Am J Transl Res 2024;16(3):873-888
www.ajtr.org /ISSN:1943-8141/AJTR0154067

Original Article

Pan-cancer analysis of HS6ST2: associations with prognosis, tumor immunity, and drug resistance

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Received October 24, 2023; Accepted February 8, 2024; Epub March 15, 2024; Published March 30, 2024

Abstract: Objectives: In this comprehensive study spanning 33 malignancies, we explored the differential expression and prognostic significance of Heparan sulfate 6-O-sulfotransferase 2 (HS6ST2). Methods: TIMER2, UALCAN, and GEPIA2 were used for the expression analysis. cBioPortal was used for mutational analysis. CancerSEA, STRING, and DAVID, were employed for the single cell sequencing data analysis, protein-protein interaction network development, and gene enrichment analyses, respectively. GSCAlite and RT-qPCR were used for drug sensitivity and expression validation analysis. Results: HS6ST2 exhibited significant ($P < 0.05$) overexpression in multiple cancers. Prognostically, elevated HS6ST2 expression was significantly associated with poor overall survival (OS) in patients with cervical squamous cell carcinoma (CESC), kidney chromophobe (KICH), lung adenocarcinoma (LUAD), and stomach adenocarcinoma (STAD), emphasizing its potential as a prognostic indicator in these cancers. Moreover, HS6ST2 expression correlated with pathological stages in CESC, KICH, LUAD, and STAD patients. Exploration of genetic alterations using cBioPortal unveiled distinct mutational landscapes, with low mutation frequencies in CESC, KICH, LUAD, and STAD. Additionally, reduced DNA methylation in CESC, KICH, LUAD, and STAD suggested a potential link between hypomethylation and heightened HS6ST2 expression. Analysis of immune cell infiltration revealed a positive correlation between HS6ST2 expression and the infiltration of CD8+ T and CD4+ T cells in CESC, KICH, LUAD, and STAD, highlighting its involvement in the tumor immunology processes. Single-cell functional states analysis demonstrated associations between HS6ST2 and diverse cellular processes. Moreover, gene enrichment analysis revealed the involvement HS6ST2 in crucial cellular activities. GSCAlite analysis underscored the potential of HS6ST2 as a therapeutic target, showing associations with drug sensitivity. Finally, experimental validation through reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and immunohistochemistry in LUAD tissues confirmed elevated HS6ST2 expression. Conclusion: Overall, this study provides a comprehensive understanding of HS6ST2 in CESC, KICH, LUAD, and STAD, emphasizing its potential as a prognostic biomarker and therapeutic target.

Keywords: Cancer, HS5ST2, prognosis, therapeutic target

https://doi.org/10.62347/NCPH5416

Introduction

Cancer stands as a prominent contributor to human mortality, exerting substantial adverse effects on both global societal well-being and economic aspects [1, 2]. Cancer arises from genetic alterations in the key genes which lead to uncontrolled cell growth [3-7]. These genetic alterations can result from various factors, including exposure to carcinogens, genetic predisposition, and lifestyle choices such as tobacco use, poor diet, and lack of physical activity, as well as infections and environmental influences [8]. Despite advancements in surgical procedures, chemoradiotherapy, targeted therapy, and immunotherapy, which have enhanced cancer treatment outcomes, the overall prognosis and survival rates for cancer patients remain suboptimal due to various factors such as drug resistance and adverse effects [9, 10]. Hence, the imperative task of identifying novel pan-cancer biomarkers and therapeutic targets arises, holding pivotal significance in the ongoing efforts to improve human health [11, 12].

The HS6ST family includes three isoforms (HS6ST1, 2, and 3) primarily responsible for sulfating heparan sulfate proteoglycans (HSPGs) [13]. Heparan sulfate 6-0-sulfotransferase 2 (HS6ST2), a member of this family, has an alternatively spliced form known as HS6ST-2S, featuring a deletion of 40 amino acids. Despite sequence variations, both enzymes facilitate the transfer of sulfate groups from adenosine 3'-phosphate, 5'-phosphosulphate (PAPS) to the 6-0 position of glucosamine residues in HSPGs [14]. Through this mechanism, HSPGs actively contribute to various biological processes such as blood clotting, cell recognition, adhesion, proliferation, and differentiation, achieved through interactions with various cytokines [15, 16]. Previous research has demonstrated the correlation of HS6ST2 with the advancement of malignant tumors, showing its up-regulation in diverse cancer types, including thyroid [17, 18], colorectal [19], pancreatic [20], ovarian [21], breast cancer [22], and chondrosarcomas [23]. However, the specific function of HS6ST2 in pan-cancer remains unclear at present to the best of our knowledge.

In this study, a comprehensive analysis of HS6ST2 was conducted, utilizing extensive cancer data from online databases and complemented by confirmatory molecular experiments. The aim was to unravel the expression patterns and biological functions of HS6ST2 in pan-cancer from various perspectives. This research not only enhanced our comprehension of HS6ST2's role in tumorigenesis but also underscored its potential as a prognostic and immunological biomarker across different types of cancer. Additionally, the investigation delved into the underlying mechanisms through which HS6ST2 operates in diverse cancer contexts.

Methodology

Pan-cancer expression analysis of HS6ST2

The Tumor Immune Estimation Resource 2.0 (TIMER2.0, http://timer.cistrome.org/) serves as a comprehensive tool for analyzing immune infiltrates in various cancer types [24]. We utilized TIMER2.0 by inputting "HS6ST2" to assess the disparity in HS6ST2 expression between tumors and adjacent normal tissues across 33 cancer types from The Cancer Genome Atlas (TCGA).

UALCAN (http://ualcan.path.uab.edu) is an interactive web tool for in-depth analysis of cancer-omics data, encompassing clinical information for 31 cancer types [25]. In this study, we evaluated HS6ST2 expression levels in both tumor and normal samples, along with tumor models categorized by different stages.

Survival prognosis analysis

GEPIA2 (http://gepia2.cancer-pku.cn/), developed by Peking University scientists, is a robust bioinformatics tool for extensive gene expression analysis. Providing accessible entry to TCGA and GTEx databases, it empowers researchers to delve into complex gene expression landscapes across diverse tissues and cancers, facilitating innovative discoveries [26]. In this study, GEPIA2 was employed to investigate the association between HS6ST2 expression and cancer prognosis, specifically overall survival (OS).

Genetic alteration and DNA methylation analysis

The cBioPortal (https://www.cbioportal.org/) was queried to identify gene alterations in HS6ST2 within TCGA PanCancer Atlas Studies (Ref). Utilizing the "Oncoprint", "Cancer Type Summary", and "Mutations" modules, we investigated genetic alterations and mutation site

details. Methylation level of HS6ST2 in cancers and corresponding normal tissues was investigated in the OncoDB database (http://ualcan.path.uab.edu/analysis.html) [27]. OncoDB is an invaluable resource in cancer research, serving as a centralized platform for oncogenic mutation data. Developed to facilitate genomic exploration, it empowers scientists to investigate the genetic alterations underlying cancer, advancing our understanding and treatment strategies in the ongoing battle against this complex disease [28].

Immunogenomic analyses

TIMER2 algorithm was applied to analyze the relationship between HS6ST2 expression and immune infiltration levels across specified cancers, using TIMER2 tool (http://timer.cistrome.org/) [24].

