Down-regulation of S1PR2 is Correlated with Poor Prognosis and Immune Infiltrates in Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma

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Research Article

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Abstract

Background

Cervical squamous cell carcinoma and cervical adenocarcinoma (CESC) are the second leading cause of deaths from malignant tumors in women, while their therapeutic and diagnostic aims are still finited. A growing body of evidence indicated that sphingosine-1-phosphate receptor 2 (S1PR2) plays essential roles in the occurrence and development about several human cancers. Nevertheless, the key mechanism and role mechanism of S1PR2 in CESC are still unclear.

Methods

we first used Tissue Expression (GTEx) and Genotypic Cancer Genome Atlas (TCGA) data to perform pan-cancer analysis on the expression and prognosis of S1PR2, and found that S1PR2 may have a potential impact on CESC. To generate a protein-protein interaction (PPI) network using the STRING database. The clusterProfiler package is used for feature-rich analysis. The Tumor IMmune Estimation Resource was used to determine the connection between S1PR2 mRNA expression and immune infiltrates.

Results

S1PR2 expression in CESC tissues was down-regulated compared with adjacent normal tissues. Kaplan-Meier analysis indicated that compared with patients with high expression of S1PR2, CESC patients with low S1PR2 expression had a worse prognosis. Reduced S1PR2 expression is associated with patients with high clinical stage, more histological types of squamous cell carcinoma, and poor primary treatment outcomes. The receiver operating characteristic curve of S1PR2 was 0.870. Correlation analysis showed that the mRNA expression of S1PR2 was related to immune infiltrates and tumor purity.

Conclusion

down-regulated S1PR2 expression is related to poor survival and immune infiltration in CESC. S1PR2 is a potential biomarker for poor prognosis and as a potential target for CESC immune therapy.

Background

Cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC) are the second most prevalent gynecological cancer [1], and bring about cancer death of women in the worldwide [2] In 2012 alone, there were 528,000 new medical cases, and 266,000 patients died in that year [3], CESC mainly occurred in patient aged 20–39 years, leading to 10 premature deaths each week [4]. The condition of the immune microenvironment is intently interrelated to the CESC development. Many studies have deeply

explored the relationship between cervical cancer and immune cells [5–7]. Immunotherapies using anti-CTLA4 [8] and anti-PD1 [9] drugs have been successful in CESC treatment. However, to date, none of them can significantly improve overall survival [10–12]. There is still an imperative need for effective diagnosis and prognostic indicators of CESC tumor occurrence and recurrence to assist treatment.

Sphingosine 1-phosphate (S1P) is a pluripotency and extensive biologically active molecule be classified to the sphingolipid family, which is a intricate lipid group appeared on all eukaryotic cells. In the past, it was thought to only perform structural functions, while recently sphingolipids are regarded as pivotal conditioners of countless cell functions in pathophysiological processes [13,14]. S1PR1, S1PR2, and S1PR3, S1P receptors 1–3, there are expressed general, while S1PR4 and S1PR5 are mainly restricted expression to central nervous system, lymphatics and hematopoietic tissues. Binding to different G proteins activates several downstream pathways that help regulate many cellular mechanisms [15]. Therefore, there has been considerable interest in the S1P/S1PRs axis as a potential therapeutic target for regulating various cellular processes. In contrast, so far, the other S1P acceptors function in the cervix has obtained rare concern. S1PR2 is the key acceptor for the occurrence and development of different genre of cancer. Even though its role varies from tissue to tissue, most data support anti-tumor function [16]. In fact, S1PR2 adjusts negatively the invasion and migration of human glioblastoma [17], melanoma [18], oral squamous cell carcinoma, and gastric cell lines [19] and cell proliferation in human kidney tumor cells [20]. In addition, the gene decomposition of S1PR2 promotes the in vivo growth of Lewis lung and melanoma cancer [21], standing by S1PR2 is a key acceptor of cell proliferation. Recently research showed that S1PR2 impeding proliferate CD4 + T cell to adjusts the epithelial barrier on intestinal epithelial cells [22]. Therefore, its features in epithelial cells still to be elaborated.

In the study, we executed survival and expression analysis of S1PR2 in various human cancers. We found that S1PR2 is down-regulated in CESC, and the down-regulation of S1PR2 is related to the adverse clinical characteristics and risk factors of CESC patients. We discovered that the decreased S1PR2 expression is interrelated to the low survival rate of CESC patients. We further explored diagnostic and prognostic value of S1PR2 and the connection between S1PR2 biomarkers and expression of immune cells, immune cell infiltration or immune checkpoints in CESC.

Materials And Methods

The Cancer Genome Atlas (TCGA) Dataset Analysis

We downloaded the expression transcriptional data of S1PR2 and the information of corresponding clinical from The Cancer Genome Atlas (TCGA) database (https://genome-cancer.ucsc.edu/). After normalizing these data, the variant expression of S1PR2 was analyzed by the R package limma [23].

GEPIA Database Analysis

GEPIA (http://gepia.cancer-pku.cn/) is a network instrument for normal or cancer gene expression profiles, Genotype Tissue Expression data and TCGA are used to analysis the interactive [24]. CESC patients survival analysis, including analyses of disease-free survival (DFS) and overall survival (OS) were performed by GEPIA .

Protein-Protein Interaction (PPI) Networks and Functional Enrichment Analysis

STRING (https://www.string-db.org/) for searching the genes of for interacting to construct PPI networks [25]. We performed with an interaction score > 0.4 of a STRING search for co-expressing genes of S1PR2 and constructed a PPI network. The “ggplot2” package was used for the “clusterProfiler” visualized and package [26] it was performed for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of co-expressing genes and Gene Ontology (GO) enrichment .

The Tumor Immune Estimation Resource (TIMER) Database

TIMER (https://cistrome.shinyapps.io/timer/) is a resource online for the immune infiltration systemic analyzed in miscellaneous cancer types [27]. The connection between S1PR2 expression level and the level of immune cell infiltration in CESC were determined by TIMER.

Statistical analyses

Statistical analyses were calculated by the above-mentioned online databases. A log rank p value of <0.05 or p value <0.05 was considered statistically significant.

