Expression and prognostic value of SULT1A2 in bladder cancer

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Abstract. Sulfotransferase Family 1A Member 2 (SULT1A2) is a protein coding gene. Several studies have reported that SULT1A2 may have a chemical carcinogenic effect if expressed as a functional protein. The present study aimed to investigate the expression and potential role of SULT1A2 in bladder cancer (BC). Data from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus databases were used to analyze SULT1A2 expression in BC. In addition, reverse transcription-quantitative PCR and western blot analyses were performed to detect SULT1A2 expression in BC cells and tissues. Immunohistochemistry analysis was performed on 100 formalin-fixed, paraffin-embedded BC tissues and corresponding adjacent normal bladder tissues (ANBTs) to verify SULT1A2 expression and determine the clinical significance of SULT1A2 in BC. Gene set enrichment analysis (GSEA) was performed to determine the potential biological processes and internal molecular mechanisms. The results demonstrated that SULT1A2 was highly expressed in BC tissues compared with ANBTs. Furthermore, high SULT1A2 expression was significantly associated with the staging of BC. Analyses of TCGA datasets and BC tissue microarray indicated that high SULT1A2 expression was significantly associated with a favorable overall survival in patients with BC. In addition, GSEA revealed pathways, diseases and biological processes associated with SULT1A2. Taken together, the results of the present study suggest that SULT1A2 acts as an oncogene in BC, and thus may serve as a biomarker for tumor staging and prognosis in patients with BC.

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Introduction

Bladder cancer (BC) is one of the most common malignancies of the genitourinary system (1). Despite the established risk factors, such as age, smoking and family history, there is an absence of early detection strategies (2). Biomarkers have potential for the diagnosis, staging, prognosis, and treatment of BC (3). However, the biomarkers currently used for BC present several limitations, such as PD-L1 immunohistochemistry lacking standard uniformity and definitions for PD-L1 testing, with the major concern being the lack of a common method for assessment and interpretation of IHC staining (4). Thus, it is important to identify novel BC biomarkers with high specificity and sensitivity.

Human cytosolic sulfotransferases (SULTs) are phase II detoxification enzymes that catalyze the biotransformation of several endogenous and exogenous substrates (5). In most cases, this reaction renders the substrate more water-soluble, resulting in excretion (6). However, in some instances, the sulfation of a molecule results in bioactivation, which induces carcinogenesis; one example is the aromatic compounds from cigarette smoke and occupational exposures, the principal exogenous risk factors for BC (7,8). By influencing DNA adduct formation, Sulfotransferase Family 1A Member 2 (SULT1A2) has been reported to induce the mutagenicity and carcinogenicity of substrates, including nitrotoluenes, 3-nitrobenzanthrone, aristolochic acids, aromatic hydroxylamine and polycyclic aromatic hydrocarbons (9-11). Several studies have demonstrated that SULT1A2 plays a role in the chemical carcinogenesis of these substrates if it is expressed as a functional protein (12,13). In addition, SULT1A2 is considered one of the five major genes associated with BC (2). Although SULT1A2 RNA has been detected in several tissues, Nowell *et al* (12) demonstrated that SULT1A2 protein expression in human tissues, including bladder tumors, was poorly detected using a SULT1A2-specific antibody. However, the expression of SULT1A2 may be misleading since the protein may be induced under certain physiological states to achieve bioactivation by sulfation. Only a few studies have investigated the tissue distribution and regulatory mechanism of SULT1A2 (12). Thus, it is important to determine whether SULT1A2 is expressed in human bladder tissues, and clarify how SULT1A2 participates and affects the occurrence and development of BC.

To the best of our knowledge, the present study was the first to demonstrate that SULT1A2 expression is upregulated in BC cells and tissues compared with normal bladder cells and tissues. The aim of the current study was to explore the feasibility of SULT1A2 as an effective biomarker for the staging and determining the prognosis of patients with BC.

Materials and methods

Clinical samples. A total of 100 formalin-fixed, paraffin-embedded (FFPE) BC tissues, and 12 frozen BC tissues and corresponding adjacent normal bladder tissues (ANBTs; >5 cm from the tumor) were collected from patients with BC who underwent radical resection, without preoperative chemotherapy or radiotherapy, at the First Affiliated Hospital of Sun Yat-sen University between February 2015 and October 2018. According to BC histopathology (14), the tissue samples were divided into non-muscle invasive cancer (NMIBC) and muscle invasive cancer (MIBC). NMIBC includes Ta stage (tumor is confined to the bladder mucosa), Tis stage (carcinoma *in situ*) and T1 stage (tumor manifests as invasion of the subepithelial connective tissue). MIBC includes T2 stage (tumor invades the muscle layer), T3 stage (tumor invades the adjacent bladder tissues) and T4 stage (tumor invades other tissues or organs). Immunohistochemistry (IHC) analysis was performed using FFPE BC tissues stored at 4˚C including 41 NMIBC and 59 MIBC samples, and corresponding ANBTs. Reverse transcription-quantitative (RT-q)PCR and western blot analyses were performed using the 12 frozen BC tissues stored at -80˚C, including six NMIBC and six MIBC samples, and corresponding ANBTs. The present study was approved by the Institutional Ethics Committee for Clinical Research and Animal Trials of the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China [approval no. (2016)067]. Written informed consent was provided by all patients prior to the study start.