Single cell sequencing

Using CancerSEA (http://202.97.205.69/CancerSEA/) [29], we explored the correlation between HS6ST2 expression and different functional status of cancer cells at the single cell levels. CancerSEA is a comprehensive resource for deciphering cancer-associated signaling pathways. By integrating omics data, it provides a systematic exploration of the molecular landscape in cancer, aiding researchers in unraveling intricate signaling networks and identifying potential therapeutic targets for precise and effective interventions.

Protein-protein interaction network and enrichment analysis

STRING (https://string-db.org/) [30] and DAVID (https://david.ncifcrf.gov/) [31] are powerful bioinformatics tools. STRING predicts protein-protein interactions, while DAVID offers functional annotation, facilitating in-depth analysis and interpretation of complex biological data for researchers. In this work, STRING was used to construct the PPI of the HS6ST2 binding partners while DAVID was utilized for the enrichment analysis.

Drug sensitivity analysis

The GSCAlite database (http://bioinfo.life.hust.edu.cn/web/GSCALite/) [32] was employed to establish the correlation between drug sensitivity and the mRNA expression of HS6ST2.

Clinical samples and reverse transcription polymerase chain reaction (RT-qPCR)

After getting approval from the ethical committee, a total of 40 matched samples of LUAD and adjacent surrounding normal tissues were obtained at the Nishtar Medical College, Multan, Pakistan. RNA was isolated from fresh tissue samples utilizing the Trizol extraction method [33]. The extracted RNA was subsequently transcribed in reverse into cDNA using a reverse transcription kit supplied by a Chinese genetic engineering company. The PCR reaction mixture underwent specific temperature and time conditions in a thermal cycler for amplification. This process included initial denaturation to separate DNA strands, annealing to enable primer binding to template DNA, and extension for polymerase synthesis of new DNA strands. A total of 45 cycles were executed with a maximum system volume of 20 µl. Fluorescence expression was quantified using the ANanoDrop3000 from Thermo Fisher Scientific, Waltham, USA. The 2-ΔΔCT method was employed to ascertain the relative expression level of HS6ST2, HS3ST1, HS2ST1, GPC3, and SDC3. The primer sequences utilized were as follows: β-actin FORWARD: 5'-CTGGCACCACACCTTCTACAATG-3', REVERSE: 5'-TGGGTCATCTTCTCACGGTTGG-3'. HS6ST2 FORWARD: 5'-GCTGCTACACTGGCGATGACTG-3', REVERSE: 5'-CCTGGCGGTTGTTGGCTAGATTG-3'. HS3ST1 FORWARD: 5'-GCGTGCTATCTGACTACACCCA-3', REVERSE: 5'-GCCTTGTAGTCCACATTGAGCC-3'. HS2ST1 FORWARD: 5'-GATCCTATTGAGAGGCTAGTTTCT-3', REVERSE: 5'-AGCACAGTCTGAGCCACCTTCT-3'. GPC3 FORWARD: 5'-CATTGGAGGCTCTGGTGATGGA-3', REVERSE: 5'-TTGTCCTTCGGAGTTGCCTGCT-3'. SDC3 FORWARD: 5'-CTCCTGGACAATGCCATCGACT-3', SDC3 REVERSE: 5'-TGAGCAGTGTGACCAAGAAGGC-3'.

Immunohistochemistry (IHC) staining analysis

In our investigation, we verified HS6ST2 expression in both LUAD tissue and corresponding adjacent non-cancerous samples. The tissues underwent formalin fixation for morphological preservation. Heat-induced epitope retrieval facilitated antigen retrieval. To minimize false positives, nonspecific binding sites were blocked with 10% goat serum. HS6ST2, HS3ST1, HS2ST1, GPC3, and SDC3 primary antibodies (Abcam, 1:200) were applied and left to incubate overnight at 4 degrees Celsius. Post-

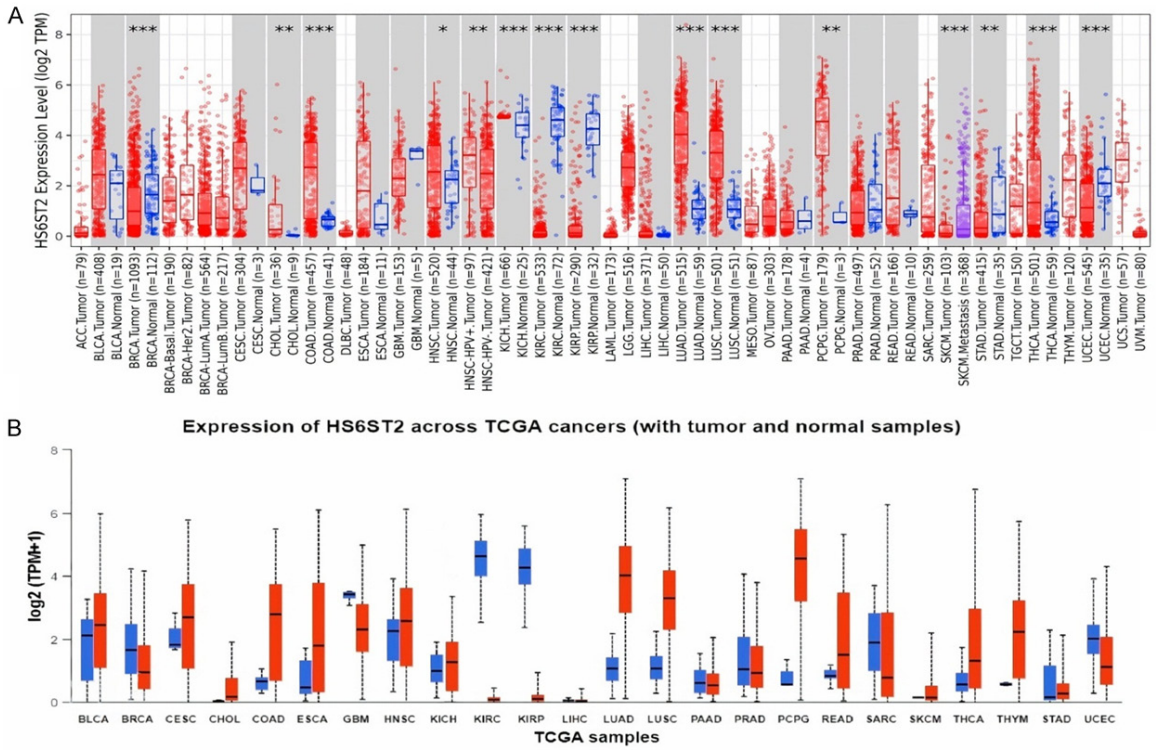

Figure 1. The pan-cancer mRNA expression status of HS6ST2. A. Analysis of HS6ST2 between tumor and non-tumor samples via the TIMER2 from TCGA database. B. Analysis of HS6ST2 between tumor and non-tumor samples via the UALCAN from TCGA database. **p*-value < 0.05, ***p*-value < 0.01, ****p*-value < 0.001. HS6ST2 = Heparan sulfate 6-O-sulfotransferase 2.

incubation, samples underwent three 5-minute PBS washes to eliminate unbound antibodies and other nonspecific substances. Following that, secondary antibodies labeled with horseradish peroxidase (HRP) (Beyotime, 1:100) were introduced and incubated for 30 minutes. Subsequent to this, samples underwent PBS washing four to eight times for 5 minutes each, eliminating unbound secondary antibodies and other nonspecific substances. DAB (3,3'-diaminobenzidine) chromogen was applied, and a reaction period of 5-15 minutes ensued. The development of a yellow color was observed under a microscope, and the reaction was promptly terminated. Ultimately, tissue sections were scrutinized under a microscope to assess the expression of the target protein.

