

Comprehensive Analysis of Potential Correlation Between Solute Carrier 1A (SLC1A) Family and Lung Adenocarcinoma

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Background: Lung adenocarcinoma (LUAD) is the most common dangerous malignant tumor and the leading cause of global cancer incidence and mortality. The Solute Carrier 1A (SLC1A) family play a significant part in cellular biological process, inflammation, and immunity. Specific functions of the SLC1A family in lung cancer are still not systematically described.

Objective: This study aimed to explore the best biological understanding of SLC1A family in lung cancer.

Methods: To study the expression and role of the SLC1A family in lung cancer, researchers used a variety of bioinformatics databases and tools.

Results: Aberrant expression of SLC1A family genes were demonstrated and analyzed the association with gender, tumor grade, cancer stages, and nodal metastasis status. The ectopic expression of SLC1A family genes has prognostic value for LUAD patients. Immune infiltration revealed a significant correlation between SLC1A family genes expression in LUAD. SLC1A family genes were involved in manifold biological processes and have different levels of DNA methylation and genetic alteration.

Conclusions: These findings suggested that members of the SLC1A family could be a potential target for the development of LUAD therapeutics as well as a reliable indicator of LUAD prognostic value.

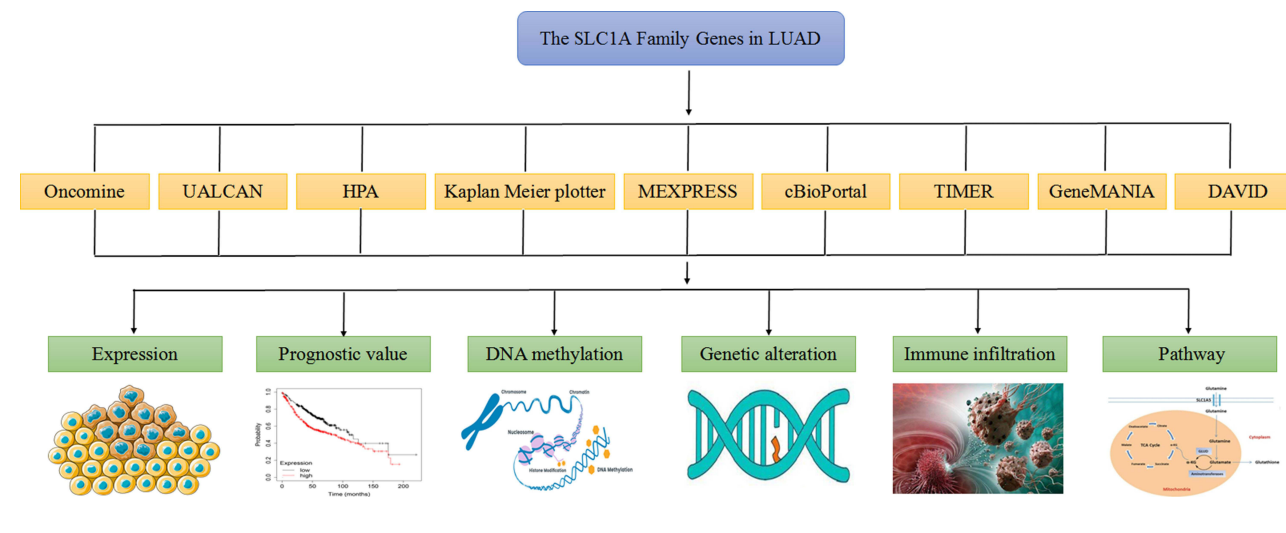
Keywords: lung adenocarcinoma, SLC1A family, expression, prognosis, bioinformatics analysis

Introduction

Lung cancer includes two major histological groups, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Lung adenocarcinoma (LUAD) is a histological subtype of NSCLC, accounting for about 50% of all lung cancer cases.¹ LUAD is the most common cancer in both men and women, and it is also the leading cause of cancer death. In recent years, many countries had reported that LUAD had the highest incidence and mortality rate, and was one of the most dangerous malignant tumors for people's health and life.² As a result, it's critical to look for potential prognostic biomarkers for early detection and novel therapeutic targets.