Cell culture. The human uroepithelial SV-HUC-1 cell line was purchased from the American Type Culture Collection and maintained in F-12K medium (Gibco, Thermo Fisher Scientific, Inc.). The human T24 and 5637 BC cell lines were purchased from the Institute of Cell Biology, Chinese Academy of Sciences(https://www.cellbank.org.cn). T24 cells were maintained in RPMI-1640, while 5637 cells were maintained in minimum essential medium (both purchased from Gibco; Thermo Fisher Scientific, Inc.). All media were supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and cultured at 37˚C, with 5% CO_2.

IHC. BC tissues and ANBTs were fixed in 4% formalin solution for 24 h at room temperature, then embedded in paraffin and sectioned (5-μm-thick). Paraffin sections were heated at 55˚C for 2 h. Prior to immunostaining, slides were dewaxed in xylene and rehydrated in alcohol, and antigen retrieval was performed by microwaving the slides in citric saline (Wuhan Promoter Biological Co., Ltd.). The slides were subsequently incubated with 3% hydrogen peroxide to inhibit endogenous peroxidase activity for 15 min at room temperature and blocked with 3% goat serum (Sigma-Aldrich; Merck KGaA) for 10 min at 37˚C. For IHC staining, the slides were incubated with primary antibody against SULT1A2 (cat. no. HPA051051; 1:200; Sigma-Aldrich; Merck KGaA) overnight at 4˚C, and subsequently incubated with secondary antibody [GTVision I; 1:500; Gene Science and Technology (Shanghai) Co., Ltd.] for 1 h at room temperature. SULT1A2 expression was detected using a DAB detection system [Gene Science and Technology (Shanghai) Co., Ltd.]. The slides were visualized and captured at x400 magnification (Axio Imager. Z2 fluorescence microscope; ZEISS).

IHC staining was assessed using a semi-quantitative scoring method (15) by recording both the area of positive staining and the staining intensity. The area of positive staining was scored as follows: 0, 0%; 1, 1-25%; 2, 26-50%; 3, 51-75% and 4, >75%. The staining intensity was defined as follows: 0, no staining; 1, weak staining; 2, moderate staining and 3, strong staining. The immunoreactivity score (IHS) was calculated by multiplying the positive area score by the staining intensity score. An IHS <8 was classified into the low expression group, while an IHS ≥8 was classified into the high expression group.

Tissue microarray. A tissue microarray from FFPE tissues containing 55 BC tissue spots (item no. HBlaU066Su01; Shanghai Xinchao Biological Technology Co., Ltd.) was used to detect SULT1A2 protein expression via IHC analysis.

RT-qPCR. Total RNA was extracted from tissues or cells using TRIzol® reagent (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol, and reverse transcribed into cDNA using the TransScript All-in-One First-Strand cDNA Synthesis SuperMix (TransGen Biotech Co., Ltd.) according to the manufacturer's protocol. qPCR was subsequently performed using the Fast SYBR Green PCR Master Mix on a Step-One Fast Real-time PCR System (both purchased from Thermo Fisher Scientific, Inc.). qPCR conditions were as follows: 95˚C for 30 sec, followed by 40 cycles at 95˚C for 5 sec, 60˚C for 34 sec and 95˚C for 15 sec. The primer sequences used for qPCR are listed in Table I. Each sample was run in triplicate. The average CT values of each target gene are then compared to the internal reference gene GAPDH CT values. The formulas were used in relative quantitative analysis: Change Fold=$2^{-\Delta\Delta CT}$; $\Delta\Delta CT=\Delta CT$ test-ΔCT con (16).

Western blotting. Both frozen tissues and cells were used for Western blotting. Total protein was extracted using RIPA buffer (cat. no. R0278; Sigma-Aldrich; Merck KGaA). Total protein was quantified using the BCA Protein Quantitation Assay kit (Takara Bio, Inc.) and 40 μg protein/lane was separated by 10% SDS-PAGE. The separated proteins were subsequently transferred onto PVDF membranes (Sigma-Aldrich; Merck KGaA) and blocked with 5% bovine serum albumin (Sigma-Aldrich; Merck KGaA) in Tris-buffered saline containing 0.1% Tween-20 for 1 h at room temperature. The membranes were incubated with primary antibodies against SULT1A2 (HPA051051; 1:200; Sigma-Aldrich; Merck KGaA) and β-actin (cat. no. 4970; 1:1,000; Cell Signaling Technology, Inc.) overnight at 4˚C. Membranes were washed with Tris-buffered saline containing 0.1% Tween-20 for 30 min and subsequently incubated with Anti-rabbit IgG, HRP-conjugated secondary antibodies (cat. no. 7074; 1:1,000; Cell Signaling Technology, Inc.) for 1 h at room temperature. Membranes

Table I. Primer sequences used for quantitative PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
SULT1A2	TACTTTGCAGAGGCACTGGG	CGCCCTGGTAGATCATGTCC
GAPDH	TGTGGGCATCAATGGATTTGG	ACACCATGTATTCCGGGTCAAT

SULT1A2, Sulfotransferase Family 1A Member 2.

were re-washed with TBST for 30 min, and protein bands were visualized using the ECL western blotting detection system (Bio-Rad Laboratories, Inc.). Protein bands were analyzed using ImageJ software (version 1.51e; National Institutes of Health).