Statistical analysis

All other analyses were performed with the use of R software (version 3.6.3). A *p*-value below 0.05 was considered to have statistical significance. Correlation analysis between two variables was conducted using the Spearman test.

Results

Differential expression of HS6ST2

We initiated a comprehensive pan-cancer analysis involving 33 malignancies sourced from the TCGA database to gain deeper insights into the expression of HS6ST2 across diverse cancer types. HS6ST2 exhibited significant (*p*-value < 0.05) overexpression in bladder urothelial carcinoma (BLCA), cholangiocarcinoma (CHOL), pheochromocytoma and paraganglioma (PCPG), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), chromophobe renal cell carcinoma (KICH), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC) (**Figure 1**). Conversely, tumor tissues exhibited reduced levels of HS6ST2

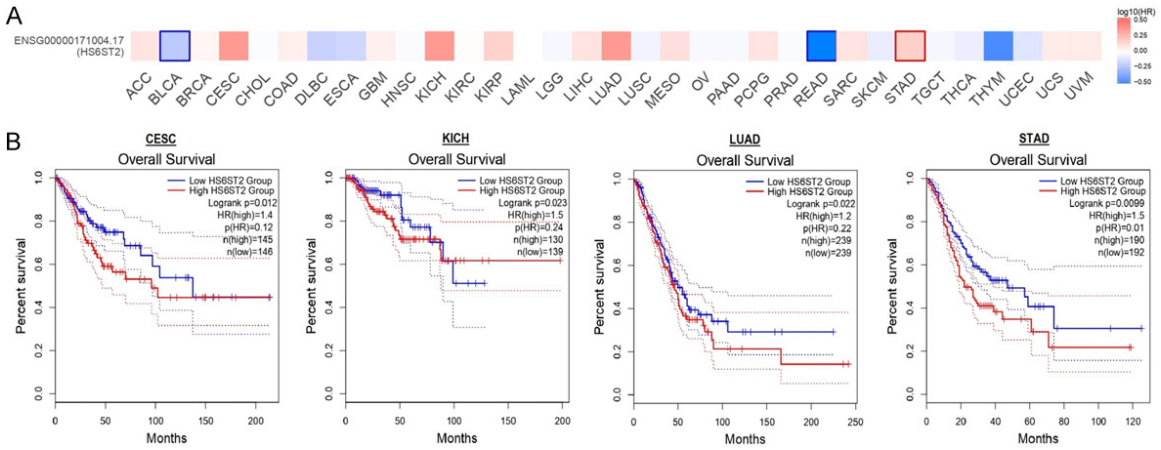

Figure 2. Illustration of the relationship between HS6ST2 expression and patient prognosis in pan-cancer. A. Displays HS6ST2 expression-based survival map across 33 cancer types. B. Shows Kaplan-Meier curves for overall survival in CESC, KICH, LUAD, and STAD based on HS6ST2 expression. Level of significance = *p*-value < 0.05. HS6ST2 = Heparan sulfate 6-O-sulfotransferase 2, CESC = cervical squamous cell carcinoma, KICH = kidney chromophobe, LUAD = lung adenocarcinoma, STAD = stomach adenocarcinoma.

compared to healthy tissues in breast invasive carcinoma (BRCA), kidney renal papillary cell carcinoma (KIRP), uterine corpus endometrial carcinoma (UCEC), and kidney renal clear cell carcinoma (KIRC) (**Figure 1**).

Prognostic significance of HS6ST2

Utilizing the mRNA expression of HS6ST2, we segregated patients into high and low expression groups, subsequently examining the relationship between HS6ST2 expression and prognosis across various tumors. Our findings indicated that elevated HS6ST2 expression correlated with significantly (*p*-value < 0.05) diminished OS in patients with CESC, KICH, LUAD, and STAD (**Figure 2**). This suggests that HS6ST2 expression closely correlates with adverse prognoses in CESC, KICH, LUAD, and STAD, highlighting its potential as a prognostic indicator in these specific cancers.

Correlation between HS6ST2 expression and pathological stages in CESC, KICH, LUAD, and STAD

Moreover, we utilized UALCAN to investigate the impact of HS6ST2 mRNA expression on patients' pathological stages. Our findings revealed a significant (*p*-value < 0.05) correlation between HS6ST2 expression and the pathological stages of patients with CESC, KICH, LUAD, and STAD (**Figure 3A-D**).

Genetic alterations and promoter methylation level of HS6ST2

To unravel the mutational landscape and biological implications of HS6ST2 in the progression of CESC, KICH, LUAD, and STAD, we delved into HS6ST2 genetic alterations using the cBioPortal database. Our analysis revealed that CESC and KICH exhibited the highest frequency of HS6ST2 mutations at 2.2%, primarily characterized as "missense mutations" (**Figure 3E**). In contrast, LUAD (1.6%) and STAD (2.1%) displayed lower frequencies of HS6ST2 mutations, predominantly in the "missense mutations" category (**Figure 3E**). This exploration sheds light on the genetic variations in HS6ST2 across different cancers, offering insights into potential mutational mechanisms contributing to disease progression in these specific malignancies.

Dysregulated DNA methylation controls are implicated in numerous diseases, notably cancer [34]. Cancer cells exhibit abnormal DNA methylation patterns, encompassing genomic hypomethylation and site-specific hypermethylation [35]. Using the OncoDB database, we investigated the promoter methylation status of HS6ST2 across various tumors. Our findings revealed that the methylation levels of HS6ST2 in CESC, KICH, LUAD, and STAD were significantly (*p*-value < 0.05) lower than those in normal tissues (**Figure 4**). This observation pro-

