Downregulation of ST3GAL5 is associated with muscle invasion, high grade and a poor prognosis in patients with bladder cancer

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Abbreviations: BC, bladder cancer; BLCA, bladder urothelial carcinoma; MIBC, muscle invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer

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Abstract. In patients with bladder cancer (BC), the association between ST3 β-galactoside α-2,3-sialyltransferase 5 (ST3GAL5) expression and clinical outcomes, particularly regarding muscle-invasive disease, high tumor grade and prognosis, remain unknown. In the present study, the expression of ST3GAL5 and its association with clinical outcomes in patients with BC was analyzed using various public bioinformatics databases. The difference in ST3GAL5 expression between BC and healthy bladder tissues was also evaluated using data from the Oncomine database, The Cancer Genome Atlas and Gene Expression Omnibus database. The differences in ST3GAL5 expression between muscle invasive BC (MIBC) and non-muscle invasive BC (NMIBC), and high- and low-grade BC were also analyzed. Furthermore, genes that were positively co-expressed with ST3GAL5 in patients with BC were identified from the intersection between the Oncomine, Gene Expression Profiling Interactive Analysis 2 and UALCAN databases. Enrichment analysis by Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, Reactome pathway enrichment analyses and a gene-concept network was performed using R package. Gene set enrichment analysis was also performed to assess the signaling pathways influenced by the high and low expression of ST3GAL5 in BC. The results indicated that ST3GAL5 expression was significantly lower in BC tissues compared with normal bladder tissues (P<0.05). Furthermore, ST3GAL5 expression in MIBC and high-grade BC was significantly lower compared with NMIBC and low-grade BC (P<0.05), respectively. The results from Kaplan-Meier survival analysis result demonstrated that ST3GAL5 downregulation was associated with poor survival in patients with BC (P<0.05). Taken together, these findings suggested that ST3GAL5 may be considered as an anti-oncogene in BC, could represent a potential predictive and prognostic biomarker for BC and may be a molecular target for tumor therapy.

Introduction

Bladder cancer (BC) is the 7th most common cancer affecting men in the world and the 11th most common cancer in the total population (1). BC affects ~3.4 million people worldwide, with 430,000 new cases diagnosed in 2015 (2). In the United States, 80,470 new cases of BC and 17,670 BC-associated mortality cases were expected to occur in 2019 (3). Furthermore, BC incidence and mortality rates vary across countries due to the differences in risk factors, detection and diagnostic practices and treatments availability (4). The most common type of BC is bladder urothelial carcinoma (BLCA), which accounts for ~90% of all cases (5). In addition, BLCA can be low grade or high grade (6). Low grade BLCA rarely results in cancer invasion in the bladder muscular wall or metastasis to other parts of the body, and patients rarely succumb to low grade BLCA; however, the majority of BLCA-associated mortality cases result from the high-grade disease (6). BC can also be stratified into muscle invasive bladder cancer (MIBC) and non-muscle invasive bladder cancer (NMIBC), according to invasion of the muscularis propria (6). In particular, ~75% of newly diagnosed BC cases are non-invasive, including Stages Ta, Tis or T1, based according to the Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC) staging system (8th edition) (4). NMIBC exhibits a high prevalence due to the long-term survival rates and the lower risk of cancer-specific mortality compared with patients with MIBC (6). Furthermore, improvements in the early detection and treatment of BC have increased patient survival status; however, BC-associated mortality remains high. It is therefore crucial to identify novel biomarkers and potential therapeutic targets to improve the clinical treatment of patients with BLCA.
ST3 β-galactoside α-2,3-sialyltransferase 5 (ST3GAL5) is a protein coding gene, which catalyzes the formation of ganglioside monosialodihexosylganglioside (GM3) (7). Ganglioside GM3 is known to participate in the induction of cell differentiation, modulation of cell proliferation, maintenance of fibroblast morphology, signal transduction and integrin-mediated cell adhesion (8). Furthermore, ganglioside GM3 is associated with numerous types of tumor, including lung cancer, brain cancer and melanomas, and was reported to significantly influence cancer development and progression (9-12). GM3 is also upregulated in several types of cancer, such as lung and brain cancer, and melanoma, and can be used as a tumor-associated carbohydrate antigen in immunotherapy (9,10). In addition, GM3 inhibits tumor cell proliferation through angiogenesis inhibition or decrease in cell motility (9,11,13). However, the expression profile and functional role of ST3GAL5 in BLCA remain unclear. Therefore, to the best of our knowledge, the present study is the first data mining study to predict the potential role of ST3GAL5 in BLCA, based on publicly available gene expression and clinical outcome databases.

