7 in GEO datasets.Expression levels of GRB7 in luminal and HER2+ BC were compared in the GSE65194, GSE162228, GSE45827 and GSE21653 datasets.Expression levels of GRB7 in HER2- and HER2+ BC were compared in the GSE19615 and GSE76275 datasets. Figure S3. Predictive analysis of GRB7 in cancer functional status. KEGG enrichment analysis of GRB7 co-expressed genes.The function of GRB7 in various tumors was analyzed using the CancerSEA database.The biological role of GRB7 in BC was analyzed from the CancerSEA database and GSEA database. Figure S4. Enrichment analysis of differential genes after knockdown of GRB7.Heatmap of the transcriptional changes.Volcano plot showing up- and downregulated genes in GRB7-depleted SK-BR-3 cells.Based on the different genes of GRB7 knockdown, the GO chord plots were visualized.Kyoto Encyclopedia of Genes and Genomespathway enrichment analysis of transcriptionally altered genes identified by RNA-seq in GRB7-deficient SK-BR-3 cells.Protein interaction network analysis of differentially expressed genes after knockdown of GRB7. Figure S5. GSEA of GRB7 with several signaling pathways. Figure S6. Correlation analysis of several TFs with GRB7. Figure S7. TCF12 deletion significantly reversed the proliferation, migration and invasion of HER2+ cells induced by GRB7 overexpression.Colony formation assay showed that GRB7 could partially reverse the inhibitory effect of TCF12 knockdown on cell proliferation. Wound healing assays andtranswell assays indicated that overexpression of GRB7 partially restored the inhibitory effect of TCF12 downregulation on cell migration and invasion. Data are shown as the mean ± SD, $N \geq 3$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$./>

Author contributions

GW and WYZ conceived the idea, analyzed the literature, and wrote the manuscript; GW, YLW, YS, NQ, BC, DFZ and LY performed the experiments. GW, YLW, MJLY and MPL collected and read the literature and revised the article; WYZ read through and corrected the manuscript. All the authors have read and approved the final manuscript.

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Availability of data and materials

All data used in this work can be acquired from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All animal experiments were performed in accordance with the Government published recommendations for the Care and Use of Laboratory Animals and were approved by Chongqing Medical University.

Consent for publication

All authors have agreed to the content of the manuscript and agree to this submission.

Competing Interests

The authors declare that they have no conflicts of interest regarding the publication of this article.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018. https://doi.org/10.3322/caac.21492.