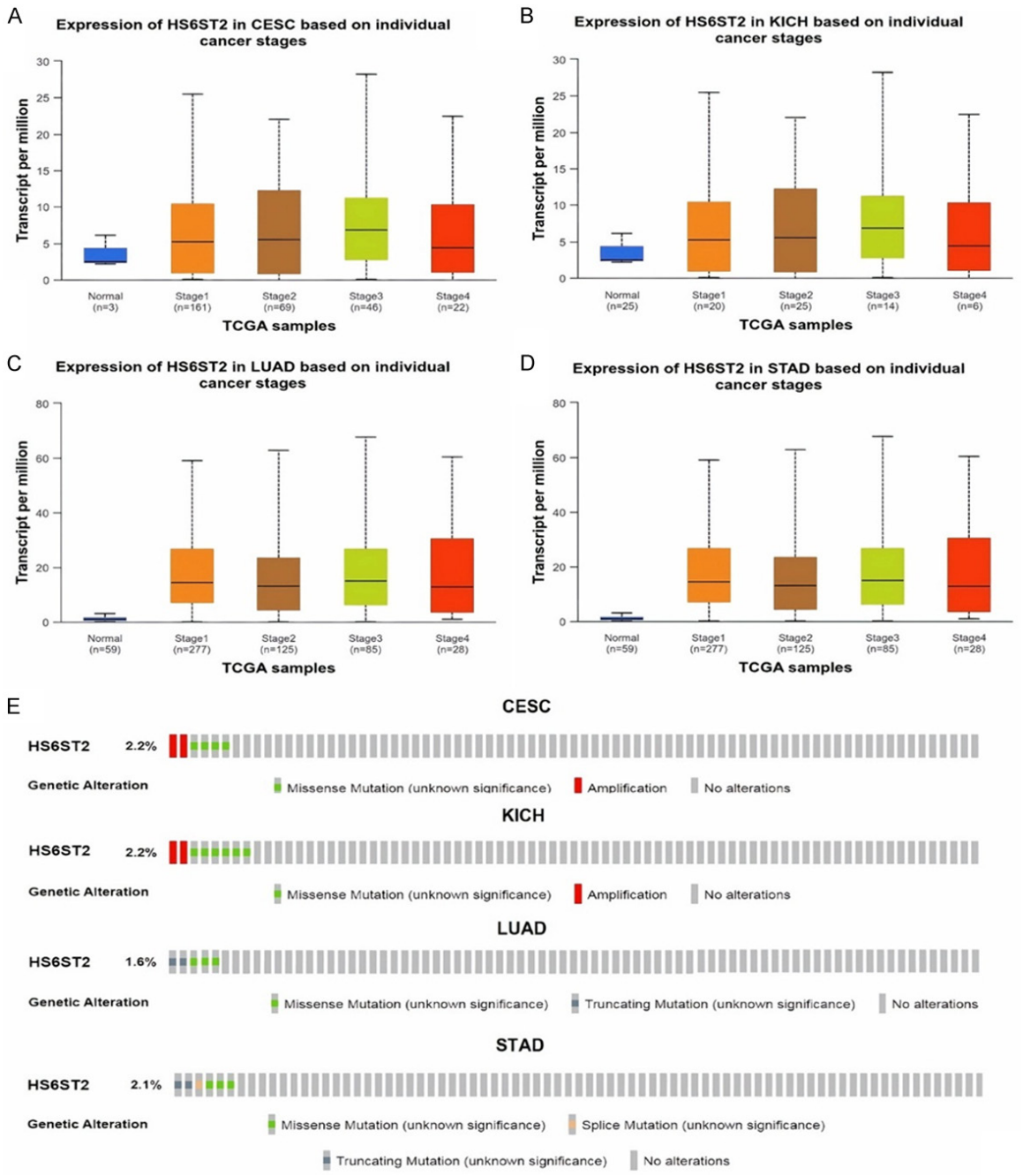

Figure 3. Depiction of the association between HS6ST2 mRNA expression and diverse pathological stages, along with mutational analysis in CESC, KICH, LUAD, and STAD. A. Showcase the correlation between HS6ST2 mRNA expression and distinct pathological stages in CESC. B. Showcase the correlation between HS6ST2 mRNA expression and distinct pathological stages in KICH. C. Showcase the correlation between HS6ST2 mRNA expression and distinct pathological stages in LUAD. D. Showcase the correlation between HS6ST2 mRNA expression and distinct pathological stages in STAD. E. Presents the frequency and types of genetic mutations in the HS6ST2 gene across these CESC, KICH, LUAD, and STAD. Level of significance = *p*-value < 0.05. HS6ST2 = Heparan sulfate 6-O-sulfotransferase 2, CESC = cervical squamous cell carcinoma, KICH = kidney chromophobe, LUAD = lung adenocarcinoma, STAD = stomach adenocarcinoma.

vides a potential explanation for the heightened expression of HS6ST2 in these specific tumors, suggesting a link between decreased methylation and increased gene expression.

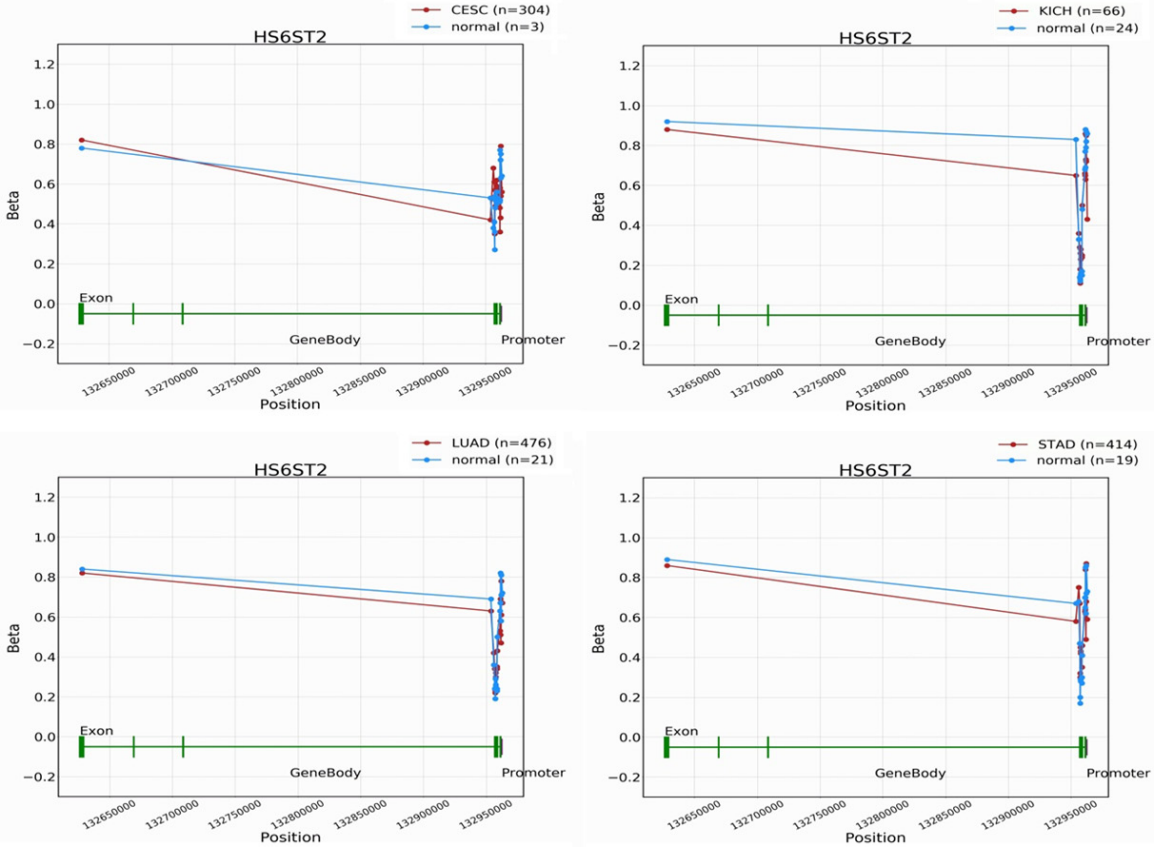

Figure 4. Depiction of correlation between HS6ST2 mRNA expression and promoter methylation level in CESC, KICH, LUAD, and STAD via the OncoDB database. Level of significance = *p*-value < 0.05. HS6ST2 = Heparan sulfate 6-O-sulfotransferase 2, CESC = cervical squamous cell carcinoma, KICH = kidney chromophobe, LUAD = lung adenocarcinoma, STAD = stomach adenocarcinoma.

