Research article

The prognostic value and immunological role of SULF2 in adrenocortical carcinoma

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ABSTRACT

Background: Adrenocortical carcinoma (ACC) represents the rare urological epithelial cancer of urinary tract, which has a large mass and is usually diagnosed at the advanced stage, thus inducing the poor prognosis. As a result, early detection and diagnosis are more important for the prognosis rather than the treatment of ACC. There is evidence supporting the association of Sulfatase2 (SULF2) with bladder cancer. However, the relationships of SULF2 with the clinical features and immune infiltration of ACC remain unclear.
Methods: This work comprehensively investigated the different expression levels of SULF2 within ACC and its prognostic significance through various databases including Gene Expression Profiling Interaction Analysis (GEPIA), Tumor Immune Estimation Resource (TIMER), The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), Kaplan–Meier (KM) plotter and UAL-CAN. Besides, SULF2 levels within different tumor and paraneoplastic tissues were examined based on Human Protein Atlas (HPA) and TIMER. Afterwards, this study identified differentially expressed genes (DEGs) in high-compared with low-SULF2-expression groups. To predict the possible interaction between SULF2 and its targets, a protein-protein interaction (PPI) network was constructed based on relevant data collected in STRING database. Besides, the SULF2 functional annotation was carried out, including Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) and GSEA. In addition, gene mutation analysis was also performed based on the cBioPortal database. The relation of SULF2 with immune infiltration was analyzed from various aspects by using the resources of various databases including TIMER, TISIDB, and GEPIA, which was first reported in this work. Finally, R package was utilized to plot the receiver operating characteristic (ROC) curves of diagnosis, time-dependent survival, and the association of SULF2 with cancer stage and the nomogram model. Finally, CellMiner dataset was adopted for SULF2 correlation as well as drug sensitivity analysis.
Results: Relative to healthy people, SULF2 level markedly elevated within ACC tissues. Besides, SULF2 up-regulation significantly predicted the dismal prognostic outcome, which may be an important prognostic factor. Afterwards, the PPI network was constructed, and the possible link of SULF2 with the corresponding targets was predicted. Besides, up-regulated SULF2 expression was tightly related to immune regulation and tumor-infiltration immune cell (TIICs), including

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$CD8^+$, $CD4^+$ and mast cells. Finally, SULF2 expression was speculated to help determine the sensitivity of certain drugs.

Conclusions: SULF2 may offer a new therapeutic target for ACC patients and become an important potential prognostic biomarker.

1. Introduction

Adrenocortical carcinoma (ACC) represents a rare while insidious endocrine disease, and its annual incidence is about 2 per million. Available data show that ACC cases have a low (< 50%) 5-year survival rate [1]. However, there are few available treatment options, and radical surgical resection is the only cure method. Even so, unfortunately, most patients who have undergone radical surgery still develop local recurrence and metastasis [2]. With regard to the prognostic factors of ACC, in addition to tumor stage, the reference value of gene sequencing and other molecular biological examinations is limited although they have been available [3,4].

As a member of the sulfatase family, Sulfatase2 (SULF2) affects heparan sulfate proteoglycan (HSPG) sulfate pattern, thus contributing to cancer progression [5]. In tumor cells, the abnormal expression of SULF2 can induce structural changes in proteoglycans, leading to abnormal tumor cell proliferation, enhanced invasion capacity and increased susceptibility to lymph node metastasis (LNM). The role of SULF2 in numerous tumor types, including bladder cancer and pancreatic cancer, has been studied investigated [6,7]. As of yet, no study has explored the relationship between SULF2 and ACC.

In the present research, SULF2 expression together with the relation with ACC patient survival was analyzed by electronic databases including Gene Expression Profiling Interaction Analysis (GEPIA), The Cancer Genome Atlas (TCGA), UALCAN datasets, as well as Kaplan–Meier (KM) plotter. Besides, relation between SULF2 and tumor-infiltrating immune cell (TIICs) under different tumor microenvironments (TME) was also evaluated based on TISIDB and Tumor Immune Estimation Resource (TIMER) databases.

2. Methods

2.1. Differential expression of SULF2 in ACC

Genotype-Tissue Expression (GTEx) database and TCGA were used as the source of research data for the web-based database GEPIA (http://gepia.cancer-pku.cn/index.html) [8]. The "DIY Expression" option in GEPIA was selected for investigating SULF2 levels among ACC cases and normal tissues. Then, SULF2 expression in ACC and normal tissues was compared using GSE14922 and GSE12368 from Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) [9]. To be specific, the former included 4 pairs of tumor and normal tissue samples, and the latter contained 12 tumor and 6 normal tissue samples. Moreover, SULF2 levels among distinct samples collected in Human Protein Atlas (HPA) (http://www.proteinatlas.org/) and Tumor Immune Estimation Resource (TIMER2.0) (http://cistrome.shinyapps.io/timer/) were compared [10,11]. Besides, differentially expressed genes (DEGs) were identified by R package in high-compared with low-SULF2-expression groups upon the thresholds of $p < 0.05$ and |log2 fold change (FC)|$\geq$1.5. Finally, R packages "ggplot2" was utilized to illustrate the results in the form of volcano plots.

2.2. UALCAN

UALCAN is a powerful network interaction platform, which allows researchers to gather valuable information and perform multiple bioinformatics analyses [12]. In this study, UALCAN was adopted to compared the expression of SULF2 in ACC according to patient's gender, individual cancer stages (stage 1, 2, 3, 4), lymph node metastasis (LNM), and TP53 mutation status.

2.3. Functional annotation analysis of protein-protein interaction (PPI) networks

STRING (http://string-db.org) is the powerful platform developed to constructed protein networks, which allows researchers to input a list of proteins by name or amino acid sequence. STRING was utilized in the present work to construct a PPI network of SULF2. Our default minimum required interaction score is 0.4. Besides, GeneMANIA (https://genemania.org) helps researchers integrate biological network to sequence genes and predict gene functions [13]. In this study, GeneMANIA was employed to construct gene interaction networks of SULF2 and predict the related functions. In addition, the relevance of nine genes associated with SULF2 was analyzed in STRING database using R package. Gene Oncology (GO) together with KEGG analysis was subsequently conducted on the nine genes associated with SULF2 and DEGs by using ggplot2 R packages. Additionally, gseKEGG and gsePathway functions in clusterProfiler were also applied in gene set enrichment analysis (GSEA).

