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ORIGINAL RESEARCH

Down-regulation and clinical significance of Sorbin and SH3 domain-containing protein 1 in bladder cancer tissues

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Abstract

Bladder cancer (BC) is a common cancer worldwide with a high prevalence. This study was conducted to elucidate the expression and clinical significance of Sorbin and SH3 domain-containing protein 1 (SORBS1) in BC as well as to explore its molecular mechanism in BC tumourigenesis. RNA-sequencing data, microarray, and Immunohistochemistry (IHC) were applied to elucidated the SORBS1 expression at multiple levels. After that, the relationship between tumour-immune infiltration and SORBS1 was also explored. Finally, SORBS1-related genes in BC were identified to perform functional enrichment analyses. The expression integration revealed that the comprehensive expression of SORBS1 at the mRNA level was −1.02 and that at the protein level was −3.73, based on 12 platforms, including 1221 BC and 187 non-BC samples. SORBS1 was negatively correlated with tumour purity (correlation = −0.342, $p < 0.001$) and positively correlated with macrophage (correlation = 0.358, $p < 0.001$). The results of enrichment analyses revealed that the most significant biological pathways of SORBS1-related genes were epithelial-mesenchymal transition. SORBS1 was significantly down-regulated in BC and may play a role as tumour suppressor. This study provides new directions and biomarkers for future BC diagnosis.

KEYWORDS

bioinformatics, biological tissues, correlation methods, proteins, RNA

1 | BACKGROUND

Bladder cancer (BC) is a common cancer worldwide with a high prevalence. The incidence of BC has increased in recent years, and there are an estimated 81,400 new cases of BC and 17,980 BC-related deaths in the United States in 2020. [1–4] The diagnosis of BC consists of either non-muscle invasive bladder cancer or muscle-invasive bladder cancer (MIBC), and the treatment of BC depends on this pathological classification. Non-muscle invasive bladder cancer is treated with

Abbreviations: AUC, area under the curve; BC, bladder cancer; BMI, body mass index; CCLE, Broad Institute Cancer Cell Line Encyclopaedia; DEGs, differentially expressed genes; EMT, epithelial-mesenchymal transition; GEO, Gene Expression Omnibus; GTEx, Genotype-Tissue Expression; HPA, The Human Protein Atlas; IHC, Immunohistochemistry; M, mean; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle invasive bladder cancer; PPAR, peroxisome proliferator-activated receptor gamma; PPI, protein-protein interaction; ROC, receiver operating characteristic; SD, standard deviation; SORBS1, Sorbin and SH3 domain-containing protein 1; TCGA, The Cancer Genome Atlas; TFs, transcription factors; TIICs, tumour-infiltrating immune cells; TIMER, The Tumour Immune Estimation Resource; TSS, transcription start site; TURBT, transurethral resection of the bladder tumour.

transurethral resection of the bladder tumour (TURBT), which can be followed by adjuvant therapy. For treatments of MIBC, radical cystectomy and cisplatin-based chemotherapy are currently the major therapeutic options. [5–12] However, for all stages, the 5-year survival rate of BC is 77%. For regional and distant metastasis BC patients, the 5-year survival rates are 36% and 5% respectively. [3] Therefore, it is necessary to seek a novel marker for BC.

Sorbin and SH3 domain-containing protein 1 (SORBS1), also called CAP/ponsin, is an important factor in signalling and stimulation of insulin. Mutations of gene SORBS1 induce insulin resistance and cause human disorders. [13–16] A few studies have explored the role SORBS1 plays in cancer. Song et al. reported that SORBS1 protein expression is lower in breast cancer cells. The SORBS1 mRNA expression also revealed a similar tendency in tissue samples. Furthermore, SORBS1 was associated with cancer cell invasive ability and clinical outcomes. [17] Yu et al. demonstrated that SORBS1 inhibited biological behaviours and metastasis of breast cancer cells through the negative regulation of miR-145-5p. Low SORBS1 expression exhibited poor prognosis. [18] However, the functions and mechanisms of SORBS1 in BC have not been explored.

In this study, mRNA expression data obtained from the Broad Institute Cancer Cell Line Encyclopaedia (CCLE), The Cancer Genome Atlas (TCGA), the Genotype-Tissue Expression (GTEx) Project, and the Gene Expression Omnibus (GEO) was used to elucidate the SORBS1 expression difference between BC tissues and non-BC tissues. Immunohistochemistry (IHC) was also utilised to further confirm the SORBS1 protein expression. The relationship between tumour-immune infiltration and SORBS1 was also explored by utilising the Tumour Immune Estimation Resource (TIMER). Genes that correlated with SORBS1 were collected through correlation analysis to explore possible functions and signalling pathways. Transcription factors (TFs) that could possibly function as gene expression regulators of SORBS1 were also predicted. In summary, this study was conducted to elucidate the expression and clinical value of SORBS1 in BC as well as to explore its molecular mechanism in BC tumourigenesis. The flowchart of the present study is shown in Figure 1.

2 | METHODS

2.1 | mRNA expression of SORBS1 in BC

To elucidate the mRNA expression of SORBS1 in BC, RNA-sequencing data of SORBS1 were obtained from CCLE, TCGA, and GTEx. [19–21] SORBS1 RNA-sequencing data in cancer cell lines was collected from CCLE. Briefly, the RNA-sequencing data of 407 BC tissues and 19 para-tumour tissues were collected from TCGA. Clinical parameters related to BC were also downloaded for further analyses. Moreover, RNA-sequencing data of 9 normal bladder tissues were downloaded from GTEx. Sequencing data from different databases were both log2(x+1) transformed and integrated with R package 'sva' and 'limma.' The prognostic value of SORBS1 was also estimated by utilising survival time data and vital status extracted from clinical information of TCGA. The BC patients were divided into low and high groups according to the median expression.

Furthermore, GEO, SRA, and ArrayExpress databases were searched to obtain the microarray that contain BC and non-BC tissues. The keywords in searching these databases were as follows: (BC OR BC OR carcinoma of urinary bladder). The microarrays were merged and normalised based on platforms. R package 'sva' and 'limma' were adopted to remove the batch effect.

