# Glut10 is a Novel Immune Regulator Involved in Lung Cancer Immune Cell Infiltration and Predicts Worse Survival When Transcriptionally Down-Regulated

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# Abstract

**Background:** GLUT10 is encoded by SLC2A10 gene. Recent investigations have shown that GLUT10 is not only involved in glucose metabolism, but also involved in the body's immune response to cancer cells. However, the correlations between GLUT10 expression and prognosis, immune cells infiltrating of different cancers remain unclear.

**Methods:** We Knocked down SLC2A10 and performed transcriptome sequencing to analyze the biological function of GLUT10. The SLC2A10 expression level was analyzed by the Oncomine databases and Tumor immune Estimation Resource (TIMER) site. We evaluated the prognostic potential of SLC2A10 in different cancers using the Kaplan-Meier plotter database and the PrognoScan online software. The correlations between SLC2A10 expression and immune infiltrates were analyzed by TIMER. In addition, correlations between SLC2A10 expression and gene marker sets of immune infiltrates were analyzed by TIMER and GEPIA.

**Results:** Knocking down of SLC2A10 widely activated immune and inflammatory signaling. SLC2A10 was abnormally expressed in several tumors. The expression level of SLC2A10 was closely correlated with cancer prognosis. Low SLC2A10 expression was related to poorer prognosis and increased malignancy of lung cancer. Lung cancer patients with low expression of SLC2A10 have a much shorter median survival time than patients with high expression of SLC2A10. SLC2A10 expression is closely related to different types of immune cell infiltration, particularly with macrophages.

**Conclusions:** By using various databases and analysis software, we found that GLUT10 is involved in tumor immunity and is expected to serve as a therapeutic target in the future. Down-regulation of SLC2A10 expression predicts poor prognosis of lung cancer. GLUT10 may be a new important immune regulating molecular thus worth further focusing.


# Introduction

SLC2A10 encodes a protein named GLUT10, which is a member of the glucose transporter family. Mutations of SLC2A10 result in hereditary arterial tortuosity syndrome (ATS). So far, the mechanism of ATS is still poorly understood [1]. Meanwhile, the exact biological function of GLUT10 is also far from clear.[2]

SLC2A10 is most highly expressed in smooth muscle cells (SMCs) and SMCs enriched organs like aorta, digestive organs, prostate, and thyroid [3]. To explore the biological function of GLUT10, we have used several effective interference sequences of SLC2A10 to knock down it in vascular smooth muscle cells. Interestingly, transcriptome experiments reveal a broad and significant immune and inflammatory signaling activation after SLC2A10 knock down. Whether and how GLUT10 plays a role in immune response has never been reported.

The impact of immune infiltration on tumors has been particularly focused in the past decades. Several established data bases, such as SEER and TCGA, with detailed survival and gene expression data are available to investigate the involvement and up-stream molecular of immune infiltration in tumors. By this research, we have analyzed the SLC2A10 expression level in main categories of tumors and found SLC2A10 was abnormally expressed in several tumors. We also analyzed the relationship between SLC2A10 expression and immune cell infiltration in tumors, as well as the relationship between SLC2A10 expression and survival. Our research reveals the loss of SLC2A10 is associated with more significant immune cell infiltration and worse survival, especially, in lung cancer.

# Methods

# Gene interference and transcriptome sequencing

shRNA was used to knock down SLC2A10 vascular smooth cells (VSMCs). The effectiveness of gene interference was tested by qPCR. Transcriptome sequencing was performed and FASTQ files from RNA-seq experiments were clipped and trimmed. KEGG enrichment analysis on differently expressed genes was performed. The rich-factor was calculated as the ratio of the number of genes in the pathway entry in the differentially expressed gene to the total number of genes in the pathway entry in all the annotated genes

# Oncomine Database Analysis

The expression level of the SLC2A10 in various types of cancers was identified in the Oncomine database (https://www.oncomine.org/resource/login.html)[4]. Oncomine, a cancer microarray database and web-based data mining platform, aims to stimulate new discovery from genome-wide expression analyses and compare the transcriptome data in most major types of cancer with respective normal tissues[5].The threshold was determined according to the following values: P-value of 0.001,fold change of 1.5,and gene ranking of all.

# PrognoScan Database Analysis

The correlation between SLC2A10 expression level and survival in various types of cancers was analyzed by PrognoScan online software (htpp://www.abren.net/http://www.abren.net/PronoScan/) [6]. Pronoscan searches for relationships between gene expression and patient prognosis, such as overall survival (OS), post progression survival (PPS)and first progression (FP), across a large collection of publicly available cancer micropylar datasets. The threshold was adjusted to a Cox-P-value < 0.05.

# Kaplan-Meier Plotter Database Analysis

The Kaplan Meier plotter is capable of assessing the effect of 54k genes (mRNA, miRNA, protein) on survival in 21 cancer types including 6234 breasts, 2190 ovarian, 3452 lung, and 1440 gastric cancers. Sources for the databases include GEO, EGA, and TCGA. The primary purpose of the tool is a meta-analysis based discovery and validation of survival biomarkers. The correlation between SLC2A10 expression and survival in gastric cancer, ovarian cancer, bladder cancer, breast cancer, esophageal adenocarcinoma, kidney renal clear cell carcinoma, liver hepatocellular carcinoma, lung cancer, pancreatic ductal adenocarcinoma, stomach adenocarcinoma and uterine corpus endometrial carcinoma was analyzed by Kaplan-Meier plotter(www.kmplot.com)[7].The hazard ratio (HR) with 95% confidence intervals and log-rank P-value were also computed.

# TIMER Database Analysis

TIMER (Tumor Immune Estimation Resource) web server is a comprehensive resource for systematical analysis of immune infiltrates across diverse cancer types(cistrome.shinyapps.io/timer)[8].The TIMER database includes 10,897 samples across 32 cancer types from The Cancer Genome Atlas (TCGA) to estimate the abundance of immune infiltrates. We analyzed SLC2A10 expression in different types of cancer and the correlation of SLC2A10 expression with the abundance of immune infiltrates, including B cells, CD4 + T cells, CD8 + T cells, neutrophils, macrophages, and dendritic cells, via gene modules. Gene expression levels against tumor purity are displayed on the left-most panel. In addition, correlations between SLC2A10 expression and gene markers of tumor-infiltrating immune cells were explored via correlation modules. The gene markers of tumor-infiltrating immune cells included markers of CD8 + T cells, T cells (general), B cells, monocytes, TAMs, M1 macrophages, M2 macrophages, neutrophils, natural killer (NK) cells, dendritic cells (DCs), T-helper 1 (Th1) cells, T-helper 2(Th2) cells, follicular helper T (Tfh) cells, T-helper 17 (Th17) cells, Tregs, and exhausted T cells. These gene markers are referenced in prior studies [9, 10]. The correlation module generated the expression scatter plots between a pair of user defined genes in a given cancer type, together with the Spearman's correlation and the estimated statistical significance. SLC2A10 was used for the x-axis with gene symbols, and related marker genes are represented on the y-axis as gene symbols. The gene expression level was displayed with log2 RSEM.

# Gene Correlation Analysis in GEPIA

Survival curves were generated by the PrognoScan and Kaplan-Meier plots. The results generated in Oncomine are displayed with P-values, fold changes, and ranks. The results of Kaplan-Meier plots, PronoScan and GEPIA are displayed with HR and P or Cox P-values from a log-rank test. The correlation of gene expression was evaluated by Spearman's correlation and statistical significance, and strength of the correlation was determined using the following guide for the absolute value:0.00–0.19 “very weak,” 0.20–0.39 “weak,” 0.40–0.59 “moderate,” 0.60–0.79 “strong,” 0.80–1.0 “very strong.” P-values < 0.05 were considered statistically significant.

