

# RETRACTED ARTICLE: ST6GAL2 Downregulation Inhibits Cell Adhesion and Invasion and is Associated with Improved Patient Survival in Breast Cancer

This article was published in the following Dove Press journal:  
OncoTargets and Therapy

Junchi Cheng<sup>1,\*</sup>  
Rong Wang<sup>1,\*</sup>  
Guansheng Zhong<sup>2</sup>  
Xi Chen<sup>1</sup>  
Yun Cheng<sup>1</sup>  
Wei Li<sup>1</sup>  
Yunshan Yang<sup>1</sup>

<sup>1</sup>Department of Medical Oncology, Cancer Hospital of the University of Chinese Academy of Sciences, Hangzhou 310000, People's Republic of China;  
<sup>2</sup>Department of Breast Surgery, The First Affiliated Hospital, Zhejiang University, Hangzhou 310003, People's Republic of China

\*These authors contributed equally to this work

**Objective:** Breast cancer is one of the most common and serious types of cancer, with a particularly unfavorable prognosis. Although downregulation of  $\beta$ -galactoside  $\alpha$  2,6-sialyltransferase 2 (ST6GAL2) has been observed in multiple cancers, the mechanism involved remains to be clarified. In this study, we focused on the potential function of ST6GAL2 in the regulation of breast cancer.

**Methods:** Flow cytometry and CCK-8 were used to measure markers of the cell cycle proliferation, adhesion, and invasion. Real-time PCR and immunohistochemistry analysis were used to detect the expression levels of ST6GAL2 in breast cancer tissues. Western blot was used to analyze the expression levels of genes correlated with focal adhesion and metastasis pathways in breast cancer cells.

**Results:** ST6GAL2 expression levels were higher in breast cancer tissues as compared to healthy tissues. ST6GAL2 expression was associated with tumor stage, survival time, and estrogen receptor (ER)/progesterone receptor (PR)/human epidermal growth factor receptor 2 (HER2) status of breast cancer patients. Silence of ST6GAL2 inhibited cancer progression by arresting cell cycle progression at G0/G1 phase and inhibiting cell adhesion and invasion. ST6GAL2 was positively correlated with focal adhesion and metastasis pathways, and its downregulation inhibited the expression of ICAM-1, VCAM-1, CD24, MMP2, MMP9, and CXCR4.

**Conclusion:** These findings indicated that ST6GAL2 might serve as a useful potential target for treatment of breast cancer.

**Keywords:** breast cancer, ST6GAL2, prognosis, adhesion, invasion

## Introduction

Worldwide, breast cancer is the most common malignancy in women and one of the leading causes of cancer deaths.<sup>1</sup> About 260,000 new cases of invasive breast cancer and 40,000 breast cancer deaths are currently projected for the United States in 2018.<sup>2</sup> Prognosticators for individuals diagnosed with breast cancer include tumor size, pathological grade, and the presence of lymph node or distant metastases.<sup>3</sup> These parameters alone or in combination enable the identification of individuals who at increased risk of dying of breast cancer and may benefit from aggressive treatment.<sup>4</sup> Despite tremendous progress in cancer therapy, breast cancer remains a major cause of mortality, a primary factor being metastasis to distant organs.<sup>6</sup> The identification of underlying molecular mechanisms allows for further precision in predicting patient survival.<sup>5</sup> Efforts to understand the genes and

Correspondence: Yunshan Yang  
Department of Medical Oncology, Cancer Hospital of the University of Chinese Academy of Sciences, 1st Banshandong Road, Hangzhou 310000, People's Republic of China  
Tel +86-57188122052  
Email yyunshan@163.com

signaling pathways that promote tumor invasion and metastasis are important for breast cancer treatment.

Sialic acids, which are commonly found in glycoconjugates, are terminal monosaccharide attached to glycan chains on the cell surface. Sialic acids decorating the cell surface are involved in many biological processes, such as cell adhesion, receptor activation, cancer progression, metastatic spread, and signal transduction.<sup>7,8</sup> ST6GAL1 is a sialyltransferase that links the sialic acid residues to terminal galactose of glycan chains by  $\alpha 2$ , 6-linkage.<sup>9</sup> It is widespread tissue-specific distribution in mammals, and the aberrant expression of ST6GAL1 is often related to poor prognoses in colon and gastric cancer and acute myeloid leukemia.<sup>10–13</sup> In contrast to ST6GAL1, ST6GAL2, a recently identified sialyltransferase, is confined to human intestine, colon, and brain.<sup>14,15</sup> A tremendous number of studies have focused on the involvement of ST6GAL2 in tumorigenesis, such as follicular thyroid carcinoma<sup>16</sup> and melanoma.<sup>17</sup> However, the role of ST6GAL2 in breast cancer is still unclear. Further, the influence of ST6GAL2 on the proliferation and metastatic potential of cancer remains to be seen.

In order to clarify the role of ST6GAL2 in tumorigenesis and malignant progression, we first investigated ST6GAL2 expression in breast cancer tissues. We report here that ST6GAL2 is frequently overexpressed in breast cancer. More importantly, those patients with elevated ST6GAL2 expression were also found to have a significantly worse prognosis. The tumorigenic role of ST6GAL2 in breast cancer cell lines contributed to breast cancer cell proliferation, adhesion, and invasion. These data suggest that ST6GAL2 is a potent oncogene and potential target for treatment of breast cancer.

## Materials and Methods

### Patients and Tissue Samples

A total of 633 breast cancer patients were recruited from The First Affiliated Hospital of Zhejiang University between January 2005 and March 2009. The samples used were not subjected to preoperative radiotherapy and/or chemotherapy. Among the 633 breast cancer patients, randomly selected 40 paired tumor and adjacent normal tissues at least 5 cm from the tumor regions. Tissue was collected for real-time PCR. Tumor tissues from the 633 breast cancer patients were collected for immunohistochemistry (IHC) as previously described.<sup>18</sup> The patients' clinical characteristics, including age, tumor site, histological type, AJCC stage, tumor stage, lymph node involvement, estrogen receptor (ER)/progesterone

receptor (PR)/human epidermal growth factor receptor 2 (HER2) status, and prognosis, were collected for statistical analysis. Ethical approval for the study was provided by the independent ethics committee of The First Affiliated Hospital of Zhejiang University. Informed and written consent was obtained from all patients according to the ethics committee guidelines.

## Cell Culture and Transfection

MDA-MB-435S, MDA-MB-231, MCF-7, ZR-75-30, and T47D are breast cancer cells obtained from Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and incubated in a humidified atmosphere at 37°C with 5% CO<sub>2</sub>. Short hairpin RNA (shRNA) for ST6GAL2 (20 nM) and control shRNA (CCACACUAGUCCUCAA) were from Genesil Biotechnology (Shanghai, China). shRNA targeting position 1133–1155 (CCACAUACGCAUCAUUA; named ST6GAL2 shRNA) of human ST6GAL2 mRNA was cloned into lentiviral vector (PLKO.1-C1). Cell transfection was performed with Lipofectamine 2000 (Invitrogen, Shanghai, China) following the manufacturer's protocol. Nonspecific shRNA was used as a negative control (NC). At 48 h after transfection breast cancer cells were transduced with lentivirus to knockdown ST6GAL2. The selective silencing of ST6GAL2 was identified by Western blot analysis.

## Reverse Transcription and Real-Time PCR

Total RNA was isolated from human breast cancer patients' tissue and transfected cells using Trizol reagent (Invitrogen, Shanghai, China). Reverse transcription reactions were performed as described.<sup>19</sup> Real-time PCR was performed using a standard SYBR Green PCR kit protocol on ABI7300 (Applied Biosystem, Shanghai, China) thermal cycler. The GAPDH RNA was used as internal controls for ST6GAL2. The 2<sup>−ΔΔCt</sup> method for relative quantification of gene expression was used to determine mRNA expression levels. The PCR primers are listed in Table 1.