2.2 | Protein expression of SORBS1 in BC

IHC was applied to evaluate the protein expression of SORBS1 through tissue array. To achieve this goal, clinical samples that included 137 BC tissues and 34 non-BC tissues were collected. The included patients were first diagnosed as BC patients and never received any surgery, chemotherapy, and radiotherapy. The study protocol was authorised by the Ethical Committee of this University, and written informed consent for the use of the tissues in this study was provided from patients. For each case, 100 stained cells from 10 representative regions were evaluated. The results of IHC were analysed by two factors: staining intensity and the number of positive cells. The staining intensity

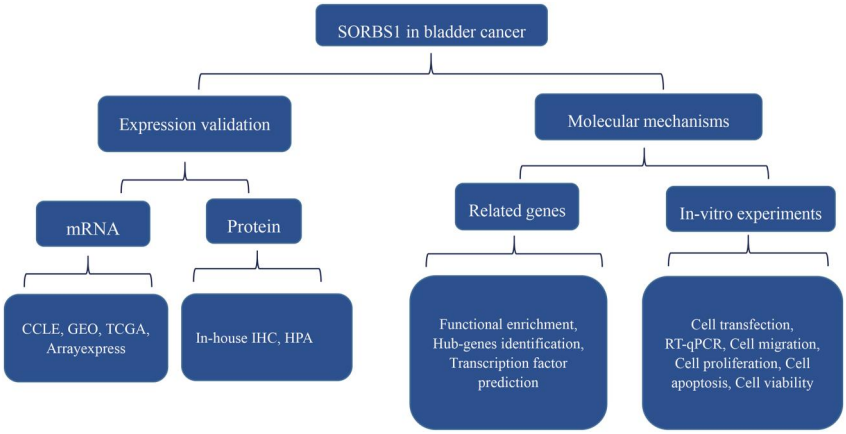

FIGURE 1 Flowchart of this study. The present study contains two parts: SORBS1 expression validation and underlying molecular mechanisms exploration.

and number of stained cells of SORBS1 were accessed as: staining intensity (0: negative; 1: weak; 2: moderate; 3: strong) and number of stained cells (0: 0%; 1: 1%–25%; 2: 26%–50%; 3: 51%–75%; 4: 76%–100%). The final score of each sample was generated by the numbers of staining intensity and number of stained cells multiplied. The expression of SORBS1 was determined based on the final score. Clinical information of included patients was also collected for further analyses [22].

Then, the protein expression of SORBS1 was further verified by using The Human Protein Atlas (HPA) (https://www.proteinatlas.org/). The protein expression was evaluated according to staining intensity and quantity.

2.3 | Expression integration of SORBS1

We conducted expression integration based on the data resources mentioned above to confirm the expression of SORBS1 in BC. The standard mean difference (SMD) of SORBS1 expression at the mRNA level was integrated. After that, SORBS1 protein expression was included to confirm the expression.

2.4 | Tumour immune estimation resource database analysis

The TIMER (cistrome.shinyapps.io/timer) is an online resource to identify the relationship between genes expression and tumour purity. [23] The correlation between SORBS1 expression and the gene markers of tumour-infiltrating immune cells (TIICs)—including macrophages, CD8+T-cells dendritic cells, CD4+T-cells, neutrophils, and B-cells—were also analysed.

2.5 | Statistical analysis

SPSS software version 22.0 was applied for the calculation of expression data. GraphPad Prism 8 was applied to plot figures. The SORBS1 expression was presented as mean (M) and standard deviation (SD) using Student's t-test. The receiver operating characteristic (ROC) curve of each study was performed to distinguish the clinical significance of SORBS1, and the area under the curve (AUC) was also calculated. SORBS1 mRNA expression was calculated based on clinical parameters collected from TCGA. The Kaplan–Meier curve was adopted to analyse the BC patient's survival data. Hazard ratio (HR) and corresponding 95% confidence interval were calculated. As for protein level, SORBS1 expression was calculated based on clinical information collected from the hospital. $p < 0.05$ was regarded as statistically significant. Stata software version 12.0 was adopted to integrate the expression data of SORBS1. A random model or fixed model was decided depending on whether heterogeneity existed ($I^2 > 50\%$ or $p < 0.05$ were considered as demonstrating that heterogeneity existed). Additionally, summarised ROC (sROC), sensitivity, and specificity were also conducted.

2.6 | Relative genes and enrichment analyses of SORBS1 in BC

Relative genes of SORBS1 in BC were identified using the Pearson correlation based on each platform. Genes were screened out when the correlation coefficient was more than 0.4 and $p < 0.05$. Differentially expressed genes (DEGs) of each platform were identified and merged using R package 'limma' and 'RobustRankAggreg.' Genes with |logFC (fold change)| > 1 were considered as DEGs. Genes significantly correlated with SORBS1 and differentially expressed in BC were considered as final SORBS1-related genes. Then SORBS1-related genes were submitted to FunRich3.1.3 to perform functional enrichment analyses. [24, 25] The protein-protein interaction was conducted by STRING (https://string-db.org/). Hub genes were identified by a plugin in Cytoscape named MCODE (http://cytoscape.org). The expression of hub genes in BC was confirmed by RNA-sequencing and microarray.

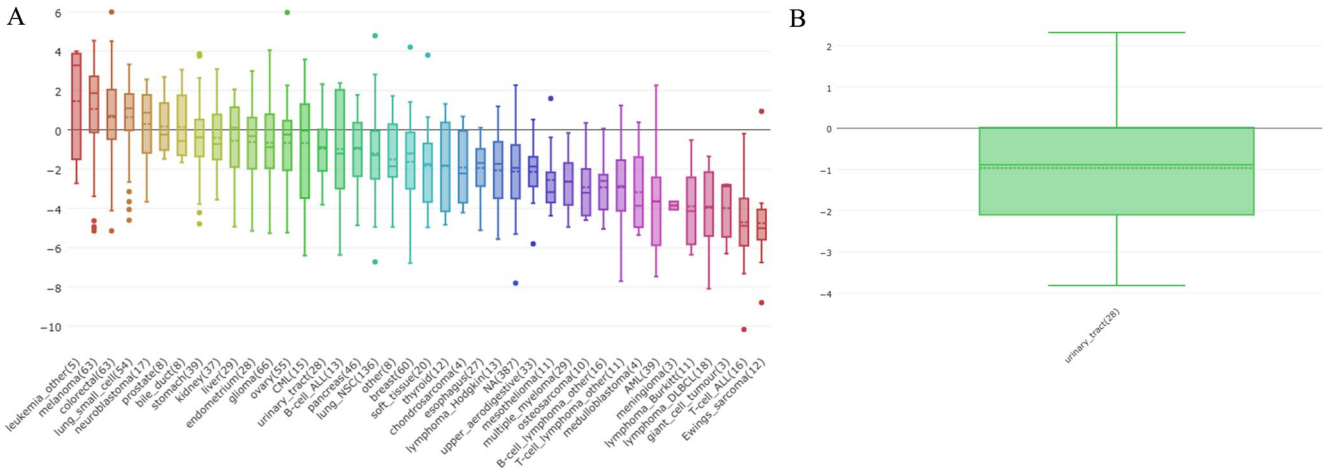

FIGURE 2 SORBS1 expression based on cancer cell line encyclopaedia (CCLE). (a) SORBS1 expression in various cancer cell lines. (b) SORBS1 expression was obviously down-regulated in urinary tract cancer cell lines.

2.7 | Transcription factors identification of SORBS1

To further explore the underlying mechanism of SORBS1 in BC, TFs of SORBS1 were predicted. Firstly, UCSC genome browser (http://genome.ucsc.edu/) was utilised for SORBS1 TFs prediction. The −2000 bp to +2000 bp region was limited in the transcription start site (TSS) out of interest. After that, JASPAR (http://jaspar.genereg.net/) was used to see the detail-binding information of those TFs.