In the present study, the expression of ST3GAL5 and its clinical outcomes were investigated in patients with BLCA using various public gene expression and survival datasets. In addition, the DNA methylation and gene expression patterns of ST3GAL5 in BLCA were analyzed. Furthermore, enrichment analyses were performed on genes that were positively co-expressed with ST3GAL5 in BLCA, and gene set enrichment analysis (GSEA) was also used. The findings from the present study hypothesized that ST3GAL5 downregulation may influence BLCA carcinogenesis, suggesting that ST3GAL5 may represent a novel therapeutic target in BLCA.

Materials and methods

Data set acquisition and processing. All data were acquired and processed from the public bioinformatics databases Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo) (14), Oncomine (www.oncomine.org) (15,16), Tumor IMmune Estimation Resource (TIMER; cistrome.shinyapps.io/timer) (17,18), Gene Expression across Normal and Tumor tissue (GENT; medical-genome.kribb.re.kr/GENT) (19,20), University of California, Santa Cruz (UCSC) Xena (xenabrowser.net) (21), Gene expression Profiling Interactive Analysis 2 (GPIA2; gepia2.cancer-pku.cn) (22) and Kaplan-Meier plotter (kmplot.com/analysis) (23). The BLCA microarray datasets GSE13507 (24), GSE120736 (25) and GSE31684 (26,27) were downloaded from the GEO database to analyze the expression of ST3GAL5. The Lee Bladder (27), Blaveri Bladder 2 (28), Sanchez-Carbayo Bladder 2 (29) and Stransky Bladder (30) datasets from the Oncomine database were extracted and processed using the R package ‘RONcomine’ v0.0.0.9 (github.com/yikeshu0611/RONcomine). The datasets from Genomic Data Commons (GDC; gdc.cancer.gov), The Cancer Genome Atlas (TCGA; cancergenome.nih.gov) and Genotype-Tissue Expression (GTEx; commonfund.nih.gov/GTEx) databases were downloaded using UCSC Xena browser tool (xenabrowser.net/). In the Oncomine database, the default settings were used and the threshold parameters were as follows: P<1x10^-4, ifold change>2 and gene rank in the top 10%. In the GENT database, data were analyzed using the Human Genome U133 Plus 2.0 Array platform (http://www.affymetrix.com/support/technical/byproduct.affx?product=bg-u133-plus).

Enrichment analysis. The Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGO) pathway enrichment analysis were determined using the R package ‘clusterProfiler’ v3.14.3 (31), and the Reactome pathway enrichment analysis was performed using the R package ‘ReactomePA’ v1.30.0 (32). Subsequently, the gene-concept network analysis were performed using the R package ‘clusterProfiler’ and ‘ReactomePA’. Microarray datasets of accession number GSE83586 (33) were downloaded from the GEO database in order to investigate the relevant signaling pathways using GSEA. According to the mean expression value of ST3GAL5 in the GSE83586 dataset, the matrix file was divided into high- and low-expression groups, and GSEA was performed using GSEA 4.02 software (34) in order to determine the KEGG pathways (c2.cp.kegg.v7.0.symbols) associated with high and low expression of ST3GAL5. Gene set permutations were performed 1,000 times for each analysis. The false discovery rate <0.25, inormalized enrichment score >1 and nominal P<0.05 were considered to indicate a statistically significant difference. Subsequently, replotting of the output from the GSEA report folder was conducted using the R package ‘Rtoolbox’ v1.4 (github.com/PeeperLab/Rtoolbox).