The members of the Solute Carrier 1A (SLC1A) family consists of five homologous glutamate transporters: EAATs (EAAT1/SLC1A3, EAAT2/SLC1A2, EAAT3/SLC1A1, EAAT4/SLC1A6 and EAAT5/SLC1A7) and two neutral amino acid transporters: ASCTs (ASCT1/SLC1A4, and ASCT2/SLC1A5). The main function of EAATs is maintaining the low synaptic concentration of glutamate, and ASCTs play an important role in tumor biology.³ Numerous studies reported the SLC1A family members' aberrant expression in various tumors and predict adverse prognosis. SLC1A1 dysregulates in lung cancer cells, and is tightly intertwined with Glutathione Synthesis.⁴ SLC1A2 enhances glutamate release from glioma cells may contribute to tumor-associated necrosis.⁵ Meanwhile, SLC1A2 expression is highly

Graphical Abstract



correlated with tumor grade in glioma patients, and play important role in controlling cell proliferation in vitro and in vivo.^{6,7} Upregulated SLC1A3 correlates with poor prognosis and activates the PI3K/AKT pathway in gastric cancer.⁸ The latest study reported that SLC1A4 is upregulated in HCC and involved in various carcinogenesis-associated signaling pathways and processes.⁹ Previous studies have shown that SLC1A5 is found to be overexpressed in multiple tumors, including breast cancer,¹⁰ endometrial carcinoma,¹¹ esophageal cancer,¹² prostate cancer,¹³ gastric cancer.¹⁴ In addition, SLC1A5 regulates glutamine uptake cancer growth and tumor development in vitro and in vivo.^{11–14} During radiation treatment, SLC1A6 is upregulated and correlated with poor prognosis in nasopharyngeal carcinoma patients.¹⁵ In a word, the members of the SLC1A genes family play an important role in multiple tumors. Nonetheless, few studies investigating the functions and prognostic values of SLC1A and LUAD on a systemic level are still lacking.

In the present study, we first comprehensive analysis to investigate the significance of the SLC1A family members expression, prognosis, and biological function in LUAD via multiple bioinformatics databases. We hope that our research will be beneficial to the diagnosis and treatment of LUAD.

Materials and Methods

Expression Analysis of SLC1A Family Members

Oncomine (<https://www.oncomine.org/resource/main.html>), an integrated online cancer microarray database, was used to compute gene expression signatures, clusters and gene-set modules, automatically extracting biological insights from 715 datasets of 86,733 samples.¹⁶ The pan-cancer studies in SLC1A family genes were selected to compare the expression levels in tumors vs normal tissues. The selection criteria for the Oncomine studies were $P < 0.05$, 1.5-fold change and expressed gene rank in the top 10% were input as our selection threshold.

UALCAN (<http://ualcan.path.uab.edu/index.html>) is a web-portal platform to facilitate the comprehensive analysis of the relative expression of a query gene in various molecular subtypes based on individual cancer stages, tumor grade, race, body weight or other clinico-pathologic parameters features using The Cancer Genome Atlas (TCGA) data.¹⁷ TCGA provides web resources including RNA-sequencing and patients' Clinical data from 33 different cancer types.¹⁸ Gene expression of the SLC1A family genes in various tumor subgroups of LUAD was estimated using UALCAN from a primary TCGA-LUAD dataset (n=574). UALCAN also discovered the effect of SLC1A gene expression levels and relative clinic-pathologic characteristics.

Differentially Expressed SLC1A Family Members at Protein Level

The Human Protein Atlas (<http://www.proteinatlas.org>) is a user-friendly online platform that contains the protein expression of in cells, tissues, and organs of the human body based on proteomics, transcriptomics, and systems biology.¹⁹ The Human Protein Atlas between normal lung and LUAD tissues was used to show Immunohistochemistry (IHC) data for Clinical potential application of distinct SLC1A family members.