2.4. Genetic mutation analysis

SULF2 mutation features were analyzed based on the cBioPortal for Cancer Genomics database (http://cbioportal.org) via genome-wide pan-cancer analysis (ICGC/TCGA, Nature 2020) from pan-cancer studies [14,15].
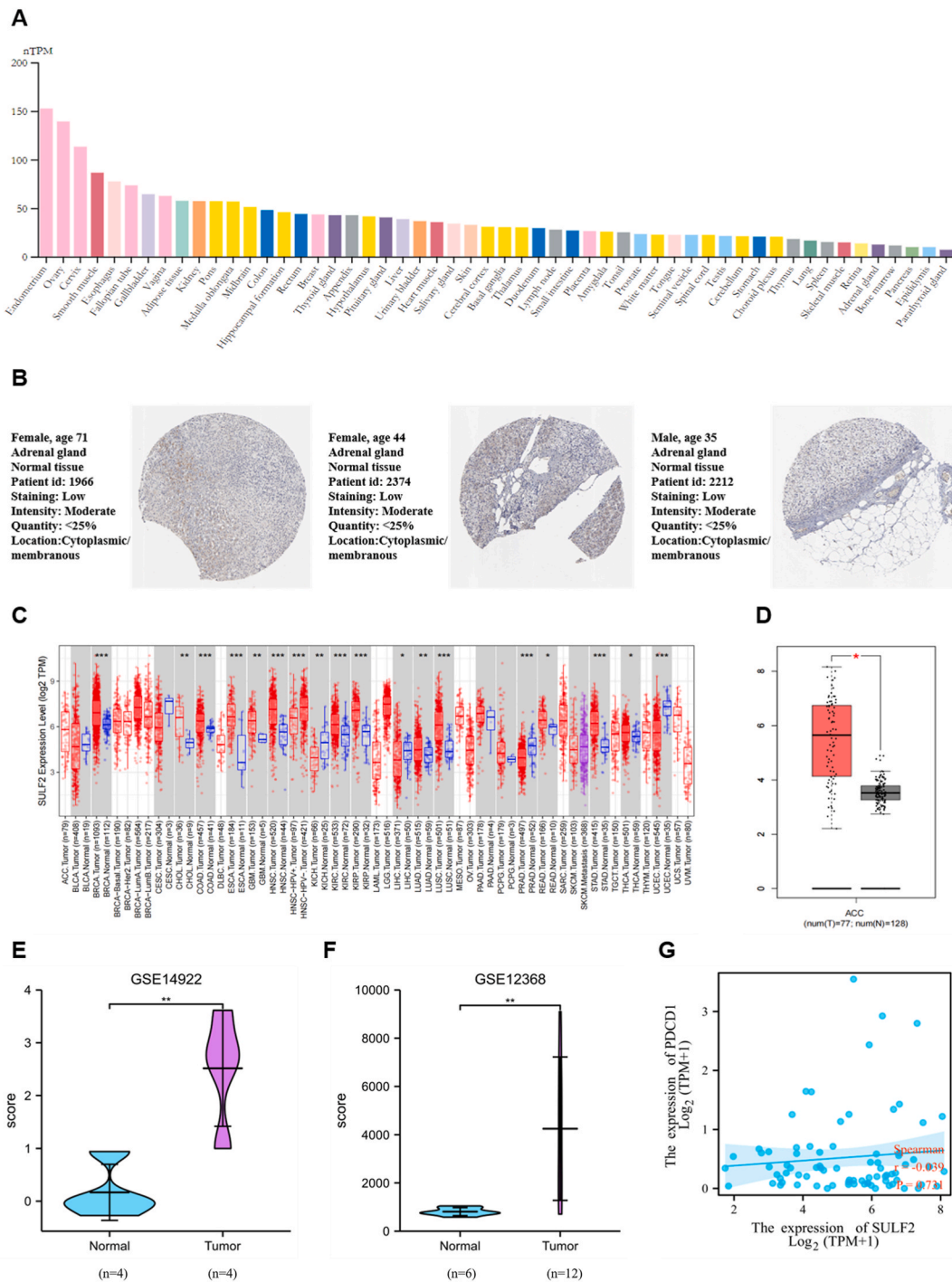
Fig. 1. Sulfatase2 (SULF2) expression levels in different human cancers. (A) Different expression level of SULF2 in normal human tissues from The Human Protein Atlas (HPA) database. (B) Immunohistochemical (IHC) staining of normal adrenal tissue SULF2 in a 71-year-old female from HPA database. (C) high expression SULF2 in different tumor types from Tumor Immune Estimation Resource (TIMER2.0) database (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). (D) Increased SULF2 in Adrenocortical carcinoma (ACC) compared with normal tissues in Gene Expression Profiling Interaction Analysis (GEPIA). (E, F) SULF2 expression was higher in ACC than in the normal tissue in GSE14922 and GSE12368 (**$p < 0.01$). (G) The correlation analysis between SULF2 and PD1 mRNA level.

2.5. SULF2 and immune infiltration

TIMER2.0 database allows for comprehensively analyzing TIICs levels in different cancer types. By applying the Gene module, users can select one or more genes and see how its or their expression is correlated with TIICs levels. In this work, relation of SULF2 level with gene markers for tumor-infiltrating lymphocytes (TILs), including B cells, CD8$^+$ T cells, Mast cell, M2 macrophages, Tregs and natural killer (NK) cells, was analyzed. TISIDB (http://cis.hku.hk/TISIDB/index.php) has been developed as the integrated web-based database to analyze tumor-immune system interconnectedness, which encompasses an extensive immune data resources [16]. It may help researchers develop new immunotherapeutic targets and forecast immunotherapeutic responses. In our study, TISIDB was chosen to explore the relations of SULF2 with immune-related molecules and cells in ACC.

2.6. Prognostic significance of SULF2 for ACC

The study analyzed whether SULF2 was associated with survival events based on a Cox proportional hazards model that estimated hazard ratios (HRs). Univariate survival analysis was first of all conducted, which obtained HRs, associated 95% confidence intervals (CIs) and *p*-values upon log-rank test. When the *p*-value was below 0.05, it indicated statistical significance of our results, which might thus be used for reference. In addition, the present work selected Kaplan–Meier plotter (http://kmplot.com/analysis/) for investigating whether SULF2 was of prognostic significance for ACC, like overall survival (OS) as well as disease-free survival (DFS). By using R packages (such as survival packages, pROC, and timeROC), this study also plotted ROC curve and time-dependent curve of diagnosis, and carried out nomogram model analysis. Source data for the above analyses were obtained from TCGA database.

2.7. Drug sensitivity analysis

Data regarding gene expression profiles together with drug sensitivity were collected in the CellMiner dataset. Later, correlation coefficients of SULF2 level with drug sensitivity were determined, and correlation tests were performed using R language. $P < 0.05$ stood for the significant correlation of the outcomes. A correlation coefficient > 0 indicated the positive gene-drug sensitivity correlation, and vice versa.
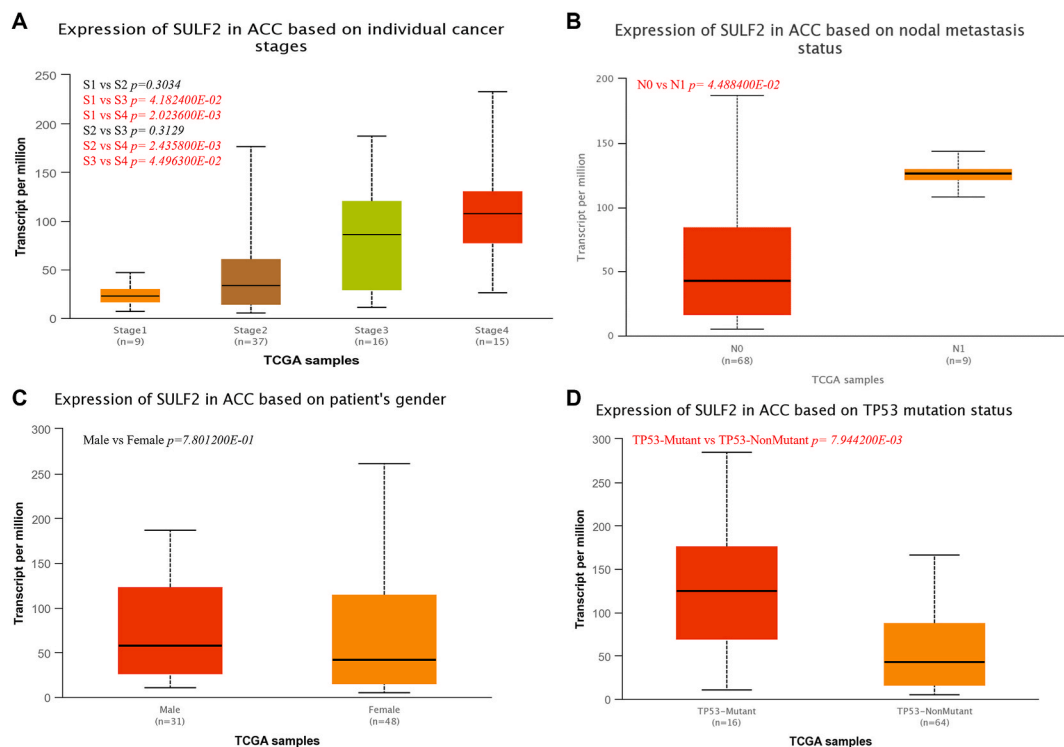

Fig. 2. Correlation between SULF2 expression level and clinicopathological parameters of Adrenocortical carcinoma through the UALCAN database. (A) Cancer stages (stage 1, 2, 3, 4). (B) Lymph node stage (N 0, 1). (C) Patient's gender (male and female). (D) TP53 mutation status (TP53-Mutant and TP53-NonMutant). S1, stage 1; S2, stage 2; S3, stage 3; S4, stage 4; ACC, adrenocortical carcinoma.