## Results

# Knocking down of SLC2A10 widely activated immune and inflammatory signaling

SLC2A10 is super highly expressed in smooth muscle cells. To investigate its biological function, we knocked it down in vascular smooth muscle cells(VSMCs) by shRNA with different interference sequences. Transcriptome sequencing was performed in cells infected by shRNA virus or control virus. KEGG analysis revealed a wide activation of immune related pathways like Toll-like receptor signaling pathway, TNF signaling pathway, RIG-I-like receptor signaling pathway, NOD-like receptor signaling pathway, Natural killer cell mediated cytotoxicity pathway, IL-17 signaling pathway, C5-Branched dibasic acid metabolism pathway, IL-17 signaling pathway, and linked to some immune related disease, like viral myocarditis, Epstein-Barr virus infection, Hepatitis C, Influenza, Autoimmune thyroid disease and Allograft rejection.)Figure 1 These results remind us a strong immune modulation function of SLC2A10.

# SLC2A10 is abnormally expressed in several tumors

To investigate the expression level of SLC2A10 in different tumors, the mRNA expression of SLC2A10 in multiple cancer types and their corresponding normal tissues were compared using Oncomine data base. SLC2A10 is expressed lower than normal tissue in colorectal cancer, head and neck cancer, liver cancer, and melanoma, while expressed higher than normal tissue in bladder cancer, brain and central nervous system cancer, breast cancer, kidney cancer, lymphoma, and sarcoma. (Fig. 2A)

Then, we used an online tool named TIMER to further confirm the expression level of SLC2A10 in tumors. The differential expression of SLC2A10 between tumor and adjacent normal tissues across all TCGA tumors were compared. SLC2A10 is lowly expressed in colon adenocarcinoma, head and neck squamous cell carcinoma, kidney chromophobe, thyroid carcinoma, and uterine corpus endometrial carcinoma, while highly expressed in bladder cancer. (Fig. 2B) The result of SLC2A10 expression level in tumors from Oncomine database and TCGA database is similar.

# Prognostic potential of SLC2A10 expression in tumors

To evaluate whether SLC2A10 impacts prognosis of cancer patients, we analyzed the correlation between SLC2A10 expression level and patients’ survival using PrognoScan online software. As it was presented in table1, SLC2A10 expression level was significantly correlated with worse survival of several cancers, including brain cancer, breast cancer, lung cancer, colorectal cancer, eye cancer, skin cancer, and soft tissue cancer, while slightly correlated with worse survival of ovarian cancer.

To further examine the prognostic potential of SLC2A10 in different cancers, Kaplan-Meier plotter database was used to evaluate the SLC2A10 prognostic value based on Affymetrix microarrays or PCR (only for lung cancer). Expression

level of SLC2A10 expression significantly correlated with worse survival of patients with gastric cancer, ovarian cancer, bladder cancer, breast cancer, esophageal adenocarcinoma, kidney renal clear cell carcinoma, liver hepatocellular carcinoma, lung cancer, pancreatic ductal adenocarcinoma, stomach adenocarcinoma, and uterine corpus endometrial carcinoma. (Fig. 3A-K)

# Down regulation of SLC2A10 strongly decreases the survival of patients with lung cancer

Among all cancer types, the survival of lung cancer patients is most strongly affected by SLC2A10 expression. Whatever type of survival (OS, PPS, FP) is considered, lower expression of SLC2A10 predicts the worst prognosis of lung cancer. Lower expression of SLC2A10 also corelated with shorter median survival months. When histological type is considered, adenocarcinoma is more significantly correlated with worse survival ($p < 0.0001$) than squamous cell carcinoma ($p = 0.046$). (Fig. 4A-E) Patients with low expression of SLC2A10 in lung cancer have much shorter median survival time than patients with high expression of SLC2A10. (Table 2).

# SLC2A10 expression correlates with immune cell infiltration in several tumors including lung cancer

Some research shows that Tumor-infiltrating lymphocyte is an independent predictor of sentinel lymph node status and an effective marker for evaluating the prognosis in some cancers [12, 13]. Therefore, we used the Gene module of TIMER to explore the correlation between gene expression and the abundance of immune infiltrates in different types of cancers. We found that SLC2A10 expression has significant correlations with tumor purity in 5 types of cancers and significant correlations with Macrophages infiltration levels in 22 types of cancers. Furthermore, the expression of SLC2A10 has significant correlations with infiltration levels of Dendritic cells in 19 types of cancers, Neutrophils in 16 types of cancers, CD8 CD8 + T cells in 13 types of cancers, CD4 CD4 + T cells and B cells both in 11 types of cancers. In these results, we further found that SLC2A10 expression has significantly associated with the level of immune infiltrates in 10 of these cancers. As the figure shows, the SLC2A10 expression level correlates with poorer prognosis and high immune infiltrates in COAD, KIRC, LGG and READ.(Fig. 5A,5C,5D,5I) SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells ($r = 0.201, p = 4.66e-05$), CD8 + T cells ($r = 0.141, p = 4.42e-03$), CD4 + T cells ($r = 0.222, p = 7.06e-06$), Macrophages ($r = 0.31, p = 1.89e-10$), Neutrophils ($r = 0.18, p = 2.95e-04$), Dendritic cells ( $r = 0.264, p = 8.09e-08$) in COAD.(Fig. 5A) SCL2A10 expression levels has significant positive correlation with infiltrating levels of CD4 + T cells ($r = 0.281, p = 7.99e-10$), Macrophages ($r = 0.263, p = 1.59e-08$), Neutrophils ($r = 0.157, p = 7.56e-04$), Dendritic cells ( $r = 0.154, p = 9.76e-04$) in KIRC.(Fig. 5C) SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells ($r = 0.415, p = 2.79e-21$), CD8 + T cells ($r = 0.365, p = 1.65e-16$), CD4 + T cells ($r = 0.297, p = 3.64e-11$), Macrophages ($r = 0.422, p = 6.96e-22$), Neutrophils ($r = 0.463, p = 1.30e-26$), and Dendritic cells ( $r = 0.445, p = 1.40e-24$) in LGG.(Fig. 5E) Similarly, SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells ($r = 0.223, p = 8.19e-03$),CD8 + T cells ($r = 0.205, p = 1.54e-02$), CD4 + T cells ($r = 0.19, p = 2.52e-02$), Macrophages ($r = 0.3, p = 3.33e-04$), Neutrophils ($r = 0.165, p = 5.26e-02$), and Dendritic cells ( $r = 0.196, p = 2.05e-02$) in READ.(Fig. 5I) In addition,SLC2A10 expression has no significant correlation with tumor purity, but has significant positive correlation with infiltration levels in HNSC,KIRP,LUAD,LUSC,PRAD and THCA.(Fig. 5B,5D,5F,5G,5H,5J) SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells ($r = 0.279, p = 5.59e-10$), CD8 + T cells ($r = 0.15, p = 1.07e-03$), CD4 + T cells ($r = 0.366, p = 1.24e-16$), Macrophages ($r = 0.388, p = 8.20e-19$), Neutrophils ($r = 0.109, p = 1.68e-02$), Dendritic cells ( $r = 0.306, p = 8.09e-12$) in HNSC.(Fig. 5B) SCL2A10 expression levels has significant positive correlation with infiltrating levels of Macrophages ($r = 0.174, p = 5.82e-03$), Neutrophils ($r = 0.135, p = 2.96e-02$), Dendritic cells ( $r = 0.145, p = 2.06e-02$) in KIRP.(Fig. 5D) SCL2A10 expression levels has significant positive correlation