2. Waks AG, Winer EP. Breast cancer treatment: a review. JAMA. 2019. https://doi.org/10.1001/jama.2018.19323.
3. Choong GM, Cullen GD, O'Sullivan CC. Evolving standards of care and new challenges in the management of HER2-positive breast cancer. CA Cancer J Clin. 2020. https://doi.org/10.3322/caac.21634.
4. Tarantino P, Viale G, Press MF, Hu X, Penault-Llorca F, Bardia A, et al. ESMO expert consensus statements (ECS) on the definition, diagnosis, and management of HER2-low breast cancer. Ann Oncol Off J Eur Soc Med Oncol. 2023;34(8):645–59.
5. Chu P-Y, Tai Y-L, Shen T-L. Grb7, a critical mediator of EGFR/ErbB signaling, in cancer development and as a potential therapeutic target. Cells. 2019. https://doi.org/10.3390/cells8050435.
6. Nadler Y, González AM, Camp RL, Rimm DL, Kluger HM, Kluger Y. Growth factor receptor-bound protein-7 (Grb7) as a prognostic marker and therapeutic target in breast cancer. Ann Oncol Off J Eur Soc Med Oncol. 2010. https://doi.org/10.1093/annonc/mdp346.
7. Pei Y-Y, Ran J, Wen L, Liu X, Xiang L, Liu W, et al. Up-regulated GRB7 protein in gastric cancer cells correlates with clinical properties and increases proliferation and stem cell properties. Front Oncol. 2022. https://doi.org/10.3389/fonc.2022.1054976.
8. Zhao H-B, Zhang X-F, Jia X-L, Wang H-B. Grb7 is over-expressed in cervical cancer and facilitate invasion and inhibit apoptosis in cervical cancer cells. Pathol Res Pract. 2017;213(9):1180–4.
9. Tang Y, Yang S, Wang M, Liu D, Liu Y, Zhang Y, et al. Epigenetically altered miR-193a-3p promotes HER2 positive breast cancer aggressiveness by targeting GRB7. Int J Mol Med. 2019. https://doi.org/10.3892/ijmm.2019.4167.
10. Zheng Y, Pei Y, Yang L, Zeng Z, Wang J, Xie G, et al. Upregulated GRB7 promotes proliferation and tumorigenesis of Bladder Cancer via Phospho-AKT Pathway. Int J Biol Sci. 2020. https://doi.org/10.7150/ijbs.49410.
11. Ling Y, Liang G, Lin Q, Fang X, Luo Q, Cen Y, et al. circCDYL2 promotes trastuzumab resistance via sustaining HER2 downstream signaling in breast cancer. Mol Cancer. 2022;21:1–6.
12. Chu P-Y, Li T-K, Ding S-T, Lai I-R, Shen T-L. EGF-induced Grb7 recruits and promotes Ras activity essential for the tumorigenicity of Sk-Br 3 breast cancer cells. J Biol Chem. 2010. https://doi.org/10.1074/jbc.C110.114124.
13. Blackledge NP, Klose RJ. The molecular principles of gene regulation by Polycomb repressive complexes. Nat Rev Mol Cell Biol. 2021. https://doi.org/10.1038/s41580-021-00398-y.
14. de Mattos K, Viger RS, Tremblay JJ. Transcription factors in the regulation of leydig cell gene expression and function. Front Endocrinol. 2022;13:881309.
15. Suter DM. Transcription factors and DNA play hide and seek. Trends Cell Biol. 2020;30(6):491–500.
16. Yi S, Yu M, Yang S, Miron RJ, Zhang Y. Tcf12, a member of basic helix-loop-helix transcription factors, mediates bone marrow mesenchymal stem cell osteogenic differentiation in vitro and in vivo. Stem Cells. 2017. https://doi.org/10.1002/stem.2491.
17. Lee C-C, Chen W-S, Chen C-C, Chen L-L, Lin Y-S, Fan C-S, et al. TCF12 protein functions as transcriptional repressor of E-cadherin, and its overexpression is correlated with metastasis of colorectal cancer. J Biol Chem. 2017. https://doi.org/10.1074/jbc.M111.258947.
18. Yang J, Zhang L, Jiang Z, Ge C, Zhao F, Jiang J, et al. TCF12 promotes the tumorigenesis and metastasis of hepatocellular carcinoma via upregulation of CXCR4 expression. Theranostics. 2019;9(20):5810.
19. Liu L, Zhou X, Cheng S, Ge Y, Chen B, Shi J, et al. RNA-binding protein DHX9 promotes glioma growth and tumor-associated macrophages infiltration via TCF12. CNS Neurosci Ther. 2023. https://doi.org/10.1111/cns.14031.
20. Qiu Y, Wang X, Sun Y, Du Y-E, Yin G, Luo H, et al. TCF12 regulates exosome release from epirubicin-treated CAFs to promote ER+ breast cancer cell chemoresistance. Biochim Biophys Acta Mol Basis Dis. 2023;1869(6):166727.
21. Cui Y, Zhao M, Yang Y, Xu R, Tong L, Liang J, et al. Reversal of epithelial-mesenchymal transition and inhibition of tumor stemness of breast cancer cells through advanced combined chemotherapy. Acta Biomater. 2022;152:380–92.