Kaplan–Meier Plotter Analysis

Kaplan-Meier Plotter (<http://kmplot.com/analysis/index.php?p=background>) is a comprehensive and user-friendly online tool to analyze the prognostic value of a particular gene, which contains the gene expression and survival information of 661 LUAD patients.²⁰ The cancer patients were divided into high and low expression groups according to the median mRNA expression values to evaluate overall survival (OS), first progression survival (FPS) and post progression survival (PPS) by Kaplan-Meier Plotter, with the hazard ratio (HR) with 95% confidence intervals (CI) and log-rank P-value. Furthermore, the Kaplan-Meier Plotter was also used to assess the relationship between mRNA expression of SLC1A family genes and other clinic-pathological variables such as gender, clinical stage, smoking history, and surgery mode.

DNA Methylation Analysis

We utilized the UALCAN to analyze promoter methylation of SLC1A family genes between the normal and primary lung tumor tissues. Also, we used the MEXPRESS to measure the correlation between DNA methylation and gene expression of SLC1A family genes from TCGA-LUAD cohorts.^{21,22}

Genetic Alteration Analysis

cBioPortal (<https://www.cbioportal.org/>) is an online open-access resource for exploring, visualizing, and analyzing multidimensional cancer genomics data.²³ The cBioPortal was used to browse, visualize, and analyze genomic profiles of SLC1A family genes in TCGA-LUAD cohorts, including mutations and probable copy-number changes (CNA). Furthermore, mRNA expression Z-scores (RNASeq V2 RSEM) and protein expression Z-scores (RPPA) were obtained. The correlation analyses of SLC1A genes family were performed using TCGA-LUAD cohorts.

Immune Infiltration Analysis

TIMER 2.0 (<http://timer.cistrome.org/>) is an online database for systematically investigating and visualizing the clinical impact of different immune cells in diverse cancer types, which incorporated 10,009 samples across 23 cancer types from TCGA.²⁴ The immune infiltration estimation of SLC1A family genes was carried out in LUAD by TIMER. All analyses were performed using Spearman correlation and a P-value less than 0.05 indicated statistical significance.

Construction of Related Genes Network

GeneMANIA (<http://www.genemania.org>) is a tool for finding other genes that are related to a set of input genes using available genomics and proteomics data.²⁵ In this study, the SLC1A genes family were submitted to the software Cytoscape version 3.8.2²⁶ to illustrate the functional association network among SLC1A family genes and their related genes using GeneMANIA Manage app. The advanced statistical options were that max resultant attributes were 20, max resultant genes were 10, and the weighing method used was automatically selected.

Functional Annotation and Pathway Enrichment Analysis

The Database for Annotation, Visualization and Integrated Discovery (DAVID, <https://david.ncifcrf.gov/>) provided a comprehensive set of functional annotation tools for investigators to understand the biological meaning of genes.²⁷ Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis functions of SLC1A family genes and their 20 related genes were enriched by DAVID. $P < 0.05$ was set as the threshold to indicate a statistically significant difference.

Results

Transcriptional Expression Studies of the SLC1A Family Members in LUAD Tissues

Oncomine examined the mRNA expression of SLC1A family members in LUAD patients in comparison to normal tissues. As shown in Figure 1 and Table 1, we found that mRNA expressions of SLC1A1 and SLC1A2 was lower in LUAD tissues compared to normal tissues with fold changes of 4.377, 3.960, 3.085, 3.826, 2.826, 1.768, 2.642 and 2.439 ($p=2.72E-11$, $2.24E-8$, $1.55E-4$, $1.02E-19$, $4.14E-7$, $5.25E-13$, $1.74E-8$, $4.09E-4$), respectively. mRNA expressions of SLC1A4 and SLC1A5 were obviously higher in LUAD tissues with fold changes of 1.904, 1.724, 1.549, 1.640, 1.586 and 1.688 ($p=2.16E-8$, $5.57E-6$, $7.10E-13$, $1.79E-10$, $3.14E-5$, $3.96E-10$), respectively.

