Breast Cancer: Targets and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

ST6GAL1 Promotes Epithelial-to-Mesenchymal Transition in Breast Cancer via the HIF-HK2 Signaling Pathway

Dongliang Ren, Dengfeng Xue, Zhichao Hou, Huijuan Xu, Xinzheng Li

Department of Breast Surgery, Second Hospital of Shanxi Medical University, Taiyuan, Shanxi Province, People's Republic of China

Correspondence: Xinzheng Li, Department of Breast Surgery, Second Hospital of Shanxi Medical University, Taiyuan, Shanxi Province, People's Republic of China, Email breast_surgery23@163.com

Background and Purpose: Breast cancer presents a substantial clinical challenge because of its complex aetiology and diverse phenotypic presentations. ST6Gal1 expression and epithelial-to-mesenchymal transition (EMT) frequently lead to metastasis, drug resistance and poor prognosis in many cancers. Nevertheless, the molecular details surrounding ST6GAL1 in the carcinogenesis of breast cancer, especially in the EMT of breast cancer, remain unclear. The objective of this study was to clarify the possible role and mechanism of ST6GAL1 in breast cancer.

Methods: PCR, WB, and IHC were employed to analyze the expression of ST6GAL1, epithelial-mesenchymal transition (EMT) markers, and components of the HIF-HK2 signaling pathway in MCF-10A, MDA-MB-231, and MCF-7 cells. Wound-healing, cell adhesion, drug resistance and extracellular matrix invasion assays were used to analyse the effects of ST6GAL1 on the biological process of breast cancer cells. The HIF-HK2 signalling pathway was also analysed.

Results: ST6GAL1 expression is increased in breast cancer. Altered expression of ST6GAL1 affects the biological function of breast cancer cells both in vitro and in vivo. ST6GAL1 knockdown inhibited EMT in breast cancer cells. ST6GAL1 Mediates the Activity of the HIF-HK2 Signalling Pathway in Breast Carcinoma Cells.

Conclusion: In our study, in vitro and in vivo models revealed that ST6GAL1 promotes malignant phenotypes in breast cancer cells and regulates the EMT process through activation of the HIF-HK2 signalling pathway.

Keywords: breast cancer, ST6GAL1, HIF-HK2 signalling pathway, EMT

Introduction

Breast cancer poses a significant global health burden and affects millions of individuals worldwide, and it presents a substantial clinical challenge because of its complex aetiology and diverse phenotypic presentations.[1,2] Despite notable advancements in the knowledge of breast cancer genetic alterations, expression patterns, and biomarkers, the treatment of breast cancer remains complex and challenging. The major reason for treatment failure is the prevailing focus on molecular classification, disregarding the impact of extensive heterogeneity and cooccurring genomic alterations on treatment outcomes. Therefore, there is a critical need to develop new targets for patients who are not aided by current treatments.

Numerous studies have shown that epithelial-to-mesenchymal transition (EMT) frequently leads to metastasis and drug resistance, which results in disease progression and poor prognosis in patients with breast cancer.[3–5] EMT is characterized by decreased expression of epithelial markers, such as E-cadherin and α3 integrin, and increased expression of mesenchymal cell markers, including N-cadherin and vimentin. While many studies have successfully focused on the genomic, epigenomic, transcriptomic, and proteomic signatures of EMT, more research on the genomics of this process, particularly the role of sialylation in EMT, is needed.

An area of innovation in the search for new targets for breast cancer has been sialylation. Sialic acid, which is positioned at the terminal end of glycan chains, has diverse structural characteristics and performs a variety of vital and absolute biological

Received: 23 July 2025
Accepted: 4 December 2025
Published: 9 December 2025

functions.[6,7] Sialyltransferase enzymes, which include 20 subtypes in humans, such as ST6GAL1-2, ST6GALNAC 1–6, ST3GAL1-6, and ST8SIA 1–6, synthesize sialylated glycans[8] and are associated with malignant transformation.[9] Numerous studies have suggested that ST6Gal1 is upregulated and associated with poor patient prognosis in numerous types of epithelial cancers, such as breast, ovarian, colon, and pancreatic adenocarcinomas.[10–14] Nevertheless, the molecular details of the role of ST6GAL1 in the carcinogenesis of breast cancer, especially in the EMT of breast cancer, remain unclear. Aberrant sialylation may affect the stability and activity of HIF-1α, thus influencing downstream processes such as glucose metabolism and EMT. The mechanisms by which ST6GAL1-mediated sialylation regulates HIF-1α signaling in breast cancer remain to be clarified.

Hypoxia, a hallmark of solid tumours,[15,16] has a substantial effect on cell proliferation, apoptosis, differentiation, metabolism, etc.[17] Hypoxia-inducible factor 1 (HIF-1) is a member of one of the most recognized pathways by which tumour cells adapt to low-oxygen tension conditions and facilitate extensive metabolic reprogramming of cancer cells.[18] The HIF-1-dependent reprogramming of glucose metabolism promotes cell survival under hypoxic conditions.[19] Studies have shown that the ST6Gal1 sialyltransferase enhances glycolytic metabolism in ovarian cancer cells[20] and protects tumour cells against hypoxia by enhancing HIF-1α signalling.[21] However, little is known about ST6Gal1 and its signalling pathway in relation to the EMT in breast cancer.

Therefore, in the present study, we determined the expression levels of ST6Gal1 in the MCF-10A, MCF-7 and MDA-MB-231 cell lines and in breast cancer tissue samples. In addition, we investigated whether ST6GAL1 participates in the regulation of EMT via the HIF-HK2 pathway and the possible mechanisms involved.

Materials and Methods

Genetic Mutation and Methylation Analysis

The genetic mutation of ST6GAL1 in breast cancer was analysed with the cBioPortal tool (https://www.cbioportal.org/). UALCAN (https://ualcan.path.uab.edu/index.html) is an online database for analysing the DNA methylation status of ST6GAL1.

Cell Culture

The human breast cancer cell lines MDA-MB-231 and MCF-7, as well as the normal breast epithelial cell line MCF-10A, were obtained from the Cell Bank of the Chinese Academy of Sciences. MCF-10A cells were cultured in specialized culture medium (Wuhan Pricella Biotechnology Co., Ltd., CM-0525). MDA-MB-231 and MCF-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Wuhan Pricella Biotechnology Co., Ltd., PM150210) supplemented with antibiotics (penicillin (100 units/mL), streptomycin (100 μg/mL), Biosharp, BL505A) and 10% foetal bovine serum (CellMax, SA101.02). All experiments were performed during the logarithmic growth phase, and the cells were incubated at 37°C in a humidified atmosphere containing 5% CO2.

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) Analysis

Total RNA was extracted from the two cell lines via an RNA extraction kit (Vazyme, R401-01). cDNA was synthesized via a PrimeScript RT premix kit (Takara, RR036Q) according to the manufacturer's instructions. The QuantStudio 1 Plus System (Applied Biosystems) was used to perform RT-qPCR for 40 cycles. Gene expression levels were calculated via the $2^{-(\Delta\Delta CT)}$ method. SYBR Green Premix Ex Taq II (Takara, RR820) was used to quantify the relative mRNA expression of ST6GAL1. The PCR primers are showed as Supplementary Table 1.