22. Chi M, Liu J, Mei C, Shi Y, Liu N, Jiang X, et al. TEAD4 functions as a prognostic biomarker and triggers EMT via PI3K/AKT pathway in bladder cancer. J Exp Clin Cancer Res. 2022. https://doi.org/10.1186/s13046-022-02377-3.
23. Liu S, Dou L, Miao M, Man X, Wei B, Jiang Z, et al. HES1-mediated down-regulation of miR-138 sustains NOTCH1 activation and promotes proliferation and invasion in renal cell carcinoma. J Exp Clin Cancer Res. 2023. https://doi.org/10.1186/s13046-023-02625-0.
24. Yang F, Ma Q, Huang B, Wang X, Pan X, Yu T, et al. CircNFATC3 promotes the proliferation of gastric cancer through binding to IGF2BP3 and restricting its ubiquitination to enhance CCND1 mRNA stability. J Transl Med. 2023. https://doi.org/10.1186/s12967-023-04235-y.
25. Henick BS, Villarroel-Espindola F, Datar I, Sanmamed MF, Yu J, Desai S, et al. Quantitative tissue analysis and role of myeloid cells in non-small cell lung cancer. J Immunother Cancer. 2022. https://doi.org/10.1136/jitc-2022-005025.
26. Zhao H, Ming T, Tang S, Ren S, Yang H, Liu M, et al. Wnt signaling in colorectal cancer: pathogenic role and therapeutic target. Mol Cancer. 2022;21(1):144.
27. Merikhian P, Eisavand MR, Farahmand L. Triple-negative breast cancer: understanding Wnt signaling in drug resistance. Cancer Cell Int. 2021;21(1):419.
28. Meurette O, Mehlen P. Notch signaling in the tumor microenvironment. Cancer Cell. 2018;34(4):536–48.
29. Xiu M-X, Liu Y-M, Kuang B-H. The oncogenic role of Jagged1/Notch signaling in cancer. Biomed Pharmacother. 2020;129:110416.
30. Shah D, Wyatt D, Baker AT, Simms P, Peiffer DS, Fernandez M, et al. Inhibition of HER2 increases JAGGED1-dependent breast cancer stem cells: role for membrane JAGGED1. Clin Cancer Res. 2018;18:4566.
31. Gómez-Archila JD, Espinosa-García AM, Palacios-Reyes C, Trujillo-Cabrera Y, Mejía ALS, González AVDA, et al. NOTCH expression variability and relapse of breast cancer in high-risk groups. Am J Med Sci. 2022;364(5):583–94.
32. Zhang M, Han Y, Zheng Y, Zhang Y, Zhao X, Gao Z, et al. ZEB1-activated LINC01123 accelerates the malignancy in lung adenocarcinoma through NOTCH signaling pathway. Cell Death Dis. 2020. https://doi.org/10.1038/s41419-020-03166-6.
33. Lambert SA, Jolma A, Campitelli LF, Das PK, Yin Y, Albu M, et al. The human transcription factors. Cell. 2018. https://doi.org/10.1016/j.cell.2018.01.029.
34. Li Y, Azmi AS, Mohammad RM. Deregulated transcription factors and poor clinical outcomes in cancer patients. Semin Cancer Biol. 2022. https://doi.org/10.1016/j.semcancer.2022.08.001.
35. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science. 1987. https://doi.org/10.1126/science.3798106.
36. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ. Strategies for subtypes–dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol Off J Eur Soc Med Oncol. 2011. https://doi.org/10.1093/annonc/mdr304.
37. von Minckwitz G, Procter M, de Azambuja E, Zardavas D, Benyunes M, Viale G, et al. Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer. N Engl J Med. 2017. https://doi.org/10.1056/NEJMoa1703643.
38. Ahmed ARH. HER2 expression is a strong independent predictor of nodal metastasis in breast cancer. J Egypt Natl Canc Inst. 2016;28(4):219–27.
39. Aversa C, Rossi V, Geuna E, Martinello R, Milani A, Redana S, et al. Metastatic breast cancer subtypes and central nervous system metastases. Breast. 2014. https://doi.org/10.1016/j.breast.2014.06.009.
40. Haran M, Chebatco S, Flaishon L, Lantner F, Harpaz N, Valinsky L, et al. Grb7 expression and cellular migration in chronic lymphocytic leukemia: a comparative study of early and advanced stage disease. Leukemia. 2004. https://doi.org/10.1038/sj.leu.2403512.
41. Wang Y, Chan DW, Liu VWS, Chiu P, Ngan HYS. Differential functions of growth factor receptor-bound protein 7 (GRB7) and its variant GRB7v in ovarian carcinogenesis. Clin Cancer Res. 2010. https://doi.org/10.1158/1078-0432.CCR-10-0018.
42. Chan DW, Hui WWY, Cai PCH, Liu MX, Yung MMH, Mak CSL, et al. Targeting GRB7/ERK/FOXM1 signaling pathway impairs aggressiveness of ovarian cancer cells. PLoS ONE. 2012. https://doi.org/10.1371/journal.pone.0052578.
43. Janes PW, Lackmann M, Church WB, Sanderson GM, Sutherland RL, Daly RJ. Structural determinants of the interaction between the erbB2