Results

Expression of S1PR2 in Pan-cancer

To make a thorough inquiry the roles of S1PR2 in carcinogenesis, first, we inquired the expression of S1PR2 in 32 human cancers. As presented in Fig. 1A, compared to normal samples, S1PR2 was markedly up-regulated in 7 of all 32 cancer types, including STAD, CHOL, ESCA, HNSC, LIHC, GBM and THCA, and was significantly down-regulated in 9 cancer types, involving KIRP, BLCA, BRCA, PRAD, KICH, KIRC, LUAD, LUSC, and CESC. However, no obviously difference expression of S1PR2 in COAD, PAAD, PCPG, READ, or UCEC was observed. Next, we used TCGA database to further verify the S1PR2 expression in these 16 types of cancer. As shown in Figs. 1B–1H, S1PR2 expression in BLCA, BRCA, CESC, LUAD, LUSC, PRAD or ESCA was reduced compared to the homologous normal contrasts. In CHOL, GBM, HNSC, LIHC, STAD, or THCA, S1PR2 was obviously increased (Figs. 1I–1N). Taken together, S1PR2 was up-regulated in CHOL, GBM, HNSC, LIHC, STAD, or THCA, and down-regulated in BLCA, BRCA, CESC, LUAD, LUSC, and PRAD, demonstrating that S1PR2 may as a key regulatory role in the carcinogenesis of 12 cancer types.

Prognostic values of S1PR2 in human cancer

We analyzed the survival of S1PR2 in HNSC, CHOL, STAD, LIHC, GBM, or THCA, and down-regulated in BLCA, BRCA, CESC, LUAD, LUSC, and PRAD was conducted. Including two prognostic indicators: one is disease-free survival (DFS) and another is overall survival (OS). As presented in For OS, low S1PR2 expression in CESC has a poor prognosis, but BLCA patients with low S1PR2 expression have a better prognosis (Fig. 2). As presented in Fig. 3, for RFS, reduced S1PR2 expression in all cancer types indicates poor prognosis for BRCA and CESC. The statistical markedly of S1PR2 in predicting the patients prognosis with other cancers has not been inquired. Integrating OS and RFS, S1PR2 can be used as disadvantageous prognostic biomarker for CESC patients.

The prospective biomarker of S1PR2 in CESC patients

We implemented ROC curve analysis to probe the values for S1PR2 in distinguishing CESC from normal samples. As presented in Fig. 4, the ROC curve analysis indicated that S1PR2 with an AUC value about 0.870 (95% CI: 0.713–1.000). With a 2.689 cutoff, S1PR2 had a specificity, sensitivity of 84.6 and 90.5%. And the negative predictive value was 27.5%, the 99.3% positive predictive value. These results indicate that S1PR2 may be a prospective biomarker for distinguishing CESC tissues from normal tissues.

Association of S1PR2 levels with clinical characteristics in CESC Patients

We implemented logistic regression analysis and Mann-Whitney U test to estimate the relationship about S1PR2 mRNA expression with the clinicopathological characteristics of CESC samples. As presented in Table 1, lower expression levels of S1PR2 were observed patients with high Clinical stage ($P = 0.015$), Histological type with more squamous cell carcinoma ($P < 0.001$), patients with worse primary therapy outcome ($P = 0.013$). Nevertheless, no markedly statistically association were discovered about S1PR2 levels with other clinicopathological characteristics, for instance, BMI ($P = 0.702$), age ($P = 0.557$), T stage ($P = 0.331$), N stage ($P = 0.060$) and M stage ($P = 0.111$).

Table 1
Clinical characteristics of the CESC patients (TCGA).

Characteristic	Low expression of S1PR2	High expression of S1PR2	p
n	153	153	
T stage, n (%)			0.331
T1	65 (26.7%)	75 (30.9%)	
T2	32 (13.2%)	40 (16.5%)	
T3	14 (5.8%)	7 (2.9%)	
T4	5 (2.1%)	5 (2.1%)	
N stage, n (%)			0.060
N0	58 (29.7%)	76 (39%)	
N1	36 (18.5%)	25 (12.8%)	
M stage, n (%)			0.111
M0	54 (42.5%)	62 (48.8%)	
M1	2 (1.6%)	9 (7.1%)	
Clinical stage, n (%)			0.015*
Stage I	77 (25.8%)	85 (28.4%)	
Stage II	30 (10%)	39 (13%)	
Stage III	33 (11%)	13 (4.3%)	
Stage IV	10 (3.3%)	12 (4%)	
Age, n (%)			0.557
<=50	97 (31.7%)	91 (29.7%)	
> 50	56 (18.3%)	62 (20.3%)	
BMI, n (%)			0.702
<=25	52 (20%)	48 (18.5%)	
> 25	78 (30%)	82 (31.5%)	
Histological type, n (%)			< 0.001***
Adenosquamous	12 (3.9%)	41 (13.4%)	

*p value < 0.05; ***p value < 0.001.

Characteristic	Low expression of S1PR2	High expression of S1PR2	p
Squamous cell carcinoma	141 (46.1%)	112 (36.6%)	
Primary therapy outcome, n (%)			0.013*
PD	18 (8.2%)	5 (2.3%)	
SD	3 (1.4%)	3 (1.4%)	
PR	4 (1.8%)	4 (1.8%)	
CR	79 (36.1%)	103 (47%)	
Age, meidan (IQR)	46 (39, 57)	48 (38, 56)	0.942
*p value < 0.05; ***p value < 0.001.			

PPI network and functional annotation about S1PR2

We implemented GO, KEGG analyses and STRING database to elevate functional annotations and PPI networks. As presented in Fig. 5A, a network of S1PR2 and its 10 coexpression genes. Figure 5B shown transforms in the biological circuit of S1PR2 were related with sphingolipid signaling pathway, and parathyriod hormone synthesis, secretion and action. The correlation analysis between S1PR2 expression and CESE co-expressed genes from TCGA is shown in Fig. 5C-L.

S1PR2 positively correlates with immune cell infiltration in CESC

We next examined the relationship between S1PR2 expression and seven tumor types of infiltrating immune cells from the TIMER database. The SCNA module shown the association between the CESC tumor immune cell infiltration and copy number of different somatic cells of RRAGB through the Wilcoxon rank sum test (Fig. 6A). Connection analyzed could supply important clues for researching the machine-processed and foundation of S1PR2. Therefore, the relationship about the level of immune cell infiltration and the expression level of S1PR2 was assess. As shown in Figs. 6B, the expression of S1PR2 was markedly positively related with immune cells, counting dendritic cell, neutrophil, CD4 + T cell, macrophage and B cell in CESC.