3 | RESULTS

3.1 | mRNA expression of SORBS1 was down-regulated in BC

SORBS1 was down-regulated in most of the cancer cell lines. The expression of SORBS1 in 28 types of urinary tract cancer cell lines was down-regulated with the average expression of −0.8869 (Figure 2). Based on the TCGA and GTEx RNA-sequencing data, we found that SORBS1 mRNA expression was significantly down-regulated in BC tissues compared to non-BC tissues (8.27 ± 1.91 vs. 13.12 ± 2.49, $p < 0.001$). Receiver operating characteristic of RNA-sequencing showed that the AUC was 0.936. Furthermore, SORBS1 expression in different clinical parameters was calculated. SORBS1 expression was decreased in non-lymphovascular invasion compared with those lymphovascular invasions (8.02 ± 1.75 vs. 8.05 ± 2.05, $p = 0.021$), decreased in low grade compared with high grade (7.21 ± 1.24 vs. 8.33 ± 1.92, $p = 0.008$), decreased in T1 + T2 compared with T3 + T4 (7.85 ± 1.80 vs. 8.62 ± 1.94, $p < 0.001$), decreased in N0 compared with N1+N2+N3 (8.11 ± 1.86 vs. 8.83 ± 1.98, $p < 0.001$), and decreased in stage I + II compared with stage III + IV (7.70 ± 1.59 vs. 8.55 ± 1.99, $p < 0.001$) (Table 1). Survival analysis revealed no significance in different prognosis indexes, including overall survival (HR = 0.809; 95CI%:0.603–1.086; $p = 0.159$), disease specific survival (HR = 0.763; 95CI%:0.534–1.089; $p = 0.136$), disease-free interval (HR = 0.921; 95CI%:0.455–1.866; $p = 0.818$), and progression-free interval (HR = 0.768; 95CI%:0.571–1.034; $p = 0.083$).

In addition, 19 microarrays were collected for further analyses. Chips from the same platforms were merged and SORBS1 expression in each platform was calculated. In this part, GPL96(GSE2361, GSE3167, GSE5287), GPL570(GSE7476, GSE31684, GSE31189, GSE2109), GPL6102(GSE37815, GSE13507, GSE37817, GSE19423), GPL14951(GSE65635, GSE86411), GPL3883 (GSE19915), GPL6791(GSE24152), GPL13497(GSE40355), GPL6884(GSE52519), GPL17586 (GSE76211), and GPL10558(GSE51843) were included and merged into 10 platforms. SORBS1 expression was significantly down-regulated in GPL570, GPL6102, GPL3883, GPL13497, and GPL6884. Additionally, GPL96 revealed a down-regulation trend in BC tissues. The ROC curves of these platforms were also plotted (Figure 3).

3.2 | Protein expression of SORBS1 was down-regulated in BC

The SORBS1 protein expression was significantly down-regulated in BC, according to our tissue array (5.06 ± 1.34 vs. 10.76 ± 2.15, $p < 0.001$). However, no significant difference of SORBS1 protein expression was found in clinical

TABLE 1 SORBS1 mRNA expression in tissues and different clinical parameters based on RNA-sequencing.

			SORBS1 mRNA expression		
Clinical parameters	**Groups**	***N***	***M***	**SD**	***p*-value**
TCGA + GTEx	BC tissues	407	8.27	1.91	<0.001
Tissues	Non-BC tissues	28	13.12	2.49	
TCGA only					
Radiation therapy	No	361	8.27	1.90	0.192
	Yes	20	7.70	1.61	
Age	≤60	107	8.14	1.85	0.443
	>60	300	8.31	1.93	
Subtype	Non-papillary	270	8.48	2.00	<0.001
	Papillary	132	7.77	1.60	
Gender	Male	301	8.29	1.89	0.680
	Female	106	8.20	1.97	
Lymphovascular invasion	No	129	8.02	1.75	0.021
	Yes	151	8.55	2.05	
Metastasis	NO	146	8.22	1.95	0.303
	YES	61	8.53	2.17	
Grade	Low	21	7.21	1.24	0.008
	High	383	8.33	1.92	
Relapse	No	251	8.09	1.92	0.064
	Yes	125	8.47	1.72	
T	T1 + T2	122	7.85	1.80	<0.001
	T3 + T4	251	8.62	1.94	
N	N0	236	8.11	1.86	<0.001
	N1+N2+N3	129	8.83	1.98	
M	M0	196	7.92	1.78	<0.001
	MX	197	8.64	1.93	
Stage	I + II	132	7.70	1.59	<0.001
	III + IV	273	8.55	1.99	

Note: The reason for the variety of case number among different clinical categories is that clinical information of some patients were incomplete in TCGA.

Abbreviations: GTEx, Genotype-Tissue Expression; *M*, mean; *N*, number; SD, standard deviation; TCGA, The Cancer Genome Atlas.