High-throughput data processing. The RNA-Seq data and clinical data for the BC samples were downloaded from The Cancer Genome Atlas (TCGA; http://gdc.cancer.gov) database. The microarray data based on the Affymetrix platform were downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/; GSE3167: Tumor, n=46; normal, n=14 and GSE68020: Tumor, n=30; normal, n=20; GSE3167: NMIBC, n=33; MIBC, n=13 and GSE120736: NMIBC, n=84; MIBC, n=61) (17,18). The data from TCGA database were log2 transformed, and the results were analyzed using Microsoft Excel 2019 (Microsoft Corporation) and GraphPad Prism 6 software (GraphPad Software, Inc.).

Detection of biological pathways and internal mechanism. 'ClusterProfiler' R language packages (version 3.8; http://www.bioconductor.org) and 'DOSE' R language packages (version 3.5; http://www.bioconductor.org) were used to perform pathway enrichment analysis and Disease Ontology (DO) annotation to investigate the pathways and biological parameters associated with SULT1A2, respectively. The samples in the GSE3167 dataset were separated into high and low expression groups, according to median SULT1A2 expression. Differentially expressed genes were determined using the 'limma' R package (version 3.12; http://www.bioconductor.org), and an adjusted P<0.05 was selected as the threshold for enriched terms. To determine the function of SULT1A2 in BC, gene set enrichment analysis (GSEA; version 2.2.4 jar software; http://software.broadinstitute.org/gsea/downloads.jsp) was performed to identify pathways that were associated with SULT1A2. The gene sets with normalized using an enrichment score of >1, P-value <0.05 and false discovery rate value <0.25, which were regarded as significantly enriched gene sets. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.ad.jp/kegg/ kegg2.html) was used to identify biological pathways and DO annotation was performed to assess the associations between genes and diseases. Gene Ontology (GO) enrichment analysis was also performed to determine the biological functions in BC, and the associated biological processes, molecular functions and cellular components were identified.

Statistical analysis. Statistical analysis was performed using SPSS 17.0 software (SPSS, Inc.), GraphPad Prism 6 software (GraphPad Software, Inc.) and R 3.5.0 software (https://www.r-project.org). The 'survminer' R package (version 0.4.8; http://www.sthda.com/english/rpkgs/survminer/) was used to draw survival curves. The Kaplan-Meier method and log-rank test were used to assess overall survival (OS) rate of patients in the high and low expression groups. Each experiment was repeated in triplicate. Data are presented as the mean ± standard error of the mean or standard deviation. Paired Student's t-test was used to compare differences between two groups, while one-way ANOVA followed by Tukey's post hoc test was used to compare differences between multiple groups. P<0.05 was considered to indicate a statistically significant difference.

Results

SULT1A2 is highly expressed in BC tissues and cells compared with normal bladder tissues and cells. To determine the clinical significance of SULT1A2 in BC, datasets from the GEO database were used to assess SULT1A2 expression. The results demonstrated that SULT1A2 expression was significantly higher in BC tissues compared with normal bladder tissues (Fig. 1A, P<0.001 and 1B, P<0.01). RT-qPCR and western blot analyses were subsequently performed to detect SULT1A2 mRNA and protein expression levels in BC cells and normal bladder cells, respectively. The results demonstrated that SULT1A2 expression was significantly higher in BC cells compared with normal bladder cells (Fig. 1C, P<0.001 and 1D, P<0.001). IHC analysis was performed to detect SULT1A2 expression in FFPE BC tissues (n=100) and corresponding ANBTs (n=100). SULT1A2 was prominently located in the cytoplasm and was moderately or highly expressed in most FFPE BC tissues, whereas it was undetected or weakly expressed in the majority of ANBTs (Fig. 1E). Collectively, these results suggest that SULT1A2 is highly expressed in BC tissues and cells compared with normal bladder tissues and cells.

SULT1A2 is associated with the staging of BC. Our studies demonstrate that SULT1A2 expression is significantly higher in BC tissues and cells compared with ANBTs and cells. Data from the GEO database was further analyzed to investigate the association between SULT1A2 expression and the clinicopathological characteristics of BC. The results demonstrated that SULT1A2 mRNA expression was significantly higher in NMIBC tissues compared with MIBC tissues (Fig. 2A, P<0.05 and 2B, P<0.01). Subsequently, reverse transcription-quantitative PCR and western blot analyses were performed to detect SULT1A2 mRNA and protein expression levels at