Western Blot Analysis

The cells were washed three times with phosphate buffer solution (PBS), and precooled radioimmunoprecipitation assay (RIPA) buffer (Beyotime, P0013B) was used to lyse the cells for 15–30 min at 4 °C. The supernatant was subsequently collected by centrifugation at 14,000×g to obtain total protein. A bicinchoninic acid (BCA) assay was used to quantify the protein concentrations. The protein was electrotransferred to a polyvinylidene difluoride (PVDF) membrane and blocked with TBST containing 5% skim milk powder for 1 hour. The membranes were then incubated with different primary antibodies overnight at 4 °C. The membranes were washed thoroughly with TBST buffer at room temperature to

remove unbound antibodies, followed by incubation (1 h at room temperature) with secondary antibodies (Wuhan BOSTER BIOLOGICAL TECHNOLOGY Co., Ltd., BA1056) (1:5,000 in TBST with 1% blocking solution). The membranes were washed thoroughly at room temperature again and developed with an enhanced chemiluminescence (ECL) kit (Beyotime Biotechnology, P0018S). Western blot images were obtained by exposing the membranes to a ChemiDoc XRS+ gel imaging system (Bio-Rad). The band intensity was quantified via image analysis software (ImageJ V1.8.0). The details of the antibodies used are shown in Supplementary Table 2.

Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis

The most relevant genes of ST6GAL1 were uploaded to the Database for Annotation, Visualization, and Integrated Discovery (DAVID, v6.8). The official gene symbol was selected as an identifier, and *Homo sapiens* was selected as the species. Finally, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis enrichment results were obtained. The RNA-seq data for the KEGG analysis was downloaded from The Cancer Genome Atlas Breast Invasive Carcinoma (TCGA-BRCA) database (https://portal.gdc.cancer.gov/).

Cell Transfection

To investigate the function of ST6GAL1, the following methods were used for cell transfection and infection experiments. First, cells were transfected with siRNA using a transfection reagent (IBSBIO, No. S01). For each experiment, 50 pmol of three independent ST6GAL1 siRNA sequences and a negative control siRNA (IBSBIO) were transfected into 1×10^5 cells. Among these, the siRNA with the most significant silencing efficiency for ST6GAL1 (siRNA #1) was selected for subsequent experiments (see Supplementary Figure 1A and Supplementary Table 2). In addition, for plasmid transfection, Select the plasmid with the highest overexpression efficiency for subsequent research (Supplementary Figure 1B). Lipofectamine™ 3000 (Invitrogen, L3000015) was used to transfect either a control plasmid or a recombinant ST6GAL1 plasmid (IBSBIO) into the cells, with 5 μg of plasmid per 1×10^5 cells in each experiment. Cells were subsequently infected at a multiplicity of infection (MOI) of 100. The siRNA sequences are showed as Supplementary Table 3.

Invasion Assay

Transwell units (Corning Costar, Tewksbury, NY, USA) were used to assess differences in the invasive ability of the cells. Matrigel (Beyotime Biotech Inc., C3017) was added to the upper chamber, and the lower chamber contained culture medium supplemented with 20% FBS. The cells (10^5 cells per unit) were incubated for 24 h at 37 °C and 5% CO^2. Then, the cells that penetrated the lower chamber were stained with crystal violet (Beyotime Biotech Inc., C0121) for 20 minutes and counted with a Leica inverted microscope.

Wound-Healing Assay

The cells (10^5 cells per well) were seeded in six-well plates and scratched with a 200 μL pipette tip when the cell density reached approximately 80%. The cells were subsequently incubated in DMEM containing 1% serum. Scratch healing was observed at 0, 6, 12, and 24 hours. ImageJ software was used to analyse and compare the speed of cell migration.

Cell Adhesion Assay

The cells ($5x10^3$ per well) were incubated at 37 °C for 1 hour, and nonadherent cells were removed via gentle washing three times with PBS. The remaining cells were then fixed with 4% formaldehyde for 30 min and stained with 100 μL of modified Giemsa staining solution (Beyotime Biotech Inc., C0131). Images of three randomly selected fields of view were captured under a 20x microscope, and finally, statistical analysis of the data was performed.

Drug Resistance Assay

The cells were inoculated at 1×10^4 per well in 96-well plates and cultured for 24 h. Then, 5-Fluorouracil (5-FU) was added at concentrations of 0, 20, 50, 70, 100, and 200 μm. After 24 h and 48 h of treatment, cell proliferation was detected via a CCK-8 assay kit (Solarbio, CA1210).

Tumourigenicity Assay

Ten female BALB/c nude mice (6 weeks old) were obtained from Cyagen Biosciences, Inc. (Guangzhou, China). MCF-7 cells were divided into two groups: a negative control group (n=5, CON group) and an experimental group (n=5, si-ST6GAL1 group). The cells were injected subcutaneously into the left backs of the mice. The tumour size and weight were recorded every 3 days. After 28 days, the mice were euthanized via pentobarbital sodium injection, and the tumours were harvested for further analysis. The animal experiments were performed in accordance with the Guidelines of the Ethics Committee of the Second Hospital of Shanxi Medical University.

Immunohistochemical (IHC) Analysis

The tissue sections were deparaffinized with xylene, and different concentrations of ethanol were used for dehydration and hydration. Then, antigen retrieval was conducted using a composite digestive solution (BOSTER, AR0022), after which peroxidase blocking solution and goat serum were used for blocking. The sections were incubated overnight at 4 °C with primary antibodies. The sections were subsequently incubated with secondary antibodies according to the manufacturer's instructions (Maxim, KIT-9922). Finally, immunoreactivity was observed via a DAB colour assay kit (zsbio, ZLI-9017), and counterstaining was performed with haematoxylin. The details of the antibodies used are shown in Supplementary Table 2.

Statistical Analysis

All the experiments described in this study were conducted at least three times. The results are displayed as the means ± standard deviations. Two groups were compared via t tests, whereas multiple group comparisons were analysed via one-way ANOVA. GraphPad Prism 8.3 (GraphPad Software, San Diego, CA, United States) was used to analyse the data and draw relevant graphs.

Results

ST6GAL1 Expression is Increased in Breast Cancer

To assess the gene changes in ST6GAL1 in breast cancer, the mutation of ST6GAL1 was assessed through the cBioPortal database. The results revealed that amplification was the main type of mutation, and the alteration frequency was approximately 3% in invasive breast carcinoma (Figure 1A). Studies have shown that promoter methylation is involved in the tumourigenesis and progression of cancers. The methylation levels of ST6GAL1 were compared between breast cancer tissues and paired normal tissues via UALCAN. The level of ST6GAL1 promoter methylation was significantly lower in breast cancer tissues (Figure 1B) (P=5.68E-10). These results suggest that there may be a significant difference in ST6GAL1 expression between breast cancer and normal tissues.

Compared with MCF-10A cells, MDA-MB-231 and MCF-7 cells presented a remarkable increase in the protein level of ST6GAL1 (Figure 1C and D). At the mRNA level, RT–PCR analysis confirmed this tendency (Figure 1E). Figure 1F shows images of IHC-stained paracancerous and breast cancer tissue sections. The paracancerous tissue samples presented weak ST6GAL1 staining, whereas the cancer tissue samples presented high ST6GAL1 staining (Figure 1G). These data indicate that ST6GAL1 may be associated with the malignant phenotype of human breast carcinoma cells.