Correlation between S1PR2 and the expression of immune cells in CESC

We firmed the correlation between S1PR2 and the expression of immune cells in CESC to make a thorough inquiry the part of S1PR2 in immune tumor using GEPIA database. Table 2 listed that S1PR2 was markedly positively related with CD8 + T cell's biomarkers (CD8A and CD8B), dendritic cell's

biomarkers (HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, CD1C, NRP1, and ITGAX), M2 macrophage's biomarkers (CD163, VSIG4, and MS4A4A), CD4 + T cell's biomarker (CD4), neutrophil's biomarkers (ITGAM and CCR7), and B cell's biomarkers (CD19 and CD79A) in CESC. The results section stand by the S1PR2 is positively related with immune cell infiltration.

Table 2
Correlation analysis between S1PR2 and biomarkers of immune cells in CESC determined by GEPIA database.

Immune cell	Biomarker	R value	p value
B cell	CD19	0.23	6.9E-05***
	CD79A	0.24	2.5E-05***
CD8 + T cell	CD8A	0.23	5.9E-05***
	CD8B	0.31	2.6E-08***
CD4 + T cell	CD4	0.29	3.5E-07***
M1 macrophage	NOS2	0.044	0.44
	IRF5	-0.092	0.11
	PTGS2	0.047	0.42
M2 macrophage	CD163	0.24	2.4E-05***
	VSIG4	0.19	7.0E-04***
	MS4A4A	0.23	3.8E-05***
Neutrophil	CEACAM8	-0.11	0.055
	ITGAM	0.32	1.0E-08***
	CCR7	0.28	4.5E-07***
Dendritic cell	HLA-DPB1	0.34	1.2E-09***
	HLA-DQB1	0.19	0.00093***
	HLA-DRA	0.3	1.2E-07***
	HLA-DPA1	0.28	6.2E-07***
	CD1C	0.16	0.0049**
	NRP1	0.15	0.0081**
	ITGAX	0.3	1.1E-07***
**p value < 0.01; ***p value < 0.001.			

Correlation between S1PR2 and immune checkpoints in CESC

PD1 and CTLA-4 are key immune checkpoints there are liable for tumor immune escape. The correlation of S1PR2 with PD1 and CTLA-4 was evaluated while premeditating the latent oncogenic role of S1PR2 in CESC. There was markedly positively related between S1PR2 with CTLA-4 and PD1 in CESC (Figs. 7A–7D). These foundings indicate that tumor immune escape could be concerned in S1PR2 intermediary carcinogenesis of CESC.

Discussion

CESC is a general tumor in women. With the upgrowth of standard treatment options for CESC patients with concurrent chemotherapy and brachytherapy, it has improved of the five-year survival rate, while the prognosis is poor [28]. nevertheless, there is a pressing need for promising prognostic biomarkers to assess pressing patients risk with metastatic CESC and corresponding effective therapeutic targets that can improve clinical outcomes.

More and more evidence shows that S1P is associated with angiogenesis, cell proliferation, chemotaxis, migration and differentiation, and is also associated with the cancer biology. These S1PRs seems to be specific of tissues, has been shown to be consist of the cell proliferation regular pattern, surviving in various cancer types. S1PR2 roles in cancer remains agonistical. According to reports, S1PR2 can act as an anti-cancer and cancer-promoting receiver. For example, in B-cell lymphoma, glioblastoma, and melanoma, S1PR2 plays an anti-cancer receiver. On the other hand, it is reported that in prostate cancer, S1PR2 plays a carcinogenic receiver. A review introduced that on the roles of S1PR2 in cancers referred to the affect of this receiver on developing of tumor and headway is the specific of cell-type, because of its taking part with the specific G proteins to regulate physiological functions [29]. Therefore, despite the existence of context-specific and controversial evidence, the knowledge of S1PR2 in CESC is still insufficient and further research is needed. In this study, we first performed a pan-cancer analyzed of S1PR2 expression used Cancer Genome Atlas (TCGA) data, and used it to verify the S1PR2 expression. Survival analysis of S1PR2 in these cancer types of interest indicated that patients of CESC with low S1PR2 expression have a poorer prognosis. And we further found down-regulated S1PR2 expression is positively correlated with patients with high clinical stages, more histological types of squamous cell carcinoma, and poor primary treatment outcomes. ROC curve analysis indicates S1PR2 might be a promising diagnostic biomarker in distinguishing CESC from normal tissues. S1PR2 might be a promising biomarker for impoverished prognosis of CESC.

S1PR2 participates in differentiation, cell proliferation, angiogenesis, migration and chemotaxis through the sphingolipid signaling pathway. TP inhibits the SPHK-S1P signaling pathway and effectively reduces the levels of S1P and the expression of SPHK1/S1PR1/S1PR2, and markedly suppressing the S1P-mediated phosphorylation activation of ERK protein in macrophages [30]. Our co-expression analysis indicated that the expression of S1PR2 was markedly related to the expression of RHOA, GNA11, GNA12,

GNAI1, GNAI2, and GNAQ. We speculate that the down-regulation of S1PR2 will effect the entire pathway, and it possibility could be examined in future findings.

Numerous studies have convinced that the tumor immune cell infiltration could effect the usefulness of immunotherapy, radiotherapy or chemotherapy and the prognostication of cancer patients [31–33]. This work shows that S1PR2 is markedly positively related to various immune cells, involving dendritic cells, macrophages, CD4 + T cells, neutrophils, and B cells in CESC. In addition, S1PR2 is also significantly positively correlated with these biomarkers of infiltrating immune cells. These results indicate tumor immune infiltration may not wholly explain the carcinogenic effects of S1PR2 mediated in HCC.

The usefulness of immunotherapy requires sufficient immune cells to infiltrate the tumor microenvironment, and relies on the full expression of immune checkpoints [34]. Therefore, this study more evaluated the related about S1PR2 with immune checkpoints. The findings indicate that S1PR2 is closely related to CTLA-4 or PD1 in CESC, showing that aiming S1PR2 may enhance the efficacious of immunotreatment in CESC.