Correlation analysis between HS6ST2 expression and immune cell infiltration

The extent of immune infiltration in the tumor microenvironment (TME) significantly influences cancer initiation, progression, and metastasis [36]. Leveraging the TIMER2.0 database, we explored the association between HS6ST2 expression and the infiltration of CD8+ T and CD8+ T immune cells within the TME. Our analysis revealed a noteworthy positive correlation (*p*-value < 0.05) between HS6ST2 expression and the infiltration levels of CD8+ T and CD4+ T immune cells in CESC, KICH, LUAD, and STAD (**Figure 5**). These findings collectively suggest a substantial involvement of elevated HS6ST2 levels in the tumor immunology processes within these specific cancers.

Functional states analysis of HS6ST2 at single cell levels

Employing CancerSEA, we delved into the single-cell functional states of HS6ST2 in CESC, KICH, LUAD, and STAD. The outcomes revealed a positive correlation between HS6ST2 and processes such as "angiogenesis, apoptosis, cell cycle, differentiation, DNA damage, DNA repair, epithelial-mesenchymal transition (EMT), hypoxia, inflammation, invasion, metastasis, proliferation, quiescence, and stemness in CESC, KICH, LUAD, and STAD (**Figure 6**)".

PPI network and gene enrichment analysis

To gain deeper insights into the molecular mechanism of HS6ST2, we conducted PPI network analysis to identify HS6ST2-binding proteins. Utilizing the STRING online tool, we identified ten HS6ST2-binding proteins, comprising those supported or predicted by experimental evidence (**Figure 7A**). The results of enrichment analysis showed that HS6ST2-binding proteins are associated with "Golgi lumen, anchored component of plasma membrane, and lysosomal leuman" etc. cellular component (CC) terms (**Figure 7B**), "Heparan sulfate

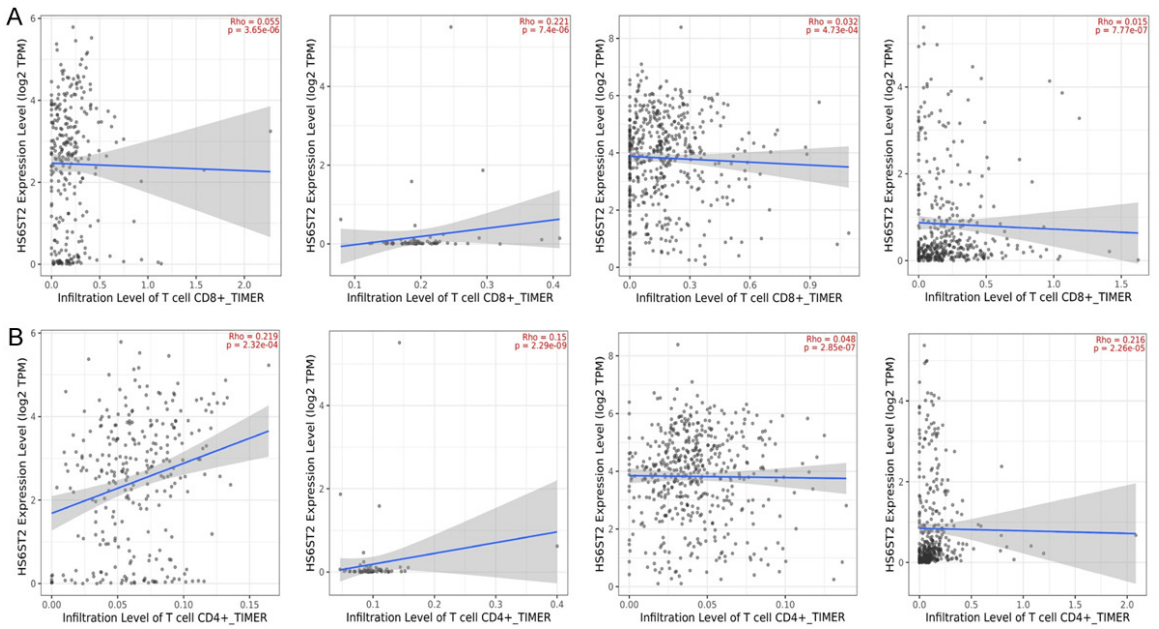

Figure 5. Illustration of the correlation between HS6ST2 expression and immune infiltrates in CESC, KICH, LUAD, and STAD. (A) Depict the correlation between HS6ST2 expression levels and the infiltration of CD8+ T cells, while (B) shows the relationship between HS6ST2 expression levels and the infiltration of CD4+ T cells in CESC, KICH, LUAD, and STAD. Level of significance = *p*-value < 0.05. HS6ST2 = Heparan sulfate 6-O-sulfotransferase 2, CESC = cervical squamous cell carcinoma, KICH = kidney chromophobe, LUAD = lung adenocarcinoma, STAD = stomach adenocarcinoma.

6-O-sulfptransferase activity and Heparan sulfate N-deacetylase activity" etc. molecular function (MF) terms (**Figure 7C**), "Heparan proteoglycan biosynthetic proc. Enzymatic modification and Heparan proteoglycan biosynthetic proc. Polysaccharide chain" etc. biological process (BP) terms (**Figure 7D**), and "Glycosaminoglycan biosynthesis-heparan sulfate/heparin, malaria, and proteoglycans in cancer signaling pathway etc. (**Figure 7E**)". Most importantly, along with HS6ST2, HS3ST1, HS2ST1, GPC3, and SDC3 genes were majorly involved in the observed dysregulated pathways.

Drug sensitivity analysis of HS6ST2

Exploration through the GSCAlite database unveiled a significant correlation between HS6ST2 mRNA expression and the predicted response to 78 anticancer treatments. HS6ST2 expression exhibited a positive association with drug sensitivity, including PIK-93, I-BET-762, BIX-02189, KIN001-236, CEP-701, KIN001-244, AZD8055, YM201636, NG-25, and KIN001-260 (**Figure 8**). Conversely, an inverse relationship was observed between HS6ST2 expression and sensitivity to four small molecules and drugs: afatinib, TAE684, and gefitinib. Based on these findings, HS6ST2 emerges as a potential therapeutic target for treating CESC, KICH, LUAD, and STAD.