## Western Blot

Cultured or transfected cells were harvest and washed twice with PBS and lysed in ice-cold radio immunoprecipitation assay buffer (RIPA, Beyotime, Shanghai, China) with freshly added 0.01% protease inhibitor cocktail (Sigma, Shanghai, China) and incubated on ice for 30 min. Cell lysis was centrifuged at 400×g for 10 min at 4°C and the supernatant (20–30 μg of protein) was run on 10% SDS-PAGE gel and

**Table 1** Primer Sequences Used in This Study

| Gene            | Sequences                  |
|-----------------|----------------------------|
| ST6GAL2-forward | 5'-CCCACGTTACACCATTCCTC-3' |
| ST6GAL2-reverse | 5'-GGTGGCTCACGCCTATAATC-3' |
| ICAM-1-forward  | 5'-AGGTGACCGTGAATGTGCTC-3' |
| ICAM-1-reverse  | 5'-AGGGAGGCGTGGCTTGT-3'    |
| VCAM-1-forward  | 5'-CAACCGTCTTGGTCAGCC-3'   |
| VCAM-1-reverse  | 5'-CTGCTCCACAGGATTTTCG-3'  |
| CD24-forward    | 5'-ATGGGCAGAGCAATGGTG-3'   |
| CD24-reverse    | 5'-GGTGGTGGCATTAGTTGGAT-3' |
| MMP2-forward    | 5'-GCTTCCAGGGCACATCC-3'    |
| MMP2-reverse    | 5'-CCTTCTGAGTTCCACCAAC-3'  |
| MMP9-forward    | 5'-TCCACCCTTGTGCTCTTCC-3'  |
| MMP9-reverse    | 5'-TCTGCCACCCGAGTGTAAAC-3' |
| CXCR4-forward   | 5'-AGTCTGGACCGCTACCTGG-3'  |
| CXCR4-reverse   | 5'-GCAAAGATGAAGTCGGGAAT-3' |
| GAPDH-forward   | 5'-CACCCACTCCTCCACCTTTG-3' |
| GAPDH-reverse   | 5'-CCACCACCCTGTTGCTGTAG-3' |

transferred by electrophoresis to a polyvinylidene fluoride membrane (Millipore, Shanghai, China). The blots were blocked with 5% skim milk, followed by incubation with antibodies specific against ST6GAL2, CXCR4, MMP2, MMP9, ICAM-1, VCAM-1, CD24 (Abcam, Cambridge, MA, USA) and GAPDH (CST, Beijing, China). Blots were then incubated with goat anti-mouse or anti-rabbit secondary antibody (Beyotime, Shanghai, China) and visualized using enhanced chemiluminescence (ECL, Thermo Scientific, Shanghai, China).

### Cell Viability Analysis

Cells were transduced with lentivirus to knockdown ST6GAL2 or a negative control, seeded in a 96-well plate ( $1 \times 10^3$  cells/well) and cultured at 37°C and 5% CO<sub>2</sub>. After incubation for 0, 12, 24, 48, and 72 h, cell viability was assessed using a CCK-8 assay kit (Beyotime, Shanghai, China). All tests were performed in triplicate for each group and the experiment was repeated at least three times.

### Cell Cycle Analysis

To determine cell cycle properties, cells were transduced with lentivirus to knockdown ST6GAL2 or a negative control for 36 h. Transduced cells were harvested by trypsinization and incubated with 100 µg/mL propidium iodide (PI) and 0.5 µg/mL RNase A for 30 min at room temperature before subjecting to fluorescence-activated cell sorting using a flow cytometer (BD biosciences, Bedford, MA).

### In vitro Adhesion Assay

Cells were transduced with lentivirus to knockdown ST6GAL2 or a negative control were digested by 0.25% trypsin (Solario, Beijing, China) and then suspended in DMEM containing 10% FBS. Cells were seeded on fibronectin-coated 12-plate microplate at a density of  $1 \times 10^5$  cells/well and then incubated for 1 h. The supernatant was discarded and cells were washed twice with phosphate-buffered saline (PBS, Gibco). Four percent paraformaldehyde (Gibco) was supplemented for 15 min and cells were stained by Giemsa (Gibco) for 30 min. Then, cells were washed several times and photographed and counted under microscope with a magnification of  $\times 200$ .

### In vitro Invasion Assay

Invasion assays were performed using Transwell chamber (Greiner Bio-One, Frickenhausen, Germany) coated with Matrigel (BD, San Diego, CA, USA) as described in the manufacturer's protocol. Cells transduced with lentivirus to knockdown ST6GAL2 or a negative control were serum-starved for 24 h and harvested by trypsinization. Equal numbers of cells ( $1 \times 10^5$ /well) in serum-free medium were seeded into the upper well of the transwell chamber. The lower chamber was filled with DMEM containing 10% FBS. After 48 h incubation, cells on the upper well were wiped off by the Q-tip. The cells attached to the lower surface were washed with PBS, fixed in 4% paraformaldehyde and stained by 0.5% crystal violet. The invaded cells from at least five random microscopic fields were photographed and counted under microscope with a magnification of  $\times 200$ .

### Bioinformatics Analysis

To validate the correlation of ST6GAL2 with signaling pathways in breast cancer, a gene set enrichment analysis (GSEA) was performed to analyze the breast cancer tumors in TCGA-KEGG database.

### In vivo Tumorigenesis in Nude Mice

A total of  $2 \times 10^6$  logarithmically growing MCF-7 cells transduced with lentivirus to knockdown ST6GAL2 or a negative control in 0.1 mL PBS were subcutaneously injected into the right armpit of 4-week-old female athymic nude mice (n = 6). The nude mice were killed and tumor tissues were excised and weighed 33 d post-injection. All animal experiments were approved by the institutional animal care and use committee at The First Affiliated Hospital of Zhejiang University. All mouse work was performed in

accordance with institutional, Institutional Animal Care and Use Committee (IACUC) and Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) guidelines.

## Statistical Analysis

Three replicates and three independent experiments were done for cell-based assays. Data were presented as mean  $\pm$  SD and analyzed with GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). Statistical analyses were performed with Student's *t* test or ANOVA. A Chi-square test was used to analyze the relationship between ST6GAL2 expression level and clinicopathological characteristics. The survival curves were estimated by the Kaplan–Meier method and the resulting curves were compared using the Log-rank test. All tests were two-tailed, and the significance level was set at \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ .

## Results

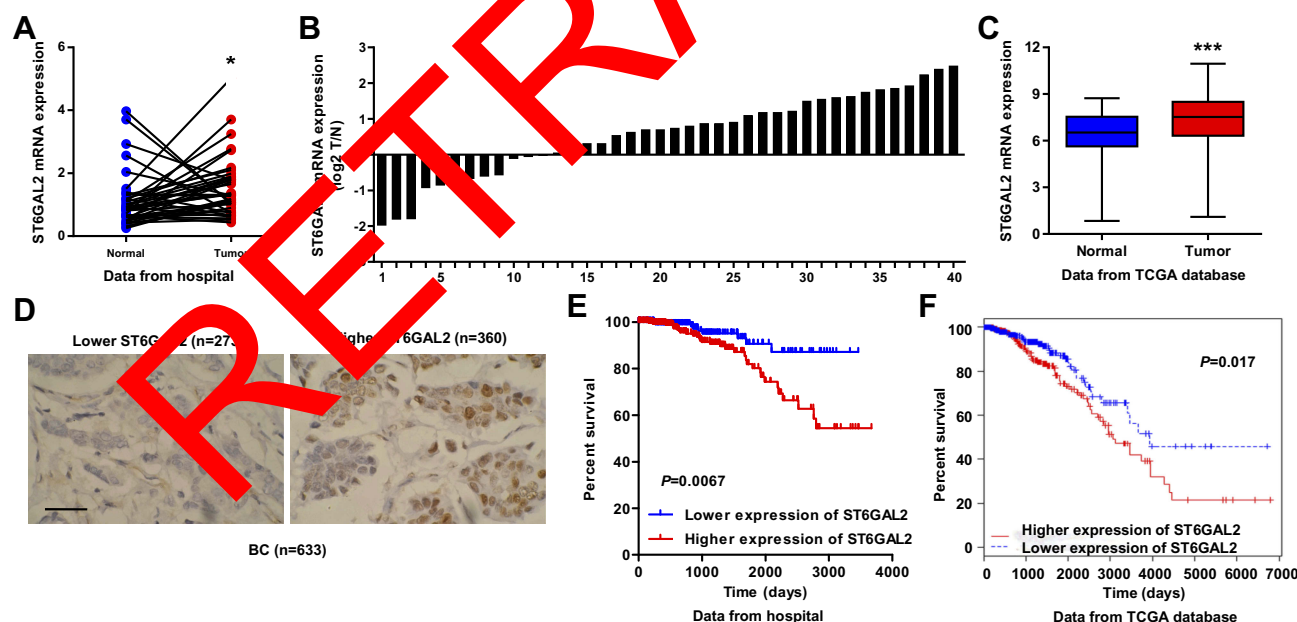
### ST6GAL2 Expression Discriminates Between Normal and Breast Cancer Tissues