Data management and statistical analysis. Cancer staging was assessed using the 8th edition of the UICC/AJCC cancer staging system. The gene expression profile and survival data were downloaded, converted, constructed and managed using Microsoft Office Excel 2016 (Microsoft Corporation). All statistical analyses were performed using R software (www.r-project.org; v3.6.1). The box plot was constructed using the R package ‘ggplot2’ v3.2.1 (35). The Cnetplot was constructed using the R package ‘clusterProfiler’ and ‘ReactomePA’. Student's t-test was used to compare the means of two independent samples, and one-way ANOVA was used to compare the means of multiple independent samples followed by Bonferroni post hoc test for multiple comparisons. Kaplan-Meier analysis and Cox proportional hazard models were used for survival analysis by using R package ‘survival’ v3.1.8 and ‘survminer’ v0.4.6. A multivariate Cox proportional hazards regression model was performed to adjust for covariate effects, and stratification analysis was used to reduce the potential confounding effect on the estimation of hazard ratio (HR). Missing data were coded and excluded from the analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of ST3GAL5 in different types of cancer. The differences in ST3GAL5 expression between various types of cancer and paired healthy tissues were compared from three independent bioinformatics databases. In the Oncomine database, the comparison between each type of cancer and healthy tissues identified the downregulation of ST3GAL5 expression in bladder, lung, ovarian, prostate and 'other' cancers, and the upregulation of ST3GAL5 expression in esophageal cancer, head and neck cancer, kidney cancer, leukemia, lymphoma,
melanoma and myeloma (Fig. 1A). ST3GAL5 expression was also analyzed in tumor and healthy tissues using TCGA data and the TIMER tool (Fig. 1B). Among the different types of cancer, 10 presented significantly lower ST3GAL5 expression, and five had significantly higher ST3GAL5 expression compared with paired healthy tissues (Fig. 1B). Furthermore, data from the GENT database indicated that ST3GAL5 expression was downregulated in certain cancer types, including bladder, blood, brain, breast, liver, ovary, prostate, stomach and testicular cancers (Fig. 1C). The three databases demonstrated the downregulation of ST3GAL5 in different cancer types. Furthermore, ST3GAL5 expression in BLCA tissues was significantly decreased in the three databases compared with paired healthy tissues.

Expression of ST3GAL5 in BLCA and healthy bladder tissues. To observe the expression of ST3GAL5 in BLCA, three independent datasets from TCGA + GTEx, Oncomine and GEO databases were analyzed. Data from the TCGA + GTEx database were acquired using the USCS Xena browser tool. Moreover, data from the Oncomine Lee Bladder dataset were extracted and processed using the R package ‘ROncomine’. The GEO datasets were acquired from the accession number GSE13507. The results demonstrated a significant downregulation of ST3GAL5 in BLCA tissues compared with healthy bladder tissues (Fig. 2A-C).

Expression of ST3GAL5 in MIBC and high-grade BLCA tissues. To further determine of ST3GAL5 expression in
association between ST3GAL5 expression and survival prognosis in patients with BLCA. To investigate the association between ST3GAL5 expression and survival prognosis in patients with BLCA, Kaplan-Meier survival analysis was performed using data from the GDC, TCGA and GEO databases via the UCSC Xena browser and Kaplan-Meier plotter web tools. The 5-year overall survival (OS), disease specific survival, progression free interval and relapse free survival were all positively associated with lower ST3GAL5 expression in patients with BLCA (Fig. 5A-E).

Meta-survival analysis of OS was performed using data from GENT2 web (gent2.appex.kr/gent2) tools and depicted as forest plots (Fig. 5F). The results demonstrated that low ST3GAL5 expression was associated with poor OS [P<0.001; HR, 2.934; 95% CI (1.916-4.493); r², 0.086; I², 0.884]. These results indicated the prognostic relevance of ST3GAL5 expression in patients with BLCA.