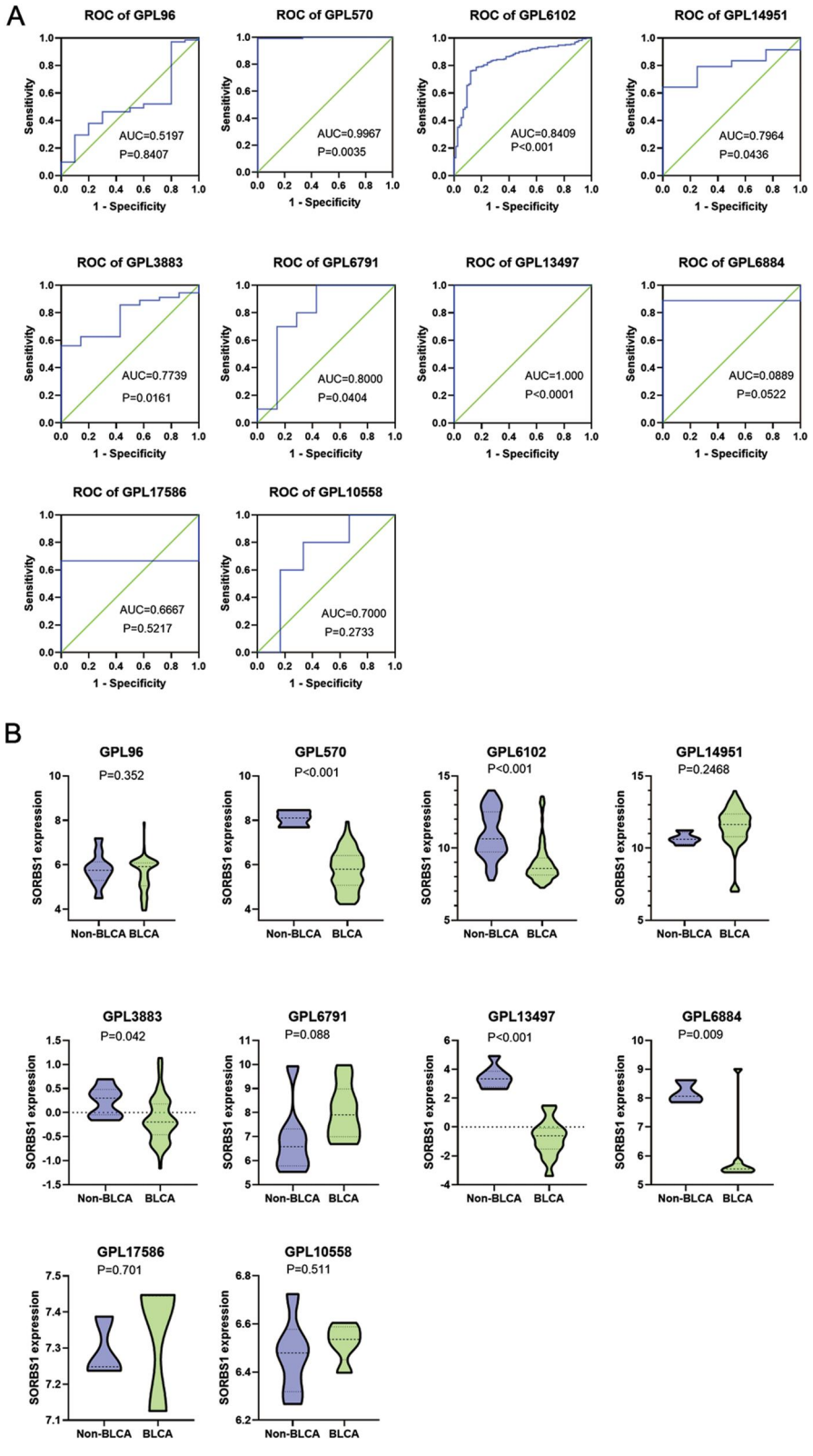

FIGURE 3 The SORBS1 expression in bladder cancer (BC) and non-BC tissues based on microarray. (a) The SORBS1 expression in BC tissues and non-BC tissues according to each platform. (b) The receiver operating characteristic (ROC) curves of SORBS1 in BC tissues and non-BC tissues according to each platform.

parameters (Table 2, Figure 4a). Moreover, remarkably down-regulation of SORBS1 protein expression was confirmed by HPA. The staining intensity and quantity of SORBS1 were obviously weaker in BC tissues (Figure 4b).

3.3 | Expression integration

The expression data from different resources were integrated to confirm the down-regulation of SORBS1 in BC. First,

TABLE 2 SORBS1 expression in different clinical parameters based on in-house IHC.

Clinical parameter	Groups	*N*	SORBS1 mRNA expression		*p*-value
			M	SD	
Tissues	BC tissues	137	5.06	1.34	<0.001
	Non-BC	34	10.76	2.15	
Age	≤60	58	5.03	1.31	0.858
	>60	79	5.08	1.37	
Gender	Male	118	5.04	1.34	0.728
	Female	19	5.16	1.38	
Grade	I + II	83	5.06	1.34	0.984
	III + IV	54	5.06	1.35	
T	T1 + T2	113	5.09	1.35	0.569
	T3 + T4	24	4.92	1.32	
N	N0	130	5.04	1.31	0.455
	N1 + N2	7	5.43	1.90	

Abbreviations: IHC, immunohistochemistry; *M*, mean; *N*, number; SD, standard deviation.

SORBS1 mRNA expression data, including RNA-sequencing and microarray, were pooled together with a random effect model. In total, 10 studies revealed a down-regulation tendency at the mRNA expression level, and the incorporative SMD reached −1.016 (95%CI: −1.746~−0.287, $p = 0.006$). Then, protein expression data were pooled together to confirm the SORBS1 expression in multiple levels. The results further confirmed the down-regulation of SORBS1, with pooled SMD reaching −3.727 (95%CI: −4.274~−3.180, $p < 0.001$, Figure 5a). In addition, SORBS1 represented clinical values with the AUC of sROC reaching 0.91 (95%CI: 0.88–0.93). The sensitivity and specificity were 0.86 (95%CI: 0.69–0.94) and 0.88 (95%CI: 0.82–0.92) (Figure 5b).

3.4 | Correlation between SORBS1 and TIICs

As shown in Figure 6, SORBS1 was negatively correlated with tumour purity (correlation = −0.342, $p < 0.001$) and positively correlated with macrophage (correlation = 0.358, $p < 0.001$). However, no significance was found between SORBS1 and other TIICs.

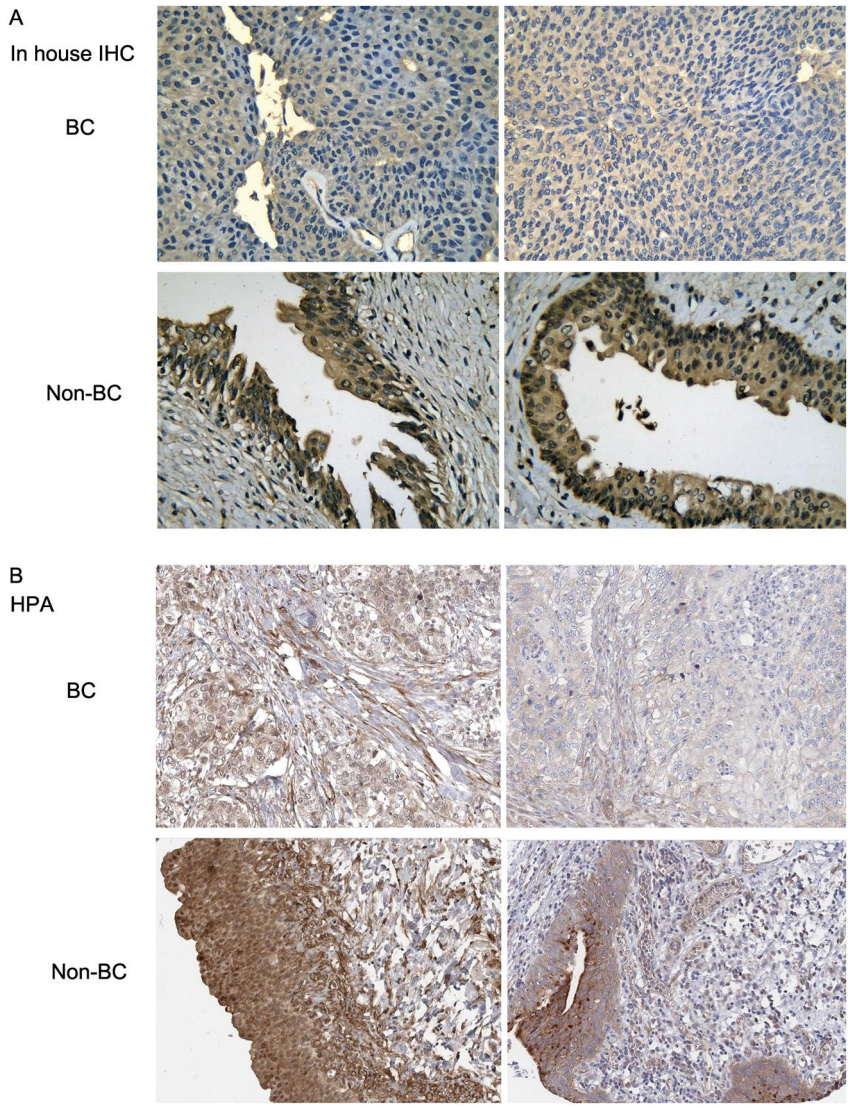

FIGURE 4 Immunohistochemistry images revealed protein expression of SORBS1 in bladder cancer (BC) and non-BC tissues. (a) BC tissues were negatively stained by immunostaining and normal tissues were positively stained based on our in-house IHC (x400). (b) Low staining in cancer tissues stained by SORBS1 and strong staining in normal tissues based on human protein Atlas (HPA) (x400).