Further analysis of the transcriptional expression of SLC1A family members in LUAD using TCGA database. The results were consistent with Oncomine database, as shown in Figure 2, the transcription expression of SLC1A1 and SLC1A2 was significantly down-regulated in LUAD tissues compared to the normal tissues (Figure 2A and B). On the contrary, the expression of SLC1A4, SLC1A5 and SLC1A7 in LUAD tissues was significantly higher than the corresponding normal tissues (Figure 2D–E and G). SLC1A3 and SLC1A6 showed no significant difference compared with normal tissues (Figure 2C and F). However, SLC1A2 was significantly down-regulated in tumor compared with the

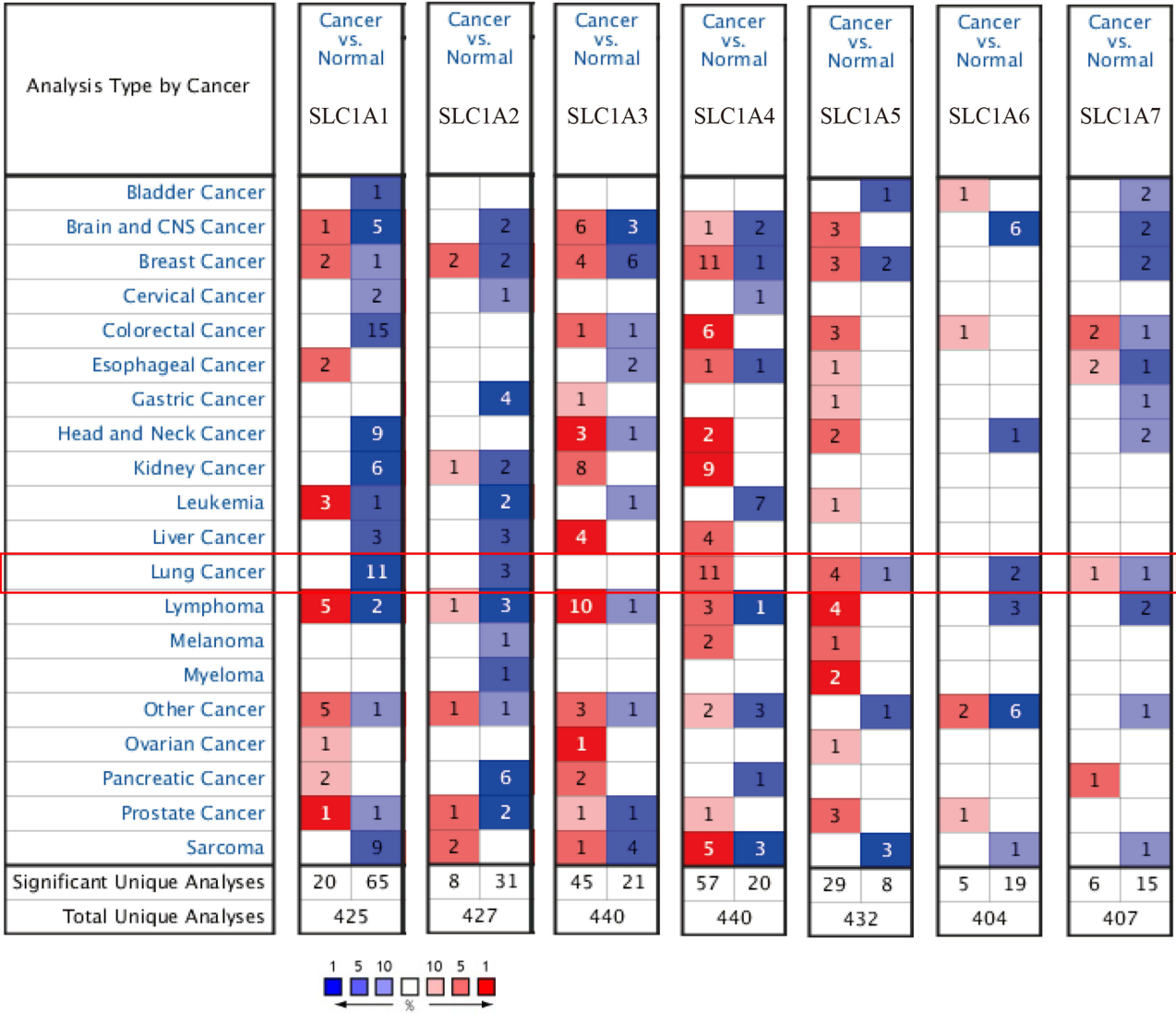


Figure 1 Transcriptional expressions of SLC1A family members in human cancers. The number in the cell represents the number of analyses that meet our threshold. Cell color is determined by the gene rank. Red: up-regulation; Blue: down-regulation.