Altered Expression of ST6GAL1 Affects the Biological Function of Breast Cancer Cells Both in vitro and in vivo

To detect the effect of ST6GAL1 in breast cancer, an ST6GAL1 knockdown cell model was established by transfecting MDA-MB-231 and MCF-7 cells (Supplementary Figure 1A). Wound healing and transwell migration assays demonstrated that the knockdown of ST6GAL1 significantly suppressed the migration of MDA-MB-231 and MCF-7 cells (Figure 2A–D). A cell adhesion assay revealed a substantial reduction in the number of MDA-MB-231 and MCF-7 cells adhering to fibronectin following ST6GAL1 knockdown (Figure 2E and F). As shown in Supplementary Figure 1C and D, we assessed the sensitivity of MDA-MB-231 cells to 5-FU. Compared with that of the control, the viability of the MDA-MB-231 transfectants was significantly lower. As shown in Figure 3, compared with those in the control cells, the

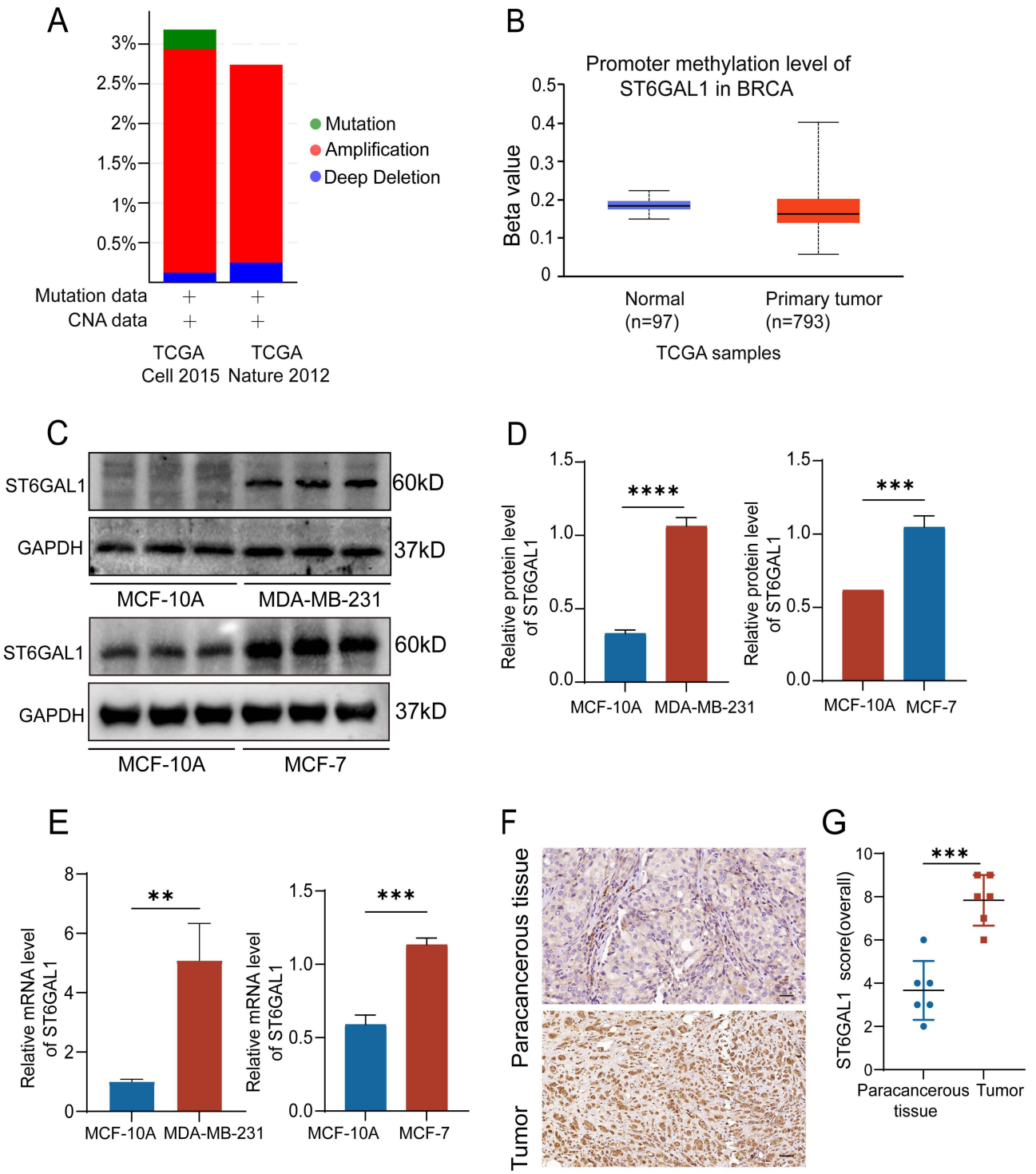

Figure 1 ST6GAL1 expression is increased in breast cancer. (**A**) Mutations in ST6GAL1 were assessed via the cBioPortal database. (**B**) The methylation levels of ST6GAL1 were compared between breast cancer tissues and paired normal tissues via UALCAN. (**C**) Western blot analysis of ST6GAL1 protein levels in MCF-10A, MDA-MB-231, and MCF-7 cells, with GAPDH as the loading control; n=3. (**D**) Quantification of ST6GAL1 protein levels in MCF-10A, MDA-MB-231, and MCF-7 cells. Data are presented as relative expression, normalized to GAPDH. (**E**) Relative mRNA expression levels of ST6GAL1 in MCF-10A, MDA-MB-231, and MCF-7 cells. Data are normalized to 18S. (**F**) Immunohistochemical staining of ST6GAL1 in para-cancerous tissue (top) and tumor tissue (bottom) from breast cancer samples. Scale bar: 20μm, n=6. (**G**) Quantification of ST6GAL1 staining intensity in para-cancerous and tumor tissues; n=6. Data are presented as the mean ± SD. **p < 0.01, ***p < 0.001, ****p < 0.0001.

expression levels of stemness-related markers (c-Myc, Oct-4, and Sox-2) were significantly lower in the MDA-MB-231-transfected and MCF-7-transfected cells. Collectively, these findings indicate that ST6GAL1 promotes the malignant phenotypes of breast cancer cells in vitro.