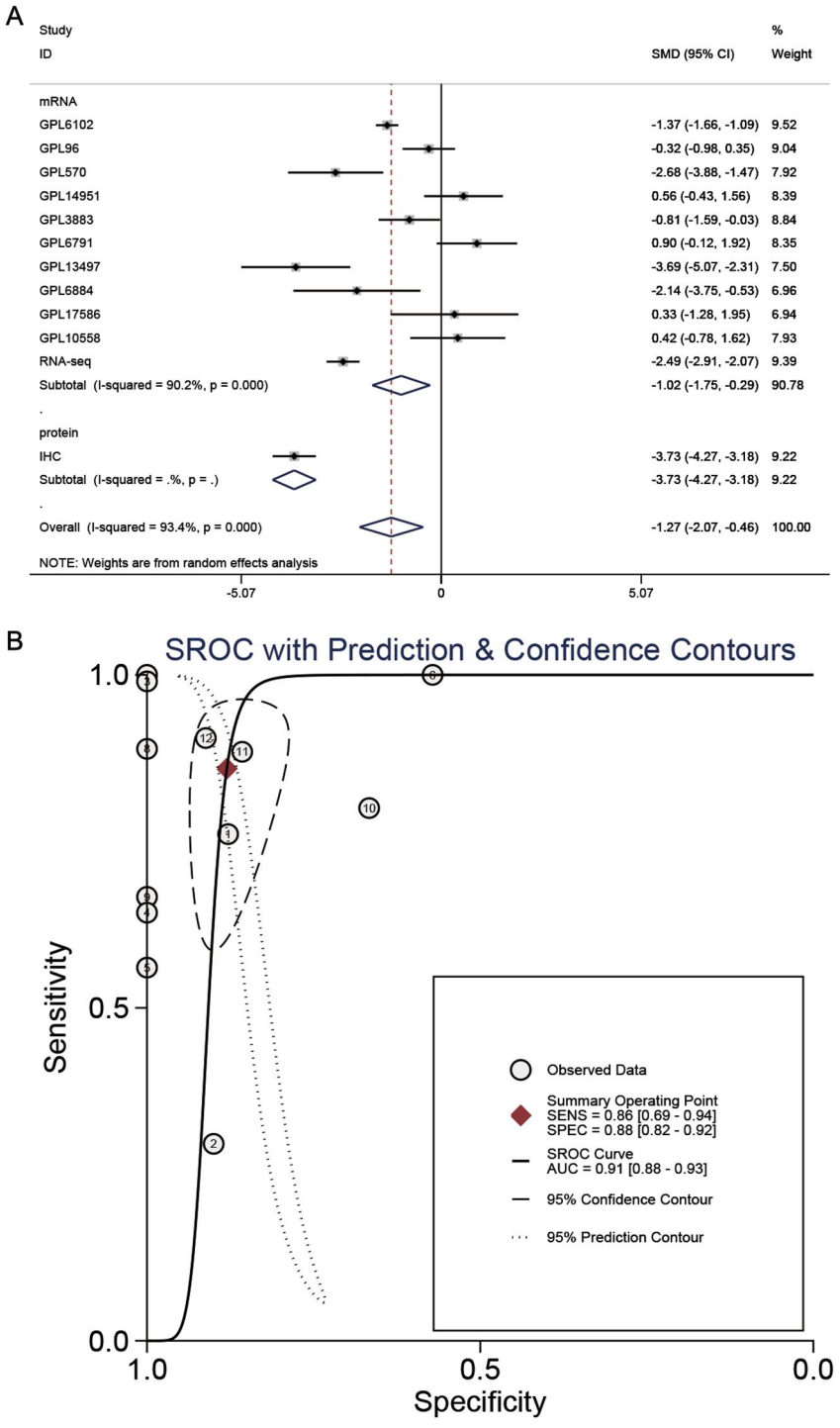

FIGURE 5 Expression integration of SORBS1. (a) The mRNA and protein expression integration of SORBS1 in bladder cancer (BC). SORBS1 was down-regulated at both mRNA and protein level. (b) sROC curve revealed the clinical significance of SORBS1 in BC.

3.5 | Functional enrichment analyses

The SORBS1-related genes were screened out. Related genes were selected when they appeared in more than eight platforms (Figure 7a). A total of 576 genes were screened, and 680 DEGs were screened out (Figure 7b). The overlapping 354 genes were considered as final SORBS1-related genes (Figure 7c). The results of enrichment analyses revealed that the most significant biological pathways of SORBS1-related genes were epithelial-mesenchymal transition (EMT) (Figure 8a). For biological process, genes were mainly enriched in signal transduction (Figure 8b). For cellular component, genes were mainly enriched in plasma membrane (Figure 8c). For molecular function, genes were mainly enriched in extracellular matrix structural constituent (Figure 8). In addition, hub genes including MYL9, MYLK, MYH11, and CALD1 were identified based on the score (Figure 9a). The expression of MYL9, MYLK, MYH11, and CALD1 was −1.436, −1.768, −1.676, and −1.477 (All $p < 0.001$) (Figure 9b).

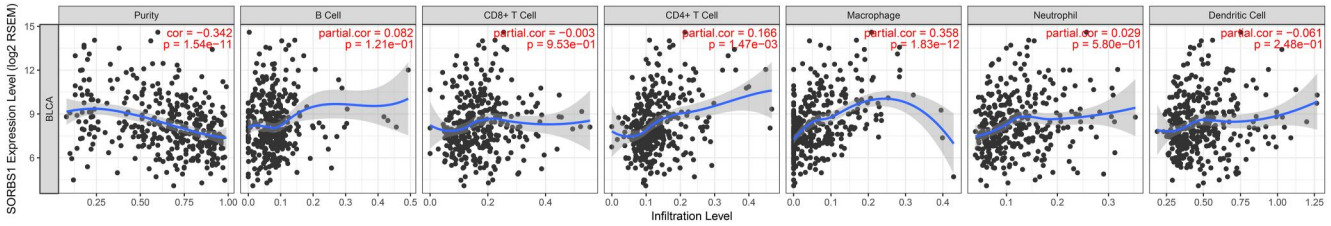

FIGURE 6 The correlation between SORBS1 and tumour-immune infiltration.