Table I The Transcription Level of SLC1A Family Genes Between LUAD and Normal Lung Tissues

	Types of Lung cancer vs Lung	Fold Change	P-value	t-Test	Reference
SLC1A1	Lung Adenocarcinoma vs Normal	-4.377	2.72E-11	-7.925	Hou
	Lung Adenocarcinoma vs Normal	-3.960	2.24E-8	-7.065	Stearman
	Lung Adenocarcinoma vs Normal	-3.085	1.55E-4	-4.259	Bhattacharjee
	Lung Adenocarcinoma vs Normal	-3.826	1.02E-19	-11.120	Landi
	Lung Adenocarcinoma vs Normal	-2.826	4.14E-7	-5.649	Su
	Lung Adenocarcinoma vs Normal	-1.768	5.25E-13	-8.106	Selamat
	Lung Adenocarcinoma vs Normal	-2.642	1.74 E-8	-7.537	Okayama
SLC1A2	Lung Adenocarcinoma vs Normal	-2.439	4.09E-4	-4.234	Garber
SLC1A3	NA	NA	NA	NA	NA
SLC1A4	Lung Adenocarcinoma vs Normal	1.904	2.16E-8	6.975	Stearman
	Lung Adenocarcinoma vs Normal	1.724	5.57E-6	5.071	Su
	Lung Adenocarcinoma vs Normal	1.549	7.10E-13	8.050	Landi
	Lung Adenocarcinoma vs Normal	1.640	1.79 E-10	9.178	Okayama
SLC1A5	Lung Adenocarcinoma vs Normal	1.586	3.14 E-5	4.876	Stearman
	Lung Adenocarcinoma vs Normal	1.688	3.96 E-10	6.809	Selamat
SLC1A6	NA	NA	NA	NA	NA
SLC1A7	NA	NA	NA	NA	NA

Abbreviations: LUAD, lung adenocarcinoma; NA, not available.

paired normal tissues of 59 LUAD patients. SLC1A3, SLC1A4, SLC1A5 and SLC1A6 were overexpression in LUAD tissues compared with their paired normal tissues ([Figure S1](#)).

Association of SLC1A Family Members' Prognostic Values in LUAD Patients with Different Clinicopathological Features

The relationship between the transcription expression of SLC1A family genes and clinicopathological features was analyzed by UALCAN. As shown in [Figure S2-S9](#), the mRNA expression of SLC1A1, SLC1A2, SLC1A4, SLC1A5 and SLC1A7 were significantly correlated with tumor stages, race, gender, smoking, tumor histological subtypes, nodal metastasis status and TP53 mutation status of LUAD patients. However, SLC1A3 and SLC1A6 were no obvious relativity with clinical factors, it may be that the transcriptional expression of SLC1A3 and SLC1A6 were no significant difference in LUAD patients ([Figure 2C](#) and [F](#)). The above results indicated that part of SLC1A family genes correlated with different clinicopathological features.

Protein Expression of Different SLC1A Family Members in Patients with LUAD

The Human Protein Atlas also looked into the protein expression of SLC1A family members in LUAD patients. As shown in [Figure 3](#), the protein expression of SLC1A1 and SLC1A6 was lower expressed in LUAD tissues compared with normal tissues. Consisted with the mRNA expression, the protein level of SLC1A4 and SLC1A5 highly expressed in LUAD tissues, while lower expressed in normal tissues. In short, the aberrant expression of SLC1A family genes in LUAD tissues both in the transcriptional and protein expressions.