To study the biological role of ST6GAL2 in breast cancer, we first used real-time PCR to detect the expression levels of ST6GAL2 in breast cancer patient tissues. We collected

tumor and adjacent normal tissues from 40 breast cancer patients at The First Affiliated Hospital of Zhejiang University. As shown in Figure 1A, ST6GAL2 mRNA level was higher in breast cancer tissues compared with adjacent normal tissues ( $P < 0.05$ ). The ST6GAL2 mRNA levels were also shown in 40 cases of breast cancer patients (Figure 1B). ST6GAL2 expression in breast cancer patients' tissues from TCGA independent database was consistent with that from The First Affiliated Hospital of Zhejiang University cohort (Figure 1C,  $P < 0.001$ ).

### Correlation Between ST6GAL2 Expression and Clinicopathological Characteristics of Breast Cancer

Using IHC detection, breast cancer patients were divided into two groups according to the expression of ST6GAL2. Patients with at least 25% of the tumor cells with positive staining were defined as ST6GAL2 high expression group and those with less than 25% of the tumor cells with positive staining were classified as ST6GAL2 low expression group (Figure 1D). Examination of the correlation between ST6GAL2 expression and clinical pathological features showed that ST6GAL2 expression was correlated with tumor stage, and ER/PR/HER2 status (Table 2).



**Figure 1** Correlation between ST6GAL2 expression and survival time of patients with breast cancer. (A, B) The expression level of ST6GAL2 detected by real-time PCR in breast cancer (n=40) and adjacent normal tissues (n=40) from patients at The First Affiliated Hospital of Zhejiang University. (C) The expression level of ST6GAL2 in breast cancer (n=1040) and adjacent normal tissues (n=112) from the TCGA database. Results are reported as mean  $\pm$  SD and analyzed with Student's *t*-test. (D) IHC staining of ST6GAL2 in breast cancer tissues from patients at The First Affiliated Hospital of Zhejiang University. Scale bars: 50  $\mu$ m. (E, F) The survival time of breast cancer specimens from patients at The First Affiliated Hospital of Zhejiang University and TCGA database was estimated by the Kaplan–Meier method using the Log-rank test. \* $P < 0.05$ , \*\*\* $P < 0.001$  compared with normal.



**Table 2** Relationship Between ST6GAL2 Expression Level and Clinicopathological Parameters of BC

| Variable          | Number of Cases | ST6GAL2      |             |         |
|-------------------|-----------------|--------------|-------------|---------|
|                   |                 | High (n=360) | Low (n=273) | P value |
| Age (years)       |                 |              |             | 0.1772  |
| ≥58               | 326             | 177          | 149         |         |
| <58               | 307             | 183          | 124         |         |
| Histological type |                 |              |             | 0.2130  |
| Ductal            | 537             | 299          | 238         |         |
| Lobular           | 64              | 43           | 21          |         |
| Other             | 32              | 18           | 14          |         |
| Tumor site        |                 |              |             | 0.8651  |
| Left              | 350             | 198          | 152         |         |
| Right             | 283             | 162          | 121         |         |
| AJCC stage        |                 |              |             | 0.4300  |
| I                 | 116             | 73           | 43          |         |
| II                | 363             | 200          | 163         |         |
| III               | 142             | 79           | 63          |         |
| IV                | 12              | 8            | 4           |         |
| Tumor stage       |                 |              |             | 0.0012  |
| T1                | 177             | 120          | 57          |         |
| T2                | 373             | 200          | 173         |         |
| T3                | 65              | 28           | 37          |         |
| T4                | 18              | 12           | 6           |         |
| Lymph node status |                 |              |             | 0.406   |
| Metastasis        | 325             | 190          | 135         |         |
| No metastasis     | 308             | 170          | 138         |         |
| ER status         |                 |              |             | <0.0001 |
| Positive          | 491             | 255          | 186         |         |
| Negative          | 142             | 55           | 87          |         |
| PR status         |                 |              |             | <0.0001 |
| Positive          | 420             | 276          | 131         |         |
| Negative          | 96              | 84           | 122         |         |
| HER2 status       |                 |              |             | 0.0332  |
| Positive          | 86              | 58           | 28          |         |
| Negative          | 547             | 302          | 245         |         |

**Note:** Differences between groups were done by the Chi-square test.

**Abbreviations:** ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor type 2.

However, we did not find any association between ST6GAL2 expression levels and other clinical pathological features including patients' age, histological type, tumor site, AJCC stage, and lymph node involvement (Table 2). The survival time of breast cancer patients showed that lower-ST6GAL2-expressing patients lived notably longer than higher-ST6GAL2-expressing patients

(Figure 1E,  $P=0.0067$ ). Similar results were found in the TCGA database (Figure 1F,  $P=0.017$ ).

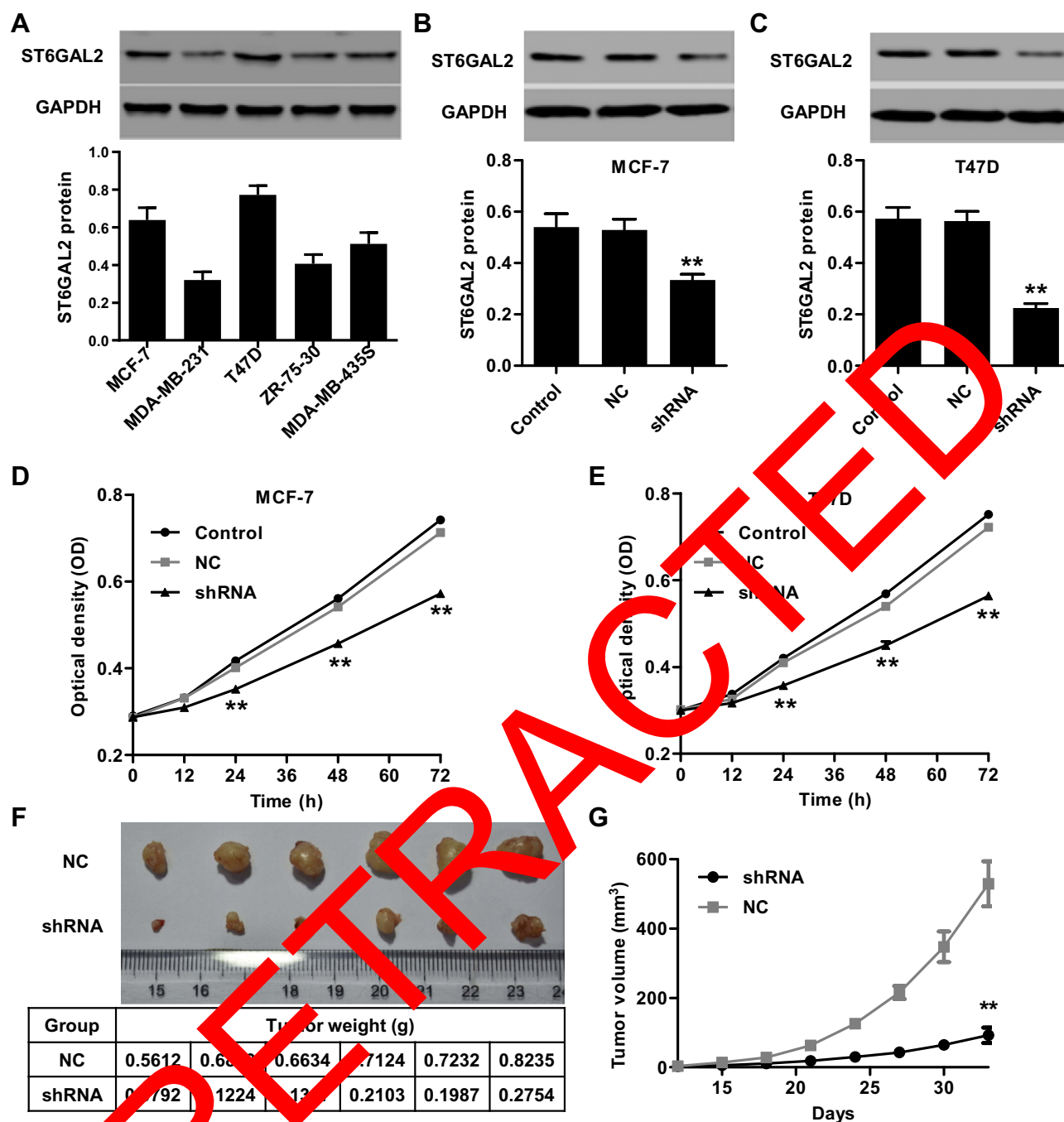
## Silencing of ST6GAL2 Represses Breast Cancer Cell Viability