Experimental validation of HS6ST2 expression

We conducted RT-qPCR experiments to assess the expression levels of HS6ST2 and its other binding partners (HS3ST1, HS2ST1, GPC3, and SDC3) majorly involved in dysregulated pathways across 20 LUAD tissues, comparing them to corresponding adjacent normal tissues. As depicted in **Figure 9A**, the mRNA expression levels of HS6ST2, HS3ST1, HS2ST1, GPC3, and SDC3 in LUAD tissues markedly overexpress as compared to the normal tissues. Additionally, we performed immunohistochemistry experiments to evaluate the HS6ST2, HS3ST1, HS-2ST1, GPC3, and SDC3 proteins expression in LUAD samples (**Figure 9B**). Immunostaining results indicated low staining intensities of HS6ST2, HS3ST1, HS2ST1, GPC3, and SDC3 in normal tissues, contrasting with LUAD tissues where medium intensities of these proteins

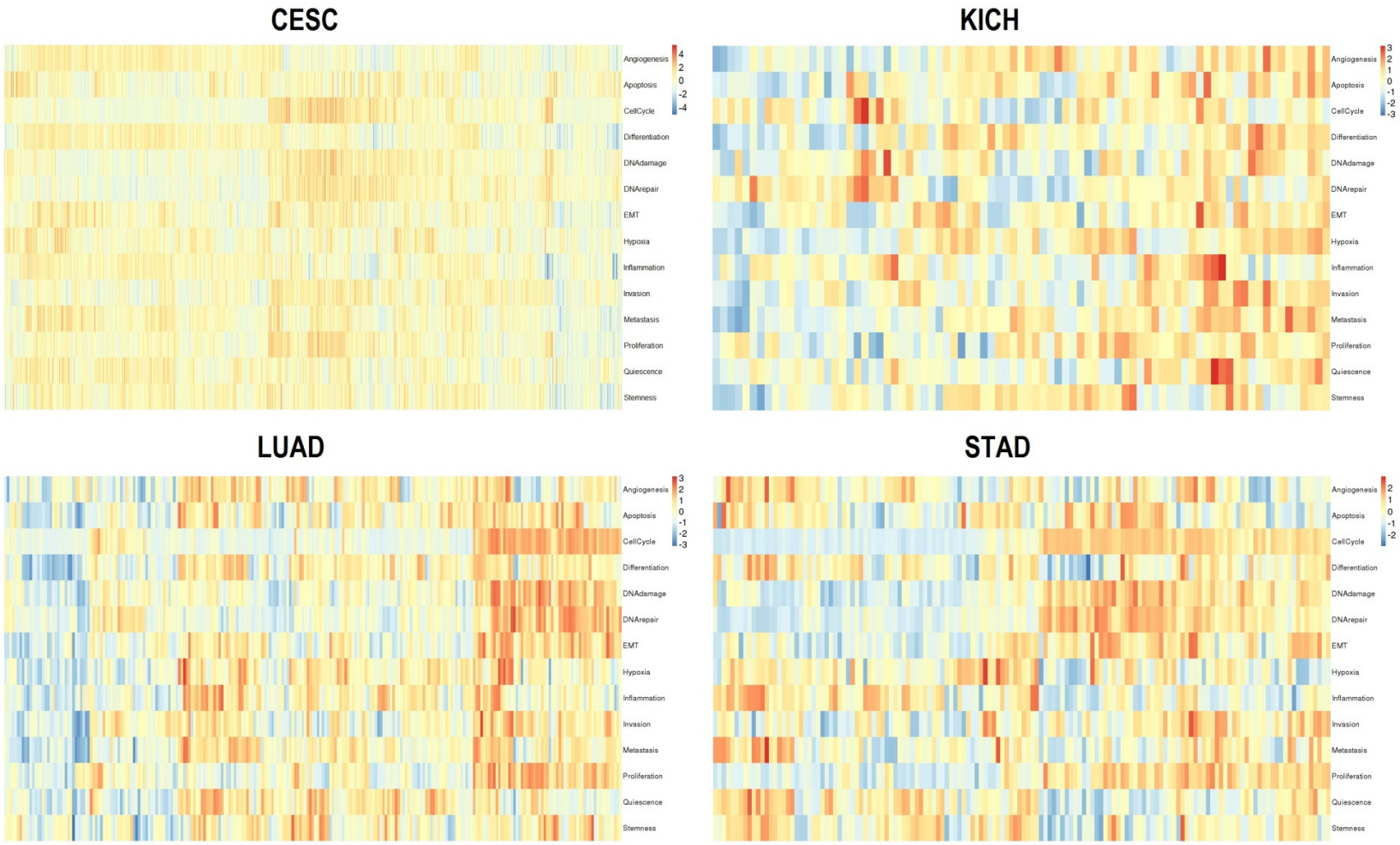

Figure 6. Demonstration of the correlation between HS6ST2 expression and diverse functional states at the single-cell level in CESC, KICH, LUAD, and STAD. Level of significance = *p*-value < 0.05. HS6ST2 = Heparan sulfate 6-O-sulfotransferase 2, CESC = cervical squamous cell carcinoma, KICH = kidney chromophobe, LUAD = lung adenocarcinoma, STAD = stomach adenocarcinoma.