In conclusion, this study clarified that S1PR2 is under-expressed in many human cancers (involving CESC) and is positively related to the poor prognosis of CESC. Furthermore, our current research results also indicate that S1PR2 may exert its anti-cancer effect by increasing the tumor immune cell infiltration and the expression of immune checkpoint. Therefore, these findings could be verified more through large-scale clinical trials and basic experiments in the future.

Conclusion

We demonstrated that S1PR2 is under-expressed in many types of human cancers, including CESC, and showed that S1PR2 represents a possible poor prognostic biomarker which could be used to identify CESC patients with poor clinical outcomes. Our research results also indicate that S1PR2 may exert its anti-cancer effect by increasing the expression of immune checkpoint and tumor immune cell infiltration. The findings could be verified through additional experiments and large-scale clinical trials.

Abbreviations

S1PR2, sphingosine 1-phosphate receptor 2, CESC,cervical squamous cell carcinoma and endocervical adenocarcinoma, TCGA, The Cancer Genome Atlas, TIMER, Tumor Immune Estimation Resource, GSEA, Gene Set Enrichment Analysis, PPI, Protein–Protein Interaction Network, DFS, disease-free survival, OS, overall survival, TNM, Tumor-node-metastases, KEGG, Kyoto Encyclopedia of Genes and Genome, 95% CI 95% confidence interval, AUC, Area under the curve, ROC, Receiver operating characteristic.

Declarations

Data availability statement

Data and material availability can be obtained from corresponding author on request.

Author contributions

LiY designed and approached this study. YuZ and LiY wrote the manuscript and analyzed data. YuZ contributed analysis tools. The manuscript reviewed by all authors.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

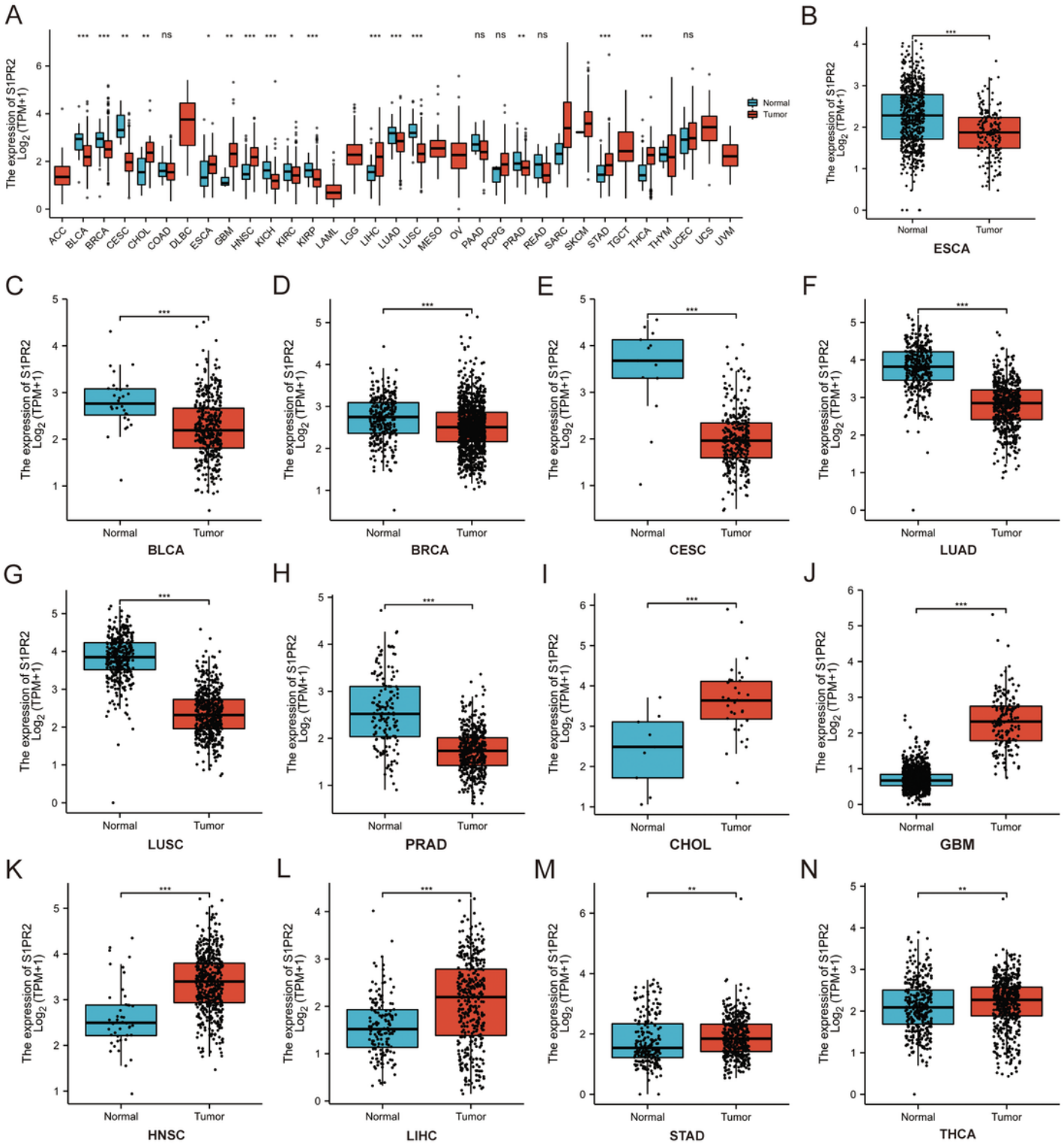

Figure 1

Expression of S1PR2 in Pan-cancer. **(A)** The expression of S1PR2 in 32 human cancers based on TCGA data of normal and cancer. **(B-N)** Compared with corresponding TCGA and GTEx normal tissues, the expression of S1PR2 in TCGA ESCA (**B**), BLCA (**C**), BRCA (**D**), CESC (**E**), LUAD (**F**), LUCS (**G**), PRAD (**H**), CHOL (**I**), GBM (**J**), HNSC (**K**), LIHC (**L**), STAD (**M**) and THCA (**N**) tissues. *p value < 0.05, **p value < 0.01, ***p value < 0.001.