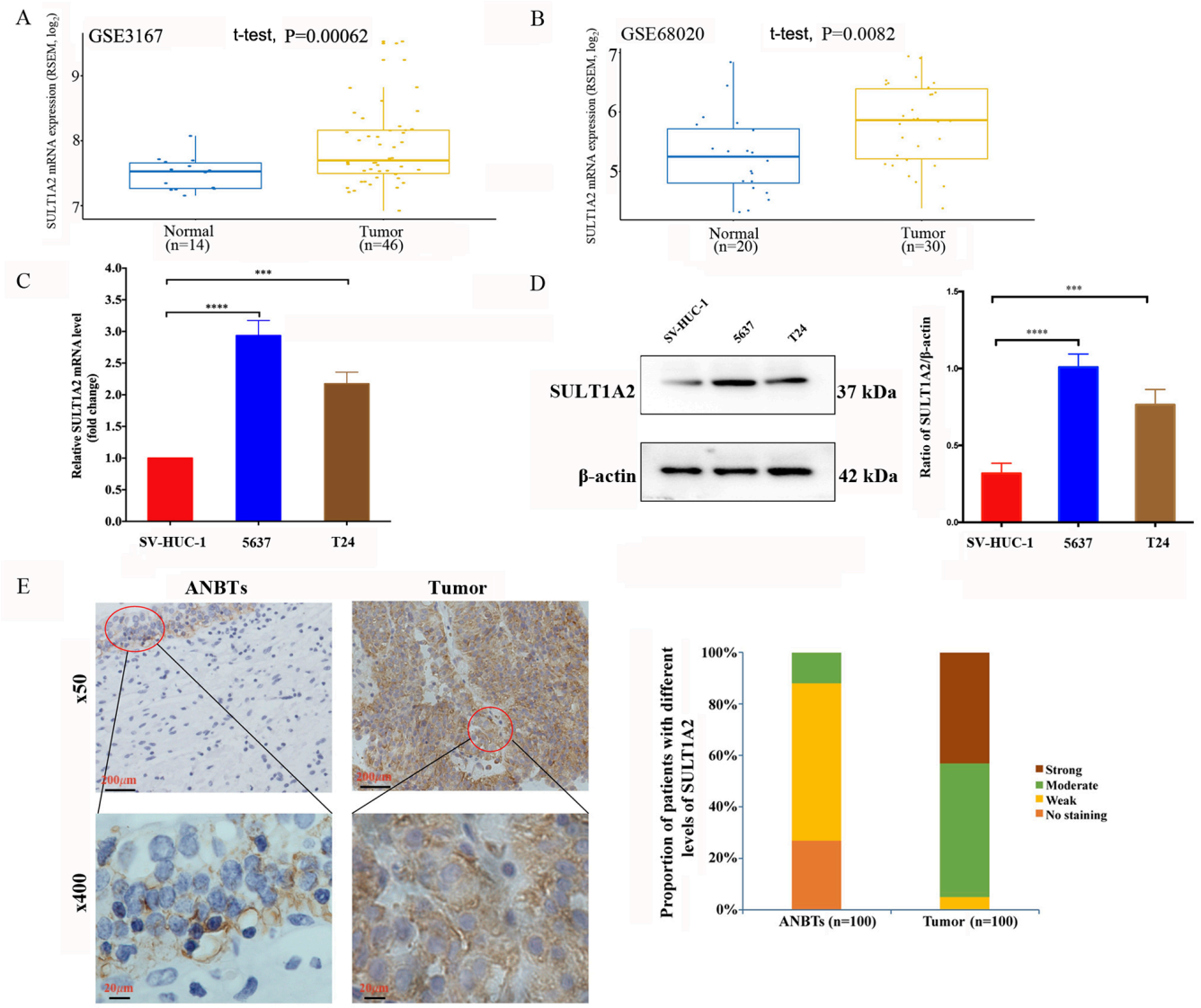

Figure 1. SULT1A2 expression is upregulated in BC tissues and cells compared with normal bladder tissues and cells. (A and B) SULT1A2 was highly expressed in BC tissues compared with normal bladder tissues according to analyses from the Gene Expression Omnibus database (GSE3167; tumor, n=46; normal, n=14 and GSE68020; tumor, n=30; normal, n=20). (C) Reverse transcription-quantitative PCR analysis was performed to detect SULT1A2 mRNA expression in BC cells and normal bladder cells. (D) Western blot analysis was performed to detect SULT1A2 protein expression in BC cells and normal bladder cells. β-actin was used as the internal control. (E) Immunohistochemistry analysis was performed to detect SULT1A2 protein expression in BC tissues. ***P<0.001; ****P<0.0001. SULT1A2, Sulfotransferase Family 1A Member 2; BC, bladder cancer; ANBTs, adjacent normal bladder tissues.

different stages of frozen BC tissues (n=12) and corresponding ANBTs (n=6). The results demonstrated that SULT1A2 mRNA and protein expression levels were significantly higher in frozen BC tissues compared with ANBTs, and SULT1A2 expression levels were significantly elevated in NMIBC tissues compared with MIBC tissues (Fig. 2C, P<0.01 and 2D, P<0.05). IHC analysis was performed to detect SULT1A2 expression in FFPE BC tissues, with NMIBC and MIBC. The results demonstrated that SULT1A2 protein expression was significantly higher in NMIBC tissues compared with MIBC tissues (Fig. 2E, P<0.01). Taken together, these results suggest that SULT1A2 expression is associated with BC stages.

High SULT1A2 expression predicts better prognosis in patients with BC. Patients in TCGA dataset were divided into two groups (high and low expression groups), based on median SULT1A2 expression. Kaplan-Meier OS curves were constructed and the log-rank test was used to determine statistical significance. The results demonstrated that patients with high SULT1A2 expression had a better prognosis for long-term survival (Fig. 3A, P<0.05). IHC analysis was performed using the BC tissue microarray (n=55) to validate the results and determine the prognostic value of SULT1A2 in patients with BC. The results demonstrated that SULT1A2 was highly expressed in 26/55 BC tissues, and patients with high SULT1A2 expression had a better prognosis for long-term survival (Fig. 3B and C, P<0.05), which was consistent with TCGA analysis. Collectively, these results suggest that SULT1A2 expression is positively associated with survival in patients with BC.