Prognostic Value of SLC1A Family Members in LUAD Patients

The prognostic value of SLC1A genes family for LUAD patients was investigated using Kaplan-Meier plotter. As shown in [Figure 4](#), low expression of SLC1A1, SLC1A2, SLC1A4 and SLC1A7 showed poorer OS, while high SLC1A5 and SLC1A6 were related to worse prognosis in LUAD patients. And SLC1A3 was not associated with OS. The median OS of SLC1A1, SLC1A2, SLC1A3, SLC1A4, SLC1A5, SLC1A6 and SLC1A7 in LUAD patients were 93.165, 103.87, 101, 100.885, 98.985, 102.585, and 103.165 months ($p=0.133, 0.0712, 0.259, 0.0225, 0.587, 0.161, 0.132$), respectively. To evaluate the association between mRNA expression of SLC1A family genes and OS in various clinic-pathological features by Kaplan-Meier Plotter, the results revealed that SLC1A family genes correlated with OS in gender, clinical

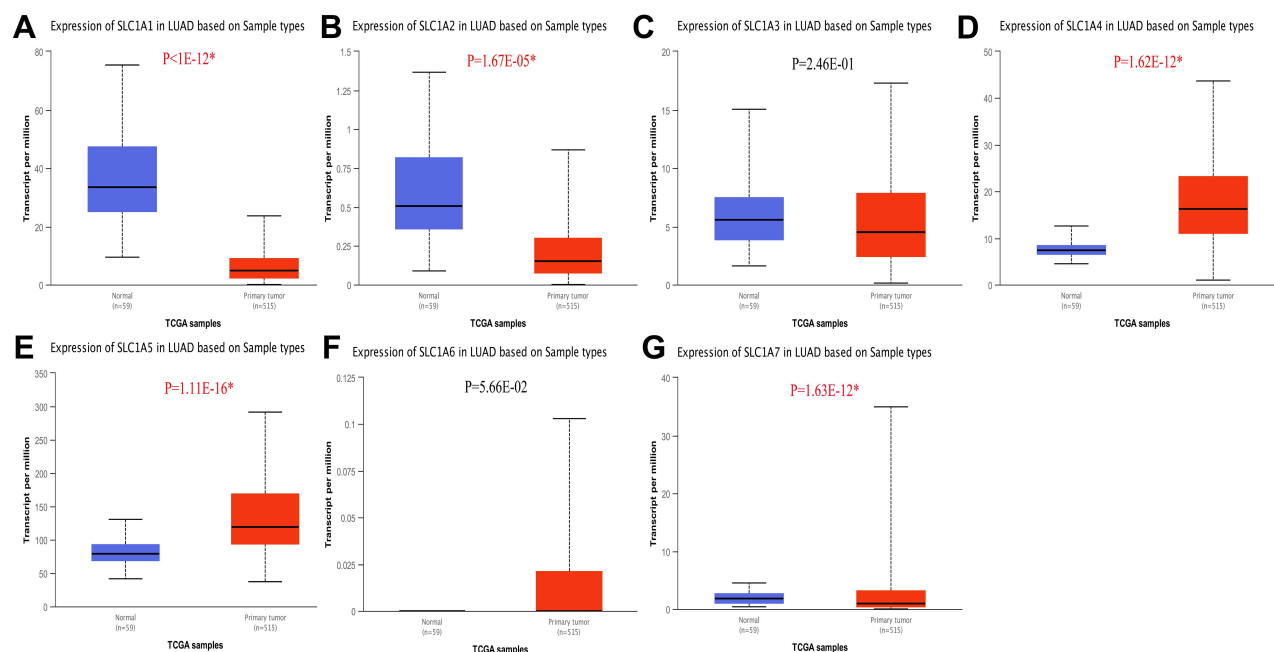


Figure 2 The expression of SLC1A family genes in LUAD patients. Expression panels for SLC1A1 (A), SLC1A2 (B), SLC1A3 (C), SLC1A4 (D), SLC1A5 (E), SLC1A6 (F) and SLC1A7 (G) based on major cancer stage comparing 59 normal individuals and data for 515 LUAD patients in TCGA database. *P<0.05 was considered statistically significant.

Abbreviation: LUAD, lung adenocarcinoma.