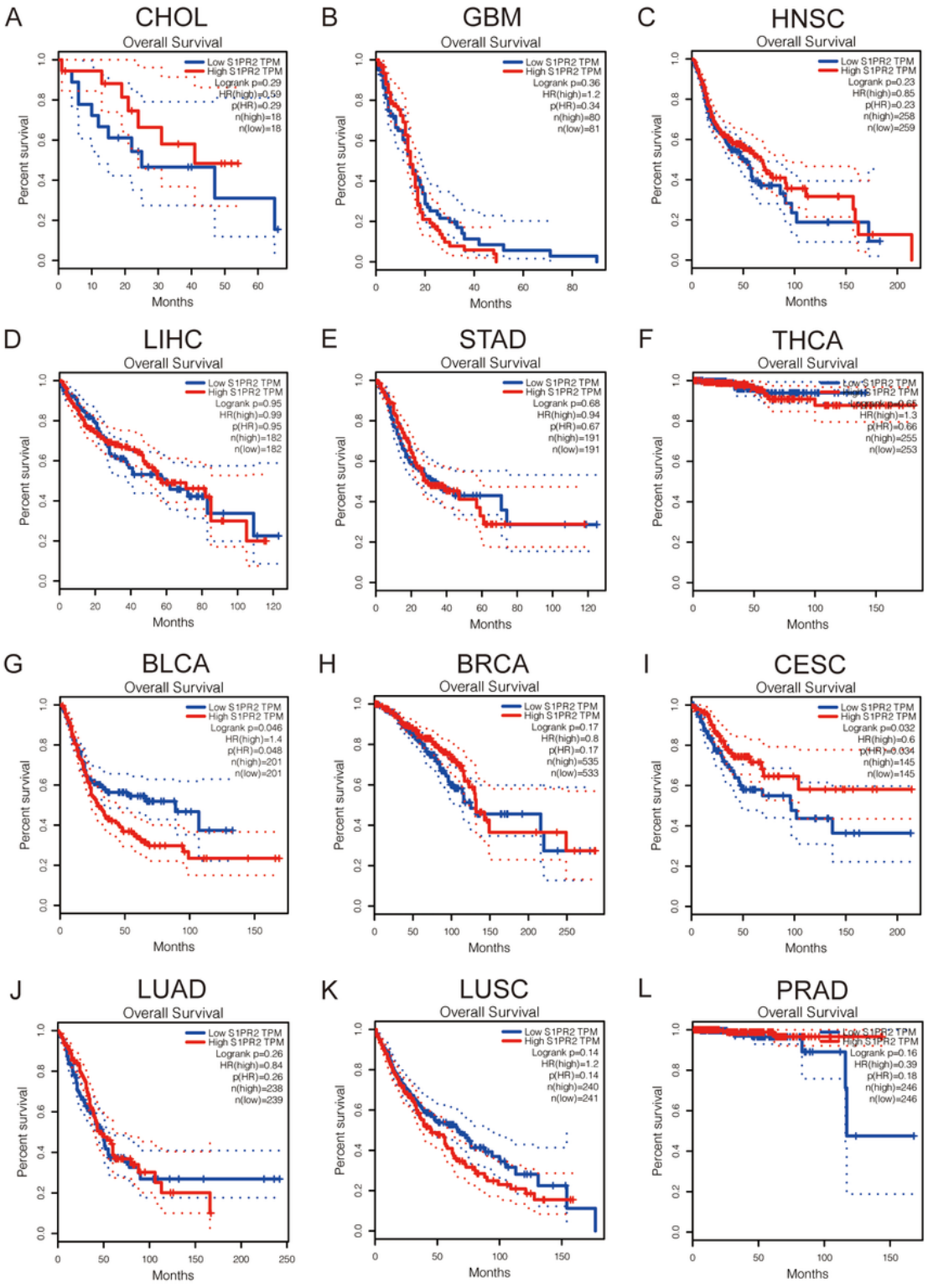

Figure 2

S1PR2 overall survival (OS) analysis in diverse human cancers established by GEPIA database. **(A-I)** The OS plot of S1PR2 in CHOL (**A**), GBM (**B**), HNSC (**C**), LIHC (**D**), STAD (**E**), THCA (**F**), BLCA (**G**), BRCA (**H**), CESC (**I**), LUAD (**J**), LUCS (**K**), and PRAD (**L**).