Having documented significant upregulation of ST6GAL2 in clinical breast cancer tissues, we also examined the expression levels of ST6GAL2 in several breast cancer cell lines, MDA-MB-435S, MDA-MB-231, MCF-7, ZR-75-30, and T47D by Western blot (Figure 2A). ST6GAL2 was expressed at higher level in MCF-7 and T47D cells compared with the three other breast cancer cell lines. MCF-7 and T47D cells were transduced with lentivirus to knockdown ST6GAL2 for a negative control. The reduction of ST6GAL2 protein levels in MCF-7 cells was  $36.7\% \pm 0.02\%$  compared with the negative control group (Figure 2B,  $P<0.01$ ). And reduction of ST6GAL2 protein levels in T47D cells was  $60.2\% \pm 0.048\%$  compared with the negative control group (Figure 2C,  $P<0.01$ ).

Cell viability was analyzed using CCK-8 assay at 0, 24, 48, and 72 h after transfection. As shown in Figure 2D and E, ST6GAL2 significantly inhibited cell viability in MCF-7 and T47D at 24, 48, and 72 h compared with negative control groups ( $P<0.01$ ). Next, we determined the effect of ST6GAL2 knockdown on the tumor growth in vivo. MCF-7 cells transduced with a lentivirus to knockdown ST6GAL2 or a negative control were subcutaneously injected into athymic nude mice and tumor volumes were measured for 33 days. As shown in Figure 2F, ST6GAL2 downregulated tumors grew slower in mice compared with the negative control tumors in mice. After 33 days, the tumor volume in ST6GAL2 downregulated mice were significantly reduced compared with those in negative control mice (Figure 2G;  $P<0.01$ ). These data suggest that inhibition of ST6GAL2 in breast cancer reduces tumor growth in nude mice.

## Silencing of ST6GAL2 Suppresses Breast Cancer Cell Cycle Progression

To further validate the cell proliferation inhibition of ST6GAL2, cell cycle progression was analyzed in MCF-7 and T47D cells. Cell cycle analysis showed that silencing ST6GAL2 notably increased the G0/G1 phase fraction and reduced the S phase fraction (Figure 3A and B,  $P<0.01$ ). Following ST6GAL2 silencing, MCF-7



**Figure 2** ST6GAL2 promotes breast cancer cell viability in vitro and tumor growth in vivo. (A) Expression of ST6GAL2 in five breast cancer cell lines detected by Western blot. (B, C) The expression of ST6GAL2 was suppressed in MCF-7 and T47D cells. MCF-7 and T47D cells were transfected with lentivirus to knockdown ST6GAL2 or with a negative control (NC), and (D, E) at 0, 12, 24, 48, and 72 h after transfection, cell viability was detected by CCK-8 assay. Results are reported as mean  $\pm$  SD (n=3). MCF-7 cells transfected with lentivirus to knockdown ST6GAL2 or NC in 0.1 mL PBS were subcutaneously injected into the right armpit of nude mice. Thirty-three days after injection, tumor weight (F) and volume (G) were measured. Results are reported as mean  $\pm$  SD (n=6). Data are statistically analyzed with (A–C) one-way or (D, E, G) two-way ANOVA followed by post-hoc Tukey's test. \*\* $P < 0.01$  compared with NC.

cells were distributed across the G0/G1 ( $58.21\% \pm 1.23\%$ ), S ( $23.8\% \pm 0.76\%$ ) and G2/M ( $11.59\% \pm 0.39\%$ ) phases. While the negative control was distributed more evenly between the G0/G1 ( $41.69\% \pm 1.09\%$ ) and S ( $37.66\% \pm 2.69\%$ ), with  $15.97\% \pm 1.04\%$  in the G2/M phase.

ST6GAL2 silencing in T47D cells showed similar results: G0/G1 ( $58.43\% \pm 1.43\%$ ), S ( $24.36\% \pm 3.03\%$ ), and G2/M ( $13.02\% \pm 2.96\%$ ). Negative control T47D cells did not favor G0/G1 ( $45.32\% \pm 0.89\%$ ) over S phase ( $35.14\% \pm 1.20\%$ ) and G2/M ( $11.78\% \pm 2.47\%$ ) phases as strongly.

These results indicated that silencing ST6GAL2 in breast cancer cells may inhibit cell proliferation by arresting cell cycle progression at G0/G1 phase.

## Silencing of ST6GAL2 Inhibits Breast Cancer Cell Adhesion and Invasion

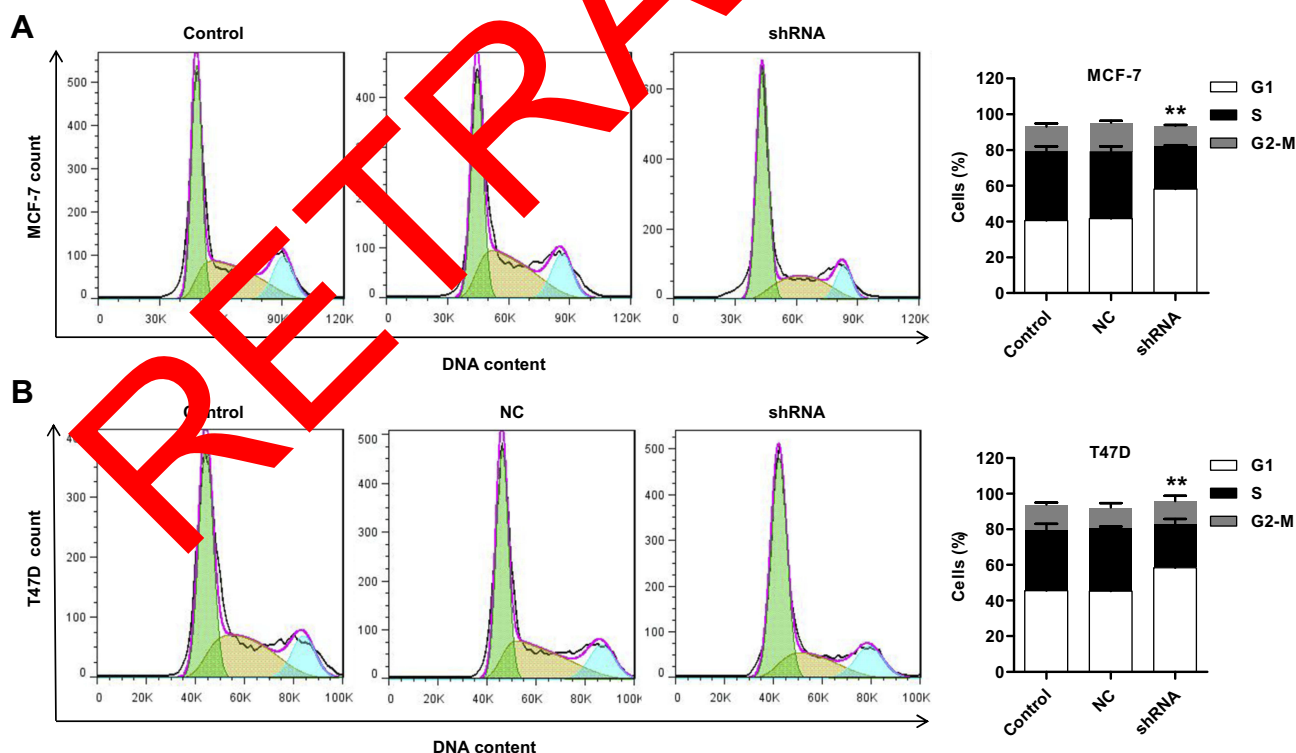
Carcinoma cell adhesion to extracellular matrix and basement membranes is regarded as an initial step in the invasion step of metastasis.<sup>20,21</sup> The effects of ST6GAL2 on breast cancer cell adhesion were identified. As shown in Figure 4A and B, ST6GAL2 silencing could notably suppress cell adhesion of MCF-7 by  $78.2\% \pm 0.085\%$  and that of T47D cells by  $73.8\% \pm 0.071\%$  compared with the negative control group ( $P < 0.01$ ). Moreover, as illustrated in Figure 4C and D, ST6GAL2 shRNA, but not negative control shRNA, effectively suppressed MCF-7 and T47D cells invasion. The number of MCF-7 and T47D cells that invaded through the filter decreased by  $55.8\% \pm 0.054\%$  and  $55.9\% \pm 0.041\%$ , respectively, when compared to cells transfected with negative control shRNA ( $P < 0.01$ ). These results indicate that ST6GAL2 is a critical mediator involved in cell adhesion.