with infiltrating levels of CD4 + T cells (r = 0.118,p = 9.52e-03), Macrophages (r = 0.176,p = 9.73e-05), Neutrophils (r = 0.175,p = 1.11e-04), Dendritic cells ( r = 0.129,p = 4.38e-03) in LUAD.(Fig. 5F) SCL2A10 expression levels has significant positive correlation with infiltrating levels of CD4 + T cells (r = 0.21,p = 3.96e-06), Macrophages (r = 0.304,p = 1.16e-11), Neutrophils (r = 0.174,p = 1.36e-04), Dendritic cells (r = 0.131,p = 4.33e-03) in LUSC.(Fig. 5G) SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells (r = 0.244,p = 5.28e-07),CD8 + T cells (r = 0.554,p = 7.50e-35), Macrophages (r = 0.354,p = 9.36e-14), Neutrophils (r = 0.207,p = 2.26e-05), Dendritic cells ( r = 0.307,p = 1.73e-10) in PRAD.(Fig. 5H) SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells (r = 0.426,p = 1.07e-22),CD4 + T cells (r = 0.562,p = 5.54e-42), Macrophages (r = 0.467,p = 9.44e-28), Neutrophils (r = 0.228,p = 3.48e-07), Dendritic cells ( r = 0.148,p = 1.12e-03) in THCA.(Fig. 5J) These findings strongly suggest that SLC2A10 plays a specific role in immune infiltration in some cancers, particularly in macrophages.

# Correlation Analysis Between SLC2A10 Expression and Immune Marker Sets

To further investigate the relationship between SLC2A10 and the diverse immune infiltrating cells, we use the Correlation Analysis Module of TIMER and GEPIA to measure the correlations between SLC2A10 and immune marker sets of various immune cells of LUAD and LUSC. We analyzed the correlations between SLC2A10 expression and immune marker genes of different immune cells, including included CD8 + T cells, T cells (general), B cells, monocytes, TAMs, M1 and M2 macrophages, neutrophils, NK cells, DCs, Th1 cells, Th2 cells, Tfh cells, Th17 cells, and Tregs, and exhausted T cells in LUAD and LUSC.(Table 3) Then, we adjusted the purity accordingly, the results revealed the SLC2A10 expression level was significantly correlated with most immune marker sets of various immune cells in LUAD and LUSC. Interestingly, we found that the expression levels of most marker set of M1 macrophages, monocyte, TAM, neutrophils, DCs, Th1 cells, Th2 cells, and Tregs have strong correlations with SLC2A10 expression in LUAD and LUSC (Table 3). Specifically, we showed COX2(PTGS2) of M1 macrophages and GATA3 of Th2 cells are significantly correlate with SLC2A10 expression in LUAD and LUSC. At same time, we found IRF5 of M1 macrophages and CCL2 of TAM significantly correlate with SLC2A10 expression in LUAD. we showed INOS(NOS2) of M1 macrophages, CD11b(ITGAM) of neutrophils, BDCA-4(NRP1) of DCs, STAT6 of Th2 cells, STAT3 of Th17 cells and TGFβ(TGFB1) of Tregs are significantly correlate with SLC2A10 expression in LUAD. We further analyzed the correlation between SLC2A10 expression and the above markers of M1 and M2 macrophages, TAMs and Th2 cells in the GEPIA database, including LUAD and LUSC. Correlation results between SLC2A10 and markers of TAMs are similar to those in TIMER (Table 4). These findings suggest that SLC2A10 may regulate macrophage polarization in LUAD and LUSC. Therefore, these results further confirm the findings that SLC2A10 is specifically correlated with immune infiltrating cells in LUAD and LUSC which suggests that GLUT10 plays a vital role in protective antigen in the lung cancer.

# Discussion

GLUT10 is encoded by SLC2A10 gene and it is a main transporter of glucose and DHA. Mutations in SLC2A10 cause arterial tortuosity syndrome (ATS).[14] SLC2A10 is expressed in various tissues such as brain, lung, adipose tissue, heart, placenta and vascular smooth muscle.[15] Gamberucci et al [16] found GLUT10 is mainly expressed in the endoplasmic reticulum. Previous studies have confirmed that GLUT10 is involved in oxidative stress, vitamin C transportation, extracellular matrix degradation, and TNF-β signaling transduction. [17, 18] Syu et al [19] found that missense mutation of SLC2A10 increases reactive oxygen species (ROS) production, mitochondrial fragmentation, cell proliferation, and cell migration. Recent research also discovered that SLC2A10 expression levels may be a useful prognostic biomarker in AML (acute myeloid leukemia).[15] However, few studies have focused on the relationship between SLC2A10 expression and tumor immune cell infiltration. Recently, we found that GLUT10 may be a new immune regulator by transcriptome sequencing and bioinformatic study. In this research, we found the expression level

of SLC2A10 is closely correlated with cancer prognosis. Low SLC2A10 expression predicts poorer prognosis and increases malignancy of lung cancer. More importantly, we found SLC2A10 was significantly associated with tumor immune infiltration, which may cause rapid tumor progression. Furthermore, our study revealed that SLC2A10 is tightly related to lung cancer prognosis, and may be an important effector for the regulation of immune cells functional status.

Firstly, we investigated the expression of SLC2A10 in various cancers using Oncomine, TCGA and TIMER datasets. We found SLC2A10 expression in carcinoma samples was significant different from the adjacent tissues. Further analysis revealed that SLC2A10 was highly expressed in bladder cancer, brain cancer, breast cancer, kidney cancer, lymphoma, and sarcoma while lowly expressed in colorectal cancer, head and neck cancer, liver cancer and melanoma compared to their adjacent normal tissue in Oncomine database. In TCGA database, the expression level of SLC2A10 was decreased in colon adenocarcinoma, head and neck squamous cell carcinoma, kidney chromophobe, thyroid carcinoma and uterine corpus endometrial carcinoma, and highly expressed in bladder cancer compared with their adjacent tissues. Although there is slight difference between results derived from different databases, the expression of SLC2A10 is different from normal tissues generally.

We also evaluated the relationship between SLC2A10 expression and cancers prognosis. We found reduced SLC2A10 expression predicts poor prognosis of a lot of cancers like brain cancer, breast cancer, lung cancer, colorectal cancer, ocular tumor, skin cancer, soft tissue cancer, bladder cancer, liver hepatocellular carcinoma, lung cancer, pancreatic ductal adenocarcinoma, stomach adenocarcinoma, and uterine corpus endometrial carcinoma, while high expression of SLC2A10 was related to poor prognosis of gastric cancer, esophageal adenocarcinoma. Remarkably, no matter what type of survival (OS, PPS, FP) were considered, the prognosis of lung cancer is significantly related to the expression level of SLC2A10. Comparing with lung squamous cell carcinoma, lower expression of SLC2A10 is more significantly correlated with poor prognosis of lung adenocarcinoma. Based on these data, we believe SLC2A10 plays an important role in cancer biology, especially in lung cancer biology.

A further important finding is SLC2A10 expression is closely related to immune infiltration in different cancers. Our analysis demonstrated that high SLC2A10 expression levels have significant positive correlation with infiltrating levels of CD4 + T cells, Macrophages, Neutrophils, Dendritic cells in lung cancer, as well as in COAD, KIRC, LGG, READ and so on. Macrophages infiltration and polarization is mostly significant influenced by SLC2A10 expression. These findings further convince us that Glut10 plays an important role in immune response. By now, few literatures have reported the immune modulation function of Glut10. Our findings may add some new knowledge to tumor immunology.