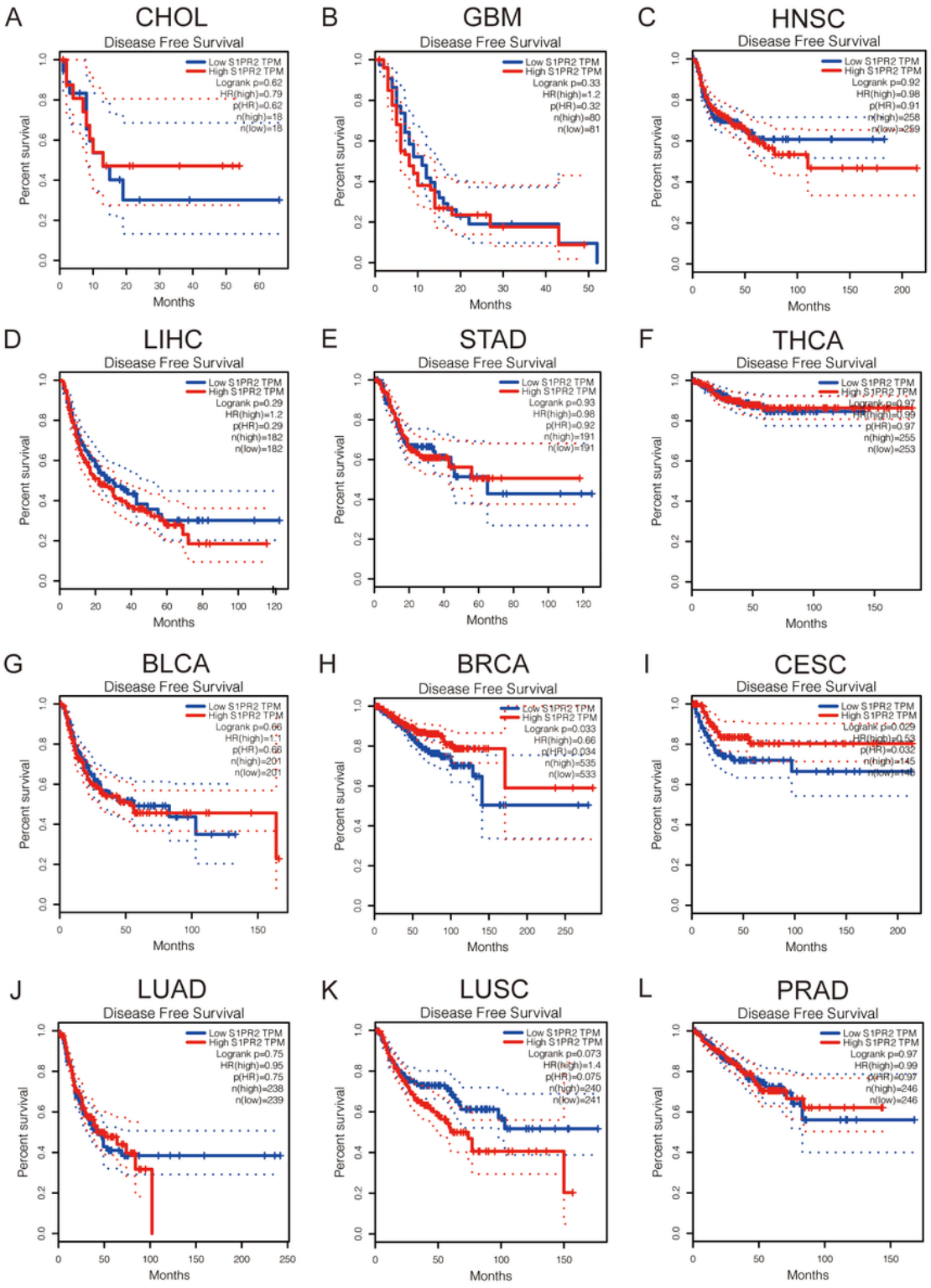

Figure 3

S1PR2 disease-free survival (RFS) analysis in diverse human cancers established by GEPIA database. **(A-I)** The RFS plot of S1PR2 in CHOL (**A**), GBM (**B**), HNSC (**C**), LIHC (**D**), STAD (**E**), THCA (**F**), BLCA (**G**), BRCA (**H**), CESC (**I**), LUAD (**J**), LUCS (**K**) and PRAD (**L**).

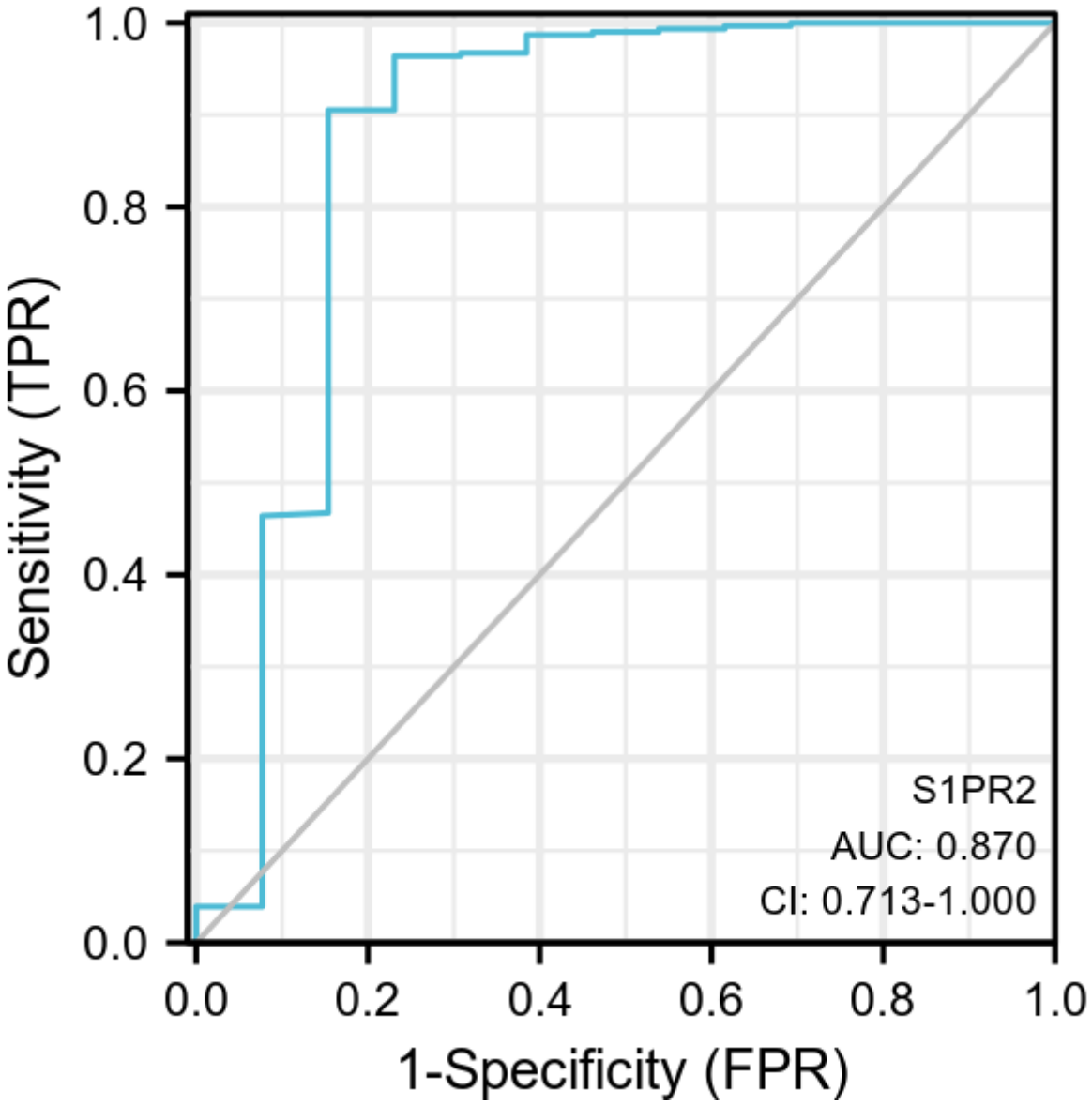

Figure 4

ROC curves for S1PR2. ROC curve for CESC patients based on S1PR2 expression.