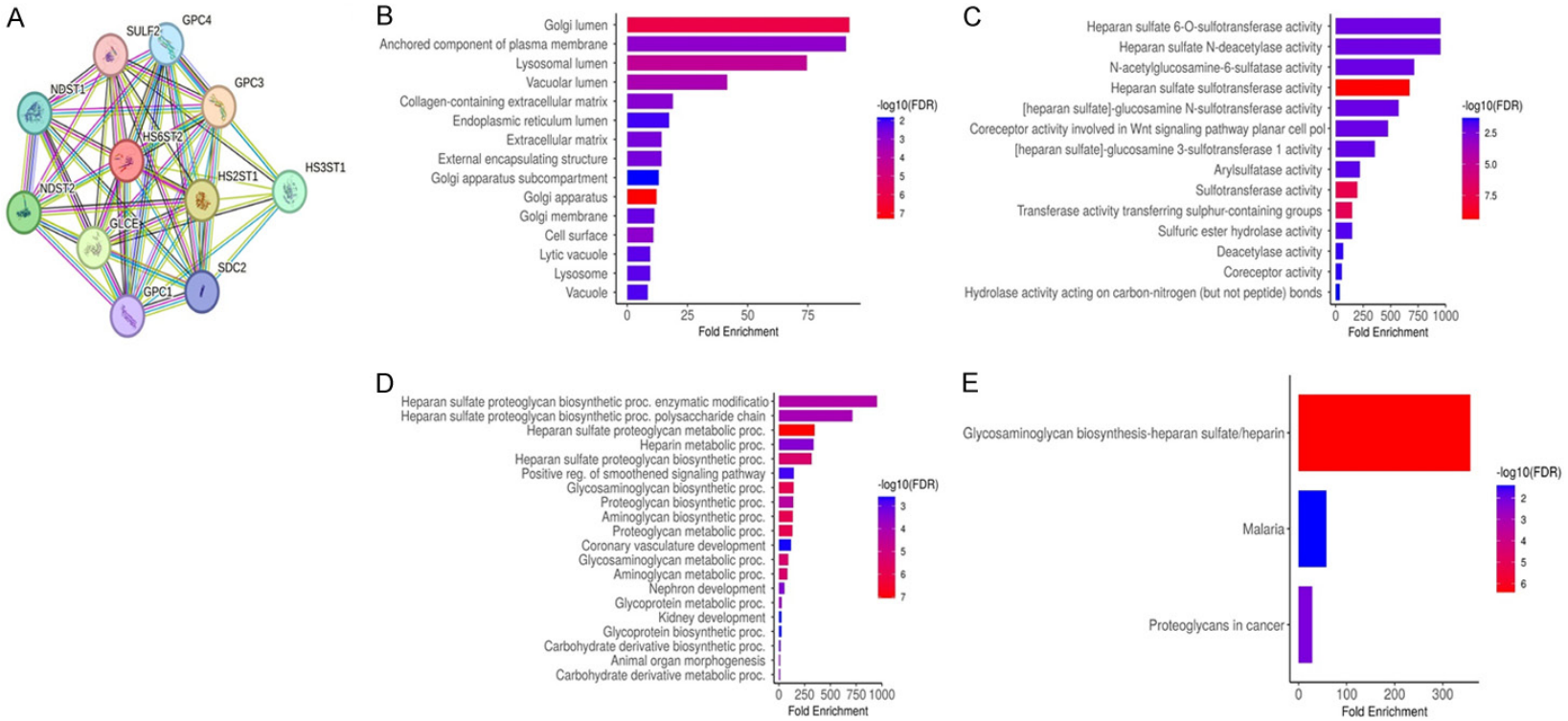

Figure 7. Protein-protein interaction (PPI) network, and gene enrichment analysis. A. A PPI of the HS6ST2-enriched genes. B. HS6ST2-enriched genes-associated CC terms. C. HS6ST2-enriched genes-associated MF terms. D. HS6ST2-enriched genes-associated BP terms. E. HS6ST2-enriched genes-associated KEGG terms. Level of significance = *p*-value < 0.05. HS6ST2 = Heparan sulfate 6-O-sulfotransferase 2, BP = Biological process, CC = Cellular components, MF = Molecular function, KEGG = Kyoto Encyclopedia of Genes and Genomes.

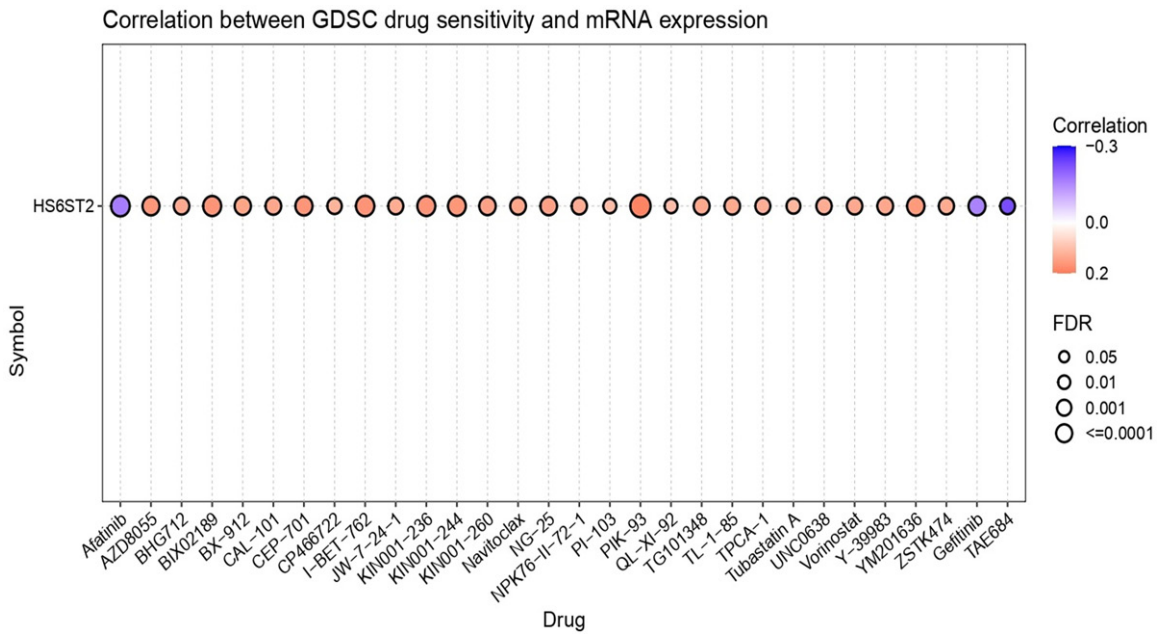

Figure 8. Illustration of the correlation between HS6ST2 expression and drug sensitivity. Positive correlation is represented by the red color, whereas negative correlation is indicated by the blue color. Level of significance = *p*-value < 0.05. HS6ST2 = Heparan sulfate 6-O-sulfotransferase 2.

were observed. These cumulative findings strongly suggest an elevated expression level of HS6ST2, HS3ST1, HS2ST1, GPC3, and SDC3 in LUAD tissues compared to adjacent normal tissues.

Discussion

HS6ST2 is an enzyme located in the Golgi apparatus, responsible for catalyzing the addition of 6-O-sulfate groups to heparan sulfate (HS) within proteoglycans (HSPGs). These HSPGs play a pivotal role in regulating various developmental processes [37, 38]. Recent compelling evidence suggests that HS6ST2 plays a pivotal role in governing angiogenesis and epithelial-mesenchymal transition (EMT) during carcinogenesis. By modulating HS 6-O-sulfation, HS6ST2 impacts angiogenic processes, specifically by triggering heparin-binding (HB)-EGF receptor signaling in ovarian cancer [39]. EMT is a crucial mechanism that prompts epithelial cells associated with tumors to adopt mesenchymal characteristics, leading to enhanced motility and decreased cell-cell contact during the initial stages of tumorigenesis [40]. In a prior investigation, it was uncovered that the activation of HS6ST2 in pancreatic cancer (PC) played a role in promoting epithelial-mesenchymal transition (EMT) and angiogenesis, influencing the progression of the disease [41]. The underlying mechanism through which HS6ST2 enhances PC carcinogenesis primarily involves the activation of the notch-signaling pathway, a mediator of EMT and angiogenesis [41]. The notch-signaling pathway is implicated in tumor cell proliferation, invasion, and the acquisition of a mesenchymal phenotype during these processes [40]. Within thyroid carcinomas, HS6ST2 has been recognized as a target gene under the influence of Twist family bHLH transcription factor 1, a crucial regulator of epithelial-mesenchymal transition (EMT) [42]. Inhibiting H6ST2 in tumor cells not only hampers cell migration, invasion, and tubule formation but also has the potential to reverse EMT [41, 43]. Significantly, previous studies demonstrated that the specific inhibition of HS6ST2, achieved through a high molecular weight Escherichia coli K5-derived heparin-like polysaccharide (K5-NSOS), successfully halted tumor progression in a mouse model of breast cancer metastasis [44]. These findings suggest that K5-NSOS holds promise as a potential anticancer agent in cancer therapy. Furthermore, a clinical study established a connection between the overexpression of HS6ST2