3. Results

3.1. High expression of SULF2 in ACC

According to analysis based on HPA database, SULF2 expression was notably lower in adrenal gland than in other tissues. Meanwhile, immunohistochemical (IHC) results of normal adrenal gland from a 71-year-old female in the database also showed the low expression levels of SULF2 (Fig. 1A and B). Thereafter, it was found that SULF2 expression was up-regulated in ACC, kidney renal clear cell carcinoma (KIRCC) and breast cancer (Fig. 1C). Besides, high SULF2 expression was observed in ACC from TCGA database by GEPIA ($p < 0.05$, Fig. 1D). By analyzing GSE14922 and GSE12368 from GEO database, the same conclusion was made (Fig. 1E and F). On the other hand, the relationship between SULF2 and PD1 expression was also analyzed, unfortunately, the result was not statistically significant (Fig. 1G).

3.2. Relationship between SULF2 expression and cancer stage

By applying UALCAN, SULF2 expression was examined in ACC based on patient's gender, individual cancer stages (stage 1, 2, 3, 4), LNM, and TP53 mutation status. The data showed that SULF2 showed high expression in intermediate-to-advanced cancers, with significant differences (Fig. 2A). Moreover, SULF2 was expressed at similarly increased levels in ACC patients developing LNM relative to those without LNM (Fig. 2B). However, SULF2 expression levels were similar between male and female ACC patients, which was of
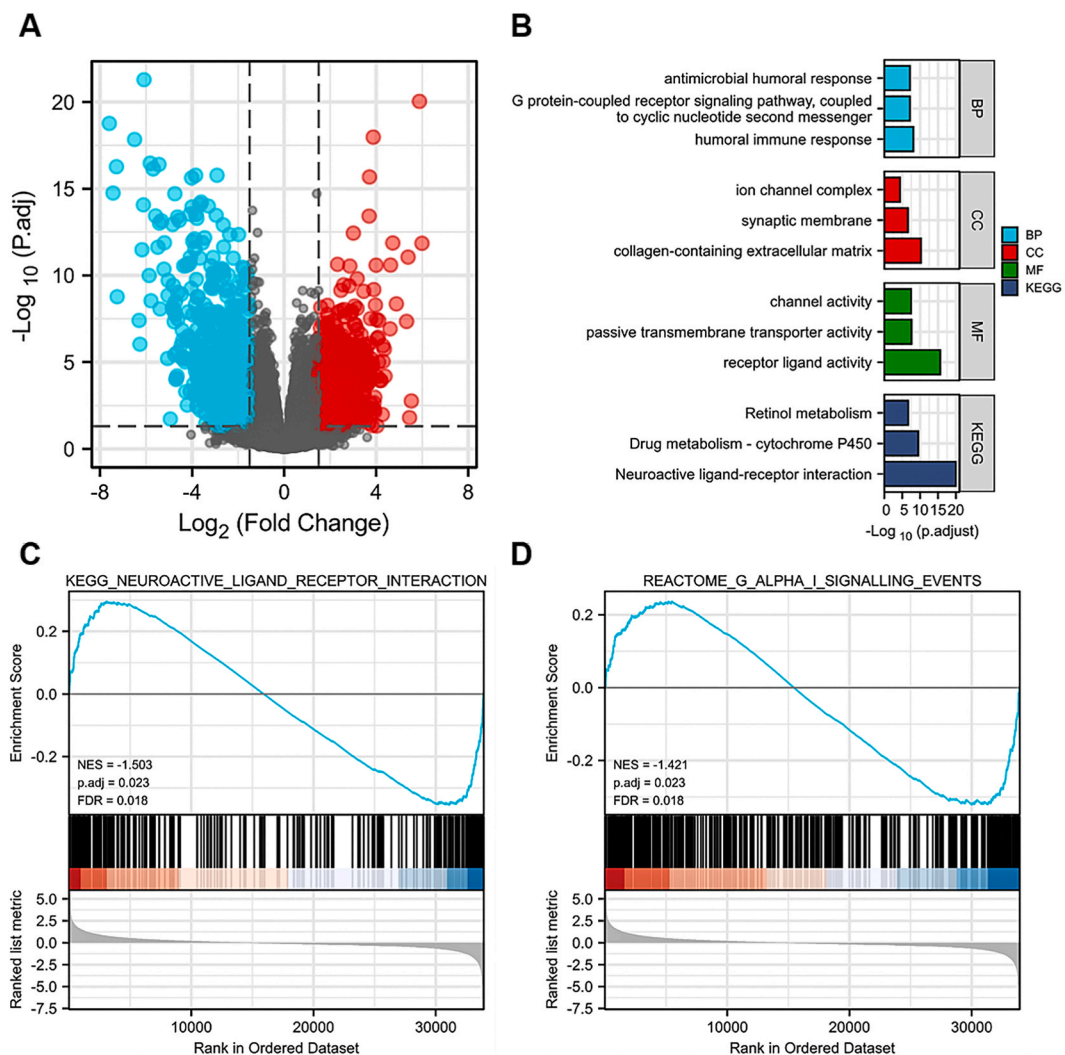

Fig. 3. Differentially expressed genes. **(A)** Volcanic plot of differentially expressed genes in the high-SULF2 expression group and the low-SULF2 expression group. **(B)** Gene Oncology and KEGG pathway. **(C)** Gene set enrichment analysis: KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTER-ACTION. **(D)** Gene set enrichment analysis: REACTOME_G_ALPHA_I_SIGALLING_EVENTS.

no statistical difference (Fig. 2C). In addition, SULF2 was expressed at higher levels in patients with TP53 mutations in the tumor than in non-mutated patients (Fig. 2D).

3.3. PPI network and functional annotation

DEGs were identified in high-compared with low-SULF2 expression groups using the R package. There were altogether 1889 DEGs obtained, which included 953 with up-regulation and 936 with down-regulation (Fig. 3A). According to Fig. 3B, such DEGs were enriched in antimicrobial humoral response and humoral immune response in the biological process (BP). Meanwhile, the enriched cellular components (CC) terms included synaptic membrane, collagen-containing extracellular matrix and ion channel complex. Furthermore, the enriched molecular function (MF) terms were channel activity, passive transmembrane transporter activity and receptor ligand activity. According to KEGG enrichment, these DEGs were enriched into retinol metabolism, drug metabolism-cytochrome P450 and neuroactive ligand-receptor interaction. As revealed by GSEA results, low-SULF2-expression patients showed significant enrichment of KEGG neuroactive ligand receptor interaction and reactome g alpha I signaling events (Fig. 3C and D). However, there was no enriched pathway in the high-SULF2-expression group.

STRING database assists in building the PPI network related to SULF2, as a result, ten functional partner genes with high connectivity were obtained from the network. In fact, HS2ST1 and ENSP00000359579 are two names of Heparan sulfate 2-O-sulfotransferase 1, therefore, 9 functional partner genes were finally obtained (Fig. 4A). Besides, GeneMANIA database-based gene-gene network
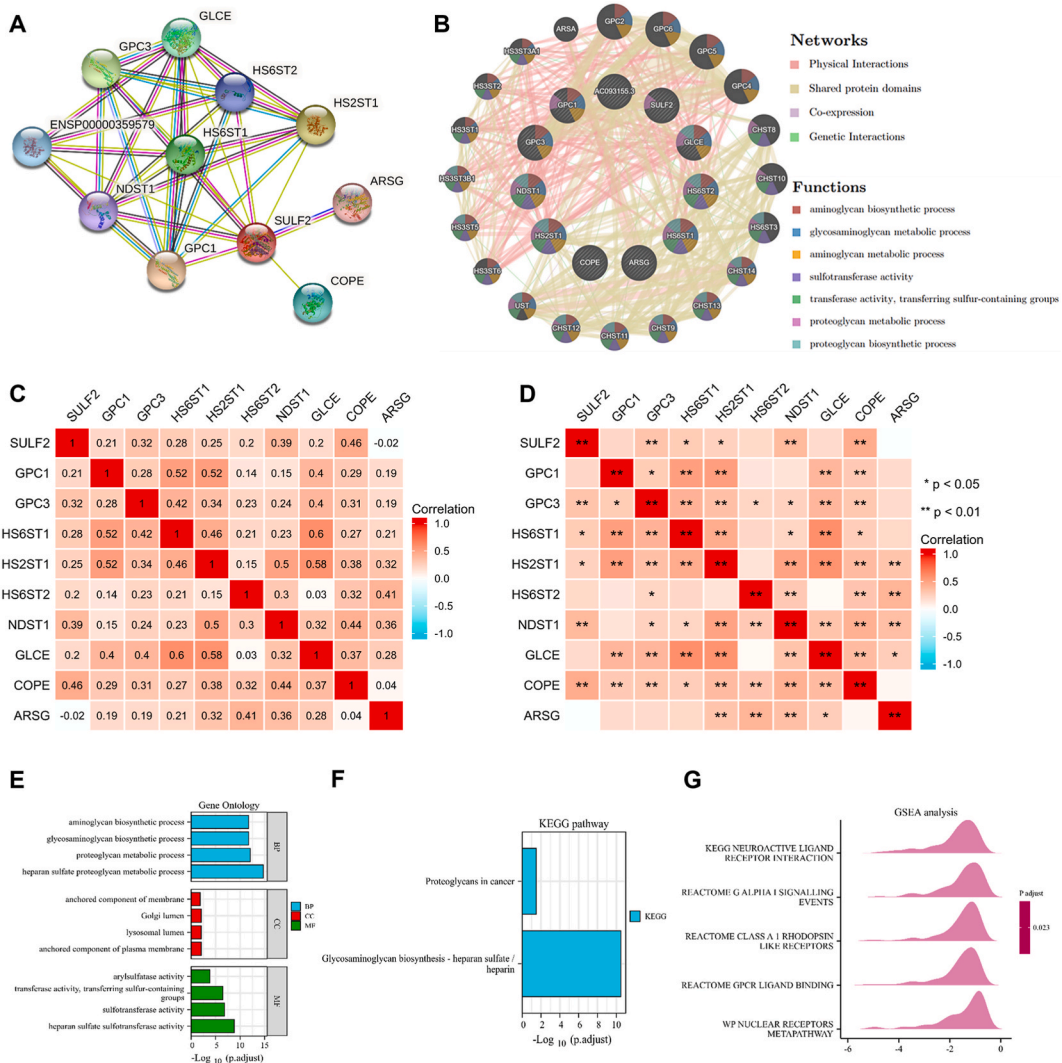