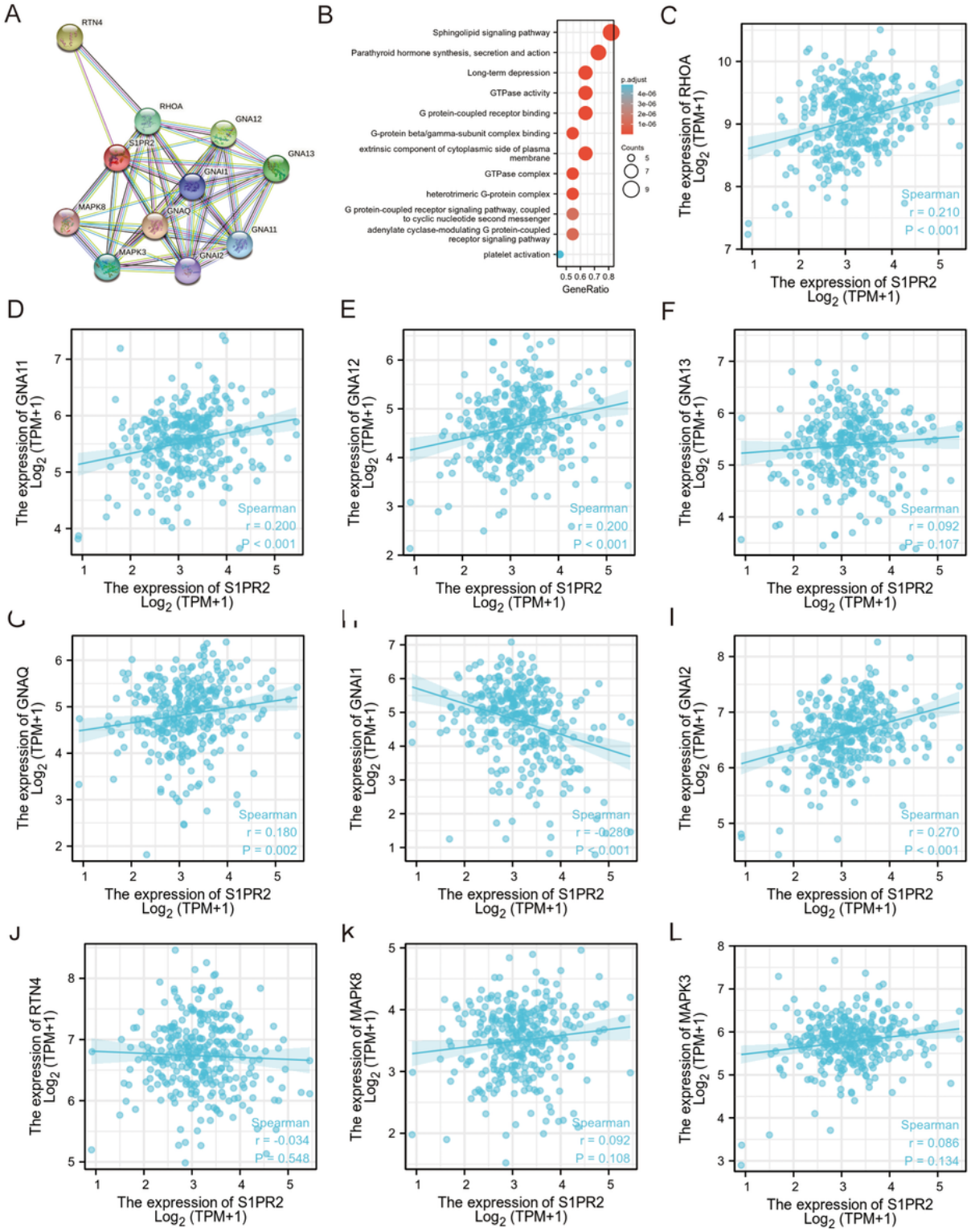

Figure 5

PPI enrichment analyzed of networks and functional. **(A)** S1PR2 network and the co-expression genes of S1PR2. **(B)** Enrichment function analyzed of 10 involved genes. **(C–L)** The correlation analyzed about the S1PR2 expression with co-expressed genes of CESC.