https://doi.org/10.2147/BCTT.S555609

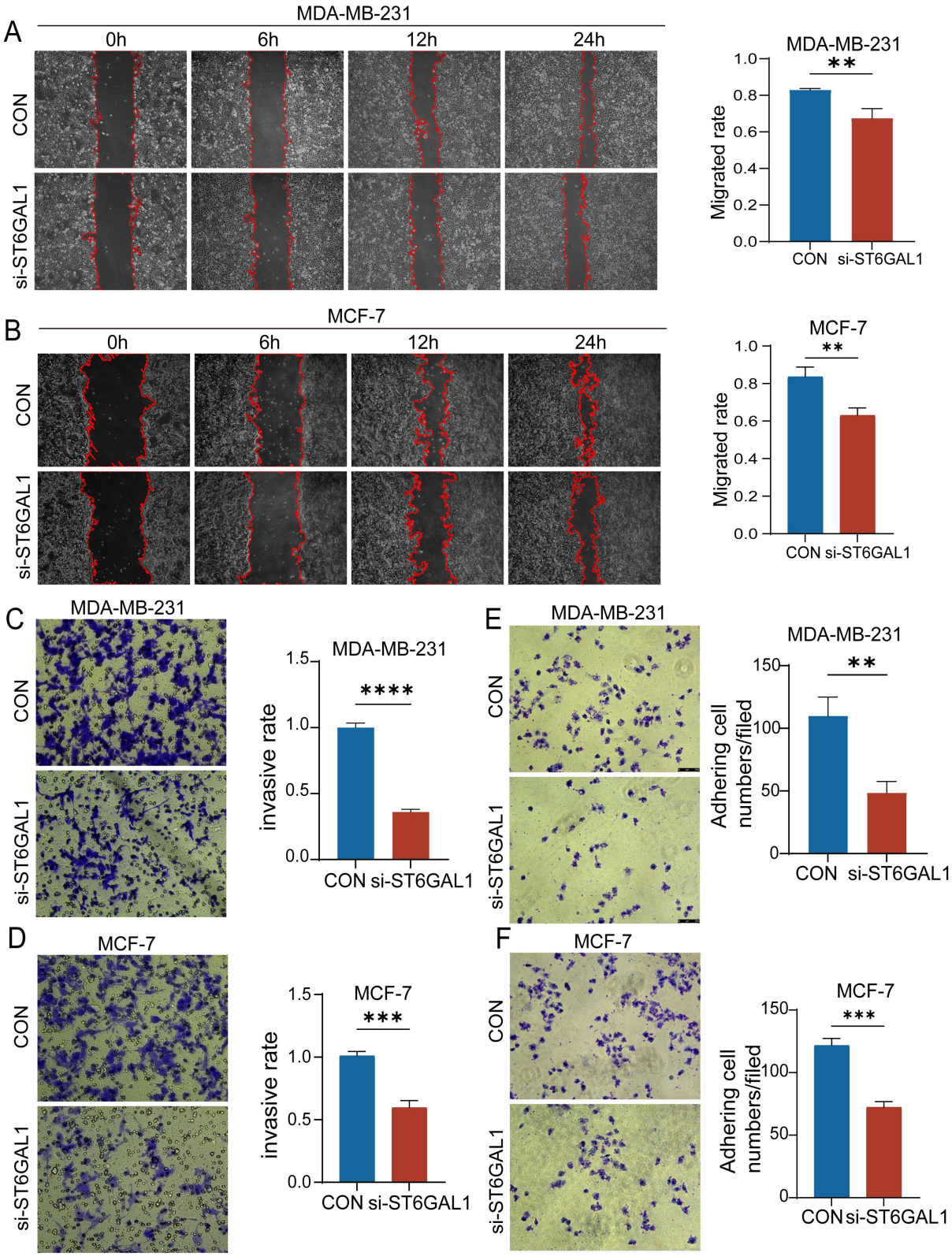

Figure 2 Effects of ST6GAL1 on the biological function of breast cancer cells in vitro. (**A** and **B**) Wound healing assay: Representative images and quantitative analysis of wound closure in MDA-MB-231 (**A**) and MCF-7 (**B**) cells at 0, 6, 12, and 24 h after transfection with si-ST6GAL1 or control (CON). Red lines indicate wound edges; n=3. (**C** and **D**) Transwell invasion assay: Representative images and quantitative analysis of invasive MDA-MB-231 (**C**) and MCF-7 (**D**) cells after ST6GAL1 silencing. Invaded cells were stained and counted under a microscope; n=3. (**E** and **F**) Cell adhesion assay: Adhesion ability of MDA-MB-231 (**E**) and MCF-7 (**F**) cells was assessed after knockdown of ST6GAL1. ST6GAL1 silencing significantly reduced the number of adhering cells per field; n=3. Data are presented as the mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

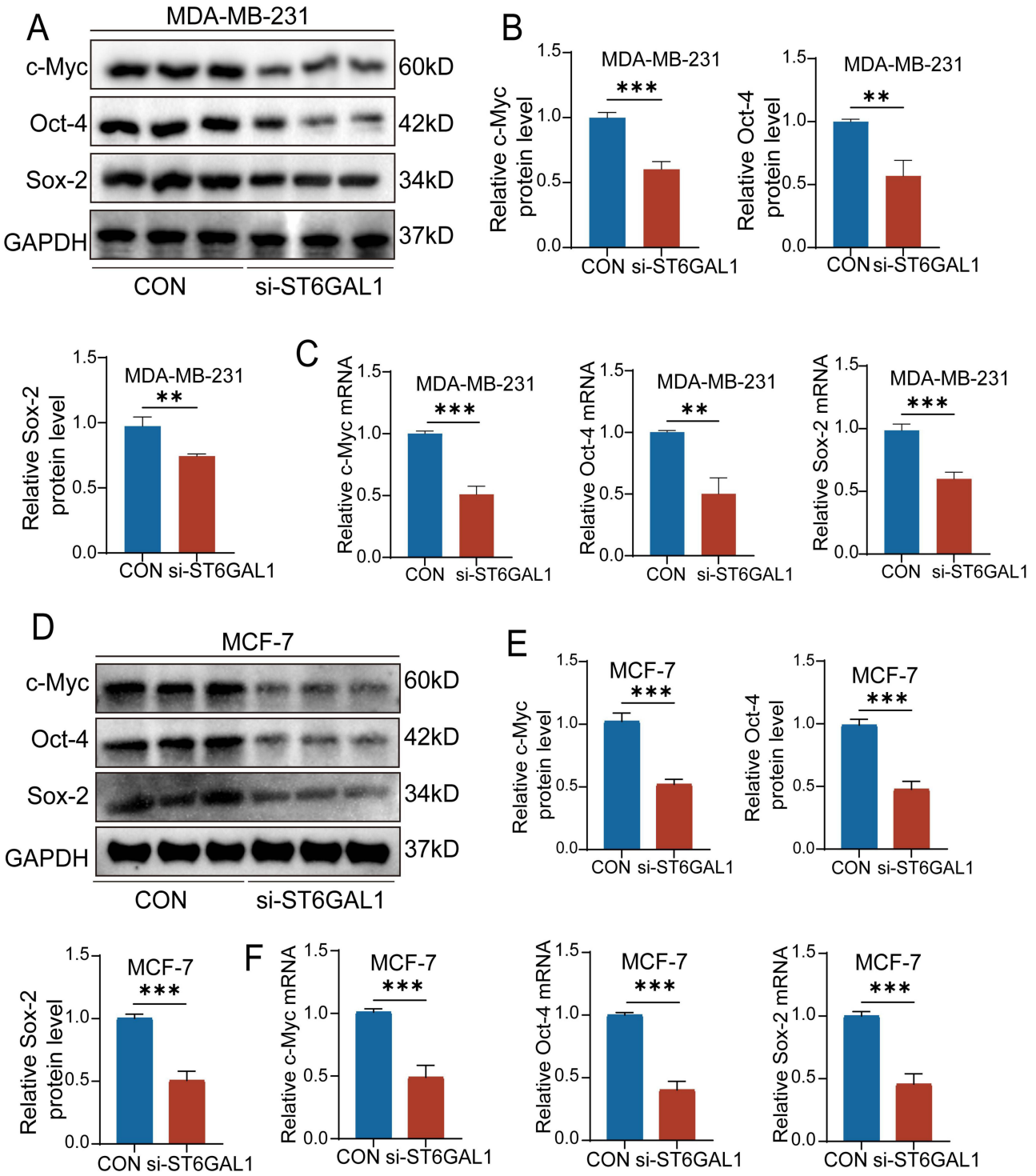

Figure 3 Effects of ST6GAL1 on the stemness of breast cancer cells. (**A** and **B**) Protein expression levels of stemness-related markers (c-Myc, Oct-4, Sox-2) in MDA-MB-231 cells, with GAPDH as the loading control. WB quantification is shown in (**B**) n=3. (**C**) mRNA expression levels of stemness-related markers (c-Myc, Oct-4, Sox-2) in MDA-MB-231 cells. Quantitative analysis of relative mRNA expression is shown in (C), with 18S as the control; n=3. (**D** and **E**) Protein expression levels of stemness-related markers (c-Myc, Oct-4, Sox-2) in MCF-7 cells, with GAPDH as the loading control. WB quantification is shown in (**E**) n=3. (**F**) mRNA expression levels of stemness-related markers (c-Myc, Oct-4, Sox-2) in MCF-7 cells, with 18S as the control; n=3. $^{**}p < 0.01$, $^{***}p < 0.001$.