It is interesting that SLC2A10 expression is significantly correlated with macrophages polarization of cancers. In addition to tumor cells, the tumor microenvironment (TME) is also composed of immune cells, fibroblasts, endothelial cells, a large number of cytokines, chemokines, and growth factors.[20] Macrophages are the most characteristic tumor infiltrating immune cells and the main component of immune cell infiltration in TME. It plays a significant and positive role in the early carcinogenesis to tumor progression and metastasis [21]. In the process of tumor progression, circulating monocytes and macrophages are actively recruited into the tumor, changing the tumor microenvironment and accelerating the tumor progression. Macrophages alter their functional phenotypes in response to various microenvironmental signals produced by tumor and stromal cells.[22] Macrophages are divided into classical activated (M1) macrophages and selective activated (M2) macrophages. The function of TAMs (tumor-associated macrophages) is very similar to M2 macrophages. Clinicopathological studies showed that TAM accumulation in tumors was associated with poor clinical outcomes [23]. Xu et al [24] reported that M2 macrophages promoted the invasion of lung cancer cells and tumor growth, M1 macrophages inhibit the proliferation and cell activity of lung cancer cells in vivo and in vitro, reduce angiogenesis, increase chemical sensitivity of lung cancer cells, and induce the

apoptosis and senescence of lung cancer cells. What is noteworthy is that we found GLUT10 expression is strongly correlated with M1 macrophages, but not M2 macrophages of lung cancer. Correlation study using GEPIA also shows M2 macrophages have a strong correlation with SLC2A10 expression in normal tissue, but not in LUAD. On the contrary, M1 macrophages were correlated with SLC2A10 expression in the LUAD tissue, but not in LUSC. Therefore, we speculated that the high expression of SLC2A10 might promote the transformation of M2 macrophages into M1 macrophages. COX2, an inducible enzyme encoded by PTGS2 gene, can be highly induced by pro-inflammatory cytokines, tumor promoter mitogen and growth factors in various cells and thus participates in various pathological processes such as inflammatory response cell proliferation and cell apoptosis. We found SLC2A10 expression level is correlated with COX2 signaling activation. It is possible that Glut10 modulate immune infiltration via COX2 pathway.

## Conclusion

By using various databases and analysis software, we found that GLUT10 is involved in tumor immunity and is expected to serve as a therapeutic target in the future. Down-regulation of SLC2A10 expression predicts poor prognosis of lung cancer. GLUT10 may be a new important immune regulating molecular thus worth further focusing.

## Declarations

Ethics approval and consent to participate:Not applicable.

Consent for publication: all authors have consent for publication.

Availability of data and materials: the data used to support the findings of this study are available from the corresponding author upon request.

Competing interests: all authors have no conflict of interests.

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Authors' contributions: Hu Z designed the study. Jian L, Zhang M, Wu Z, Hu X, Ren Z, and Wang Z performed bioinformatic study. Wu Q, Min X, Li B performed gene interference and transcriptome study. Hu Z, Jian L, Zhang M, and Wu Q wrote the manuscript.

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# Tables

**TABLE 1** Correlation analysis between SLC2A10 expression level and patients' survival in PrognoScan .

| Cancer type | Survival type | N | Corrected P-value |
|---|---|---|---|
| Brain cancer(astrocytoma) | OS | 70 | 0.0206463 |
| Brain cancer(glioblastoma) | OS | 74 | 0.0156356 |
| Brain cancer(glioma) | OS | 67 | 0.00165497 |
| Brain cancer(meningioma) | OS | 200 | 0.0946148 |
| Breast cancer | DMFS | 155 | 0.0505604 |
| Breast cancer | OS | 145 | 0.00571245 |
| Colorectal cancer | DFS | 177 | 0.0431384 |
| Colorectal cancer | DSS | 226 | 0.0916554 |
| Colorectal cancer | DFS | 63 | 0.0836614 |
| Eye cancer(uveal melanoma) | DMFS | 82 | 9.17763E-05 |
| Lung cancer(adenocarcinoma) | OS | 204 | 0.099865 |
| Lung cancer(adenocarcinoma) | RFS | 278 | 0.0273683 |
| Ovarian cancer | OS | 110 | 0.0610717 |
| Ovarian cancer | OS | 110 | 0.093969 |
| Ovarian cancer | PFS | 38 | 0.074999 |
| Skin cancer(melanoma) | OS | 140 | 0.013884 |
| Soft tissue cancer(liposarcoma | DRFS | 77 | 0.0148117 |

OS: Overall Survival; DMFS: Distant Metastasis Free Survival; DFS: Disease Free Survival; DSS:Disease Specific Survival; RFS: Relapse Free Survival; PFS: Progression Free Survival; DRFS: Distant Recurrence Free Survival

**TABLE 2** The survival time of lung cancer patients with low and high expression of SLC2A10.

| **Histology** | **Survival type** | **Low slc2a10 (months)** | **High slc2a10 (months)** |
|---|---|---|---|
| Lung cancer | OS | 59 | 95 |
| Squamous cell carcinoma | OS | 37.63 | 67 |
| Adenocarcinoma | OS | 79.87 | 136.33 |
| Lung cancer | PPS | 15 | 24.4 |
| Lung cancer | FP | 50 | 104.9 |