Functional analysis of SULT1A2. To determine the function of SULT1A2 in BC, gene set enrichment analysis was

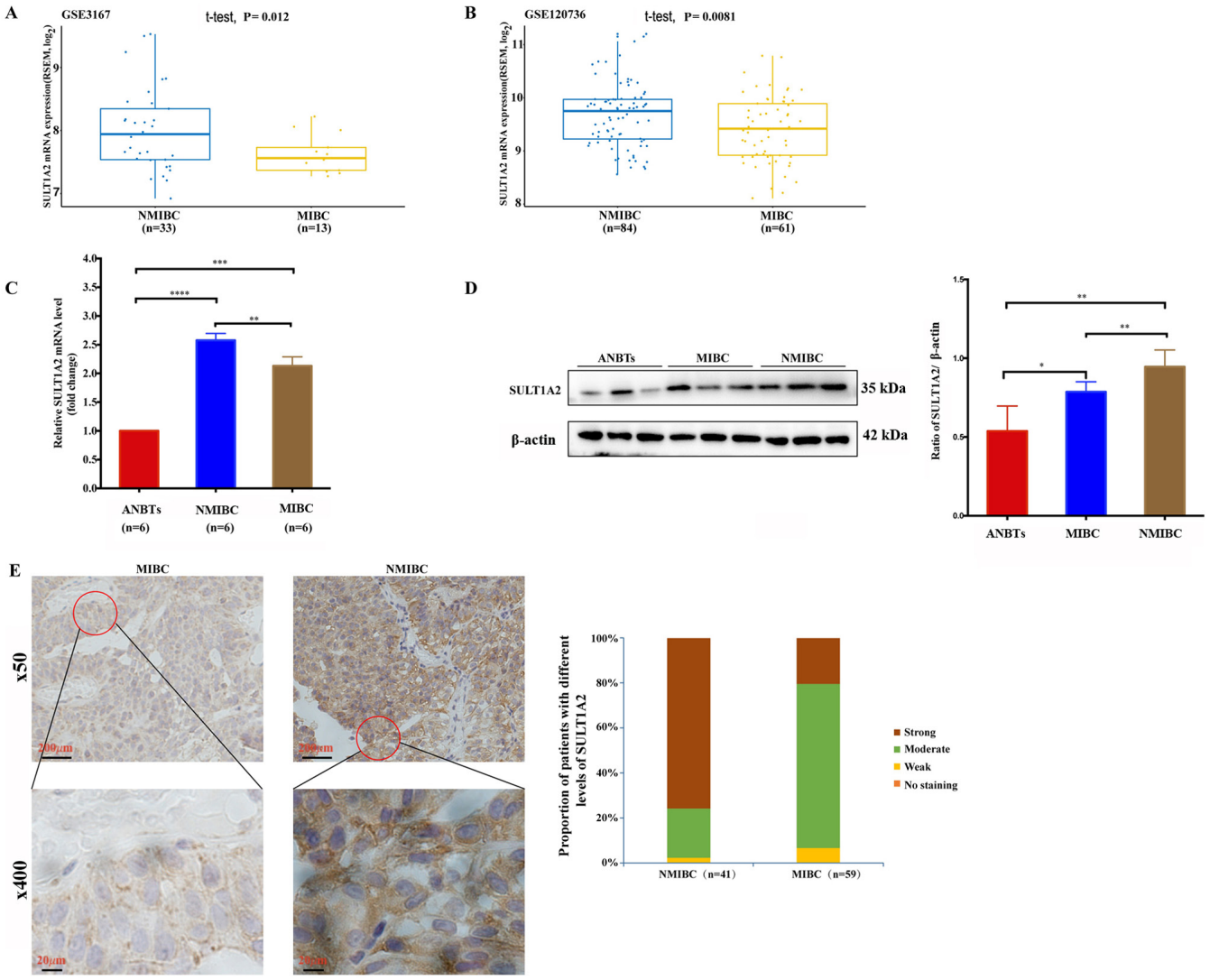

Figure 2. SULT1A2 expression is upregulated in NMIBC tissues compared with MIBC tissues. (A and B) SULT1A2 was highly expressed in NMIBC tissues compared with MIBC tissues according to analyses from the Gene Expression Omnibus database (GSE3167; NMIBC, n=33; MIBC, n=13 and GSE120736; NMIBC, n=84; MIBC, n=61). (C) Reverse transcription-quantitative PCR analysis was performed to detect SULT1A2 mRNA expression in NMIBC and MIBC tissue samples. (D) Western blot and (E) immunohistochemistry analyses were performed to detect SULT1A2 protein expression in NMIBC and MIBC tissue samples. β-actin was used as the internal control. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. SULT1A2, Sulfotransferase Family 1A Member 2; NMIBC, non-muscle invasive cancer; MIBC, muscle invasive cancer; ANBTs, adjacent normal bladder tissues.

performed to identify pathways that are associated with SULT1A2. BC-related pathways were associated with SULT1A2, including the PI3K-Akt signaling pathway (P=0.000141804), pathways in cancer (P=0.000132979), the MAPK signaling pathway (P=0.000145243) and human papillomavirus (HPV) infection (P=0.000144092) (Fig. 4A). Previous studies have demonstrated that these pathways are closely associated with the occurrence and development of BC (19,20). In addition, other important pathways associated with SULT1A2 were also discovered (Fig. 4B). To further confirm the molecular functions of SULT1A2, enriched DO and GO terms were identified. DO analysis demonstrated that BC is one of the most closely associated diseases to SULT1A2 (Fig. 4C). GO analysis demonstrated that SULT1A2 variation in BC results in changes in biosynthetic processes (P<0.001), the regulation of the metabolic processes (P<0.001), the nucleus (P<0.001) and DNA binding (P<0.05) (Fig. 4D).