Enrichment analysis genes co-expressed with ST3GAL5 in BLCA samples. The top 250 genes that were positively co-expressed with ST3GAL5 in BLCA samples were identified using Oncomine Stransky Bladder dataset and GEPIA2 and UALCAN web tools. The genes common to these three databases were selected for further analysis. In total, 33 genes were identified as positively co-expressed with ST3GAL5 in BLCA samples. The names of the genes were as follows: [methyltransferase like 7A, SMAD6, ATPase phospholipid transporting 8B1, sortilin related receptor 1, trafficking o-octanoyltransferase, tubulin tyrosine ligase like 3, aldehyde sequence similarity 13 member A, ID2, carbonyl reductase 4, glycerol-3-phosphate dehydrogenase 1 like, carnitine arachidonate 5-lipoxygenase, PPFIA binding protein 2, transmembrane transporter family (SLC) 14 member 1 (Kidd blood group), arachidonate 5-lipoxygenase, PPFIA binding protein 2, transmembrane protein 63A, 4-aminobutyrate aminotransferase, intraflagellar transport protein 5-9, oviductal glycoprotein 1, family with sequence similarity 13 member A, ID2, carnitine reductase 4, glycerol-3-phosphate dehydrogenase 1 like, carnitine O-octanoyltransferase, tubulin tyrosine ligase like 3, aldehyde dehydrogenase 4 family member A1 and malic enzyme 3].

Subsequently, GO, KEGG and Reactome pathway enrichment analyses, and gene-concept network analysis were performed with ST3GAL5 and the 33 positively

Association between ST3GAL5 expression and clinicopathological characteristics of patients with BLCA. The present study investigated the association between ST3GAL5 mRNA expression, promoter methylation level and the clinicopathological characteristics of patients with BLCA from the TCGA-BLCA dataset by using the Xena web tool. Compared with healthy bladder tissues, the expression of ST3GAL5 was downregulated in tissues from primary tumors, Stage IV cancer, extreme weight, smoking for >15 years, non-papillary tumors and nodal metastasis status N1 (Table II). Furthermore, ST3GAL5 expression was downregulated in male and female patients with BLCA. However, the level of ST3GAL5 promoter methylation in patients with BLCA was significantly increased, regardless of patient clinicopathological characteristics, including cancer stage, ethnicity, sex, age, weight, smoking status, nodal metastasis status and histological subtype compared with healthy patients (Fig. 4). Therefore, it was hypothesized that decreased ST3GAL5 promoter methylation may be positively associated with numerous clinicopathological characteristics of patients with BLCA.

Association between ST3GAL5 expression and survival prognosis in patients with BLCA. To investigate the association between ST3GAL5 expression and survival prognosis in patients with BLCA, Kaplan-Meier survival analysis was performed using data from the GDC, TCGA and GEO databases. The results demonstrated that ST3GAL5 downregulation was associated with lower ST3GAL5 expression in MIBC and high-grade BLCA, four individual datasets from the Oncomine database were analyzed (Table I). The results from meta-analysis demonstrated that ST3GAL5 was significantly downregulated in MIBC across the four datasets (Table I). In these four datasets, ST3GAL5 was significantly downregulated in MIBC and high-grade BLCA (Fig. 3A-a1-4 and B-a1-4).

The data acquired from the GEO datasets GSE120736, GSE31684 and GSE13507 also presented significantly lower expression of ST3GAL5 in MIBC and high-grade BLCA (Fig. 3A-b1-3 and B-b1-3, respectively). In addition, decreased expression of ST3GAL5 in high-grade BLCA tissues was reported in the GDC + TCGA BLCA datasets, using the USCS Xena browser tool (Fig. 3C). Taken together, these results demonstrated that ST3GAL5 downregulation was associated with MIBC and high-grade BLCA.

Microarray analysis from the GENT2 database demonstrated that ST3GAL5 downregulation was associated with lower ST3GAL5 expression in MIBC and high-grade BLCA (Fig. 3A-b1-3 and B-b1-4). In addition, decreased expression of ST3GAL5 in high-grade BLCA tissues was reported in the GDC + TCGA BLCA datasets, using the USCS Xena browser tool (Fig. 3C). Taken together, these results demonstrated that ST3GAL5 downregulation was associated with MIBC and high-grade BLCA.
Table I. Comparison of ST3 β-galactoside α-2,3-sialyltransferase 5 across four datasets in the downregulation analysis from the Oncomine database.