Fig. 4. Enrichment analysis of SULF2 in adrenocortical carcinoma. **(A)** SULF2-interaction proteins in ACC from STRING database. **(B)** The gene-gene network of SULF2 performed by GeneMANIA database. **(C, D)** The relevance between SULF2 and functional partner genes. **(E)** Gene Oncology. **(F)** KEGG pathway. **(G)** Gene set enrichment analysis (GSEA). ACC, adrenocortical carcinoma.

analysis revealed interaction of SULF2 with 30 candidate target genes (Fig. 4B). Excitingly, SULF2 was strongly linked with nine functional partner genes, which displayed significantly positive correlation with each other (Fig. 4C and D). As shown in Fig. 4E, the GO analysis results revealed the involvement of SULF2 and its partner in BPs including "glycosaminoglycan biosynthetic process", "aminoglycan biosynthetic process", "heparan sulfate proteoglycan metabolic process", and "proteoglycan metabolic process". The CC terms enriched included "anchored component of membrane", "Golgi lumen", "lysosomal lumen" and "anchored component of plasma membrane". The enriched MF terms were "arylsulfatase activity", "heparan sulfate sulfotransferase activity", "sulfotransferase activity" and "transferase activity, transferring sulfur-containing groups". Moreover, as demonstrated by KEGG enrichment, these genes were enriched into proteoglycans in cancer and glycosaminoglycan biosynthesis-heparan sulfate/heparin (Fig. 4F). Our GSEA was conducted by using TCGA-derived RNAseq data and the results showed obvious enrichment of KEGG neuroactive ligand receptor interaction and reactome g alpha I signaling events pathways (Fig. 4G).

3.4. Genetic alteration analysis of SULF2 in ACC

The present work attempted to explore the mutational signature of SULF2 in ACC based on the cBioPortal tool, as a results, the genetic alteration frequency of SULF2 was lower than 6% in ACC (Fig. 5A). Fig. 5B displays the mutation spots of SULF2 in ACC. As shown in Fig. 5C–E, there was no significant difference in OS ($p = 0.905$), progression-free survival (PFS) ($p = 0.624$) or disease-specific survival (DSS) ($p = 0.766$) in SULF2-altered ACC group compared with SULF2-unaltered group. In conclusion, SULF2 gene alterations may not be associated with the development of ACC.

3.5. Immune correlation analysis

Immune infiltration is involved in the occurrence and development of tumors. According to our results, SULF2 was associated with the infiltration levels of TILs based on TISIDB database (Fig. 6A). Besides, as shown in Fig. 6B, SULF2 up-regulation led to the decreased TIL infiltration levels, such as Mast cell (rho = −0.367) and NK cell (rho = −0.289). However, SULF2 up-regulation showed positive relation to infiltration levels of CD4$^+$ Th1 cell (rho = 0.451) and B cell (rho = 0.24). The statistical results showed that all P-values were less than 0.05. Therefore, it was reasonable to believe that SULF2 showed close relation with tumor immunity, which might affect ACC
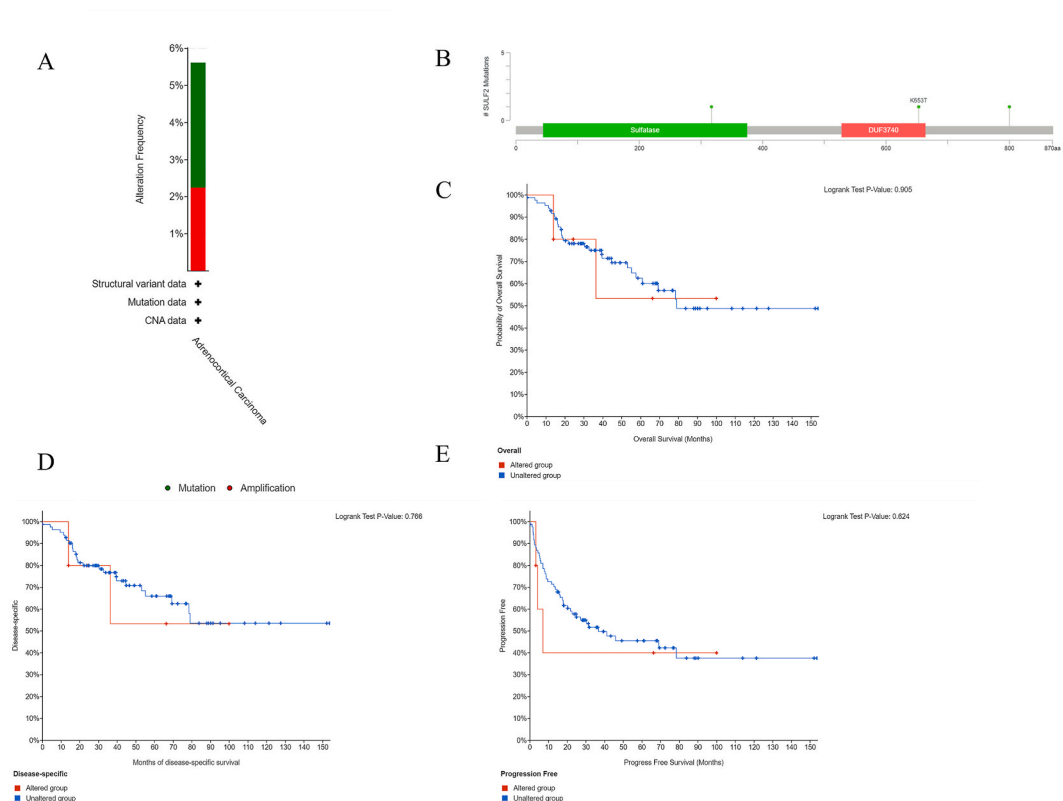