receptor and the Src homology 2 domain of Grb7. J Biol Chem. 1997. https://doi.org/10.1074/jbc.272.13.8490.
44. Lim RCC, Price JT, Wilce JA. Context-dependent role of Grb7 in HER2+ve and triple-negative breast cancer cell lines. Breast Cancer Res Treat. 2014. https://doi.org/10.1007/s10549-014-2838-5.
45. Pero SC, Oligino L, Daly RJ, Soden AL, Liu C, Roller PP, et al. Identification of novel non-phosphorylated ligands, which bind selectively to the SH2 domain of Grb7. J Biol Chem. 2002. https://doi.org/10.1074/jbc.M111816200.
46. Giricz O, Calvo V, Pero SC, Krag DN, Sparano JA, Kenny PA. GRB7 is required for triple-negative breast cancer cell invasion and survival. Breast Cancer Res Treat. 2012. https://doi.org/10.1007/s10549-011-1822-6.
47. Tanaka S, Pero SC, Taguchi K, Shimada M, Mori M, Krag DN, et al. Specific peptide ligand for Grb7 signal transduction protein and pancreatic cancer metastasis. J Natl Cancer Inst. 2006. https://doi.org/10.1093/jnci/djj105.
48. Luo C, Balsa E, Perry EA, Liang J, Tavares CD, Vazquez F, et al. H3K27me3-mediated PGC1α gene silencing promotes melanoma invasion through WNT5A and YAP. J Clin Invest. 2020. https://doi.org/10.1172/JCI130038.
49. Chen Y-F, Yang C-C, Kao S-Y, Liu C-J, Lin S-C, Chang K-W. MicroRNA-211 enhances the oncogenicity of carcinogen-induced oral carcinoma by repressing TCF12 and increasing antioxidant activity. Cancer Res. 2016. https://doi.org/10.1158/0008-5472.CAN-15-1664.
50. Gao S, Bian T, Su M, Liu Y, Zhang Y. miR-26a inhibits ovarian cancer cell proliferation, migration and invasion by targeting TCF12. Oncol Rep. 2020. https://doi.org/10.3892/or.2019.7417.
51. Gao S, Bian T, Zhang Y, Su M, Liu Y. TCF12 overexpression as a poor prognostic factor in ovarian cancer. Pathol Res Pract. 2019;215(9):152527.
52. Li X-P, Jia Y-L, Duan Y-Q, Zhao Y, Yin X-L, Zhen S-M, et al. Circular RNA hsa_circ_0002938 (circCRIM1) promotes the progression of esophageal squamous cell carcinoma by upregulating transcription factor 12. Neoplasma. 2023. https://doi.org/10.4149/neo_2023_220823N857.
53. Tian Y, Zhou J, Chai X, Ping Z, Zhao Y, Xu X, et al. TCF12 activates TGFB2 expression to promote the malignant progression of melanoma. Cancers (Basel). 2023. https://doi.org/10.3390/cancers15184505.
54. Nieto MA, Huang RYJ, Jackson RA, Thiery JP. EMT: 2016. Cell. 2016. https://doi.org/10.1016/j.cell.2016.06.028.
55. Yang J, Antin P, Berx G, Blanpain C, Brabletz T, Bronner M, et al. Guidelines and definitions for research on epithelial-mesenchymal transition. Nat Rev Mol Cell Biol. 2020;21(6):341–52.
56. Gao T, Li J-Z, Lu Y, Zhang C-Y, Li Q, Mao J, et al. The mechanism between epithelial mesenchymal transition in breast cancer and hypoxia microenvironment. Biomed Pharmacother. 2016. https://doi.org/10.1016/j.biopha.2016.02.044.
57. Pastushenko I, Blanpain C. EMT transition states during tumor progression and metastasis. Trends Cell Biol. 2019. https://doi.org/10.1016/j.tcb.2018.12.001.
58. Ingthorsson S, Andersen K, Hilmarsdottir B, Maelandsmo GM, Magnusson MK, Gudjonsson T. HER2 induced EMT and tumorigenicity in breast epithelial progenitor cells is inhibited by coexpression of EGFR. Oncogene. 2016. https://doi.org/10.1038/onc.2015.489.
59. Qiu Y, Yang L, Liu H, Luo X. Cancer stem cell-targeted therapeutic approaches for overcoming trastuzumab resistance in HER2-positive breast cancer. Stem Cells. 2021. https://doi.org/10.1002/stem.3381.

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