| Dataset                  | FC     | P-value         | Gene rank | MIBC | NMIBC |
|--------------------------|--------|-----------------|-----------|------|-------|
| Sanchez-Carbayo Bladder 2| -9.324 | 2.43x10^-9      | 315 (in top 3%) | 32   | 25    |
| Blaveri Bladder 2        | -3.622 | 2.86x10^-9      | 66 (in top 2%)  | 62   | 126   |
| Stransky Bladder         | -5.276 | 3.02x10^-10     | 3 (in top 1%)   | 22   | 19    |
| Lee Bladder              | 2.627  | 2.43x10^-12     | 7 (in top 1%)   | 81   | 28    |

Meta-analysis: Median Rank=36.5, P=2.64x10^-7. FC, fold-change; MIBC, muscle invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer.
Table II. Association between ST3 β-galactoside α-2,3-sialyltransferase 5 expression and clinicopathological characteristics of patients with bladder urothelial carcinoma.

| Parameter                        | Sample (n) | Expression value | P-value |
|----------------------------------|------------|------------------|---------|
|                                  |            | Mean  | SD   | t-test or ANOVA | Multiple comparisons |
| Sample type                      |            |       |      |                |                     |
| Healthy                          | 21         | 9.772 | 1.979 | 0.023          |                     |
| Primary tumor                    | 408        | 8.855 | 1.792 |                |                     |
| Cancer stage                     |            |       |      | 1.88x10^-4     |                     |
| Healthy                          | 21         | 9.772 | 1.979 |                |                     |
| Primary tumor                    |            |       |      |                |                     |
| Stage I                          | 4          | 10.241| 1.705| 1.000          |                     |
| Stage II                         | 130        | 9.470 | 1.839| 1.000          |                     |
| Stage III                        | 140        | 8.644 | 1.761| 0.067          |                     |
| Stage IV                         | 134        | 8.432 | 1.676| 0.014          |                     |
| Ethnicity                        |            |       |      | 0.121          |                     |
| Healthy                          | 21         | 9.772 | 1.979|                |                     |
| Primary tumor                    |            |       |      |                |                     |
| Caucasian                        | 347        | 8.852 | 1.817| 0.151          |                     |
| African-American                 | 21         | 9.147 | 1.745| 1.000          |                     |
| Asian                            | 40         | 8.715 | 1.829| 0.192          |                     |
| Sex                              |            |       |      | 0.067          |                     |
| Healthy                          | 21         | 9.772 | 1.979|                |                     |
| Primary tumor                    |            |       |      |                |                     |
| Male                             | 302        | 8.821 | 1.846| 0.064          |                     |
| Female                           | 106        | 8.945 | 1.718| 0.174          |                     |
| Age, years                       |            |       |      | 0.018          |                     |
| Healthy                          | 21         | 9.772 | 1.979|                |                     |
| Primary tumor                    |            |       |      |                |                     |
| 21-40                            | 2          | 10.690| 0.396| 1.000          |                     |
| 41-60                            | 106        | 9.156 | 1.813| 1.000          |                     |
| 61-80                            | 253        | 8.700 | 1.760| 0.095          |                     |
| >80                              | 47         | 8.919 | 2.024| 0.737          |                     |
| Weight                           |            |       |      | 0.012          |                     |
| Healthy                          | 21         | 9.772 | 1.979|                |                     |
| Primary tumor                    |            |       |      |                |                     |
| Normal weight                    | 140        | 9.144 | 1.860| 1.000          |                     |
| Extreme weight                   | 124        | 8.509 | 1.828| 0.035          |                     |
| Obese                            | 75         | 8.961 | 1.670| 0.720          |                     |
| Extreme obese                    | 10         | 8.986 | 1.889| 1.000          |                     |
| NA                               | 59         |       |      |                |                     |
| Smoking habits                   |            |       |      | 0.028          |                     |
| Healthy                          | 21         | 9.772 | 1.979|                |                     |
| Primary tumor                    |            |       |      |                |                     |
| Non-smoker                       | 12         | 9.061 | 1.967| 1.000          |                     |
| Smoker                           | 109        | 9.075 | 1.797| 1.000          |                     |
| Reformed smoker 1 (<15 years)    | 72         | 8.947 | 1.716| 0.700          |                     |
| Reformed smoker 2 (>15 years)    | 113        | 8.509 | 1.888| 0.039          |                     |
| NA                               | 102        |       |      |                |                     |
| Nodal metastasis status          |            |       |      | 0.004          |                     |
| Healthy                          | 21         | 9.772 | 1.979|                |                     |
| Primary tumor                    |            |       |      |                |                     |
| N0                               | 237        | 9.033 | 1.857| 0.721          |                     |
| N1                               | 46         | 8.177 | 1.759| 0.008          |                     |
co-expressed genes by using the R packages ‘clusterProfiler’ and ‘ReactomePA’. GO terms functional enrichment analysis was performed with ST3GAL5 and its associated genes to determine the functions associated with biological processes (BP), molecular functions (MF) and cellular components (CC). ST3GAL5 and its co-expressed genes were predominantly associated with ‘coenzyme metabolic process’, ‘organic hydroxy compound metabolic process’, ‘negative regulation of osteoblast differentiation’, ‘renal system development’, ‘tertiary granule lumen’, ‘tertiary granule’, ‘ficolin-1-rich granule lumen’, ‘coenzyme binding’, ‘NAD binding’ and ‘cofactor binding’ (Fig. 6A and B).