OS: Overall Survival; PPS: Post Progression Survival; FP: First Progression

**TABLE 3** Correlation analysis between SLC2A10 expression level and relate genes and markers of immune cells in TIMER.

| Decscription | Gene markers | LUAD | | | | LUSC | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | None | | purity | | None | | Purity | |
| | | Cor | P | Cor | P | Cor | P | Cor | P |
| CD8+ T cell | CD8A | -0.073 | 0.0985 | -0.107 | 0.0179 | -0.004 | 0.929 | -0.021 | 0.649 |
| | CD8B | -0.057 | 0.195 | -0.078 | 0.0822 | 0.081 | 0.0697 | 0.066 | 0.153 |
| T cell (gengeral) | CD3D | -0.061 | 0.169 | -0.095 | 0.0342 | -0.04 | 0.368 | -0.066 | 0.15 |
| | CD3E | -0.042 | 0.339 | -0.077 | 0.0893 | 0.017 | 0.696 | -0.001 | 0.985 |
| | CD2 | -0.04 | 0.361 | -0.068 | 0.132 | -0.012 | 0.792 | -0.032 | 0.0481 |
| B cell | CD19 | 0.011 | 0.808 | -0.013 | 0.772 | 0.049 | 0.274 | 0.034 | 0.475 |
| | CD79A | -0.005 | 0.913 | -0.032 | 0.473 | 0.064 | 0.152 | 0.053 | 0.252 |
| Monocyte | CD86 | 0.072 | 0.105 | 0.069 | 0.128 | 0.098 | 0.0281 | 0.106 | 0.0206 |
| | CD115(CSF1R) | 0.124 | * | 0.126 | * | 0.12 | * | 0.132 | * |
| TAM | CCL2 | 0.201 | *** | 0.208 | *** | 0.1 | 0.0247 | 0.107 | 0.0199 |
| | CD68 | 0.134 | * | 0.134 | * | 0.038 | 0.402 | 0.037 | 0.416 |
| | IL10 | 0.061 | 0.164 | 0.061 | 0.176 | 0.109 | 0.0147 | 0.115 | 0.012 |
| M1 Macrophage | INOS(NOS2) | 0.052 | 0.24 | 0.056 | 0.241 | 0.24 | *** | 0.238 | *** |
| | IRF5 | 0.15 | ** | 0.154 | ** | 0.058 | 0.192 | 0.068 | 0.138 |
| | COX2(PTGS2) | 0.222 | *** | 0.208 | *** | 0.276 | *** | 0.275 | *** |
| M2 Macrophage | CD163 | 0.087 | 0.049 | 0.084 | 0.0625 | 0.138 | * | 0.144 | * |
| | VSIG4 | 0.057 | 0.199 | 0.061 | 0.174 | 0.089 | 0.0471 | 0.092 | 0.0439 |
| | MS4A4A | 0.026 | 0.561 | 0.027 | 0.548 | 0.082 | 0.0656 | 0.085 | 0.0627 |
| Neutrophils | CD66b(CEACAM8) | 0.025 | 0.579 | 0.029 | 0.517 | 0.12 | * | 0.107 | 0.0189 |
| | CD11b(ITGAM) | 0.133 | * | 0.136 | * | 0.154 | ** | 0.17 | ** |
| | CCR7 | 0.008 | 0.85 | -0.011 | 0.801 | 0.087 | 0.0529 | 0.079 | 0.0856 |
| Natural Killer cell | KIR2DL1 | -0.131 | * | -0.148 | * | -0.028 | 0.535 | -0.047 | 0.308 |
| | KIR2DL3 | -0.083 | 0.0613 | -0.081 | 0.0718 | 0.054 | 0.227 | 0.049 | 0.285 |
| | KIR2DL4 | -0.014 | 0.743 | -0.033 | 0.459 | -0.056 | 0.209 | -0.071 | 0.119 |
| | KIR3DL1 | -0.127 | * | -0.132 | * | 0.031 | 0.485 | 0.019 | 0.686 |
| | KIR3DL2 | -0.038 | 0.387 | -0.053 | 0.236 | -0.066 | 0.142 | -0.082 | 0.0748 |
| | KIR3DL3 | -0.041 | 0.348 | -0.036 | 0.431 | -0.068 | 0.127 | -0.075 | 0.104 |
| | KIR2DS4 | -0.061 | 0.166 | -0.066 | 0.142 | 0.041 | 0.363 | 0.033 | 0.466 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dendritic cell | HLA-DPB1 | 0.03 | 0.492 | 0.018 | 0.695 | 0.045 | 0.32 | 0.035 | 0.447 |
| | HLA-DQB1 | 0.008 | 0.857 | 0.005 | 0.92 | 0.023 | 0.605 | 0.012 | 0.786 |
| | HLA-DRA | 0.025 | 0.578 | 0.014 | 0.754 | 0.029 | 0.518 | 0.021 | 0.652 |
| | HLA-DPA1 | 0.056 | 0.203 | 0.047 | 0.295 | 0.053 | 0.24 | 0.045 | 0.326 |
| | BDCA-1(CD1C) | 0.055 | 0.215 | 0.058 | 0.202 | 0.059 | 0.187 | 0.059 | 0.198 |
| | BDCA-4(NRP1) | 0.034 | 0.44 | 0.04 | 0.381 | 0.308 | *** | 0.327 | *** |
| | CD11c(ITGAX) | 0.125 | * | 0.121 | * | 0.139 | * | 0.161 | ** |
| Th1 | T-bet(TBX21) | -0.075 | 0.0877 | -0.102 | 0.0231 | -0.006 | 0.901 | -0.021 | 0.654 |
| | STAT4 | 0.094 | 0.0324 | 0.083 | 0.0665 | 0.156 | ** | 0.172 | * |
| | STAT1 | 0.066 | 0.133 | 0.055 | 0.223 | 0.025 | 0.572 | 0.014 | 0.756 |
| | IFN-γ(IFNG) | -0.123 | * | -0.145 | * | -0.112 | 0.0122 | -0.129 | * |
| | TNF-α(TNF) | 0.12 | * | 0.136 | * | 0.053 | 0.234 | 0.05 | 0.275 |
| Th2 | GATA3 | 0.231 | *** | 0.235 | *** | 0.265 | *** | 0.253 | *** |
| | STAT6 | 0.044 | 0.322 | 0.048 | 0.287 | 0.164 | ** | 0.161 | ** |
| | STAT5A | 0.139 | * | 0.139 | * | 0.141 | * | 0.148 | * |
| | IL13 | 0.006 | 0.899 | 0.006 | 0.899 | -0.09 | 0.0444 | -0.1 | 0.0289 |
| Tfh | BCL6 | 0.103 | 0..0199 | 0.092 | 0.0417 | 0.126 | * | 0.125 | * |
| | IL21 | -0.081 | 0.0655 | -0.095 | 0.0348 | -0.032 | 0.48 | -0.029 | 0.534 |
| Th17 | STAT3 | 0.087 | 0.0487 | 0.08 | 0.0768 | 0.231 | *** | 0.233 | *** |
| | IL17A | -0.06 | 0.175 | -0.056 | 0.217 | -0.106 | 0.0171 | -0.112 | 0.0147 |
| Treg | FOXP3 | 0.059 | 0.18 | 0.053 | 0.24 | 0.091 | 0.0428 | 0.09 | 0.0486 |
| | CCR8 | 0.037 | 0.405 | 0.029 | 0.522 | 0.105 | 0.0187 | 0.104 | 0.0234 |
| | STAT5B | 0.054 | 0.217 | 0.052 | 0.247 | 0.113 | 0.0113 | 0.131 | * |
| | TGFβ(TGFB1) | 0.142 | * | 0.132 | * | 0.199 | *** | 0.201 | *** |
| T cell exhaustion | PD-1(PDCD1) | 0.014 | 0.751 | -0.014 | 0.756 | 0.022 | 0.624 | 0.011 | 0.819 |
| | CTLA4 | -0.056 | 0.203 | -0.085 | 0.0598 | 0.008 | 0.867 | -0.004 | 0.926 |
| | LAG3 | -0.023 | 0.609 | -0.047 | 0.297 | -0.051 | 0.257 | -0.069 | 0.132 |
| | TIM-3(HAVCR2) | 0.06 | 0.177 | 0.057 | 0.207 | 0.028 | 0.537 | 0.021 | 0.649 |
| | GZMB | -0.08 | 0.069 | -0.113 | 0.0122 | -0.093 | 0.0374 | -0.12 | * |

LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TAM,tumor-associated macrophage; Th, T helper cell; Tfh, Follicular helper T cell; Treg, regulatory T cell; Cor,R value of Spearman's correlation; None, correlation without adjustment. Purity, correlation adjusted by purity. *P<0.01; **P<0.001; ***P<0.0001.

**TABLE 4** Correlation analysis between SLC2A10 expression level and relate genes and markers of macrophages and Th2 cell in GEPIA.

| Description | Gene markers | LUAD | | | | LUSC | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Tumor | | Normal | | Tumor | | Normal | |
| | | R | P | R | P | R | P | R | P |
| TAM | CCL2 | 0.18 | *** | -0.048 | 0.72 | 0.055 | 0.22 | -0.19 | 0.19 |
| | CD68 | 0.12 | 0.011 | -0.27 | 0.038 | -0.0021 | 0.96 | 0.17 | 0.23 |
| | IL10 | 0.054 | 0.24 | -0.23 | 0.077 | 0.073 | 0.11 | -0.013 | 0.93 |
| M1 Macrophage | INOS(NOS2) | 0.076 | 0.096 | 0.49 | *** | 0.25 | *** | 0.42 | * |
| | IRF5 | 0.14 | * | -0.17 | 0.2 | 0.05 | 0.27 | 0.15 | 0.28 |
| | COX2(PTGS2) | 0.21 | *** | 0.059 | 0.66 | 0.24 | *** | -0.099 | 0.49 |
| M2 Macrophage | CD163 | 0.045 | 0.32 | -0.41 | * | 0.031 | 0.49 | 0.28 | 0.047 |
| | VSIG4 | 0.047 | 0.3 | -0.34 | * | 0.025 | 0.58 | -0.057 | 0.69 |
| | MS4A4A | 0.00046 | 0.99 | -0.41 | * | 0.015 | 0.74 | 0.15 | 0.3 |
| Th2 | GATA3 | 0.22 | *** | 0.43 | ** | 0.23 | *** | 0.47 | ** |

LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TAM,tumor-associated macrophage; Th2, T helper cell 2. Tumor, correlation analysis in tumor tissue of TCGA. Normal,correlation analysis in tumor tissue of TCGA. *P<0.01; **P<0.001; ***P<0.0001.