Discussion

Although SULT1A2 can catalyze the bioactivation of several procarcinogens (12), a previous study has suggested that the SULT1A2 transcript has a splicing defect that may prevent it from becoming translated into protein (21), in which case SULT1A2 is considered a pseudogene. Another study has screened several cytosolic fractions from different tissues, including tumors, and SULT1A2 expression was undetected (12). In addition, a molecular epidemiological study has concluded that SULT1A2 has no association with the risk of liver, colon, lung, oral, gastric, renal, cervical or breast cancer (22). SULT1A2 does not appear to play a role in carcinogenesis and cancer development; however, it is associated with the early onset of breast cancer and mediated biotransformation in the breast (12). Ongoing research conducted by the present authors has demonstrated that SULT1A2 expression significantly changes in BC, but not in other SULT isoforms (data not shown). The present

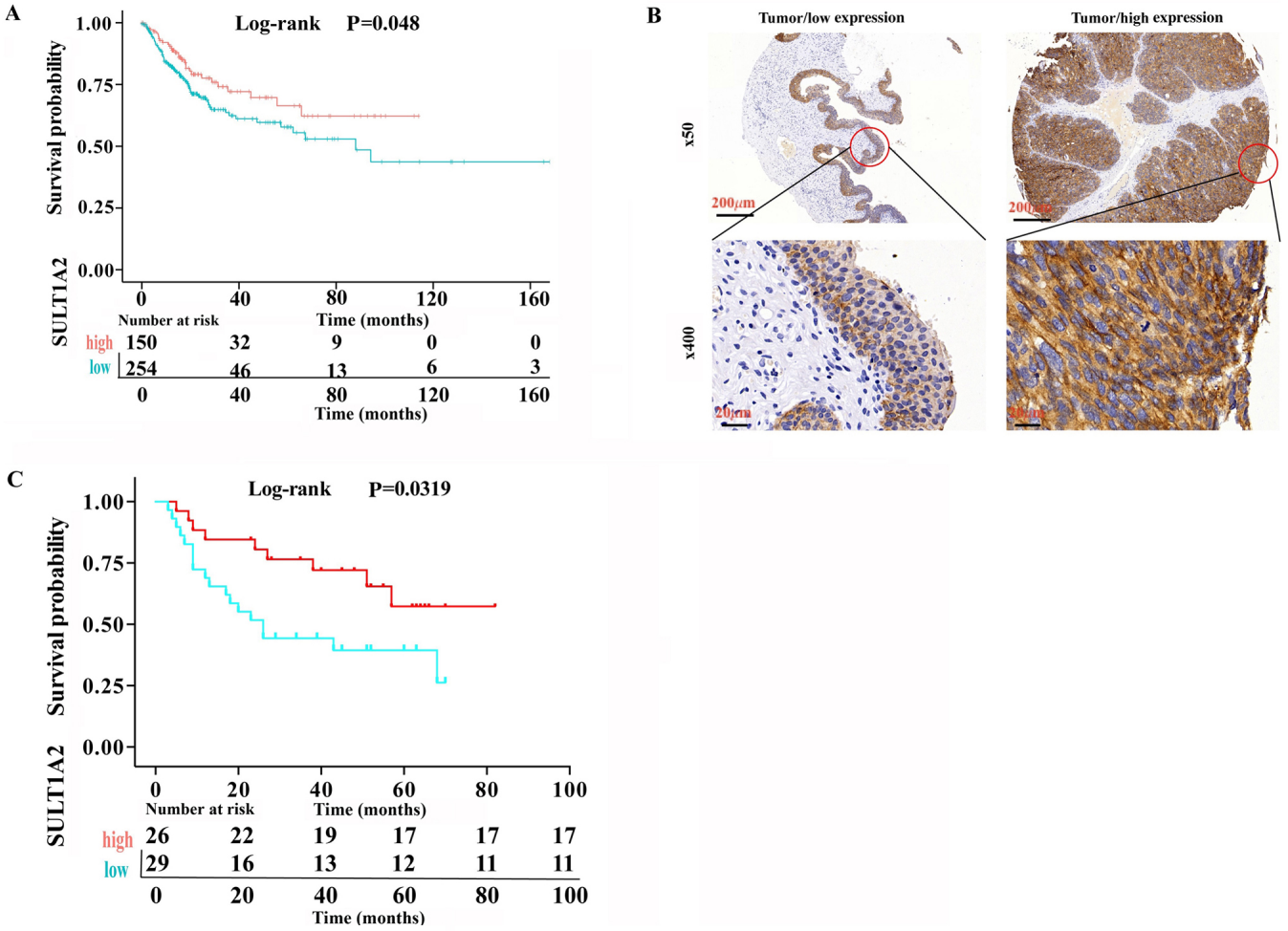

Figure 3. Prognostic value of SULT1A2 in clinical applications. (A) OS curves for patients with BC, with low or high SULT1A2 expression levels based on The Cancer Genome Atlas dataset. (B) Low and high SULT1A2 expression levels in tumor tissues. (C) OS analysis based on the BC tissue microarray using the Kaplan-Meier method. SULT1A2, Sulfotransferase Family 1A Member 2; OS, overall survival; BC, bladder cancer.