## ST6GAL2-Stimulated Breast Cancer Cell Motility Is Mediated Through the Focal Adhesion and Metastasis Pathways

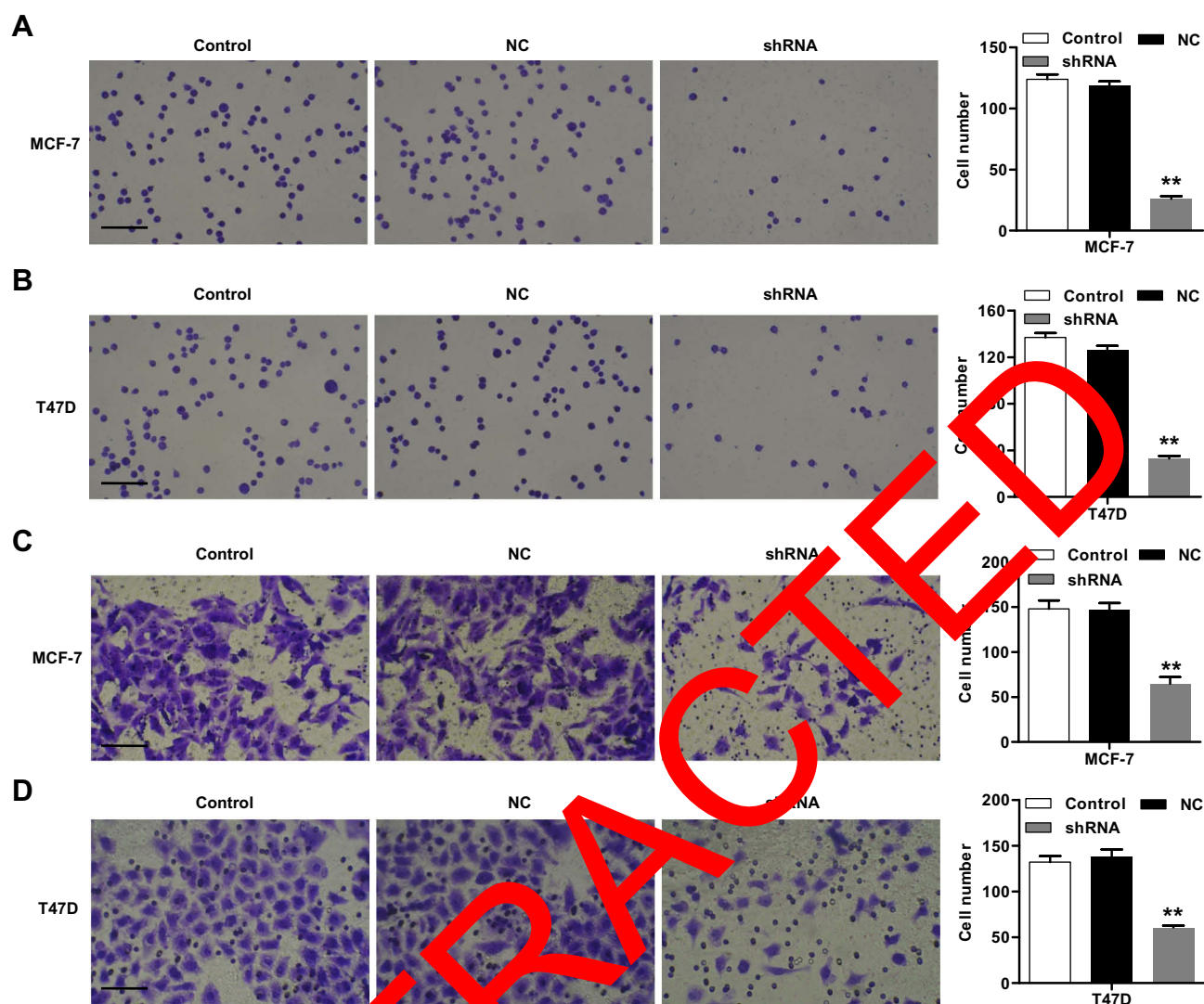
Having documented significant decrease in adhesion and invasion of breast cancer cells transduced with lentivirus to knockdown ST6GAL2, we further confirmed the correlation of ST6GAL2 with breast cancer cell motility. GSEA demonstrated that the focal adhesion and metastasis pathways were partially regulated by ST6GAL2 (Figure 5A and D,  $P < 0.01$ ). In order to probe the ST6GAL2-associated pathways in breast cancer cells, the expression of related genes involved in these pathways were determined by real-time PCR and Western blot analysis in MCF-7 and T47D cells. As shown in Figure 5B–F, knockdown of ST6GAL2 in MCF-7 and T47D cells downregulated the mRNA and protein expression of ICAM-1, VCAM-1, E-cadherin, MMP2, MMP9, and CXCR4 ( $P < 0.05$ ,  $P < 0.01$ ). These results indicate that the motility promoting function of ST6GAL2 most likely acts through regulating the focal adhesion and metastasis-related proteins.

## Discussion

Cell surface carbohydrate structures containing sialic acid play a crucial role in cellular interaction mechanisms such



**Figure 3** ST6GAL2 promotes breast cancer cell cycle progression. MCF-7 and T47D cells were transduced with lentivirus to knockdown ST6GAL2 or with an NC, and (A, B) cell cycle profile of MCF-7 and T47D was analyzed using flow cytometry. Results are reported as mean  $\pm$  SD ( $n=3$ ) and statistically analyzed with two-way ANOVA followed by post-hoc Tukey's test. \*\* $P < 0.01$  compared with NC.