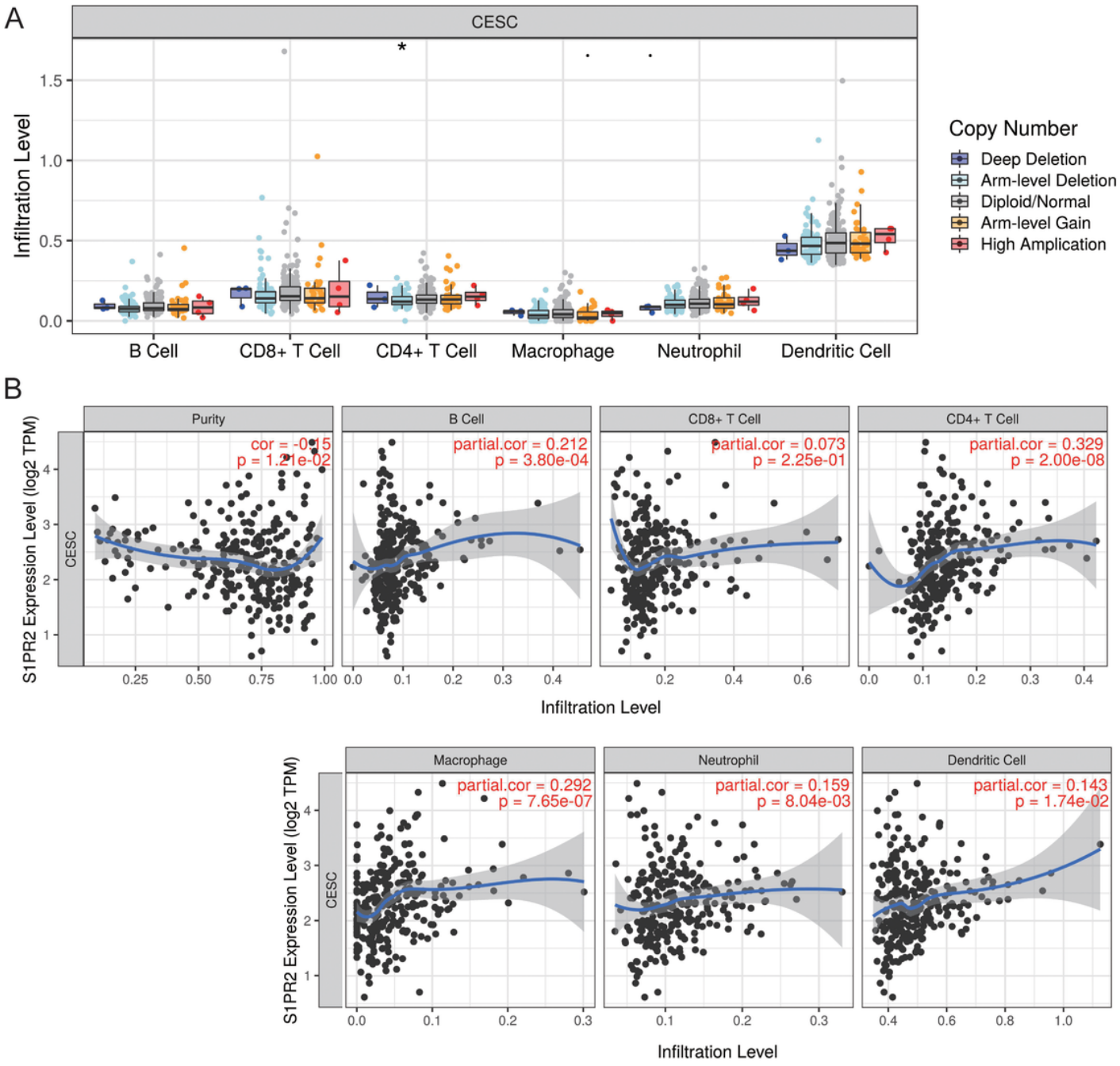

Figure 6

The correlation between immune cell infiltration and the level of S1PR2 in CESC. **(A)** Different kinds immune cell infiltration levels under different S1PR2 copy numbers of CESC. **(B)** The relationship of the expression level of S1PR2 with dendritic cell, neutrophil, macrophage, CD4+ T cell, CD8+ T cell , or B cell infiltration level of CESC.

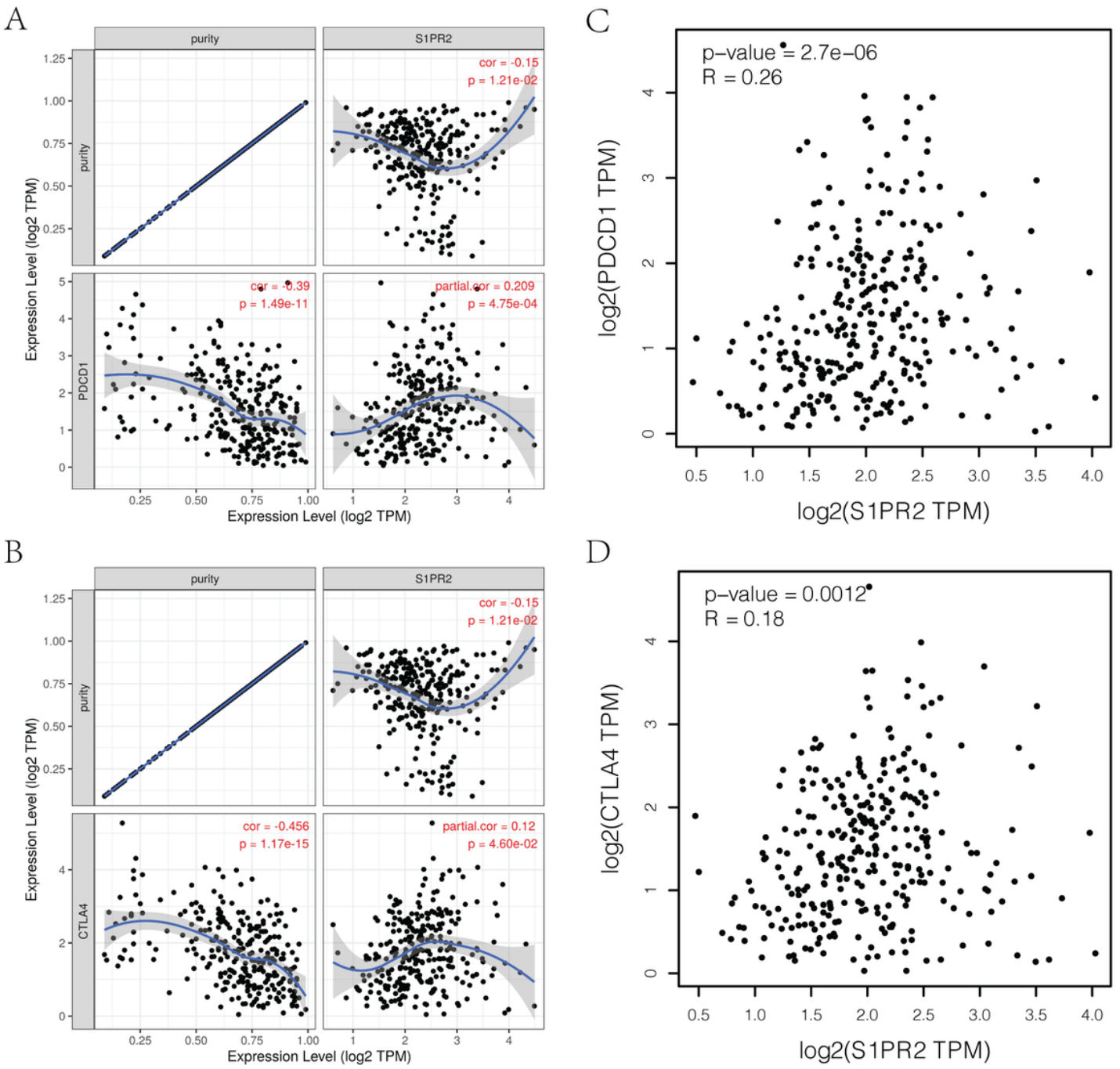

Figure 7

Relationship in CESC of the expression of S1PR2 with the expression of PD-1 and CTLA-4. Using TIMER to analyzed the Spearman relationship of S1PR27 with PD-1 (**A**), CTLA-4 (**B**) expression in CESC adjusted by purity. The expression relationship between S1PR2 and PD1 (**C**), CTLA-4 (**D**) in CESC determined by GEPIA database.