# Figures

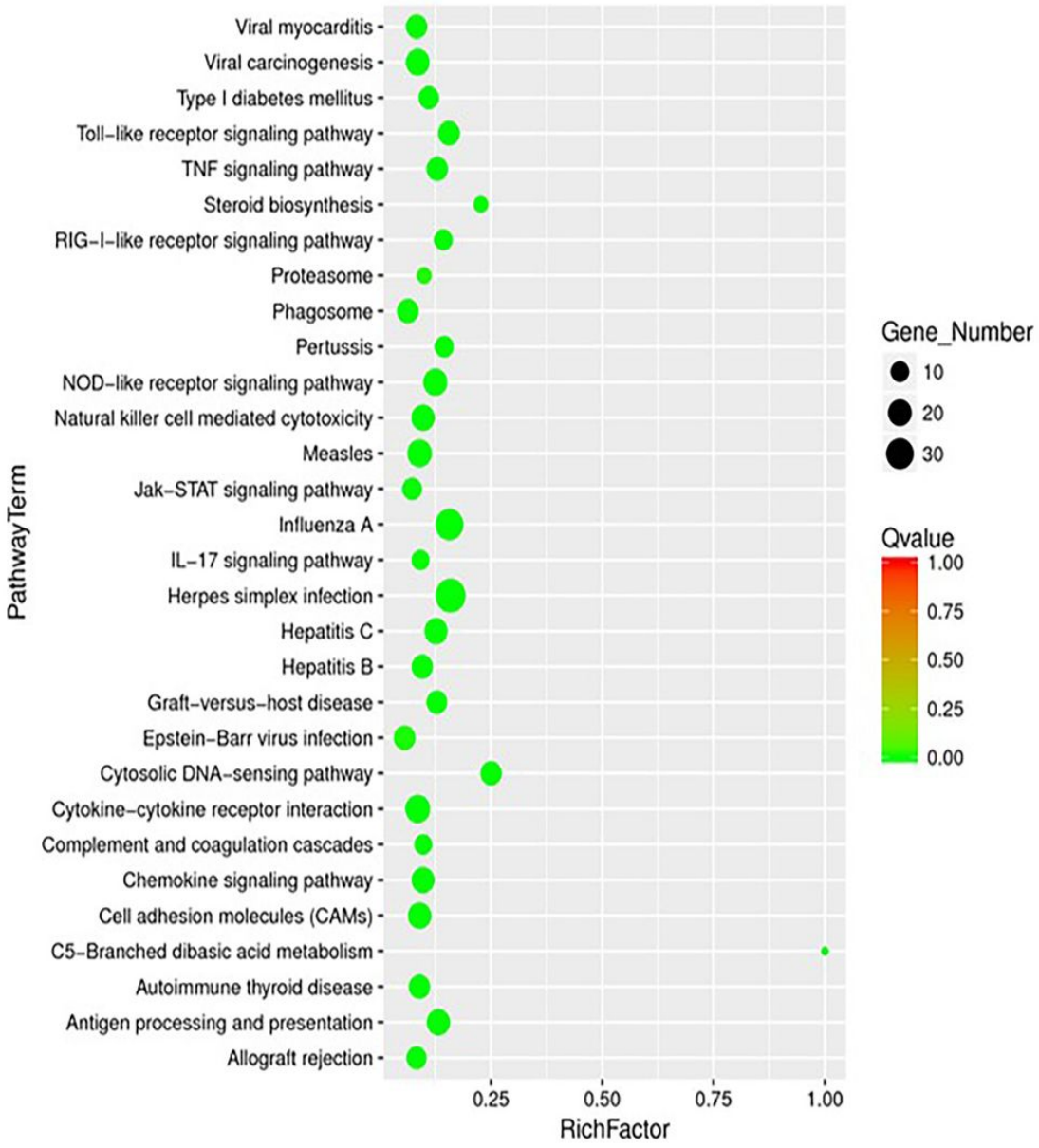
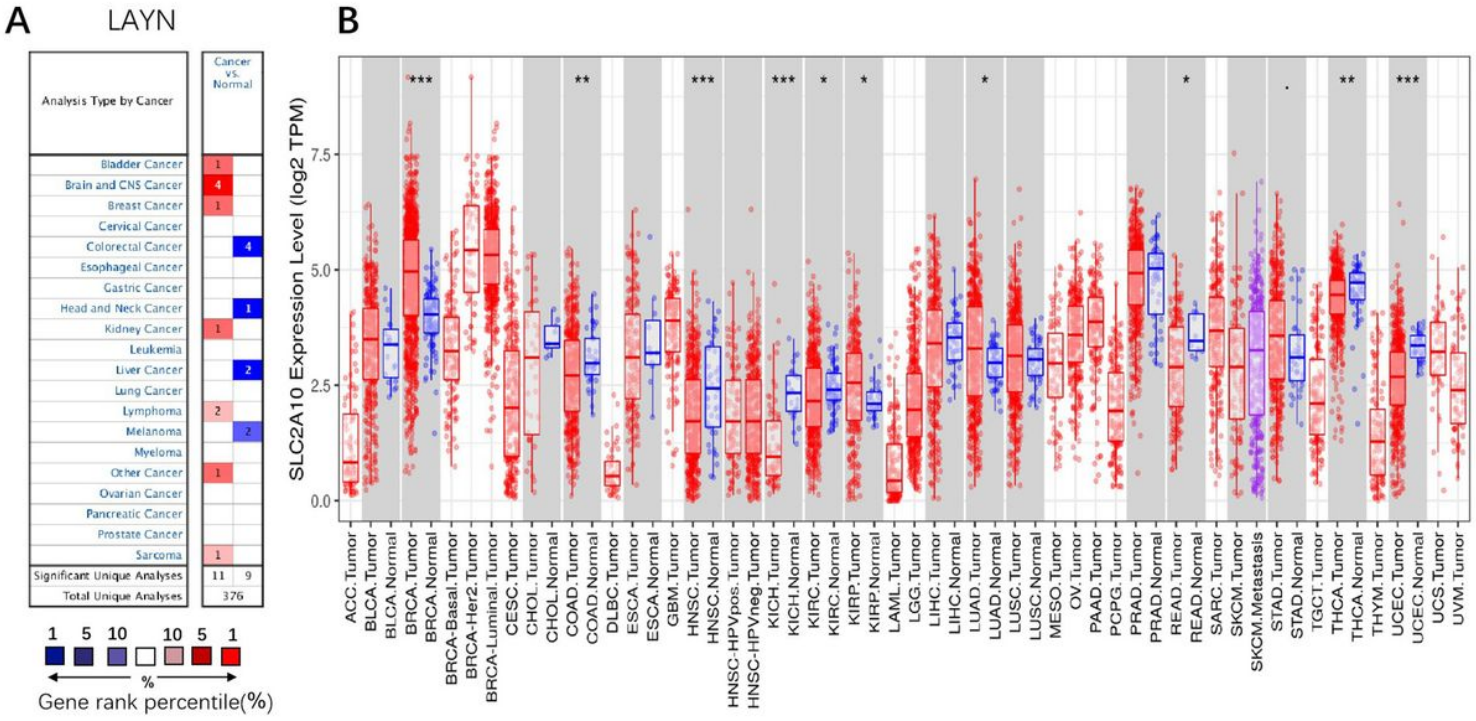