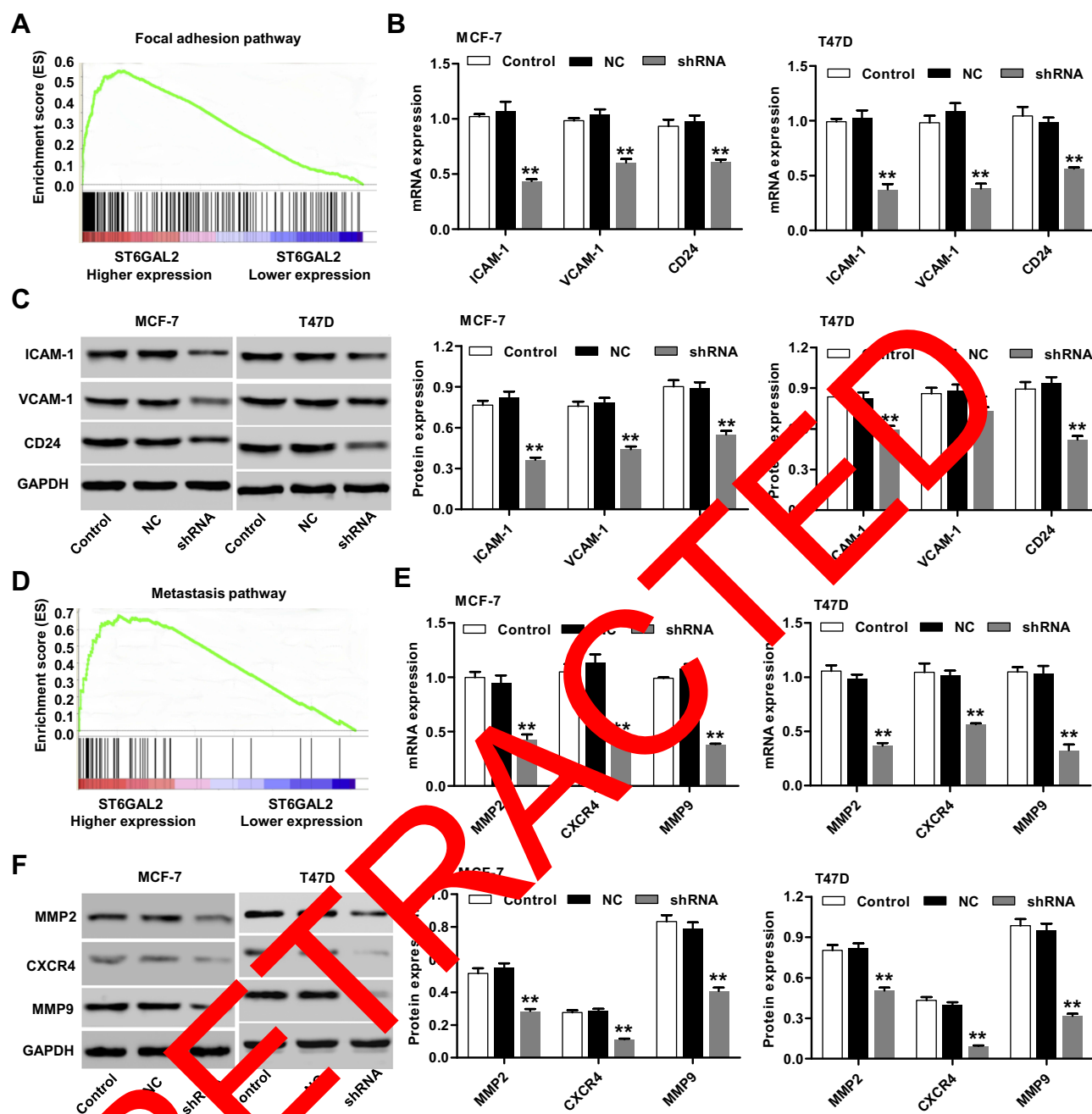
**Figure 4** Effect of ST6GAL2 knockdown on cell adhesion and invasion of breast cancer cell lines. MCF-7 and T47D cells were transduced with lentivirus to knockdown ST6GAL2 or with an NC. (A, B) Adhesion analysis was performed in MCF-7 and T47D cells; (C, D) Matrigel transwell analysis was performed to determine the invasion of MCF-7 and T47D cells. Scale bars: 50  $\mu$ m. Results are reported as mean  $\pm$  SD (n=3) and statistically analyzed with one-way ANOVA followed by post-hoc Tukey's test. \*\*P<0.01 compared with NC.

as cell-to-cell, cell-to-matrix, and cell-to-protein recognition.<sup>22</sup> ST6GAL2 is a sialyltransferase of increasing concern in carcinogenesis. Recently, some signs of progress have been gained in revealing the mechanism of ST6GAL2 in cancer.<sup>23,24</sup> Our study continued to investigate the role of ST6GAL2 in breast cancer and its possible mechanisms. Aberrant expression of sialylated glycans has been detected in cancers of pancreas, gastric, breast, bladder, brain, and colon.<sup>25–27</sup> However, data concerning the expression level of ST6GAL2 and its relevance to clinicopathologic behavior of breast cancer is unclear. In this study, we measured the expression profile of ST6GAL2 in breast cancer tissues from The First Affiliated Hospital of Zhejiang University and TCGA database using real-

time PCR analysis. Breast cancer tissues showed higher expression levels of ST6GAL2 compared to the normal breast tissues. Paradoxically, ST6GAL2 expression was upregulated in follicular thyroid carcinoma tissues,<sup>16</sup> but down-regulated in liver cancer tissues compared with non-cancer tissues.<sup>28</sup>

We next focused on the potential relationship between ST6GAL2 and the clinicopathological characteristics and survival rates of breast cancer patients. ST6GAL2 expression is significantly correlated with tumor stage, ER/PR/HER2 status, and the prognosis of patients with breast cancer. Previous studies have demonstrated that ST6GAL2 is upregulated in invasive ductal carcinoma compared to ductal carcinoma in situ, even in invasive





**Figure 5** GSEA enrichment plots of focal adhesion and metastasis pathways. **(A, D)** Genes in the focal adhesion and metastasis pathways showed significant enrichment in ST6GAL2 high versus low breast cancer. The top portion of the figure plots the enrichment scores (ES) for each gene, whereas the bottom portion of the plot shows the value of the ranking metric moving down the list of ranked genes. MCF-7 and T47D cells were transduced with lentivirus to knockdown ST6GAL2 or a negative control (NC). **(B, C)** Real-time PCR and Western blot analysis identified significant decrease in ICAM-1, VCAM-1, and CD24 expression in MCF-7 and T47D cells; **(E, F)** real-time PCR and Western blot analysis identified significant decrease in MMP2, MMP9, and CXCR4 expression in MCF-7 and T47D cells. Results are reported as mean  $\pm$  SD (n=3) and statistically analyzed with two-way ANOVA followed by post-hoc Tukey's test. \* $P < 0.05$ , \*\* $P < 0.01$  compared with NC.

HR (hormone receptors, including ER and PR)+/HER2- ductal carcinoma, but not in HR+/HER2+ ductal carcinoma,<sup>18</sup> suggesting the involvement of ST6GAL2 in the progression of the HER2+ subtype of breast cancer. Tumor subtype is an important prognostic factor for breast cancer survival, but its importance depends on the definition of subtype to some extent. T1 tumors have the same

risk of mortality regardless of ER/PR/HER2 subtype, and ER and PR negativity play a stronger role in survival than HER2 positivity.<sup>29</sup> However, HER2-positive T1 tumors have a significant risk of relapse when compared with HER2-negative tumors,<sup>30,31</sup> indicating that even small HER2-positive tumors, regardless of their ER and PR status, also need treatment. Here, the risk of mortality for

the breast cancer patients with higher ST6GAL2 levels is higher than those with lower ST6GAL2 levels regardless of tumor stage and ER/PR/HER2 status.

The precise molecular mechanisms behind the altered expression of ST6GAL2 in breast cancer are unclear. In order to further investigate the role of ST6GAL2 in breast cancer carcinogenesis, we evaluated the effect of ST6GAL2 expression on breast cancer cell viability and cell cycle progression. The results suggest that cells with a stable knockdown of ST6GAL2 proteins showed a significant inhibition of cell viability and arrest of cell cycle at G0/G1 phase, compared with the negative control group. In previous studies, it reported that ST6GAL2 promotes cell proliferation of follicular thyroid carcinoma,<sup>16</sup> induces apoptosis in mammalian cells,<sup>32</sup> and involves in multiple effects of Taxol on modulation of the cell growth, cell cycle, and apoptosis in cervical cancer.<sup>33</sup>

The hallmark of malignancy is the ability of cancer cells to invade distant tissue. Local invasion begins when cancer cells adhere to the basement membrane and invade the interstitial matrix. Previous studies have reported that ST6GAL1 promotes cell migration and invasion by activating PI3K/Akt signaling,<sup>34</sup> and ST6GALNAC2 regulates invasion and metastasis of breast cancer cells.<sup>16</sup> Here, we found that knockdown of ST6GAL2 dramatically suppressed adhesion and invasion of MCF-7 and T47D cells.