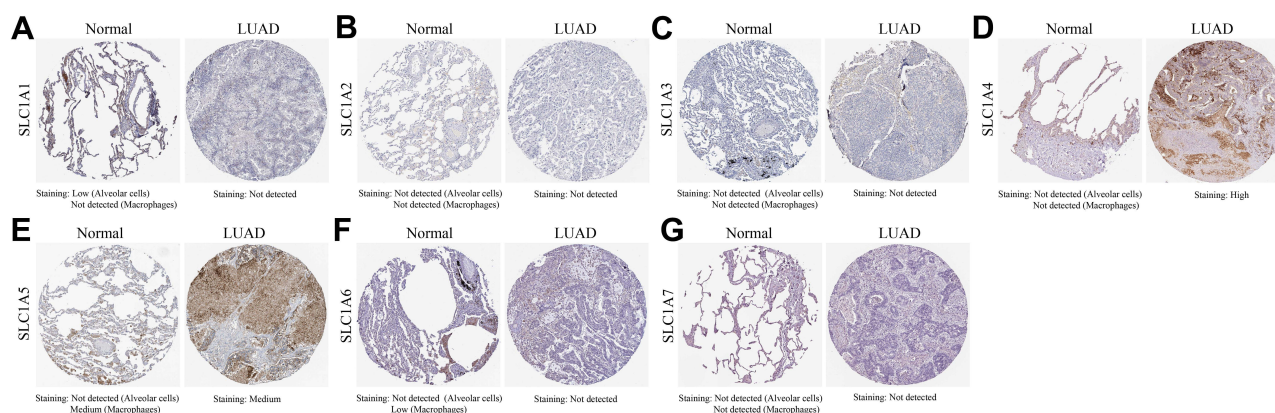


Figure 3 The immunohistochemistry expression pattern of SLC1A family members in LUAD tissues and normal lung tissues. Protein Expression for SLC1A1 (A), SLC1A2 (B), SLC1A3 (C), SLC1A4 (D), SLC1A5 (E), SLC1A6 (F) and SLC1A7 (G) obtain from Human Protein Atlas database.

Abbreviation: LUAD, lung adenocarcinoma.

stage, smoking history, and surgery method (Tables 2–345). Figure 5 shows a negative link between high SLC1A5 and improved FPS in LUAD patients, but high SLC1A1, SLC1A2, and SLC1A7 exhibited a positive correlation with favorable FPS. The results showed that the high expression of SLC1A1 and SLC1A4 had a good PPS in Figure 6.

DNA Methylation of SLC1A Family Genes in LUAD Patients

We analyzed the methylation level of SLC1A family genes promoter according to TCGA-LUAD dataset. It was found that there was no significant promoter methylation level of SLC1A1, SLC1A4 and SLC1A7 between normal and LUAD tissues (Figure 7A, D and G). Compared with normal tissues, the promoter methylation level of SLC1A2 and SLC1A3 in LUAD tissues was significantly higher (Figure 7B and C). And the promoter methylation level of SLC1A5 and SLC1A6 was significantly lower (Figure 7E and F). Using the MEXPRESS database, we discovered that the expression of SLC1A