Furthermore, the KEGG pathways analysis for ST3GAL5 and its co-expressed genes demonstrated their association with ‘transforming growth factor (TGF)-β signaling pathway’, ‘carbon metabolism’, ‘alanine, aspartate and glutamate metabolism’, ‘peroxisome’, ‘glycosphingolipid biosynthesis-ganglioside series’, ‘fatty acid biosynthesis’, ‘2-oxocarboxylic acid metabolism’ and ‘signaling pathways regulating pluripotency of stem cells’ (Fig. 6C and D).

Next, Reactome pathway analysis of ST3GAL5 and its co-expressed genes identified highlighted their association with ‘protein localization’, ‘neutrophil degranulation’, ‘peroxisomal protein import’, ‘fatty acid metabolism’, ‘metabolism of vitamins and cofactors’, ‘interaction with cumulus cells and the zona pellucida’, ‘phenylalanine and tyrosine metabolism’ and ‘interleukin (IL)-4 and IL-13 signaling’ (Fig. 6E and F). All these pathways may therefore be associated with BLCA tumor progression and tumorigenesis.

**GSEA analysis between high and low ST3GAL5 expression in BLCA.** To further identify the signaling pathways that are differentially activated in BLCA, GSEA was performed to investigate the difference between high- (n=124) and low-ST3GAL5 (n=183) expression groups by using the GEO dataset GSE83586. Three tumor-associated pathways were identified as significantly associated with the downregulation of ST3GAL5 expression in BLCA tissues, including ‘NOD-like receptor (NLR) signaling pathway’, ‘cytokine-cytokine receptor interaction’ and ‘Janus kinase (JAK)-STAT signaling pathway’ (Table III; Fig. 7).

**Discussion**

BC is the most common malignancy of the urinary system, and ~90% of BC cases are urothelial carcinoma (5). Furthermore, BC can be low grade or high grade and can also be divided into MIBC and NMIBC; low grade BC rarely invades the muscular wall of the bladder and patients rarely succumb to low grade BC, while high grade BC is more likely to result in mortality (6). Furthermore, patients with NMIBC exhibit a favorable outcome (5-year overall survival of 95 vs. 69% in MIBC) (36). However, 70% of patients with BC will experience recurrence following initial treatment (surgery, radiotherapy or chemotherapy), including 30% out of the 70% of patients presenting with muscle invasive disease (37). In addition, cancer recurrence and progression lead to a higher disease stage, ending therefore in a less favorable outcome (38).

To the best of our knowledge, ST3GAL5 expression and its effect on muscle invasion, cancer grade and prognosis in patients with BLCA have not yet been investigated. The present study investigated therefore the potential role of ST3GAL5 in BLCA. In this study, bioinformatics analysis of multiple independent public databases was performed. The results demonstrated that ST3GAL5 was downregulated in various types of cancer, including BC, and that its expression in BLCA tissues was lower compared with healthy bladder tissues. In addition, ST3GAL5 downregulation was positively associated
with muscle invasion, high grade and a poor prognosis in patients with BLCA. Collectively, these findings indicated that ST3GAL5 may be considered as a tumor suppressor gene in BLCA, and may therefore inhibit the progression of BC to MIBC and high grade BLCA. These results also highlighted the potential role of ST3GAL5 as a therapeutic target in BC. However, further investigation is required to determine the underlying mechanisms of ST3GAL5 in BC progression and in the prognosis of patients with BC.