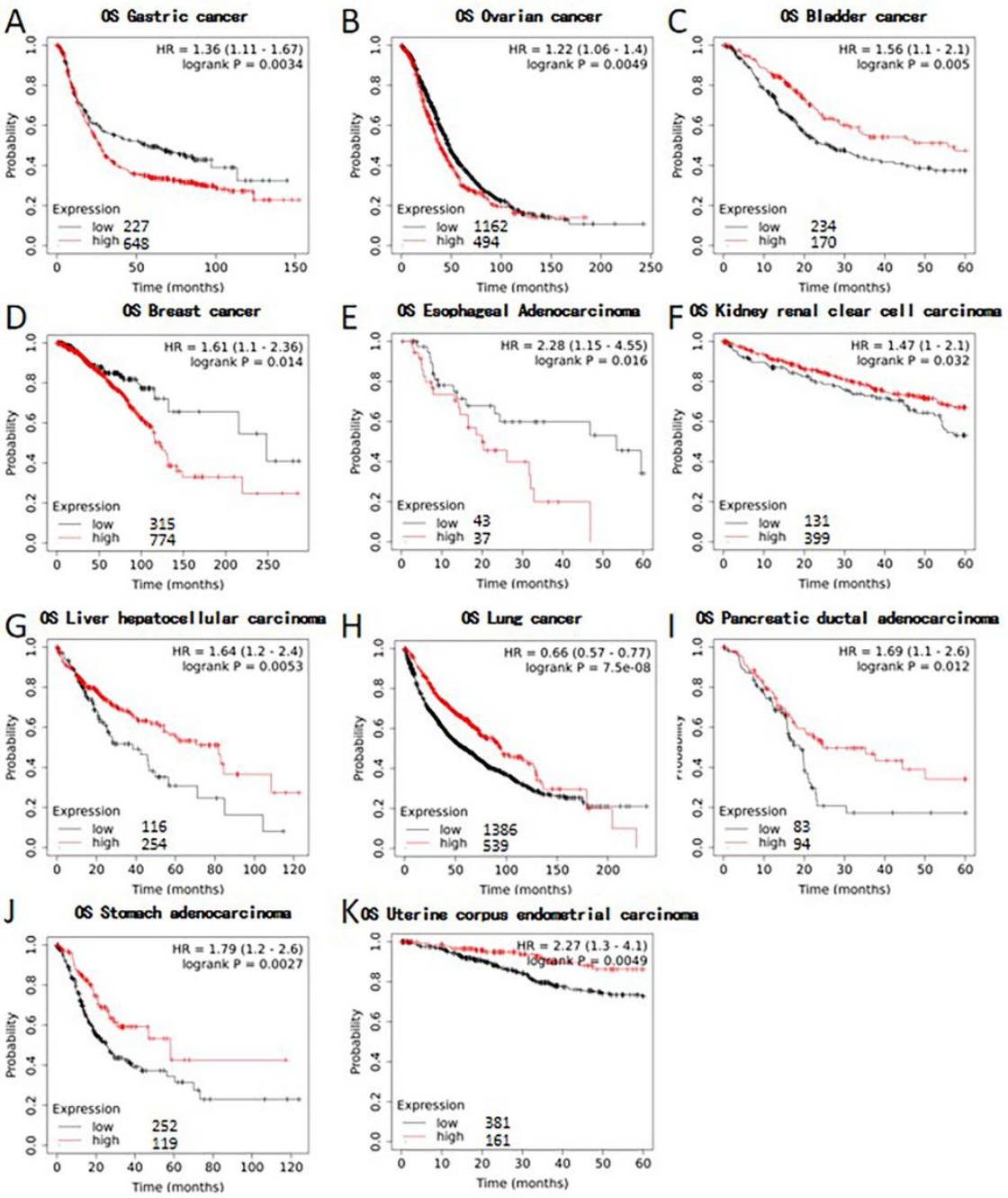

**Figure 1**

KEGG enrichment analyses of knock down of SLC2A10.



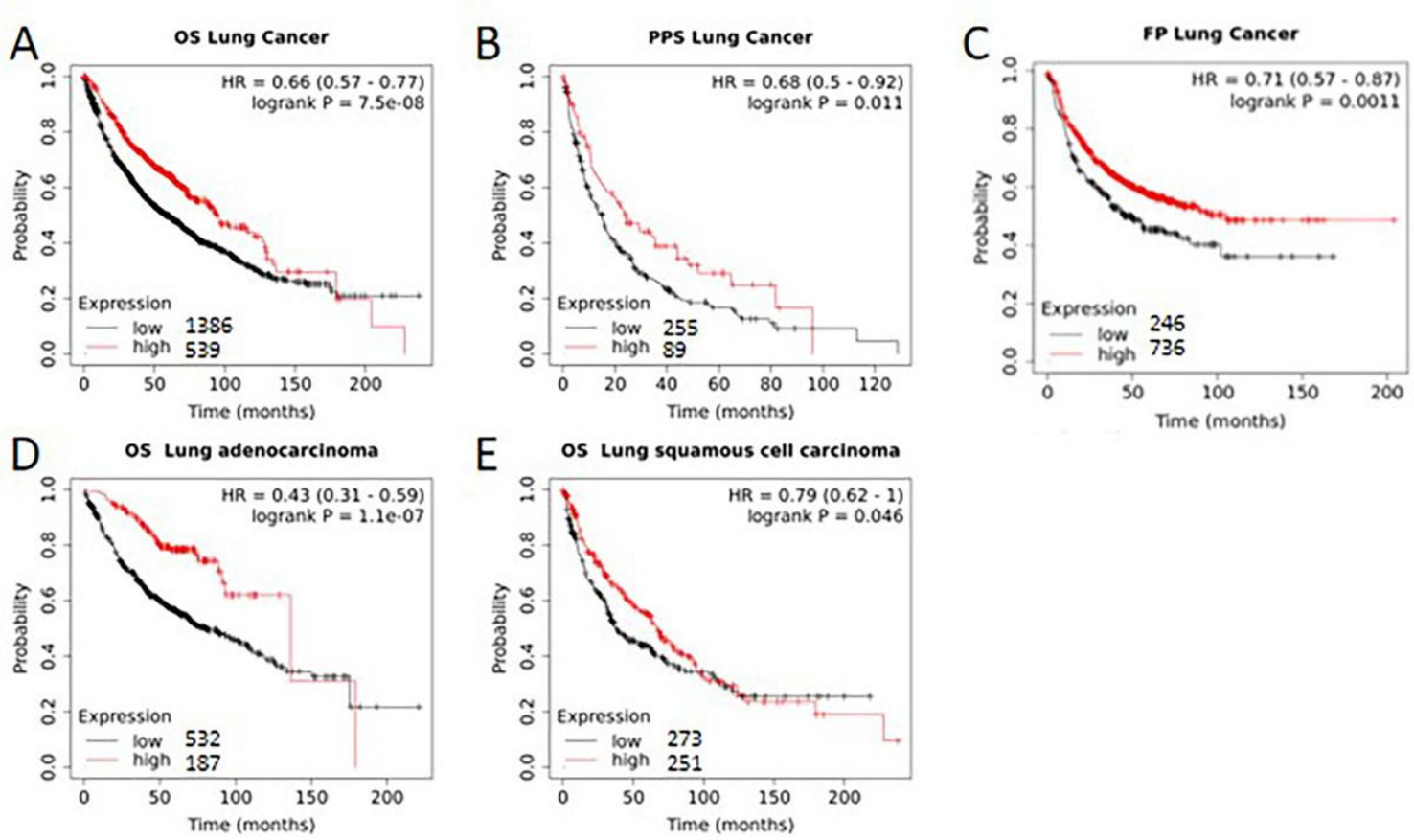

## Figure 2

SLC2A10 expression levels in different types of human cancers. (A) Increased or decreased SLC2A10 in data sets of different cancers compared with normal tissue in the Oncomine database. (B) Human SLC2A10 expression levels in different tumor types from TCGA database were determined by TIMER (*P< 0.05, **P< 0.01, ***P< 0.001).