The ability of cancer cells to migrate is essential to their ability to metastasize. GSEA analysis showed a positive correlation between ST6GAL2 expression and the four adhesion and metastasis pathways in breast cancer tissues. ST6GAL2 positively regulates the expression of genes related to these two pathways in breast cancer cells, including ICAM-1, VCAM-1, CD24, MMP2, MMP9, and CXCR4. Taken together, these data suggest that inhibition of ST6GAL2 can potentially suppress adhesion and invasion in breast cancer cell lines. Our results correlate with previous reports indicating that ST6GAL2 promotes cell migration and invasion of follicular thyroid carcinoma,<sup>16</sup> and regulates cell invasion of melanoma.<sup>17</sup> ST6GAL1 may mediate the invasiveness and tumorigenicity of non-small cell lung cancer cells, via the Notch1/Hes1/MMPs pathway.<sup>35</sup> CD24, a sialoglycoprotein cell adhesion molecule that promotes adhesion and metastasis, was up-regulated in colon cancer patients with higher ST6GAL1 levels<sup>36</sup> and modulated chemosensitivity of breast cancer cells to 5-fluorouracil.<sup>37</sup> CD44<sup>+</sup>CD24<sup>-</sup> is a well-known surface marker for breast cancer stem cells responsible for tumor formation, infinite growth, recurrence, and metastasis,<sup>38</sup>

suggesting an important role of CD24 in the breast cancer metastasis, chemosensitivity, and stemness.

Similarly, ST6GAL2 may regulate adhesion and invasion of breast cancer cells via CD24, MMP2, and MMP9 directly or indirectly. Aberrant sialylation is correlated with the invasive potential of various types of cancer. Previous studies suggest that sialylation may function as an internal factor, regulating the invasion and chemosensitivity of hepatocellular carcinoma, probably through ST6GAL1 or ST8SIA2 regulation of the activity of the PI3K/Akt pathway.<sup>39</sup> ST6GAL1 mediates inhibition of colorectal cancer metastasis through stabilizing ICAM-1 via sialylation.<sup>40</sup> Although there is still controversy regarding the contribution of ICAM-1 expression to tumor progression, a decrease of ICAM-1 may be one of the mechanisms by which tumor cells escape cell-mediated cytotoxicity and lysis by the host cellular immune system.<sup>41</sup> Activation of VCAM-1 increased breast cancer cell motility and promoted chemoresistance to doxorubicin and cisplatin, suggesting a possible mechanism of VCAM-1 activation facilitating breast cancer progression.<sup>42</sup> According to the important role of these proteins in the regulation of breast cancer metastasis, chemoresistance, and the immune system, we indicate that ST6GAL2 may regulate the cellular phenotypes of breast cancer through mediating these protein expressions.

However, the ST6GAL2-mediated network might be much more complex than previously appreciated. Identification of all important targets and understanding the relevant molecular pathways in various physiological and pathologic conditions will be of critical importance to understanding the biological functions of ST6GAL2. Taken together, these studies may explain why ST6GAL2 overexpression is associated with a poor prognosis of breast cancer patients and make a strong case for the role of ST6GAL2 in cancer.

Although increased ST6GAL2 mRNA levels were increased in breast cancer patients, the transcriptional regulation of ST6GAL2 in breast cancer is still unknown. NF- $\kappa$ B and NRSF are likely to act as transcriptional repressors, whereas neuronal-related development factors Sox5, Pura, and Olf1, are likely to act as transcriptional activators of ST6GAL2, suggesting that ST6GAL2 transcription could be potentially activated for specific neuronal functions.<sup>43</sup> Additionally, HCP5 acts as a powerful regulator in follicular thyroid carcinoma and that it functions as a ceRNA by sponging miR-22-3p, miR-186-5p, and miR-216a-5p, which activate ST6GAL2.<sup>16</sup> Therefore, additional studies to investigate the molecular mechanisms of both the cause and

effect of altered expression of ST6GAL2 in the development and/or progression of breast cancer are essential.

## Conclusions

ST6GAL2 is overexpressed in breast cancer tissues and reduced expression of ST6GAL2 can influence multiple biological progresses of breast cancer cells such as the cell cycle, cell viability, adhesion, and invasion. It likely achieves this through regulating focal adhesion and metastasis pathways and their associated proteins. ST6GAL2 can serve as a biomarker for breast cancer and inhibition of ST6GAL2 has strong potential as a treatment strategy for breast cancer.

## Acknowledgment

This study was supported by the National Science Foundation of China (No. 81602583).

## Disclosure

The authors declare that they have no competing interests.

## References

- Althuis MD, Dozier JM, Anderson WF, Devesa SS, Brint LA. Global trends in breast cancer incidence and mortality 1973–1997. *Int J Epidemiol.* 2005;34(2):405–412. doi:10.1093/ije/dyh414
- Berland LL, Monticciolo DL, Flores EJ, Malakoff J, Ye H, Dyer J. Relationships between health care disparities and coverage policies for breast, colon, and lung cancer screening. *J Am Coll Radiol.* 2019;16(4 Pt B):580–585. doi:10.1016/j.jacr.2019.03.025
- Bundred N. Prognostic and predictive factors in breast cancer. *Cancer Treat Rev.* 2001;27(3):135–142. doi:10.1053/cov.2000.0207
- Wiseman SM, Makretsov N, Nissen TC, et al. Coexpression of the type 1 growth factor receptor family members EGF-R-1, HER-2, and HER-3 has a synergistic negative prognostic effect on breast carcinoma survival. *Cancer.* 2005;107(5):1770–1777. doi:10.1002/(ISSN)1097-0142
- Montazeri M, Montazeri M, Montazeri M, Beigzadeh A. Machine learning models in breast cancer survival prediction. *Technol Health Care.* 2016;28(1):31–42. doi:10.3233/THC-151071
- Almendoz V, Kim HJ, Chen X, et al. Genetic and phenotypic diversity in breast tumor metastases. *Cancer Res.* 2014;74(5):1345–1348. doi:10.1158/0008-5472.CAN-13-2357-T
- Varki NM, Varki A. Diversity in cell surface sialic acid presentations: implications for biology and disease. *Lab Invest.* 2007;87(9):851–857. doi:10.1038/labinvest.3700656
- Hedlund M, Ng E, Varki A, Varki NM.  $\alpha$ 2-6-linked sialic acids on N-glycans modulate carcinoma differentiation in vivo. *Cancer Res.* 2008;68(2):388–394. doi:10.1158/0008-5472.CAN-07-1340
- Millflores-Flores L, Millán-Pérez L, Santos-López G, Reyes-Leyva J, Vallejo-Ruiz V. Characterization of P1 promoter activity of the  $\beta$ -galactoside  $\alpha$ 2, 6-sialyltransferase I gene (sialt 1) in cervical and hepatic cancer cell lines. *J Biosci.* 2012;37(2):259–267. doi:10.1007/s12038-012-9194-6
- Nakano M, Saldanha R, Göbel A, Kavallaris M, Packer NH. Identification of glycan structure alterations on cell membrane proteins in desoxyepothilone B resistant leukemia cells. *Mol Cell Proteomics.* 2011;10(11):M111–009001. doi:10.1074/mcp.M111.009001
- Swindall AF, Bellis SL. Sialylation of the Fas death receptor by ST6Gal-I provides protection against Fas-mediated apoptosis in colon carcinoma cells. *J Bio Chem.* 2011;286(26):22982–22990. doi:10.1074/jbc.M110.211375
- 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
- Gretschel S, Haensch W, Schlag PM, Kemmner W. Clinical relevance of sialyltransferases ST6GAL-I and ST3GAL-III in gastric cancer. *Oncology.* 2002;65(2):139–145. doi:10.1159/000072339
- Dalziel M, Huang RY, Dall'Olio F, Morris JR, Taylor-Papadimitriou J, Lau JT. Mouse ST6Gal sialyltransferase gene expression during mammary gland lactation. *Glycobiology.* 2006;16(3):407–412. doi:10.1093/glycob/11.5.407
- Petit D, Mir A-M, Petit J-M, et al. Molecular phylogeny and functional genomics of  $\beta$ -Galactoside  $\alpha$ 2-6-sialyltransferases that explain ubiquitous expression of ST6GAL1 gene in amniotes. *J Bio Chem.* 2010;285(49):38399–38414. doi:10.1074/jbc.M110.163931
- Liang L, Xu J, Wang M, et al. lncRNA HIF5 promotes follicular thyroid carcinoma progression via miRNAs sponge. *Cell Death Dis.* 2018;9(3):375. doi:10.1038/s41419-017-0382-7
- Agrawal A, Fontanals-Chen B, Sokolova E, et al. A systems biology approach identifies ST8 as a driver of melanoma metastasis. *Cancer Cell.* 2017;31(6):804–819.e807. doi:10.1016/j.ccr.2017.05.007
- Castellana B, Escuin J, Peiró G, et al. ASPN and GJB2 are implicated in the mechanisms of invasion of ductal breast carcinomas. *J Cancer.* 2012;3:175. doi:10.7150/jca.4120
- Tominaga K, Kondo C, Johmura Y, Nishizuka M, Imagawa M. The novel gene had104, containing a fibronectin type III domain, has a significant role in adipogenesis. *FEBS Lett.* 2004;577(1–2):49–54. doi:10.1016/j.febslet.2004.09.062
- Xu M, Qian G, Xie F, et al. Expression of epithelial cell adhesion molecule associated with elevated ductular reactions in hepatocellular carcinoma. *Clin Res Hepatol Gastroenterol.* 2014;38(6):699–705. doi:10.1016/j.clinre.2014.04.015
- Yamada S, Utsunomiya T, Morine Y, et al. Expressions of hypoxia-inducible factor-1 and epithelial cell adhesion molecule are linked with aggressive local recurrence of hepatocellular carcinoma after radiofrequency ablation therapy. *Ann Surg Oncol.* 2014;21(Suppl 3):S436–S442. doi:10.1245/s10434-014-3575-z
- Coutinho PM, Deleury E, Davies GJ, Henrissat B. An evolving hierarchical family classification for glycosyltransferases. *J Mol Biol.* 2003;328(2):307–317. doi:10.1016/S0022-2836(03)00307-3
- Seales EC, Jurado GA, Brunson BA, Wakefield JK, Frost AR, Bellis SL. Hypersialylation of  $\beta$ 1 integrins, observed in colon adenocarcinoma, may contribute to cancer progression by up-regulating cell motility. *Cancer Res.* 2005;65(11):4645–4652. doi:10.1158/0008-5472.CAN-04-3117
- Krzewinski-Recchi MA, Julien S, Juliant S, et al. Identification and functional expression of a second human  $\beta$ -galactoside  $\alpha$ 2, 6-sialyltransferase, ST6Gal II. *Eur J Biochem.* 2003;270(5):950–961. doi:10.1046/j.1432-1033.2003.03458.x
- Pérez-Garay M, Arteta B, Llop E, et al.  $\alpha$ 2, 3-sialyltransferase ST3Gal IV promotes migration and metastasis in pancreatic adenocarcinoma cells and tends to be highly expressed in pancreatic adenocarcinoma tissues. *Int J Biochem Cell Biol.* 2013;45(8):1748–1757. doi:10.1016/j.biocel.2013.05.015
- Jun L, Yuanshu W, Yanying X, et al. Altered mRNA expressions of sialyltransferases in human gastric cancer tissues. *Med Oncol.* 2012;29(1):84–90. doi:10.1007/s12032-010-9771-1
- Bos PD, Zhang XH-F, Nadal C, et al. Genes that mediate breast cancer metastasis to the brain. *Nature.* 2009;459(7249):1005–1009. doi:10.1038/nature08021