ST6GAL1 Knockdown Inhibited EMT in Breast Cancer Cells

Given that ST6GAL1 expression is increased in breast cancer, we hypothesized that ST6GAL1 might be involved in the biological process of EMT in breast cancer cells. The results of the WB and RT–PCR assays revealed that Si-ST6GAL1

https://doi.org/10.2147/BCTT.S555609

treatment promoted the expression of E-cadherin but inhibited the expression of vimentin (Figure 4). To determine whether ST6GAL1 participates in the EMT process in vivo, xenograft transplantation was performed. As shown in Figure 5A–D, compared with those in the control group, the tumour volume and weight were significantly lower in the Si-ST6GAL1-transfected group. The expression trends of E-cadherin and vimentin in vivo were consistent with the RT–PCR and WB results (Figure 5E and F). These results indicate that ST6GAL1 participates in the regulation of EMT.

ST6GAL1 Mediates the Activity of the HIF-HK2 Signalling Pathway in Breast Carcinoma Cells

To elucidate the precise mechanism underlying the regulation of the EMT by ST6GAL1 in breast cancer, we first conducted KEGG pathway analysis on the RNA-seq data, which revealed a significant overlap between genes targeted by the HIF signalling pathway and genes with dysregulated ST6GAL1 expression (Figure 6A). Considering the critical role of the HIF signalling pathway, we detected the expression of HIF-1α and HK2 via WB. Compared with those of the control, the expression levels of HIF-1α and HK2 were significantly lower when ST6GAL1 was knocked down (Figure 6B and C). In vivo, the same result was observed through IHC analysis (Figure 6G and H). To further determine the role of the HIF-HK2 signalling pathway, a specific inhibitor of HIF-1α (Lificiguat) was selected to treat MDA-MB-231 cells. As shown in

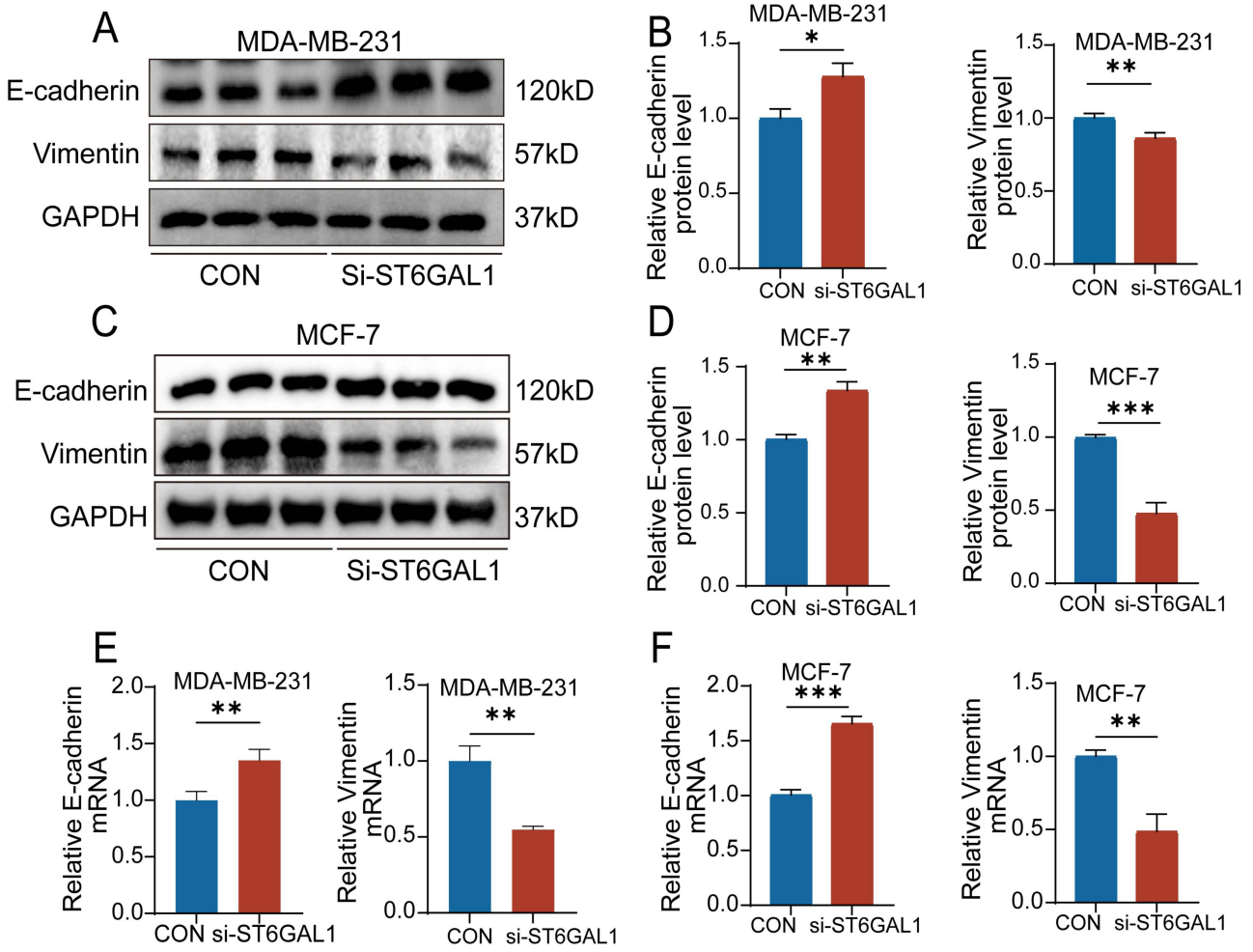

Figure 4 ST6GAL1 knockdown inhibited EMT in breast cancer cells. (**A** and **B**) Protein expression levels of EMT-related markers (E-cadherin and vimentin) in MDA-MB-231 cells, with GAPDH as the loading control. WB quantification is shown in (**B**) n=3. (**C** and **D**) Protein expression levels of EMT-related markers (E-cadherin and vimentin) in MCF-7 cells, with GAPDH as the loading control. WB quantification is shown in (**D**) n=3. (**E**) mRNA expression levels of EMT-related markers (E-cadherin and vimentin) in MDA-MB-231 cells, with 18S as the control; n=3. (**F**) mRNA expression levels of EMT-related markers (E-cadherin and vimentin) in MCF-7 cells, with 18S as the control; n=3. Data are presented as the mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.