study aimed to investigate the role of SULT1A2 in BC using cancer cells, FFPE cancer tissues and frozen cancer tissues. The results demonstrated that SULT1A2 mRNA and protein expression levels were significantly higher in BC cells and tissues compared with normal bladder cells and corresponding ANBTs. In addition, SULT1A2 expression levels were significantly higher in NMIBC tissues compared with MIBC tissues. Notably, patients with high SULT1A2 protein expression have a good prognosis for long-term survival. Analyses using TCGA and GEO databases indicated that SULT1A2 mRNA expression was higher in early stage BC compared with advanced stage BC, and patients with high SULT1A2 mRNA expression had a better prognosis for long-term survival than those with low SULT1A2 expression. These results confirm that SULT1A2 is expressed in human bladder tissues, particularly in BC tissues, and SULT1A2 expression is associated with the classification and prognosis of BC.

The results of the present study demonstrated that SULT1A2 is activated during all phases of BC and that activation of SULT1A2 is a frequent event in tumor progression and metastasis. Given that metastatic disease is the principal cause of mortality in patients with cancer (23), a better understanding of tumor invasion and metastasis is essential to identify novel therapeutic targets. Increasing evidence suggest that the pathways in cancer not only have a direct role in tumor invasion by degrading extracellular matrix protein, but they also play an important role in maintaining the tumor microenvironment, thus promoting tumor growth (24,25). A previous study demonstrated that PI3K and MAPK signaling, belonging to the pathways in cancer, are one of the three main pathways frequently dysregulated in BC (1). Activation of the PI3K/Akt and MAPK signaling pathways mediates BC invasion (26). A previous study has implicated that the p38 MAPK and PI3K/AKT signaling pathways may be responsible for MMP-2/-9 expression regulating the migratory/invasive capacity of BC cells (27). The MAPK signaling pathway also affects the invasive ability of human BC cells via the downstream signal AP-1, impeding the transition of cells from the G_1 phase to the S phase, and mediating epithelial-to-mesenchymal transition (28,29). Whole-genome and RNA sequencing identified potential therapeutic targets in 69% of BCs, including 42% with targets in the PI3K/AKT pathway and 45% with targets in the MAPK pathway (1). Activation of the epidermal growth factor receptor (EGFR) and downstream signaling pathways, including PI3K/Akt and MAPK, induces resistance to EGFR-targeted therapy in BC (26). Although chromosomal alterations involved in the PI3K/AKT signaling pathway play a major role in the effectiveness of targeted therapy (30),