Fig. 5. Mutation feature of SULF2 in ACC from TCGA cohort based on the cBioPortal database. **(A)** The alteration frequency with mutation type of SULF2 in ACC samples from TCGA cohorts. **(B)** Mutation sites of SULF2 in ACC from TCGA cohort. **(C)** K-M survival analysis of OS with or without SULF2 alteration. **(D)** K-M survival analysis of disease-specific survival with or without SULF2 alteration. **(E)** K-M survival analysis of progress-free survival with or without SULF2 alteration. ACC, adrenocortical carcinoma. OS, overall survival.
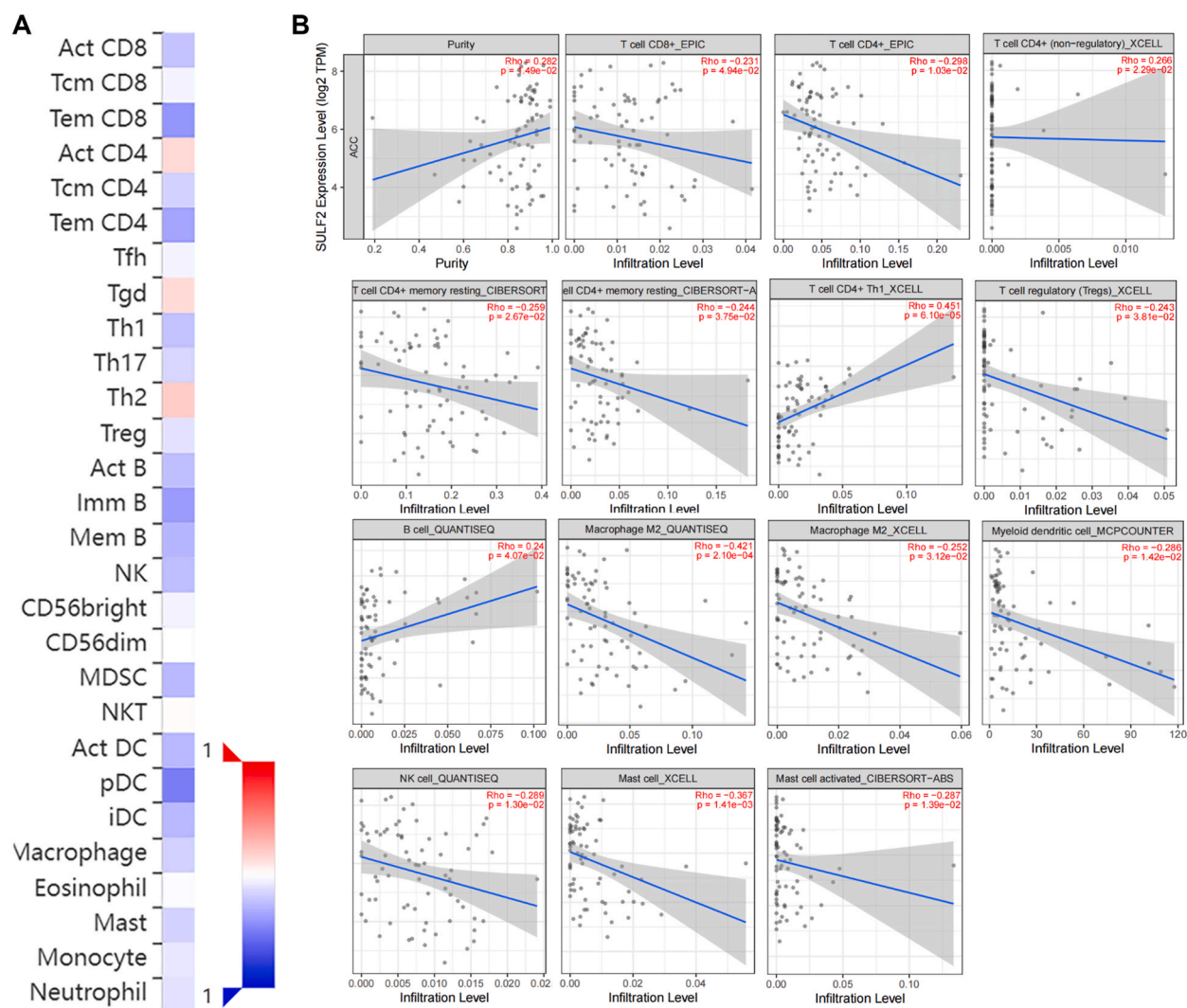
Fig. 6. Correlation of SULF2 expression with immune infiltration in ACC. **(A)** Correlation between the expression of SULF2 and the abundance of TILs in ACC through TISIDB database. **(B)** Correlation of SULF2 expression with infiltration levels of TILs in ACC available at TIMER2.0 database. ACC, adrenocortical carcinoma. TILs, tumor-infiltrating lymphocytes.

development. The expression of SULF2 was associated with multiple immune molecules. Based on our research results, SULF2 was related to several kinds of immunoinhibitors, such as PVRL2 (rho = 0.495, p = 4.74e-06), IL10RB (rho = 0.314, p = 0.00507) and CSF1R (rho = −0.272, p = 0.0157) (Fig. 7A). Besides, SULF2 expression was related to immunostimulators, including PVR (rho = 0.454, p = 3.23e-05), TNFSF13 (rho = −0.445, p = 4.7e-05), HHLA2 (rho = −0.424, p = 0.000115) and CD28 (rho = −0.387, p = 0.000465) (Fig. 7B). Therefore, it was suggested that SULF2 might be involved in facilitating immune surveillance escape of tumors.
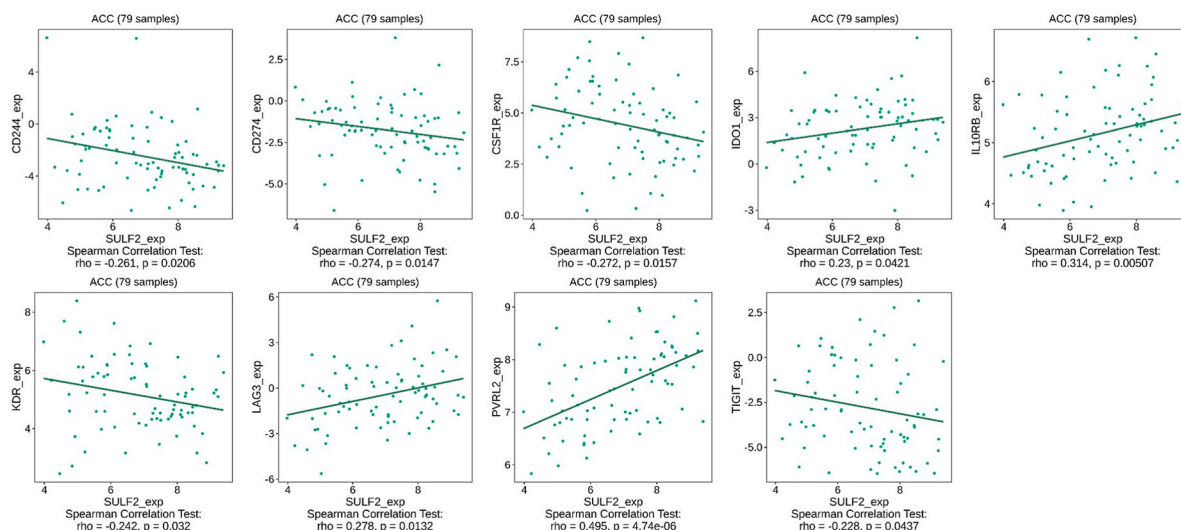
3.6. Correlation of SULF2 expression with chemokines and receptors

Chemokines and receptors play important role in tumor immune process. Based on our results, SULF2 was related to chemokines and receptors. For example, SULF2 level was tightly related to CCL8 (rho = −0.434, p = 7.72e-05), XCL1 (rho = −0.332, p = 0.0029) and CCL2 (rho = −0.264, p = 0.019) (Fig. 8A). Meanwhile, SULF2 expression was also closely associated with receptors, including CCR2 (rho = −0.317, p = 0.0046), CCR6 (rho = −0.348, p = 0.00179) and CXCR6 (rho = −0.347, p = 0.00185) (Fig. 8B).