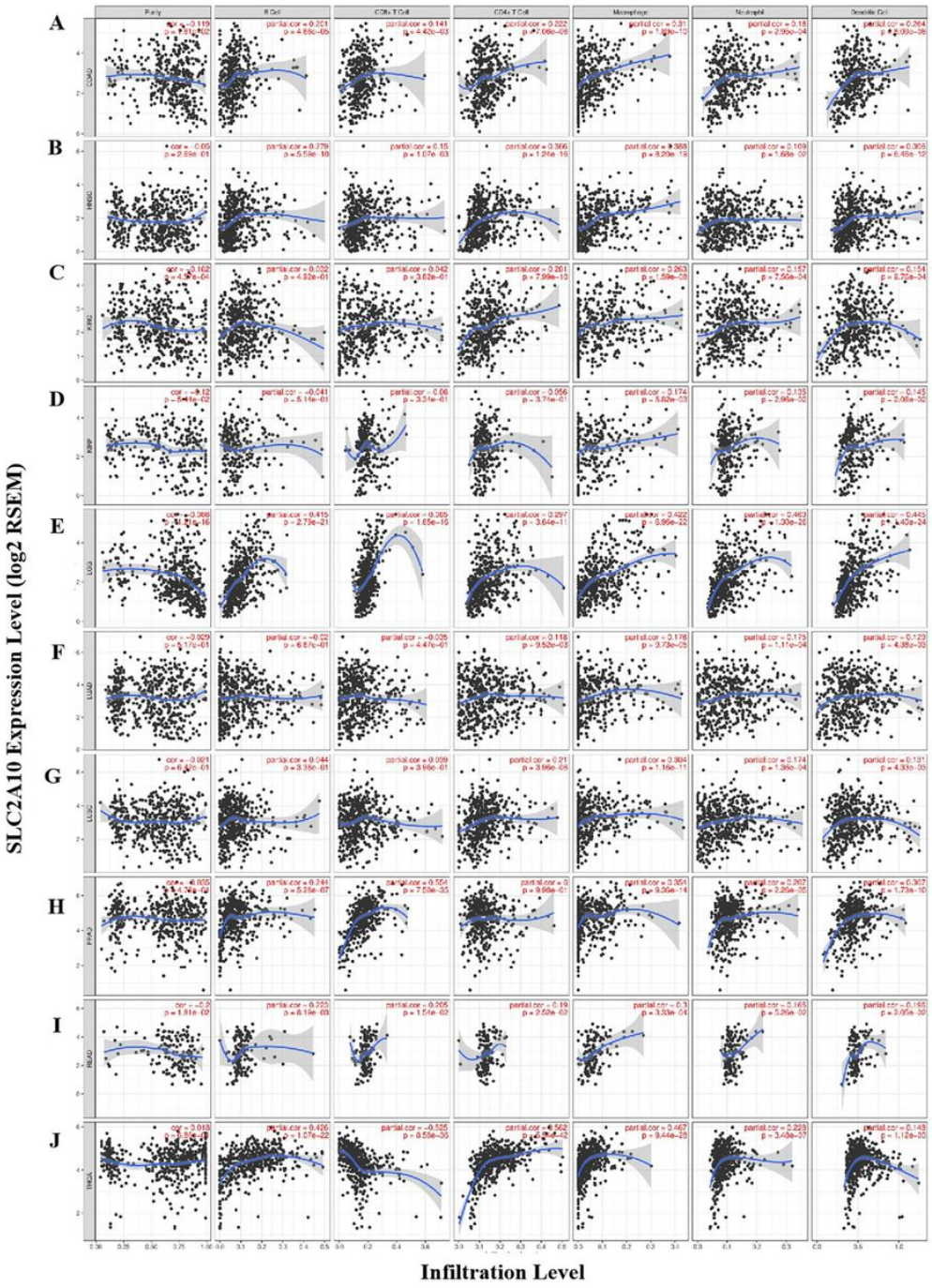

## Figure 3

kaplan-Meier survival curves comparing the high and low expression of SLC2A10 in different types of cancer in the Kaplan-Meier plotter databases(A-K). (A) OS survival curves of gastric cancer. (B) OS survival curves of ovarian cancer. (C) OS survival curves of bladder cancer. (D) OS survival curves of breast cancer. (E) OS survival curves of esophageal adenocarcinoma. (F) OS survival curves of kidney renal clear cell carcinoma. (G) OS survival curves of liver hepatocellular carcinoma. (H) OS survival curves of lung cancer. (I) OS survival curves of pancreatic ductal adenocarcinoma. (J) OS survival curves of stomach adenocarcinoma. (K) OS survival curves of uterine corpus endometrial carcinoma. OS, overall survival.




## Figure 4

kaplan-Meier survival curves comparing the high and low expression of SLC2A10 in histological type of lung cancer in the Kaplan-Meier plotter databases(A-E). (A) OS survival curves of lung cancer. (B) PPS survival curves of lung cancer. (C) FP survival curves of lung cancer. (D) OS survival curves of lung adenocarcinoma. (E) OS survival curves of lung squamous cell carcinoma.




## Figure 5

Correlation of SLC2A10 expression with immune infiltration level in COAD (colon adenocarcinoma), HNSC (head and neck cancer), KIRC (kidney renal clear cell carcinoma), KIRP (kidney renal papillary cell carcinoma), LGG (lower grade glioma), LUAD (lung adenocarcinoma), LUSC (lung squamous cell carcinoma), PRAD (prostate adenocarcinoma), READ (rectum adenocarcinoma) and THCA (thyroid carcinoma). (A) SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells (B)SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells, Macrophages, Neutrophils, and Dendritic cells in HNSC. (C)SCL2A10 expression levels has significant positive correlation with infiltrating levels of CD4+ T cells, Macrophages, Neutrophils, and Dendritic cells in KIRC. (D)SCL2A10 expression levels has significant positive correlation with infiltrating levels of Macrophages, Neutrophils, and Dendritic cells in KIRP. (E)SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells, CD8+ T cells, CD4+ T cells, Macrophages, Neutrophils, and Dendritic cells in LGG. (F)

SCL2A10 expression levels has significant positive correlation with infiltrating levels of CD4+ T cells, Macrophages, Neutrophils, and Dendritic cells in LUAD. (G)SCL2A10 expression levels has significant positive correlation with infiltrating levels of CD4+ T cells, Macrophages, Neutrophils, and Dendritic cells in LUSC. (H)SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells, CD8+ T cells, Macrophages, Neutrophils, and Dendritic cells in PRAD. (I)SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells, CD8+ T cells, CD4+ T cells, Macrophages, Neutrophils, and Dendritic cells in READ. (J)SCL2A10 expression levels has significant positive correlation with infiltrating levels of B cells, CD4+ T cells, Macrophages, Neutrophils, and Dendritic cells in READ.