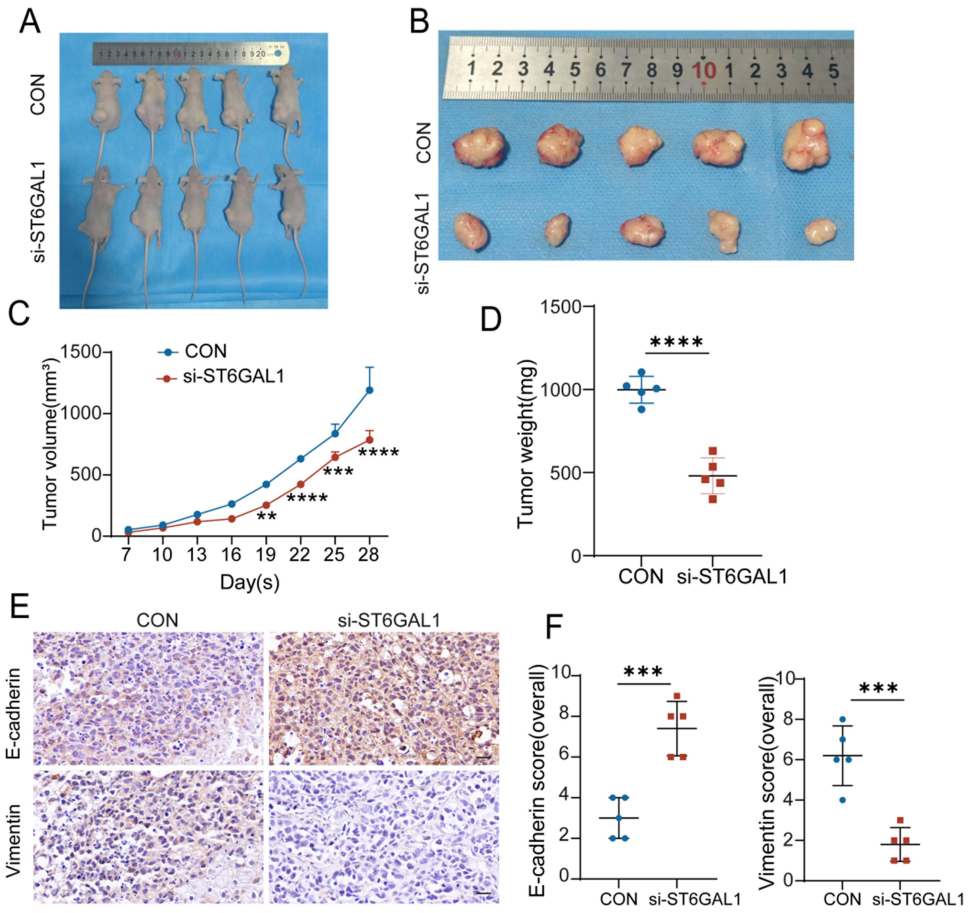

Figure 5 ST6GAL1 knockdown inhibits EMT in vivo in breast cancer mouse models. (**A**) Images of mouse models showing tumor formation in CON and si-ST6GAL1 groups; n=5. (**B**) Post-excision images of tumors from the CON and si-ST6GAL1 groups; n=5. (**C**) Tumor volume changes over time in different treatment groups, with the si-ST6GAL1 group showing a significant inhibition in tumor growth; n=5. (**D**) Final tumor weight in the si-ST6GAL1 group is significantly lower than in the CON group; n=5. (**E**) Immunohistochemical staining results showing differences in E-cadherin (top) and Vimentin (bottom) expression between the CON and si-ST6GAL1 groups. Scale bar: 20μm, n=5. (**F**) Statistical analysis showing significantly higher E-cadherin and lower Vimentin scores in the si-ST6GAL1 group compared to the CON group. Data are presented as the mean ± SD. **p < 0.01, ***p < 0.001, ****p < 0.0001.

Figure 6D–E, when ST6GAL1 was knocked down, there was a statistically significant change in the expression of E-cadherin and vimentin compared with that in the control group. However, this difference disappeared when HIF was inhibited. In summary, our findings revealed that ST6GAL1 regulates the EMT process in breast cancer cells through activation of the HIF-HK2 pathway. For further mechanistic investigation, regarding your question about how ST6GAL1 affects the HIF-1α pathway, we employed cycloheximide (CHX) to inhibit intracellular new protein synthesis and observed the degradation rate of HIF-1α by upregulating and downregulating ST6GAL1 expression. Our experiments demonstrated that ST6GAL1 overexpression slowed down HIF-1α degradation, while ST6GAL1 knockdown accelerated HIF-1α degradation, indicating that ST6GAL1 regulates the HIF-1α-HK2 pathway by slowing down HIF-1α degradation (Figure 6F).

Discussion

Many studies have established that breast cancer is a heterogeneous disease. As the subject of intense study, the process of EMT plays a critical role in tumour initiation, progression, cancer stem cell renewal and therapy resistance.[22,23] Intensive research is currently underway to identify the signalling pathways and molecular mechanisms that underlie EMT. To date, the cell signalling pathways regulating EMT include transforming growth factor-β (TGF-β), Wnt/β-catenin, and Notch.[24,25] The ultimate goal of the present study was to investigate the possible new mechanism by which ST6Gal1 regulates EMT in breast cancer cells.