3.7. Diagnostic and prognostic significance of SULF2 for ACC

By integrating age, T stage, and gender, a nomogram model was constructed for predicting 3- and 5-year survival of ACC (Fig. 9A). When SULF2 expression was added to the nomogram model, it was found that the model could be used to guide the prediction of 2-, 3-, and 5-year survival probabilities of tumor patients. And the survival probability was significantly associated with SULF2 expression

A Immunoinhibitor
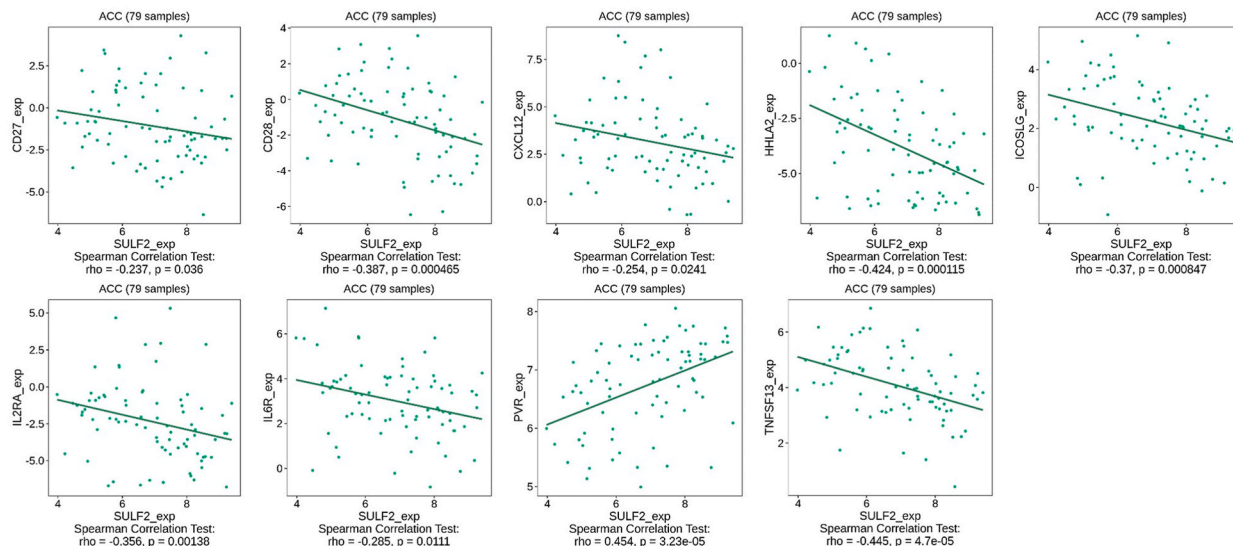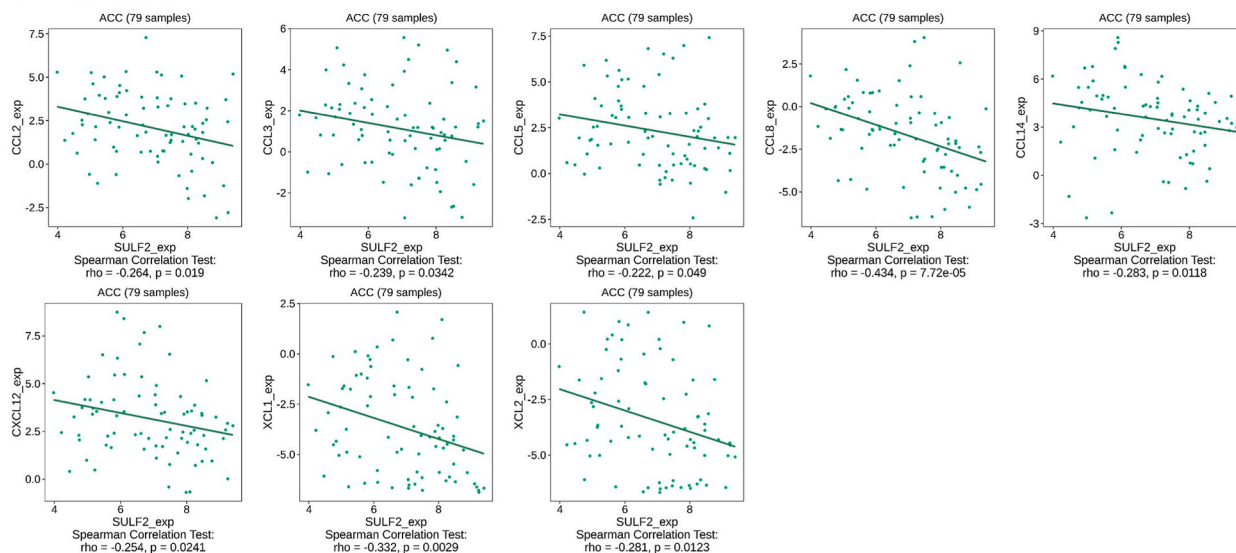

B Immunostimulator

Fig. 7. The correlation of SULF2 expression with immunomodulators in ACC. (A) Correlation between the expression of SULF2 and immune inhibitors through TISIDB database. (B) Correlation of SULF2 expression with immune stimulators in ACC available at TISIDB database. ACC, adrenocortical carcinoma.

(Fig. 9B). Based on TCGA database, univariate as well as multivariate regression revealed that SULF2 was positively correlated with HRs of ACC, and it was a valuable prognostic factor (Fig. 9C and D). According to the diagnostic ROC curve, SULF2 exhibited a good ability to discriminate tumor from healthy samples (AUC = 0.864) (Fig. 10A). Moreover, the time-dependent survival ROC curve based on SULF2 expression was used for predicting 1-, 3-, and 5-year survival of ACC cases. All these AUC values were found to be above 0.7, suggesting good predictive power of SULF2 expression (Fig. 10B). As revealed by KM curve analysis, ACC patients who had SULF2 up-regulation exhibited the poorer OS (p = 3.3e-05) and DFS (p = 0.00023) (Fig. 10C and D).

3.8. The association of the SULF2 expression level with drug sensitivity

Data regarding gene expression profiles along with drug sensitivity were collected in CellMiner. Thereafter, correlation coefficient of SULF2 level with drug sensitivity was determined by R language. Thereafter, 15 SULF2-related drugs were chosen based on R-value, as a result, SULF2 expression showed positive relation to tumor cell sensitivity to drugs like LY-2835219, Rapamycin, Everolimus, Midostaurin and Idelalisib, but the opposite was true in some drugs such as Fludarabine, DACARBAZINE and Raltitrexed (Fig. 11A-O). Therefore, it was speculated that SULF2 expression might help determine the sensitivity of certain drugs based on these results.
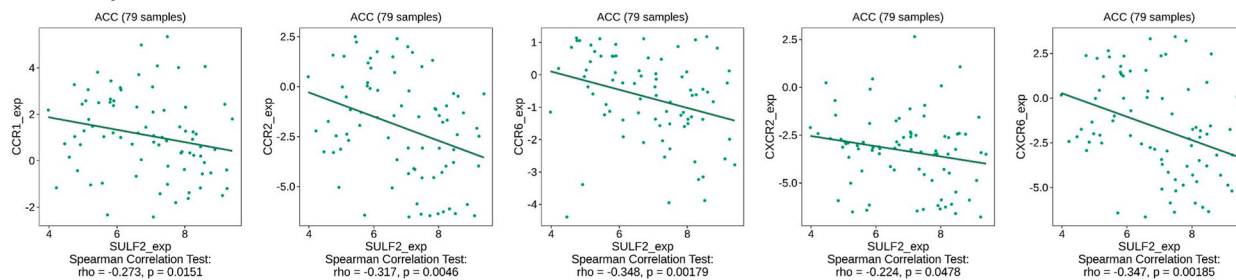
A Chemokine

B Receptor

Fig. 8. Correlation between SULF2 expression and chemokine and receptor in ACC. (A) Correlation between the expression of SULF2 and chemokine through TISIDB database. (B) Correlation of SULF2 expression with receptor in ACC available at TISIDB database. ACC, adrenocortical carcinoma.

4. Discussion

Nowadays, there are studies revealing the relationship between genes and tumors development [17,18]. The current research is the first to analyze SULF2 expression and its prognostic value of ACC through a series of bioinformatics approaches. Our study results suggested a possible link between high SULF2 expression and poor prognosis of ACC. In addition, this study also first suggested that SULF2 was strongly related to infiltration level of numerous immune-related molecules and cells in ACC. According to univariate as well as multivariate regression results, SULF2 expression was positively correlated with the HRs of ACC. Therefore, our research indicated the new and important functions of SULF2, which might affect the survival and prognosis of ACC patients by participating in immune infiltration.

SULF2, a member of sulfatase family, targets 6-O-sulfate groups on glucosamine residues in heparan sulfate (HS) chains while regulating a variety of molecular processes in the TME [19]. The signaling ligand-receptor interactions will be altered when the extracellular matrix (ECM) components of HS are affected by SULF2 [20]. Moreover, SULF2 can modify HS-mediated pathway by regulating HSPG expression [21,22]. Previous studies have shown that SULF2 expression increased within different tumor cells like bladder cancer, lung cancer and hepatocellular carcinoma, and that the high SULF2 expression level was related to poor prognosis [6, 23,24]. Until now, whether SULF2 is associated with tumor immunity and its specific significance in ACC remain unclear, which deserves further research.