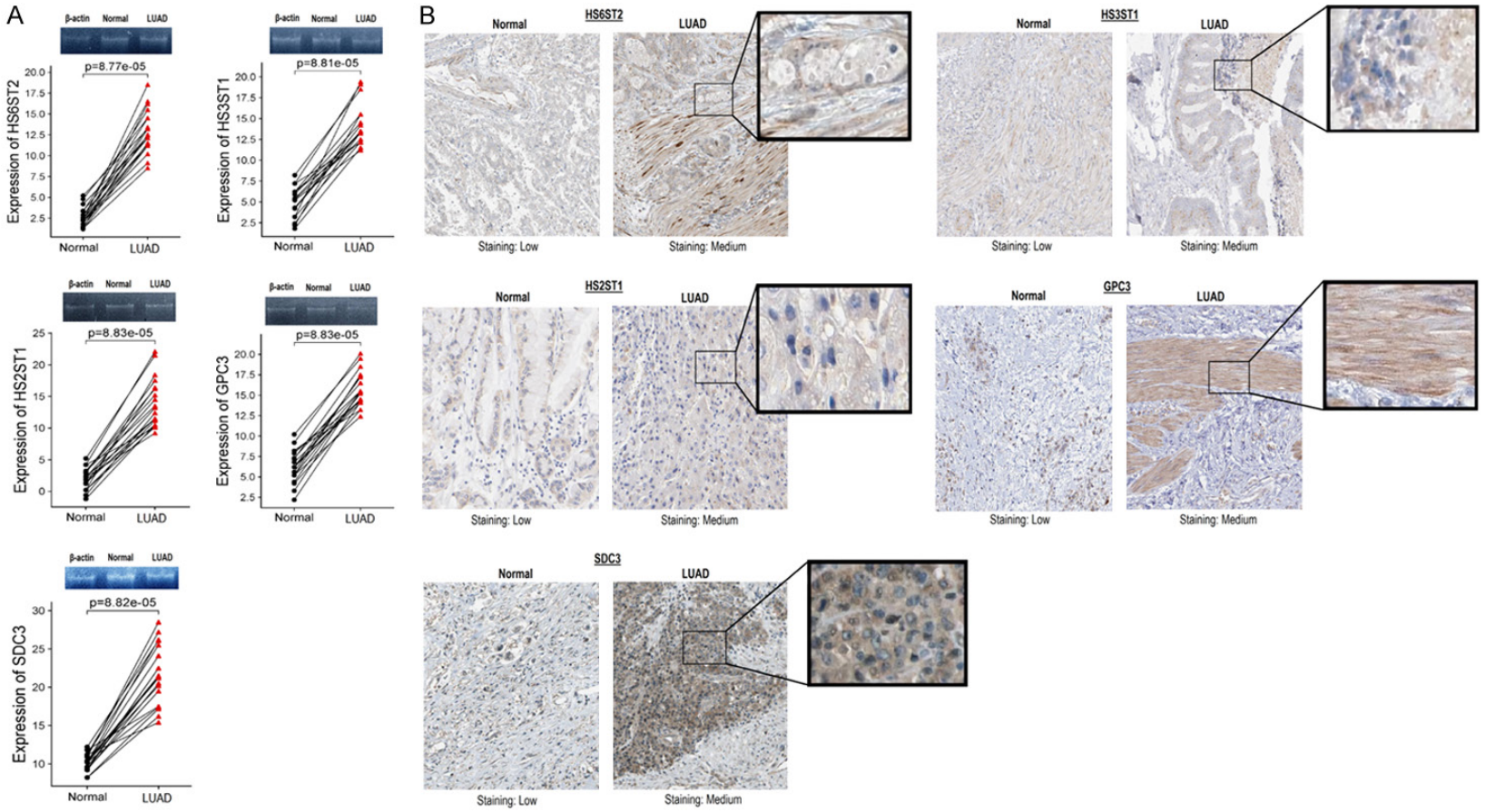

Figure 9. Validating the expression of HS6ST2, HS3ST1, HS2ST1, GPC3, and SDC3 in LUAD clinical samples. A. RT-qPCR assessed the mRNA levels of HS6ST2, HS3ST1, HS2ST1, GPC3, and SDC3 in LUAD and normal tissues. B. Illustrates representative images displaying various IHC staining intensities of HS6ST2, HS3ST1, HS2ST1, GPC3, and SDC3 in LUAD and normal tissues. Level of significance = *p*-value < 0.05. LUAD = Lung adenocarcinoma, RT-qPCR = reverse transcription-quantitative polymerase chain reaction.

and colorectal cancer (CRC), indicating its potential as a prognostic marker for adverse outcomes in CRC patients [45]. However, the role of HS6ST2 across various cancers remains significantly unexplored. To our knowledge, this study marks the inaugural exploration of HS6ST2 expression in pan-cancer and its clinical implications in cancer development and progression.

Bioinformatics analyses and molecular experiments unveiled a notable up-regulation of HS6ST2 in 14 types of tumor tissues compared to adjacent non-tumor samples, with a significant *p*-value (< 0.05). Additionally, elevated HS6ST2 expression correlated with poorer overall survival (OS) in CESC, KICH, LUAD, and STAD. Furthermore, HS6ST2 expression exhibited a positive association with pathological stages in patients afflicted with CESC, KICH, LUAD, and STAD Mutation analysis revealed a low frequency of genetic mutations in the HS6ST2 gene across CESC, KICH, LUAD, and STAD samples, indicating the genetic stability of this gene. DNA methylation, a prevalent form of epigenetic modification known to modulate gene expression by altering chromatin structure, DNA stability, and DNA conformation, plays a crucial role in various tumorigenic processes [5, 46-51]. In this investigation, evidence suggested a down-regulation in the DNA methylation level of HS6ST2 in CESC, KICH, LUAD, and STAD, aligning with the observed increase in HS6ST2 expression. Further exploration of gene alterations in HS6ST2 and the interplay between DNA methylation and HS6ST2 expression in cancer warrants attention in future studies.

The tumor immune microenvironment (TIME), a vital component of the tumor microenvironment (TME) primarily comprising immune cells, plays a crucial role in cancer progression [52, 53]. Identifying novel targets for immunotherapy is imperative to enhance clinical outcomes, and the influence of HS6ST2 on the TIME has been minimally explored to date. Infiltrating immune cells demonstrate close correlations with tumor growth, metastasis, and invasion [54, 55]. For instance, cancer-associated fibroblasts, activated by the tumor, can facilitate tumor development by secreting various cytokines or metabolites. Additionally, they create barriers by shaping the extracellular matrix, inhibiting the efficacy of drugs and immune cells [56]. This study analyzed the influence of HS6ST2 on the immune infiltration of CD8+ T and CD4+ T cells. The findings demonstrated a significant positive association between HS6ST2 expression and the infiltration of both CD8+ T and CD4+ T cells. Overall, our study implies the potential efficacy of targeting HS6ST2 in immunotherapy to improve the well-being of cancer patients.

Despite advancements in technologies and personalized therapeutics, drug resistance remains a significant challenge for researchers in both laboratory and clinical settings. New strategies to combat drug resistance, such as restoring the function of tumor suppressor genes [57] and utilizing RNA interference [58], have been developed. In our investigation, we explored the correlation between HS6ST2 and the sensitivity of various anti-cancer medications. The results indicated that heightened HS6ST2 expression was associated with reduced sensitivity to numerous drugs, suggesting its potential involvement in medication resistance. Conversely, increased HS6ST2 expression was linked to enhanced sensitivity to afatinib, gefitinib, and TAE684. This finding suggests that manipulating HS6ST2 expression could be a viable strategy to improve the efficacy of anticancer drugs.

Conclusion

In summary, HS6ST2 exhibited significant overexpression in CESC, KICH, LUAD, and STAD tissues. The up-regulation of HS6ST2 was linked to more aggressive pathological stages and poorer prognosis in patients with CESC, KICH, LUAD, and STAD. Collectively, these findings suggest that HS6ST2 could potentially serve as a therapeutic target and valuable prognostic biomarker for patients with CESC, KICH, LUAD, and STAD. However, additional research is necessary before considering clinical implications.

Acknowledgements

The authors extend their appreciation to the Researchers Supporting Project number (RSP2024R376) King Saud University, Riyadh, Saud Arabia.

Disclosure of conflict of interest

None.

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