https://doi.org/10.2147/BCTT.S555609

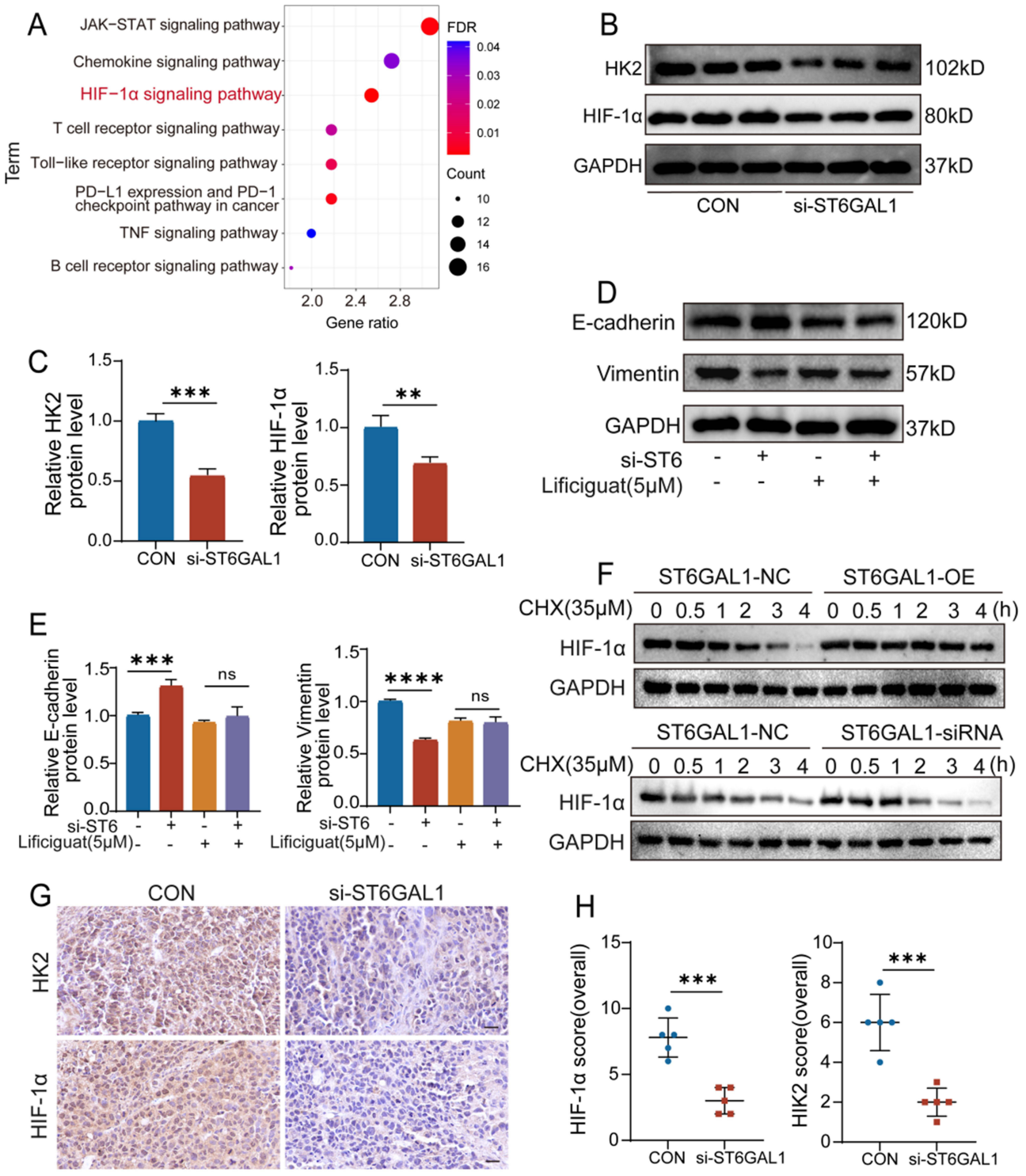

Figure 6 ST6GAL1 Mediates the Activity of the HIF-HK2 Signalling Pathway in Breast Carcinoma Cells. (**A**) KEGG pathway analysis revealed the signalling pathways related to ST6GAL1. (**B** and **C**) Protein expression levels of the HIF-HK2 signalling pathway in MDA-MB-231 cells; n=3. (**D** and **E**) Protein expression levels of EMT-related markers (E-cadherin and vimentin) in MDA-MB-231 cells when HIF-1α was inhibited; n=3. (**F**) After intervention of tumor cells with CHX, which inhibits all intracellular protein synthesis, the degradation rate of HIF-1α protein is measured following ST6GAL1 overexpression (OE) or knockdown. (**G**) Expression of HK2 (top) and HIF-1α (bottom) in tumors from the si-ST6GAL1 group showing significant changes compared to the CON group. Scale bar: 20μm, n=5. (**H**) Statistical analysis reveals significantly lower HIF-1α and HK2 scores in the si-ST6GAL1 group compared to the CON group; n=3. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Currently, many researchers have shown that the glycosylation status is closely related to cancer; however, the functional role of specific glycosyltransferases in carcinogenesis remains unclear. Gene mutations and DNA methylation can affect gene expression, cellular function and tumour occurrence.[26–28] In our study, we observed that the mutation of ST6GAL1 was amplified mainly in breast cancer and that the methylation levels were markedly lower than those in normal tissues. These results indicate that ST6GAL1 plays an essential role in tumour occurrence at both the genetic and epigenetic levels. Compared with that in normal thyroid tissue, the expression of ST6GAL1 was increased in all examined thyroid tumours.[29] However, Zhou et al reported that ST6Gal1 may participate in the inhibition of oncogenesis and malignant progression by interacting with and targeting NCAM1 in glioma.[30] To date, the involvement of ST6GAL1 in the development of breast cancer has not been widely studied. The data shown here indicate that the expression profile of the ST6GAL1 gene was altered between MCF-10A and MDA-MB-231 cells via real-time PCR and Western blot analysis and that the downregulation of ST6GAL1 expression was associated with increased cell migration, invasion and drug resistance. Taken together, these results support and emphasize that the altered expression of ST6GAL1 could be very important as an indicator and functional regulator of breast cancer.

In addition, we investigated the new molecular mechanism by which ST6GAL1 affects EMT in breast cancer. We found that ST6GAL1 was related to high activation of EMT. In line with our observations, Lu et al reported that ST6GAL1 promoted EMT in breast cancer.[31] However, to the best of our knowledge, the value of ST6GAL1 as a regulator of EMT in breast cancer patients has not been systematically evaluated. According to the literature, HK2, a hypoxia-inducible transcription factor 1α (HIF-1α)-dependent gene, can stimulate the production of lactic acid in the hypoxic environment of tumour cells.[32–34] Zheng et al reported that the HIF-1α signalling axis participated in the EMT of lung cancer.[35] The HIF pathway is activated in rapidly growing tumours and induces the expression of genes that drive anaerobic metabolism.[21] In our study, we found that ST6GAL1 regulates the EMT process in breast cancer cells through the activation of the HIF-HK2 signalling pathway.

Despite the advances made in this study, several inevitable limitations should be acknowledged. First, as this is a preliminary investigation, only five mice were used per group in the in vivo experiments, which may limit the statistical power of our analyses. Second, although we have provided some initial findings regarding the HIF-1α pathway, the detailed mechanisms underlying its regulation were not fully elucidated in this work, and further comprehensive studies are warranted to address these aspects in the future. Looking ahead, we plan to increase the sample size and conduct in-depth investigations into the interactions between ST6GAL1 and the HIF-1α pathway, with the aim of providing a more comprehensive understanding of their roles in tumorigenesis and progression.

Conclusions

In conclusion, despite much evidence that tumour-associated glycosyltransferases contribute to the malignant phenotype, the mechanistic role of ST6GAL1 in modulating cell signalling remains a markedly underinvestigated area of breast cancer. In our study, in vitro and in vivo models revealed that ST6GAL1 promotes the malignant phenotypes of breast cancer cells and regulates the EMT process through activation of the HIF-HK2 signalling pathway. Our study demonstrates a previously unrecognized function of ST6GAL1 through HIF-1α. These results shed new light on the molecular mechanisms associated with hypoxia in breast cancer and may facilitate the design of future therapeutic strategies.

Data Sharing Statement

All data supporting the findings from this study are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

The study was approved by Ethics Committee of the Second Hospital of Shanxi Medical University ((2021) YX (No.205)). The details of any images, videos, recordings, etc can be published, and that the person(s) providing consent have been shown the article contents to be published.

Author Contributions

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

Funding

This work is supported by the Natural Science Foundation of Shanxi Province (No:202103021223441), The Science Popularization and Propaganda Special Project of Shanxi Province (No:202204091001024), The Physician Research Project of Shanxi Medical Association (No:YSXH-QL2023RX006).

Disclosure

The authors declare that they have no conflicts of interest in this work.