The association between ST3GAL5 expression, promoter methylation level and the clinicopathological characteristics of patients with BLCA was examined using TCGA data from the Xena browser. The results demonstrated that ST3GAL5 expression was downregulated in high stages and moderate nodal metastasis status compared with healthy bladder tissues. However, the level of ST3GAL5 promoter methylation was significantly decreased in BCLA tissues compared with healthy bladder tissues regardless of the patients’ clinicopathological characteristics, including cancer stage, ethnicity, sex, age, weight, smoking status, nodal metastasis status and histological subtype. Furthermore, analysis of ST3GAL5 expression and DNA methylation status indicated that ST3GAL5 gene expression may be associated with certain CpG island sites. CpG islands are CG-rich stretches in the genome concentrated near transcription start sites; in normal cells they are protected and therefore are in a non-methylated state, but in tumors they are specifically methylated. These findings suggested therefore that ST3GAL5 promoter methylation may be associated with the clinicopathological characteristics of patients with BC.

ST3GAL5 is a protein that catalyzes the formation of ganglioside GM3 (7). ST3GAL5 is upregulated in several types of cancer, such as lung and brain cancer, and melanoma, and can serve as a tumor-associated carbohydrate antigen in immunotherapy for cancer (9,10). Furthermore, ST3GAL5, which encodes GM3, inhibits tumor cell proliferation through angiogenesis inhibition or decrease in cell motility (9). Previous
studies reported that ST3GAL5 exerts some anti-proliferative effects in colon cancer (39), breast cancer (40,41), liver cancer (42) and other types of tumor (9,10). Although some studies demonstrated that ST3GAL5 has anti-tumor effects in human bladder cancer (11,14,39,43), the underlying mechanisms remain unknown. Furthermore, it was reported that ST3GAL5 effects could be associated with tumor cell apoptosis and angiogenesis inhibition (9,12,44). However, the expression profile and functional role of ST3GAL5 in BLCA remain unknown.

In the present study, the biological effect of ST3GAL5 in BLCA was investigated by using bioinformatics analysis of multiple public databases. Co-expressed genes that were positively associated with ST3GAL5 expression were identified in three public databases, and intersecting genes from all databases were considered to be significantly co-expressed genes. R packages were then used to identify the signaling pathways associated with the genes that were positively co-expressed with ST3GAL5 in BLCA samples. Furthermore, from the perspective of functional classification, GO enrichment analysis of BP, CC and MF was performed on ST3GAL5 and its co-expressed genes. The results from KEGG pathway analysis revealed that the ‘TGF-β signaling pathway’ was significantly associated with ST3GAL5 expression. The deregulation of this pathway has been reported to result in tumor progression (45). In healthy and early-stage cancer, such as breast and prostate cancer cells, the TGF-β pathway...
exerts tumor-suppressive properties; however, its activation in late-stage cancer can promote tumor progression, via metastasis and chemoresistance (45,46). Furthermore, the dual function and pleiotropic nature of TGF-β signaling makes of it a challenging target; therefore, careful therapeutic dosage of TGF-β drugs and careful patient selection are required (46). In the present study, although ST3GAL5 expression was downregulated in BLCA tissues compared with healthy bladder tissues, ST3GAL5 expression was significantly downregulated in high grade and advanced stage BLCA in multiple databases. The significant downregulation in high grade and advanced stage BLCA may due to the associated activation of ST3GAL5 and its co-expressed genes following the increase in TGF-β signaling transduction. Another pathway associated with ST3GAL5 expression was ‘carbon metabolism’. Cells require one-carbon units for nucleotide synthesis, methylation and reductive metabolism, which support the high proliferative rate of cancer cells (47). A previous study reported that
polymorphisms in one-carbon metabolism and susceptibility to BC suggested that variation in glutathione synthesis may contribute to the risk of BC (48). In the present study, Reactome pathway analysis demonstrated that the main pathway associated with ST3GAL5 expression was ‘protein localization’, and previous studies reported that changes in subcellular localization of tumor-associated proteins can influence protein structure and biological function, which are associated with tumorigenesis, tumor progression and patient prognosis (49-52). Another pathway associated with ST3GAL5 expression was ‘neutrophil degranulation’. Neutrophils have been shown to be the first responders to inflammation and infection (53). The role of neutrophils in cancer is multifactorial, but is not fully understood. Furthermore, neutrophils reflect a state of host inflammation, which is a hallmark of cancer (54), and can participate in different stages of the oncogenic process including tumor initiation, growth, proliferation and metastasis (55,56). Neutrophil granule proteins released upon cell activation have also been associated with tumor progression, and this differential granule mobilization may allow neutrophils and possibly associated cancer cells to exit the bloodstream and enter inflamed and infected tissues (53). Since neutrophils are immune cells, tumor immunity must also be considered in order to predict the prognosis of patients with BC. Takeuchi et al (55), reported that the Tumour-associated macrophage polarized M2 phenotype influences microangiogenesis, pathological outcome, tumor grade and tumor invasiveness in BC. In the present study, GO analysis of the BP and MF domains identified co-enzyme involvement in BP and MF, suggesting that co-enzymes may serve an important role in the tumorigenesis and tumor progression of BC. However, further in vitro and in vivo studies are required to elucidate the biological role of ST3GAL5 in BC. Taken together, these findings highlighted the important role of ST3GAL5 and its co-expressed genes in various carcinogenic processes.