First, SULF2 levels within ACC and normal tissues were investigated through TCGA, GEO, TIMER and GEPIA databases. As a result, SULF2 showed remarkably differential expression among tumor and healthy tissues in multiple human malignancies. Similarly, SULF2 expression notably increased in ACC compared with para-cancerous samples. Besides, the increased SULF2 expression was consistent with PD1, but unfortunately, there was no statistically significant relationship between them. According to these findings, SULF2 expression might be the possible auxiliary diagnostic reference for ACC. On the other hand, the up-regulated SULF2 expression level was markedly related to the poorer outcome of ACC in stages 1 and 2, 3, 4, N 0 and 1. Furthermore, based on KM plotter analysis, SULF2 up-regulation predicted the poor OS and DFS of ACC. The above results powerfully confirmed our hypothesis that SULF2 was
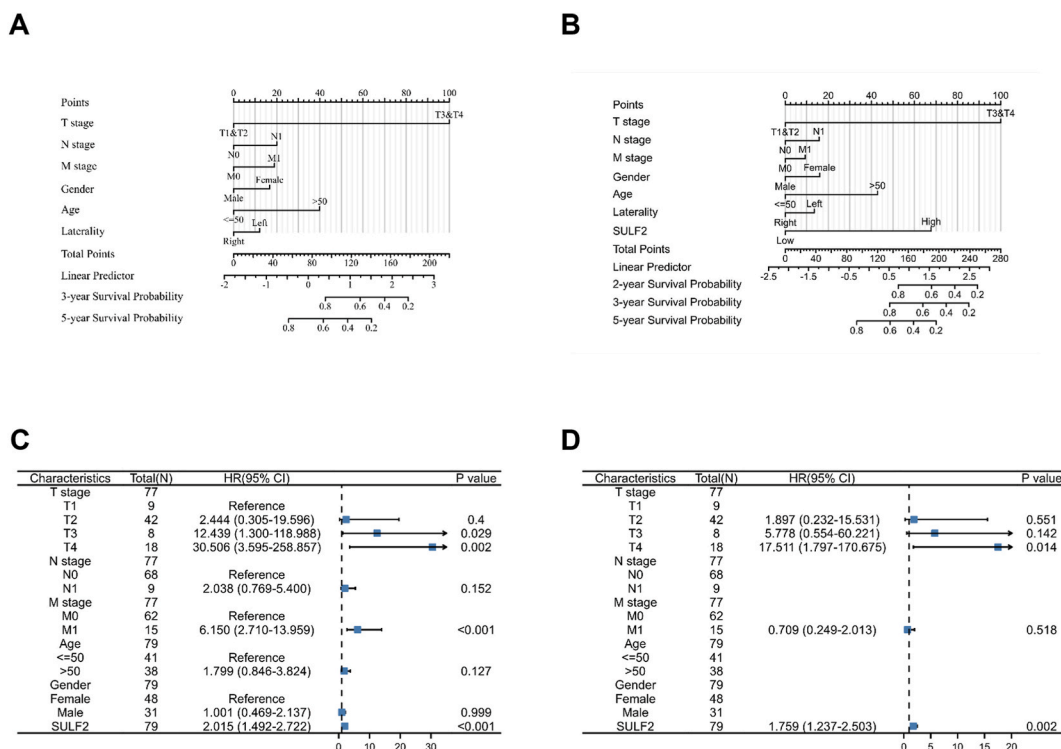
A

B

C

Characteristics	Total(N)	HR(95% CI)		P value
T stage	77			
T1	9	Reference		
T2	42	2.444 (0.305-19.596)		0.4
T3	8	12.439 (1.300-118.988)		0.029
T4	18	30.506 (3.595-258.857)		0.002
N stage	77			
N0	68	Reference		
N1	9	2.038 (0.769-5.400)		0.152
M stage	77			
M0	62	Reference		
M1	15	6.150 (2.710-13.959)		<0.001
Age	79			
<=50	41	Reference		
>50	38	1.799 (0.846-3.824)		0.127
Gender	79			
Female	48	Reference		
Male	31	1.001 (0.469-2.137)		0.999
SULF2	79	2.015 (1.492-2.722)		<0.001

D

Characteristics	Total(N)	HR(95% CI)		P value
T stage	77			
T1	9			
T2	42	1.897 (0.232-15.531)		0.551
T3	8	5.778 (0.554-60.221)		0.142
T4	18	17.511 (1.797-170.675)		0.014
N stage	77			
N0	68			
N1	9			
M stage	77			
M0	62			
M1	15	0.709 (0.249-2.013)		0.518
Age	79			
<=50	41			
>50	38			
Gender	79			
Female	48			
Male	31			
SULF2	79	1.759 (1.237-2.503)		0.002

Fig. 9. Nomogram and Cox hazard analysis of SULF2 in ACC. (A) Nomogram model, based on clinicopathologic factors to predict survival probability at 3-, and 5-years. (B) Nomogram model, integrating clinicopathologic factors and SULF2 level to predict survival probability at 2-, 3-, and 5-years. (C) Single-factor cox analysis of ACC. (D) Multivariate cox analysis of ACC.

likely to be a prognostic biomarker in ACC. SULF2 expression performed well in distinguishing cancer from healthy samples and predicting the long-term survival rates, suggesting that it was the possibly useful biomarker used to diagnose and predict the prognostic of ACC.

GPC3, NDST1 and COPE were identified as the functional partner genes for SULF2. As revealed by our GO and KEGG analyses, they were enriched in heparan sulfate proteoglycan metabolic process, glycosaminoglycan biosynthesis-heparan sulfate/heparin, and heparan sulfate sulfotransferase activity. Previous studies have clearly stated that extracellular vesicle-mediated communication is very important for pathological process in tumor [25]. Many important extracellular vesicle-mediated communication processes like uptake and biogenesis, are under the regulation of HSPG [26]. Based on these results, it is reasonable to believe that GPC3, NDST1, COPE and SULF2 have important synergistic roles in the pathogenesis of ACC. Further, our GSEA results showed that neuroactive ligand receptor interaction and reactome g alpha I signaling events pathways were obviously enriched by these genes. Studies reveal that neuroactive ligand receptor interaction is associated with diffuse intrinsic pontine glioma and granulosa cell tumor development [27,28]. Therefore, the neuroactive ligand receptor interaction may be linked with ACC pathogenesis. Additionally, infiltration of immune cells has a key role in carcinogenesis [29]. SULF2, a novel immunomodulators, has positive modulatory effects on antigen delivery and cytophagy of immune cells [30]. Xuping Niu et al. discovered that SULF2 was up-regulated in dermal mesenchymal stem cells, which affected the inflammatory microenvironment via multiple pathways, including regulation of immune cell proliferation, differentiation, migration and recruitment [31,32]. But so far, it remains unknown whether SULF2 expression is involved in immune infiltration in ACC. This study first illustrated that SULF2 was related to immune infiltration level in ACC. Based on our analyses, SULF2 expression was obviously correlated with immune cells like CD4$^+$T cells, CD8$^+$ T cells, and NK cell. Meanwhile, the increased SULF2 expression was related to diverse kinds of immuneinhibitors, immunestimulators, chemokines and receptors suggesting that SULF2 had a critical effect on immune regulation in ACC. According to our PPI network and functional annotation results, SULF2 and interacting genes were related to the underlying tumor biological processes and contributed to tumorigenesis and progression. Besides, PD1, an important immune checkpoint component, has been recognized as an important target for tumor immunotherapy [33,34]. This study disclosed that SULF2 expression was associated with PD1 and immune-related receptors and molecules. Therefore, immunotherapy may be useful for ACC and more efforts are needed to explore the molecular mechanism. On the other hand, we found that TP53 mutation status was related to tumor development and prognostic outcome [35,36]. Moreover, SULF2 levels remarkably increased in ACC patients with TP53 mutation compared with in non-mutated cases, with the difference being statistically significant. It suggests that there may be a link between SULF2 and TP53, which deserves further study. Finally, SULF2 expression was markedly related to several drugs, which provides some guidance for clinical treatment of the disease.