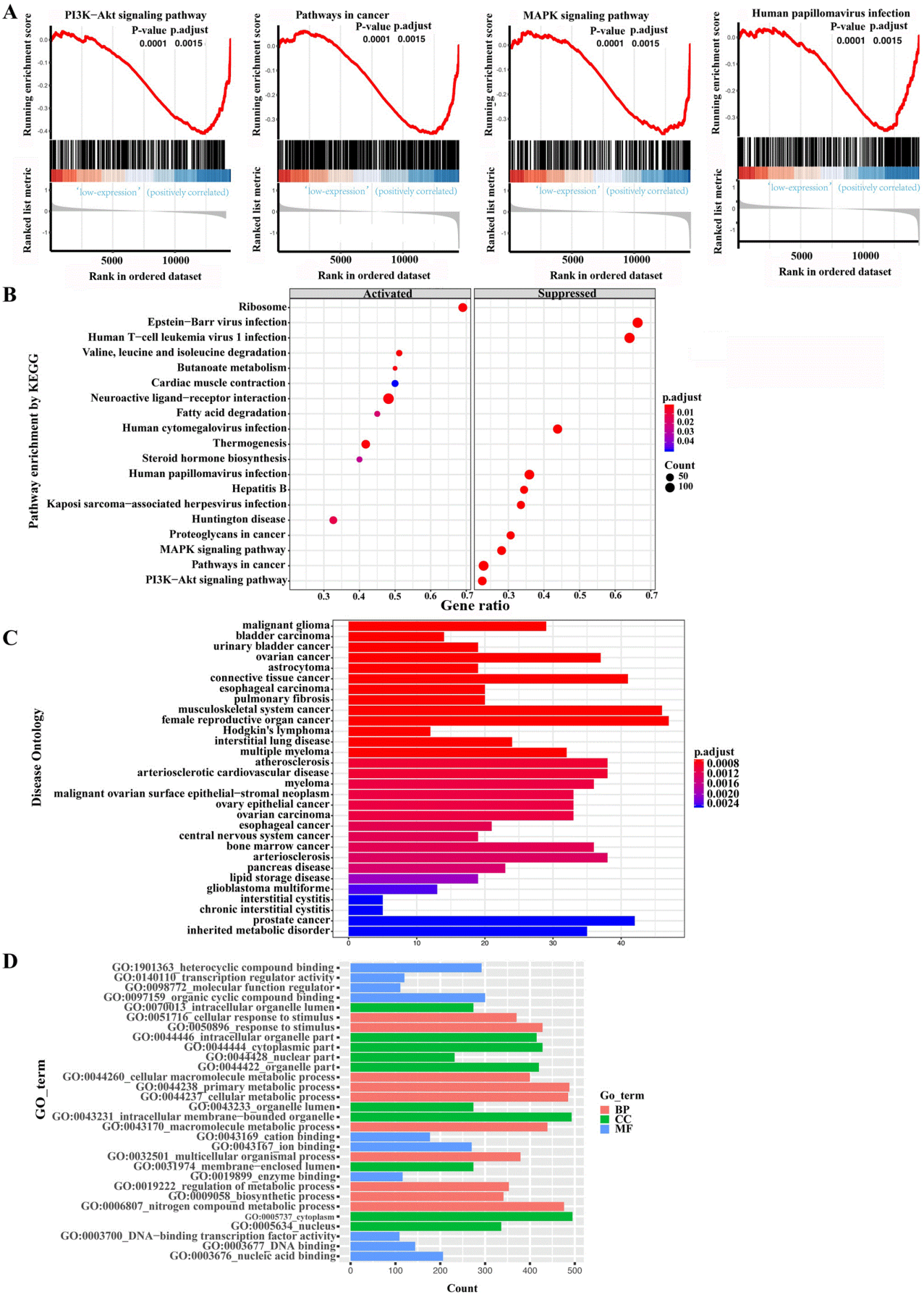

Figure 4. Functional analysis of SULT1A2. (A) Gene set enrichment analysis plots; four key bladder cancer-related pathways were associated with SULT1A2. (B) Other important pathways associated with SULT1A2. (C) Disease Ontology enrichment analysis. (D) GO enrichment analysis. BP, MF and CC are presented. SULT1A2, Sulfotransferase Family 1A Member 2; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; BP, biological processes; MF, molecular functions; CC, cellular components.

targeting the two major signaling pathways remains an important therapeutic approach for BC.

The results of the present study suggest that the pathways in cancer, the MAPK signaling pathway, and the PI3K/Akt signaling pathway have a negative association with the SULT1A2 gene in BC, which indicates that SULT1A2 may be a protector by decreasing the proliferation and metastasis of cancer cells via downregulation of these signaling pathways. GO enrichment and KEGG pathway analyses were performed to elucidate the carcinogenesis and progression of BC. SULT1A2 may have two roles in BC by affecting biosynthetic processes, metabolic process, the nucleus and DNA binding through the pathways of cancer, including the MAPK and PI3K/Akt signaling pathways. The current study speculated that when SULT1A2 expression increases to a certain threshold, carcinogenesis is activated; if it sufficiently increases to reach another threshold, a protective effect is activated. This can explain the upregulated expression of SULT1A2 in BC, in which SULT1A2 expression is higher in the early/noninvasive stage compared with the advanced/invasive stage. Given that the early stage of BC recurs in 50-70% of patients, the effective therapeutic control of cancer recurrence is required at an early stage (31). Thus, SULT1A2 may be used as a novel therapeutic target in early BC.

The results of the present study demonstrated that SULT1A2 is associated with HPV infection. HPV is a risk factor for penile cancer; however, its role in BC remains unclear. High-risk HPVs are the primary causative agents of carcinomas (32). The prevalence of high-risk HPV in BC varies, particularly in Moroccan patients, with the highest prevalence of 52.4% (33). However, clinical trials have reported that HPV is not associated with the risk of BC (34,35). Recently, Weinstein *et al* (36) identified that high-risk HPV may play a role in the development of a small percentage of BCs. It was demonstrated that only one BC tissue expressed HPV16, a high-risk HPV (36), in 122 cases; however, the HPV16 virus integrated into an apoptosis-regulating gene (BCL2L1) and induced it to be amplified and significantly overexpressed (1). Moonen *et al* (37) demonstrated a positive association between cancer stage and high-risk HPV DNA. Given that SULT1A2 is closely associated with BC, it was speculated that SULT1A2 may be involved in the development of BC via HPV infection. However, further studies are required to verify this assumption.

The present study is not without limitations. First, SULT1A2 expression was assessed in 100 FFPE BC tissues and corresponding ANBTs at the protein level but not the genomic level. If high-throughput data processing were used in the 100 samples and corresponding ANBTs, the results would be more reliable. Secondly, the present study failed to exhibit the results of some of the SULT1A2 regulatory pathways. The research is ongoing (data not shown), which will be time consuming and require additional financial resources. Based on the present study, SULT1A2 plays a protective role in the development of BC; however, the cell phenotype was not verified. In addition, further studies are required to assess the effect of overexpressing SULT1A2 on the proliferation and invasion of BC cells.

In conclusion, the results of the present study demonstrated that SULT1A2 was highly expressed in BC tissues and significantly associated with the staging of BC. In addition, high SULT1A2 expression was significantly associated with overall survival of patients with BC, and SULT1A2 was associated with BC-related pathways and biosynthetic processes. Thus, SULT1A2 may act as an oncogene in BC, and serve as a biomarker for tumor staging and prognosis in patients with BC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JS designed the present study. YC performed the experiments. YC and QO acquired and analyzed the data. YC drafted and revised the manuscript for important intellectual content. All authors have read and approved the final manuscript. JS and YC confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Institutional Ethics Committee for Clinical Research and Animal Trials of the First Affiliated Hospital of Sun Yat-sen University [approval no. (2016)067, Guangzhou, China]. Written informed consent was provided by all patients prior to the study start.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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