Table III. Gene set enrichment analysis in the group with low expression levels of ST3 β-galactoside α-2,3-sialyltransferase 5 in bladder urothelial carcinoma.

| Gene set name                                           | NES       | NOM P-value | FDR       |
|---------------------------------------------------------|-----------|-------------|-----------|
| KEGG_SYSTEMIC_LUPUSERYTHEMATOSUS                       | 1.788003  | 0.00396     | 0.212486  |
| KEGG_AUTOIMMUNE_THYROID_DISEASE                        | 1.596979  | 0.027237    | 0.242572  |
| KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS                  | 1.593668  | 0.028689    | 0.212411  |
| KEGG_ALLOGRAFT_REJECTION                               | 1.579419  | 0.044747    | 0.208527  |
| KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_ CHONDROITIN_SULFATE | 1.577063  | 0.026923    | 0.189083  |
| KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY               | 1.534106  | 0.035644    | 0.198596  |
| KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION            | 1.502448  | 0.042969    | 0.213385  |
| KEGG_JAK_STAT_SIGNALING_PATHWAY                        | 1.500872  | 0.042718    | 0.201288  |

Gene sets with |NES|>1, NOM P<0.05 and FDR<0.25 were considered as significant. NES, normalized enrichment score; NOM, nominal; FDR, false discovery rate; JAK, Janus kinase; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Figure 7. GSEA analysis between high and low expression of ST3GAL5 in BLCA. In total, three tumor-related signaling pathways were identified as positively associated with ST3GAL5 downregulation in BLCA. (A) NOD-like receptor signaling pathway. (B) Cytokine-cytokine receptor interaction. (C) JAK-STAT signaling pathway. JAK, Janus kinase; BLCA, bladder urothelial carcinoma; ST3GAL5, ST3 β-galactoside α-2,3-sialyltransferase 5; ES, enrichment score; NES, normalized enrichment score; NOM, nominal; FDR, false discovery rate.
The immune status of patients with BC. The JAK-STAT pathway with lower ST3GAL5 expression, suggesting that ST3GAL5

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The datasets generated and/or analyzed during the present study are available in the GEO (www.ncbi.nlm.nih.gov/geo), Oncomine (www.oncomine.org) and TGCA (cancergenome.nih.gov) repositories.

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Authors' contributions

JL and QW participated in the design of the present study, performed the statistical analysis and drafted the manuscript. SO, ZN and GD performed the study and collected background information and data. SO was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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