Nonetheless, certain limitations should be noted in this analysis. For example, the data were mostly sourced from online databases
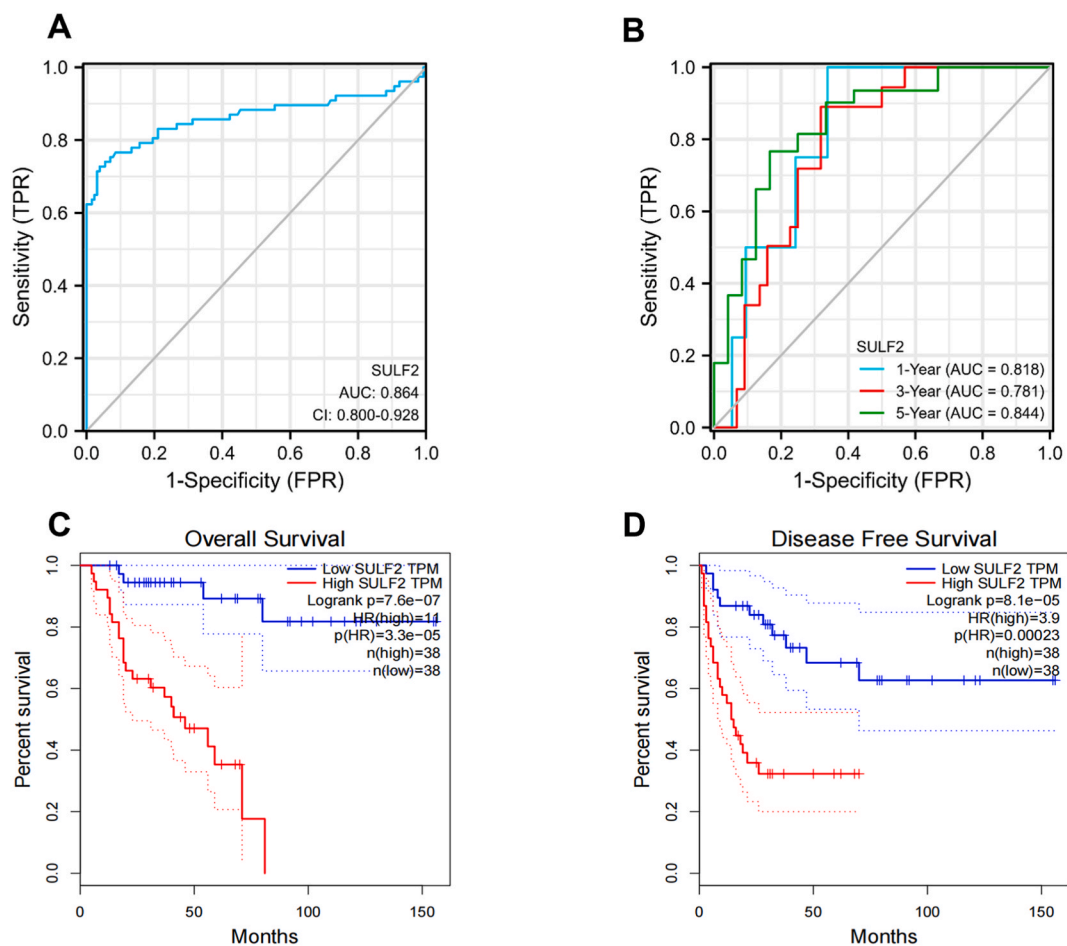
Fig. 10. ROC analysis and Kaplan-Meier survival curves. (A) The ROC curve of diagnosis to distinguish tumor from normal tissue. (B) Time-dependent survival ROC curve analysis to predict 1-, 3- and 5-year survival rates. (C, D) The overall survival and disease-free survival curves comparing patients with high and low SULF2 expression in ACC.

and our results might be affected by subsequent data updates, which might possibly introduce bias. Besides, because of the rarity of the disease, not enough cases were obtained to complete the experimental validation.

5. Conclusion

According to our results, SULF2 shows abnormal expression in ACC, which predicts the dismal prognostic outcome. Meanwhile, this study is the first to illustrate that SULF2 expression is closely related to immune system, indicating its possible involvement in immune infiltration of tumors, which sheds novel lights on diagnosing and treating ACC.

Author contribution statement

Jiusong Yan and Xiaodu Xie: Conceived and designed the experiments; wrote the paper. They are co-first authors.

Qinke Li and Peihe Liang: Performed the experiments; analyzed and interpreted the data.

Junyong Zhang and Guangyong Xu: Contributed reagents, materials, analysis tools or data. They were responsible for the final review of the paper who are co-corresponding authors of this paper.
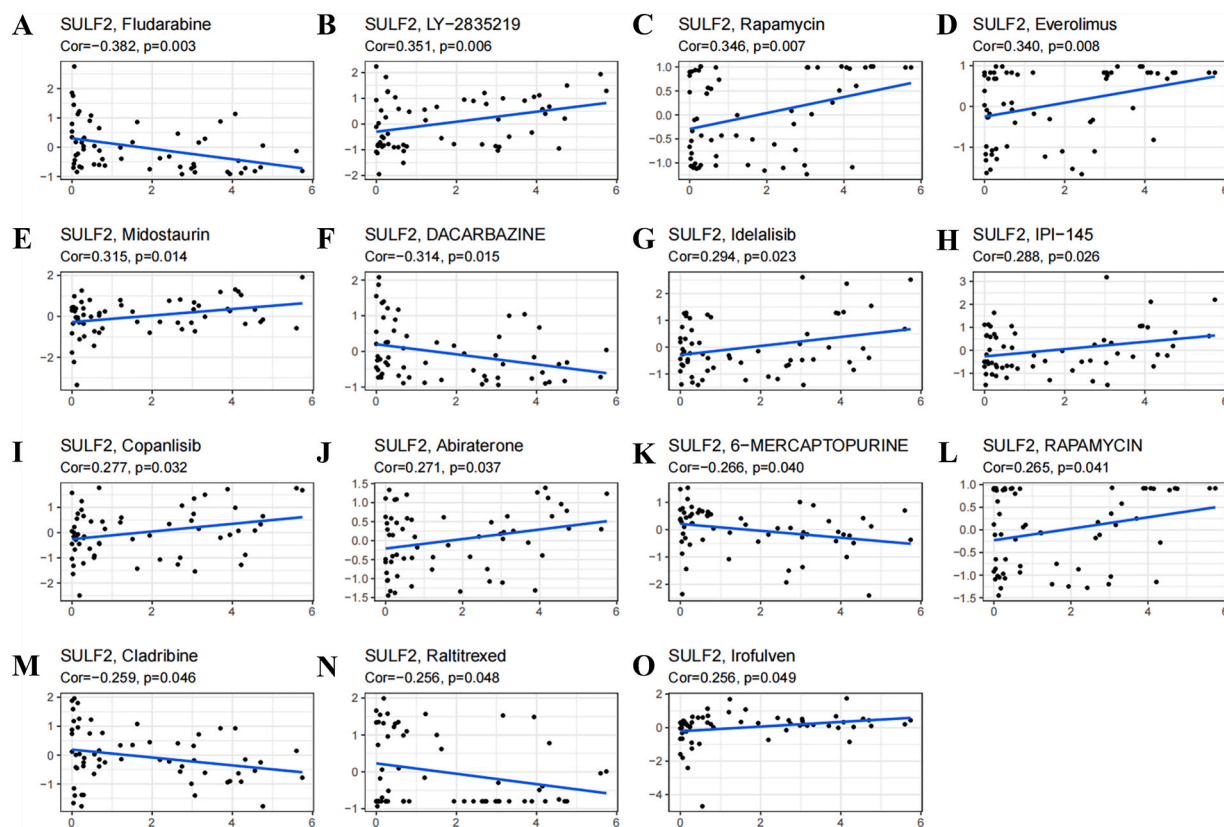
Funding statement

Fig. 11. The relationship between SULF2 expression levels and drug sensitivity. (A–O) The correlation between SULF2 expression and Fludarabine, LY-2835219, Rapamycin, Everolimus, Midostaurin, DACARBAZINE, Idelalisib, IPI-145, Copanlisib, Abiraterone, 6-MERCAPTOPURINE, RAPAMYCIN, Cladribine, Raltitrexed and Irofulven.

Data availability statement

Data can be obtained from corresponding authors upon on request.

Declaration of interest statement

All authors declared no competing interest.

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