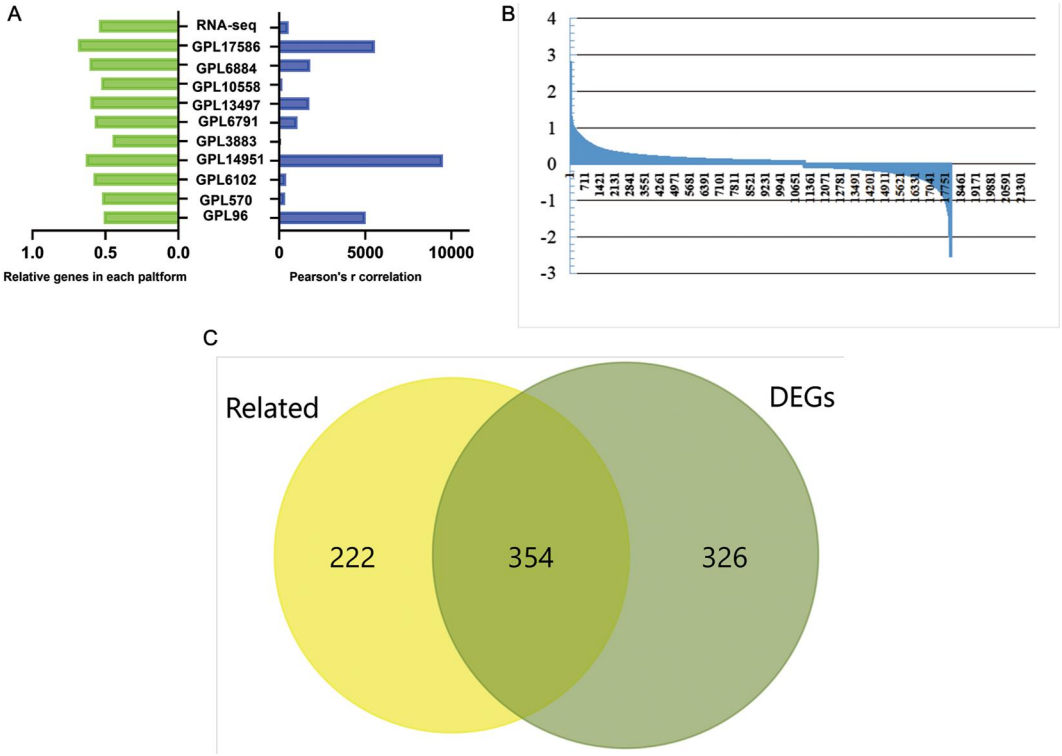

FIGURE 7 Identification of SORBS1-related genes. (a) SORBS1-related genes in each platform. (b) Differentially expressed genes (DEGs) from each platform. (c) Final SORBS1-related genes identification using Venn diagram.

3.6 | Identification of SORBS1 transcription factors

Transcription factors including USF1, CTCF, and STAT1 were identified by ChIP-seq data from the UCSC Genome Browser. As shown in Figure 10a, they could take effect in regulating SORBS1 expression while binding to the −2000 bp to +2000 bp region in TSS. Moreover, binding information details were obtained from JASPAR (Table 3, Figure 10b).

4 | DISCUSSION

SORBS1 is an important factor in insulin signalling. Lin et al. reported that SORBS1 was associated with insulin resistance and played a protective role for both obesity and diabetes. [26] Yang et al. reported that SORBS1 was related to body mass index. [13] A study from Germain et al. reported that SORBS1 may play a role in diabetic nephropathy. [27] SORBS1 also played important roles in other human diseases, including cerebral infarction, chronic obstructive pulmonary disease, hypertension, and polycystic ovary syndrome. [14, 28–30] In summary, SORBS1 plays a critical role in various human diseases. Hong et al. reported that SORBS1 expression was down-regulated in non-small cell lung cancer. [31] Aakula et al. reported that SORBS1 showed a down-regulation tendency in prostate cancer. [32] Western blotting and IHC also revealed that SORBS1 was down-regulated in colorectal cancer, and down-regulated SORBS1 was correlated with the tumour differentiation stage. [33] As for gastric cancer, Gong et al. discovered that SORBS1 was down-regulated in cancerous tissues. The SORBS1 expression was also significantly associated with the prognosis of gastric cancer patients. [34] However, no studies have been conducted to evaluate the expression and clinical significance of SORBS1 in BC.

In this study, we collected RNA-sequencing, microarray, and IHC data to elucidate the final SORBS1 expression in BC. Based on large enough samples analysis and multiple

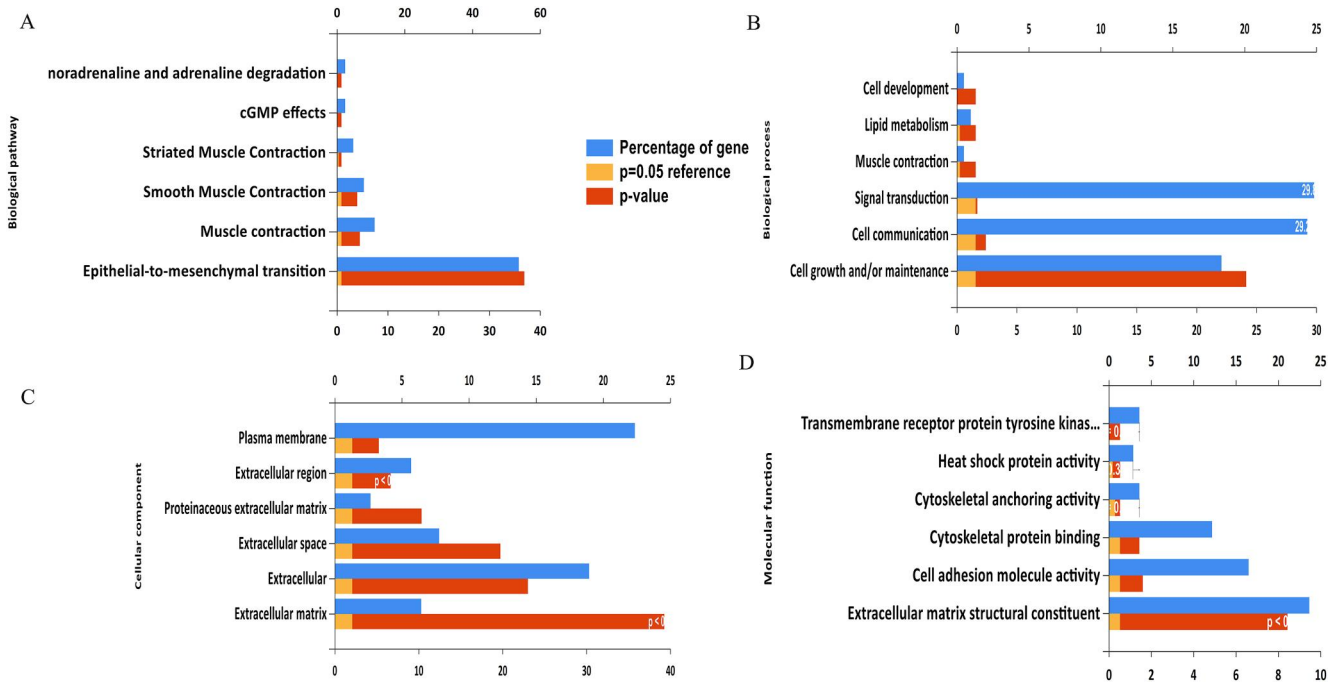

FIGURE 8 Functional enrichment analyses of SORBS1-related genes. (a) Biological pathways of SORBS1-related genes. (b) Biological process of SORBS1-related genes. (c) Cellular component of SORBS1-related genes. (d) Molecular function of SORBS1-related genes.

expression levels, we found that SORBS1 was significantly down-regulated in BC compare to non-cancerous tissues. Clinical significance of SORBS1 in the treatment of BC was also explored. Therefore, we concluded that SORBS1 played an important role in the occurrence of BC.