References

1. Bencina G, Chami N, Hughes R, et al. Breast cancer-related mortality in Central and Eastern Europe: years of life lost and productivity costs. *J Med Econ*. 2023;26(1):254–261. doi:10.1080/13696998.2023.2169497
2. DeSantis CE, Ma J, Gaudet MM, et al. Breast cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(6):438–451. doi:10.3322/caac.21583
3. Tan Z, Boyapati K, Tressler CM, Jenkinson NM, Glunde K. Glutamine transporter SLC38A3 promotes breast cancer metastasis via Gsk3β/β-catenin/EMT pathway. *Cancer Lett*. 2024;1(586):216653. doi:10.1016/j.canlet.2024.216653
4. Li H, Hou M, Zhang P, et al. Wedelolactone suppresses breast cancer growth and metastasis via regulating TGF-β1/Smad signaling pathway. *J Pharm Pharmacol*. 2024;76(8):1038–1050. doi:10.1093/jpp/rgae065
5. Li X, Lewis MT, Huang J, et al. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. *J Natl Cancer Inst*. 2008;100(9):672–679. doi:10.1093/jnci/djn123
6. Büll C, Stoel MA, den Brok MH, Adema GJ. Sialic acids sweeten a tumor's life. *Cancer Res*. 2014;74(12):3199–3204. doi:10.1158/0008-5472.CAN-14-0728
7. Ghosh M, Hazarika P, Dhanya SJ, Pooja D, Kulhari H. Exploration of sialic acid receptors as a potential target for cancer treatment: a comprehensive review. *Int J Biol Macromol*. 2024;257(Pt 1):128415. doi:10.1016/j.ijbiomac.2023.128415
8. Varki A. Sialic acids in human health and disease. *Trends Mol Med*. 2008;14(8):351–360. doi:10.1016/j.molmed.2008.06.002
9. Marcos NT, Pinho S, Grandela C, et al. Role of the human ST6GalNAc-I and ST6GalNAc-II in the synthesis of the cancer-associated sialyl-Tn antigen. *Cancer Res*. 2004;64(19):7050–7057. doi:10.1158/0008-5472.CAN-04-1921
10. Schultz MJ, Holdbrooks AT, Chakraborty A, et al. The tumor-associated glycosyltransferase ST6Gal-I regulates stem cell transcription factors and confers a cancer stem cell phenotype. *Cancer Res*. 2016;76(13):3978–3988. doi:10.1158/0008-5472.CAN-15-2834
11. Swindall AF, Londoño-Joshi AI, Schultz MJ, Fineberg N, Buchsbaum DJ, Bellis SL. ST6Gal-I protein expression is upregulated in human epithelial tumors and correlates with stem cell markers in normal tissues and colon cancer cell lines. *Cancer Res*. 2013;73(7):2368–2378. doi:10.1158/0008-5472.CAN-12-3424
12. Lise M, Belluco C, Perera SP, Patel R, Thomas P, Ganguly A. Clinical correlations of alpha2,6-sialyltransferase expression in colorectal cancer patients. *Hybridoma*. 2000;19(4):281–286. doi:10.1089/027245700429828
13. Recchi MA, Hebbar M, Hornez L, Harduin-Lepers A, Peyrat JP, Delannoy P. Multiplex reverse transcription polymerase chain reaction assessment of sialyltransferase expression in human breast cancer. *Cancer Res*. 1998;58(18):4066–4070.
14. Hsieh CC, Shyr YM, Liao WY, et al. Elevation of β-galactoside α2,6-sialyltransferase 1 in a fructoseresponsive manner promotes pancreatic cancer metastasis. *Oncotarget*. 2017;8(5):7691–7709. doi:10.18632/oncotarget.13845
15. Schito L, Rey S. Cell-autonomous metabolic reprogramming in hypoxia. *Trends Cell Biol*. 2018;28(2):128–142. doi:10.1016/j.tcb.2017.10.006
16. Xie H, Simon MC. Oxygen availability and metabolic reprogramming in cancer. *J Biol Chem*. 2017;292(41):16825–16832. doi:10.1074/jbc.R117.799973
17. Wigerup C, Påhlman S, Bexell D. Therapeutic targeting of hypoxia and hypoxia-inducible factors in cancer. *Pharmacol Ther*. 2016;164:152–169. doi:10.1016/j.pharmthera.2016.04.009
18. Semenza GL. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. *J Clin Invest*. 2013;123(9):3664–3671. doi:10.1172/JCI67230
19. Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab*. 2006;3(3):187–197. doi:10.1016/j.cmet.2006.01.012
20. Jones RB, Silva AD, Ankenbauer KE, et al. Role of the ST6GAL1 sialyltransferase in regulating ovarian cancer cell metabolism. *Glycobiology*. 2023;33(8):626–636. doi:10.1093/glycob/cwad051
21. Jones RB, Dorsett KA, Hjelmeland AB, Bellis SL. The ST6Gal-I sialyltransferase protects tumor cells against hypoxia by enhancing HIF-1α signaling. *J Biol Chem*. 2018;293(15):5659–5667. doi:10.1074/jbc.RA117.001194
22. Singh A, Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. *Oncogene*. 2010;29(34):4741–4751. doi:10.1038/onc.2010.215
23. Shibue T, Weinberg RA. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. *Nat Rev Clin Oncol*. 2017;14(10):611–629. doi:10.1038/nrclinonc.2017.44

24. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol*. 2014;15(3):178–196. doi:10.1038/nrm3758
25. Zhang YE, Stuelten CH. Alternative splicing in EMT and TGF-β signaling during cancer progression. *Semin Cancer Biol*. 2024;101:1–11. doi:10.1016/j.semcancer.2024.04.001
26. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, LA D Jr, Kinzler KW. Cancer genome landscapes. *Science*. 2013;339(6127):1546–1558. doi:10.1126/science.1235122
27. Klutstein M, Nejman D, Greenfield R, Cedar H. DNA Methylation in Cancer and Aging. *Cancer Res*. 2016;76(12):3446–3450. doi:10.1158/0008-5472.CAN-15-3278
28. Wang W, Li S, Huang Y, Guo J, Sun L, Sun G. Comprehensive analysis of the potential biological significance of cuproptosis-related gene LIPT2 in pan-cancer prognosis and immunotherapy. *Sci Rep*. 2023;13(1):22910. doi:10.1038/s41598-023-50039-x
29. Gunjača I, Benzon B, Pleić N, et al. Role of ST6GAL1 in thyroid cancers: insights from tissue analysis and genomic datasets. *Int J Mol Sci*. 2023;24(22):16334. doi:10.3390/ijms242216334
30. Zhou Y, Wu Y, Shen D, et al. Evaluation of ST6Gal1 expression and clinicopathological significance in human glioma. *Clin Neuropathol*. 2024;43(4):113–126. doi:10.5414/NP301631
31. Lu J, Isaji T, Im S, et al. β-Galactoside α2,6-sialyltranferase 1 promotes transforming growth factor-β-mediated epithelial-mesenchymal transition. *J Biol Chem*. 2014;289(50):34627–34641. doi:10.1074/jbc.M114.593392
32. Tirpe AA, Gulei D, Ciortea SM, Crivii C, Berindan-Neagoe I. Hypoxia: overview on hypoxia-mediated mechanisms with a focus on the role of HIF genes. *Int J Mol Sci*. 2019;20(24):6140. doi:10.3390/ijms20246140
33. Xu F, Yan JJ, Gan Y, et al. miR-885-5p negatively regulates warburg effect by silencing hexokinase 2 in Liver cancer. *Mol Ther Nucleic Acids*. 2019;18:308–319. doi:10.1016/j.omtn.2019.09.002
34. Wu J, Chai H, Xu X, Yu J, Gu Y. Histone methyltransferase SETD1A interacts with HIF1α to enhance glycolysis and promote cancer progression in gastric cancer. *Mol Oncol*. 2020;14(6):1397–1409. doi:10.1002/1878-0261.12689
35. Zheng Y, Ji H, Yi W, et al. PRMT5 facilitates angiogenesis and EMT via HIF-1α/VEGFR/Akt signaling axis in lung cancer. *Aging*. 2023;15(13):6163–6178. doi:10.18632/aging.204826