28. Zhang D, Liu E, Kang J, Yang X, Liu H. MiR-3613-3p affects cell proliferation and cell cycle in hepatocellular carcinoma. *Oncotarget*. 2017;8(54):93014–93028. doi:10.18632/oncotarget.21745
29. Parise CA, Caggiano V. Risk of mortality of node-negative, ER/PR/HER2 breast cancer subtypes in T1, T2, and T3 tumors. *Breast Cancer Res Treat*. 2017;165(3):743–750. doi:10.1007/s10549-017-4383-5
30. Curigliano G, Viale G, Bagnardi V, et al. Clinical relevance of HER2 overexpression/amplification in patients with small tumor size and node-negative breast cancer. *J Clin Oncol*. 2009;27(34):5693–5699. doi:10.1200/JCO.2009.22.0962
31. Gonzalez-Angulo AM, Litton JK, Broglio KR, et al. High risk of recurrence for patients with breast cancer who have human epidermal growth factor receptor 2-positive, node-negative tumors 1 cm or smaller. *J Clin Oncol*. 2009;27(34):5700–5706. doi:10.1200/JCO.2009.23.2025
32. Grimm S. Dissecting mitochondrial apoptosis pathways by gain-of-function cell culture screens. *Mitochondrion*. 2013;13(3):189–194. doi:10.1016/j.mito.2012.06.001
33. Gao X, Wang X. Effects of taxol on proliferation, apoptosis, and mRNA expression of  $\alpha 2$ , 6-sialic acid and ST6Gal in cervical carcinoma cell line U14. *Chin J Pathophysiol*. 2017;33(6):1038–1042.
34. Lu J, Isaji T, Im S, et al. Beta-galactoside  $\alpha 2$ ,6-sialyltransferase 1 promotes transforming growth factor-beta-mediated epithelial-mesenchymal transition. *J Biol Chem*. 2014;289(50):34627–34641. doi:10.1074/jbc.M114.593392
35. Yuan Q, Chen X, Han Y, et al. Modification of  $\alpha 2$ ,6-sialylation mediates the invasiveness and tumorigenicity of non-small cell lung cancer cells in vitro and in vivo via notch1/Hes1/MMPs pathway. *Nov*. 2018;143(9):2319–2330.
36. Venturi G, Ferreira IG, Pucci M, et al. Impact of sialyltransferase ST6GAL1 overexpression on different colon cancer cell types. *Glycobiology*. 2019;29:684–695. doi:10.1093/glycob/cwz053
37. Onishi H, Suyama K, Yamasaki A, et al. CD24 modulates chemosensitivity of MCF-7 breast cancer cells. *Anticancer Res*. 2017;37(2):561–565. doi:10.21873/anticancer
38. Tang H, Song C, Ye F, et al. miR-200c suppresses stemness and increases cellular sensitivity to trastuzumab in HER2+ breast cancer. *J Cell Mol Med*. 2019;23(12):8114–8127. doi:10.1111/jcmm.14681
39. Zhao Y, Li Y, Ma H, et al. Modification of sialylation mediates the invasive properties and chemosensitivity of human hepatocellular carcinoma. *Mol Cell Proteomics*. 2014;13(2):520–536. doi:10.1074/mcp.M113.034025
40. Zhou L, Zhang S, Zou X, et al. The beta-galactoside  $\alpha 2$ ,6-sialyltransferase 1 (ST6GAL1) inhibits colorectal cancer metastasis by stabilizing intercellular adhesion molecule-1 via sialylation. *Cancer Manag Res*. 2019;11:6185–6199. doi:10.2147/CMAR.S208631
41. Park J, Hwang JY, Thore A, et al. AF1q inhibited T cell attachment to breast cancer cell by attenuating intracellular adhesion molecule-1 expression. *J Cancer Metastasis Treat*. 2019;5:1.
42. Wang PC, Weng CC, Hsu SS, et al. Activation of VCAM-1 and its associated molecule CD44 lead to increased malignant potential of breast cancer cells. *Int J Mol Sci*. 2014;15(3):3560–3579. doi:10.3390/ijms15033560
43. Lehoux S, Groux-Dupont S, Cazet A, et al. Transcriptional regulation of the human ST6GAL2 gene in cerebral cortex and neuronal cells. *Glycoconj J*. 2010;27(1):99–114. doi:10.1007/s10719-009-9260-y

## OncoTargets and Therapy

### Publish your work in this journal

OncoTargets and Therapy is an international, peer-reviewed, open access journal focusing on the pathological basis of all cancers, potential targets for therapy and treatment protocols employed to improve the management of cancer patients. The journal also focuses on the impact of management programs and new therapeutic

agents and protocols on patient perspectives such as quality of life, adherence and satisfaction. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/oncotargets-and-therapy-journal>

Dovepress