The function of SORBS1 has been studied in some kinds of cancer. In MCF10A, MDA-MB-231, and HBL100 breast cancer cell lines, cells with decreased SORBS1 expression showed higher motility. Increased SORBS1 expression suppressed the invasive and migratory abilities of SUM159 cells. Furthermore, the mouse cancer model was used to conduct the in vivo experiments to confirm the tumour-suppressor role of SORBS1. The in vivo results were consistent with that of the in vitro results. In the breast cancer cell lines, results of immunofluorescent staining showed that the knockdown of SORBS1 induced EMT. Besides, SORBS1 also inhibited the JNK signalling pathway, which promoted the invasion and migration of breast cancer cells. Moreover, in the breast cancer cell lines, SORBS1 promoted the cisplatin-related drug sensitivity. Researchers observed breast cancer patients that were treated with cisplatin to evaluate the correlation between SORBS1 expression and the patients' prognoses. Results showed that patients with lower SORBS1 exhibited shorter OS. In vitro experiments showed that SORBS1 was negatively regulated by miR-142-5p in breast cancer cells by conducting the luciferase reporter assay. MiR-142-5p promoted the migration, invasion, and proliferation of breast cancer cells via targeting SORBS1. The SORBS1-aberrant expression could reverse the promotion effect of miR-142-5p. The peroxisome proliferator-activated receptor gamma (PPAR) signalling pathway regulated tumour progression and development in different types of breast cancer; SORBS1 was involved in the PPAR signalling pathway and could be a critical biomarker. [17, 18, 35, 36] Thus, SORBS1 may play a tumour-suppressor role in the pathogenesis of breast cancer and could be a therapeutic target in cisplatin-based chemotherapy. The functional enrichment analyses of SORBS1 in colorectal cancer showed that SORBS1 was associated with the PPAR signalling pathway. Besides, SORBS1 was related to actin binding in the peritoneal metastatic cell lines of colorectal cancer. [33, 37] In gastric cancer, SORBS1 was a hub gene in the gastric cancer progression module. The mTOR inhibitor Sirolimus was identified as an essential drug for the gastric cancer progression module. Results of in vitro experiments confirmed the function of Sirolimus in inhibiting gastric cancer progression, which also revealed that SORBS1 may be a target in cancer therapy. [34] In conclusion, SORBS1 may serve as a suppressor in tumourigenesis and could also be a potential target in chemotherapy, especially in cisplatin-based chemotherapy. Thus, exploring the clinical significance and molecular mechanism of SORBS1 in BC is necessary.

To explore the possible mechanisms of SORBS1 in BC, the SORBS1-related genes were identified from RNA-sequencing and microarray. Functional enrichment analyses of these SORBS1-related genes showed that they were mainly enriched in the EMT program. As previously described, SORBS1 suppressed the EMT program in breast cancer cells. [17] The EMT program is a biological program that converts epithelial cells to mesenchymal cells in tissues and developmental stages. The EMT program is critical in different kinds of pathological processes, especially in cancer pathogenesis. The functions and

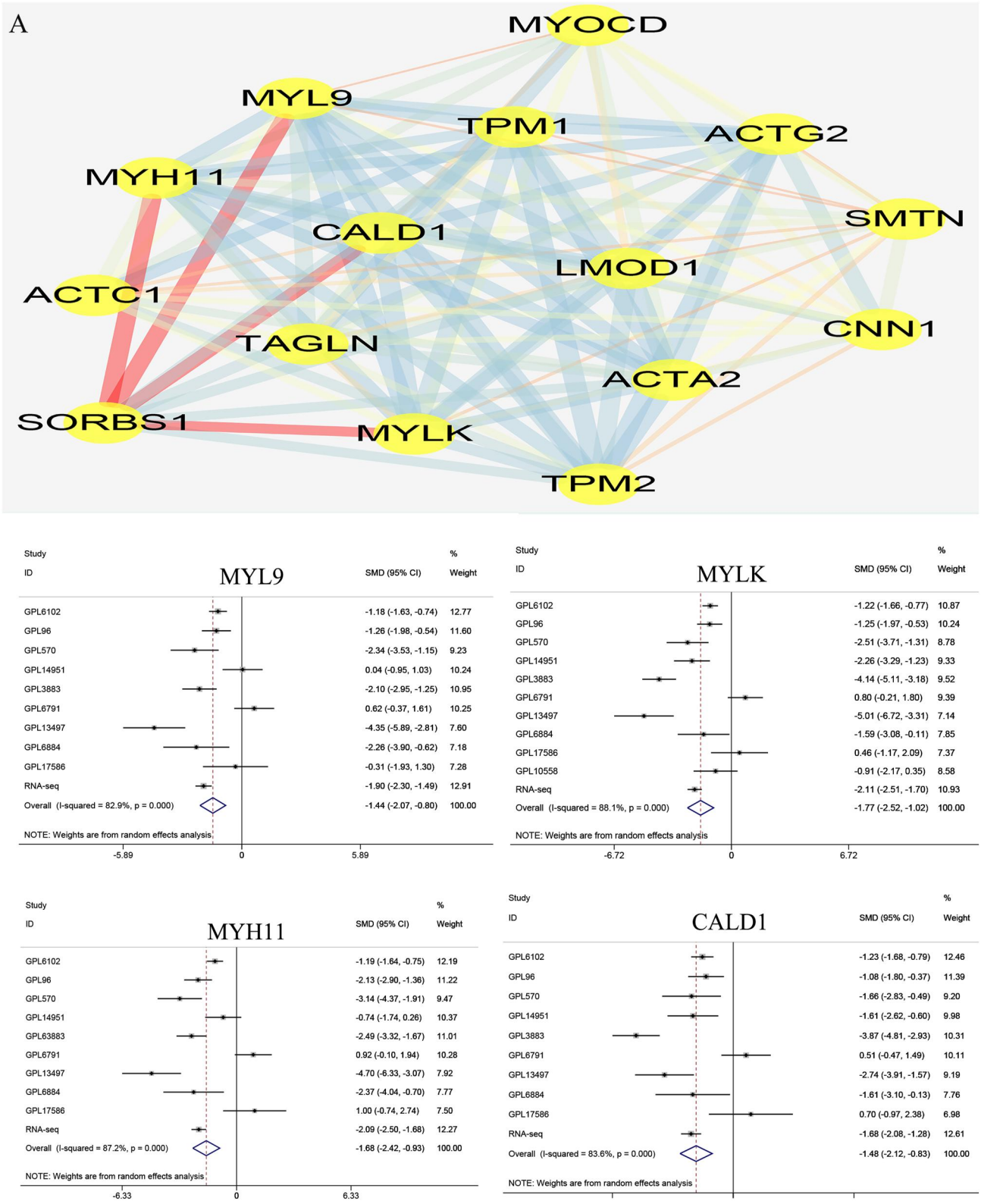

FIGURE 9 Protein-protein interaction (PPI) network of SORBS1-related genes. (a) Interaction between SORBS1 and its related genes. (b) Expression of hub genes in bladder cancer (BC) and non-BC tissues. BC: bladder cancer.

mechanisms of the EMT program were studied in various cancers. As reported, the EMT program facilitated the cancer metastasis, improved the tumour heterogeneity, and increased the therapeutic resistance. [38] The importance of the EMT program has been well explored, and the function of SORBS1 in suppressing the EMT program has also been verified in

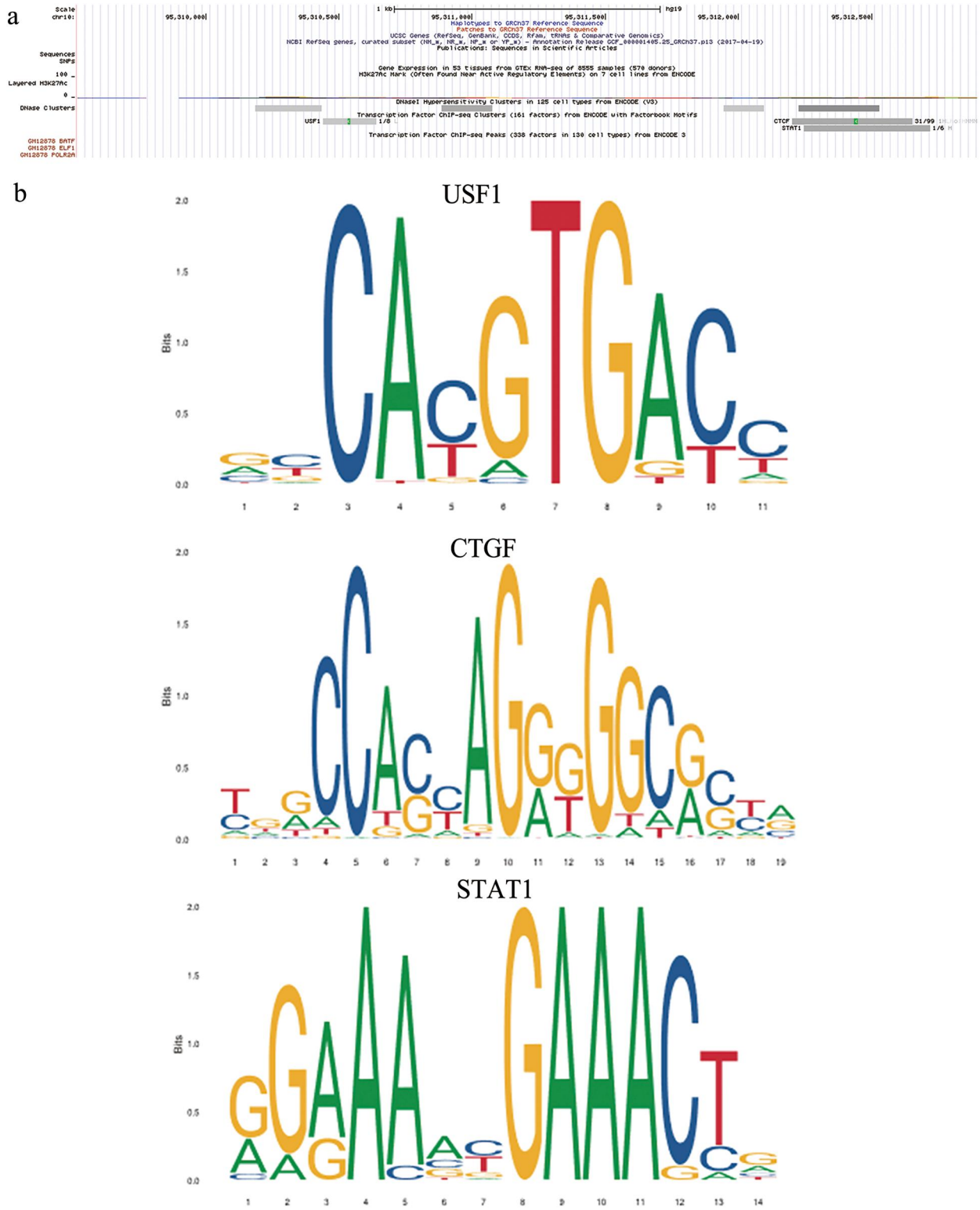

FIGURE 10 Transcription factors (TFs) prediction of SORBS1. (a) TFs located in the transcription start site (TSS) of SORBS1. (b) Motif logo of predicted TF. AbbreviationsTSS, TSS.

breast cancer cell lines. Thus, SORBS1 may also serve as a tumour suppressor by inhibiting the EMT program in BC. However, in vitro and in vivo experiments are needed to confirm the relationship between SORBS1 and the EMT program. Furthermore, considering the core molecules of the EMT program consist of EMT-activating TFs and their downstream target genes, three TFs of SORBS1 including USF1, CTCF, and STAT1 were identified. [39, 40].

TABLE 3 Information on binding sites of predicted TFs in SORBS1 TSS.

TF	Score	Relative score	Sequence ID	Strand	Predicted sequence
USF1	8.05685	0.879008823	>chr10: 95,309,271-95,312,771	+	ACCAGGTGGTC
CTCF	11.8824	0.840859362	>chr10: 95,309,271-95,312,771	+	TACACAGCAGAGGACACCA
STAT1	15.126	0.904728967	>chr10: 95,309,271-95,312,771	−	AGAAAAGGAAACAG

Abbreviation: TFS, transcription factors; TSS, transcription start site.

In summary, SORBS1 was significantly down-regulated in BC at both mRNA and protein levels. Moreover, SORBS1 may function as a tumour suppressor via inhibiting the EMT program and could be a potential target in BC treatment.

AUTHOR CONTRIBUTIONS

Sheng-Hua Li prepared the manuscript, experiments, and statistical analysis. Gao-Qiang Zhai conducted statistical analysis, data collection, and manuscript preparation. Rong-Quan He conducted experiments and statistical analysis. Gang Chen, Shi-Shuo Wang, and Jia-Lin Liu helped with literature search, manuscript preparation, and experiments. Ji-Wen Cheng and Hai-Biao Yan guided the manuscript editing and revisions. Zhi-Guang Huang prepared the study designation and experiments guidance.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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