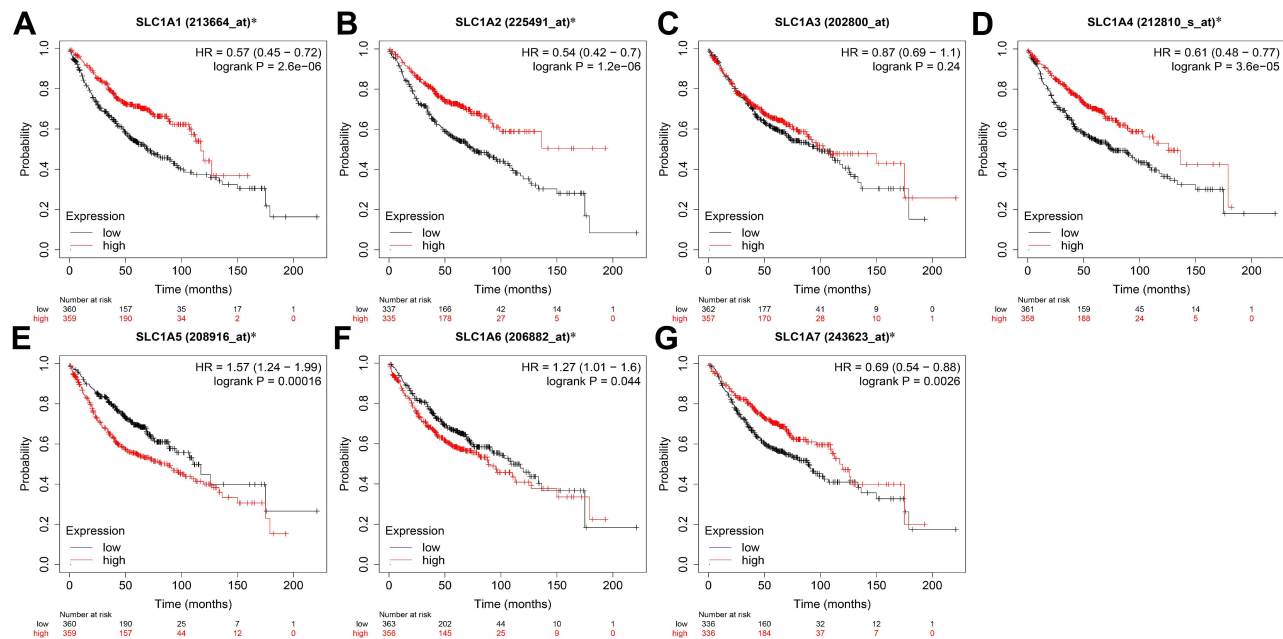


Figure 4 The correlation between expression level of SLC1A family genes and overall survival in LUAD patients. Overall survival curves for SLC1A1 (A), SLC1A2 (B), SLC1A3 (C), SLC1A4 (D), SLC1A5 (E), SLC1A6 (F) and SLC1A7 (G) in ESCA patients. *P<0.05 was considered statistically significant. The red line indicates high expression, and the black line indicates low expression.

Abbreviation: LUAD, lung adenocarcinoma.

family genes was positively linked with the methylation signal levels of most methylation probe sites in the promoters of SLC1A family genes (Figure S10A-G). This indicated that there may be a potential correlation between the methylation of gene promoter and the expression of SLC1A family genes.

Table 2 The Relationship Between SLC1A Family Genes and OS in Different Gender of LUAD Patients

Genes	Gender	Cases	HR (95% CI)	P-value
SLC1A1	Female	317	0.45(0.3–0.66)	4.1e-5***
	Male	344	0.67(0.48–0.93)	0.0161*
SLC1A2	Female	317	0.41(0.26–0.64)	5.0e-5***
	Male	344	0.5(0.35–0.71)	7.7e-5***
SLC1A3	Female	317	0.79(0.54–1.16)	0.2304
	Male	344	1.01(0.73–1.4)	0.936
SLC1A4	Female	317	0.49(0.32–0.72)	0.0003***
	Male	344	0.77(0.55–1.06)	0.11
SLC1A5	Female	317	1.52(1.04–2.24)	0.0302*
	Male	344	1.36(0.97–1.9)	0.07
SLC1A6	Female	317	1.52(1.04–2.24)	0.0302*
	Male	344	1.36(0.97–1.9)	0.07
SLC1A7	Female	317	0.6(0.4–0.9)	0.0119*
	Male	344	0.72(0.51–1.01)	0.0531

Notes: *p<0.05, ***p<0.001.

Abbreviations: LUAD, lung adenocarcinoma; OS, overall survival; HR, hazard ratio; CI, confidence interval.

Table 3 The Relationship Between SLC1A Family Genes and OS in Different Stages of LUAD Patients

Genes	Stage	Cases	HR (95% CI)	P-value
SLC1A1	I	370	0.41(0.27–0.61)	9.8e-6***
	II	136	0.49(0.3–0.82)	0.0051**
	III	24	1.22(0.45–3.29)	0.6912
	IV	4	-	-
SLC1A2	I	370	0.33(0.21–0.52)	4.4e-7***
	II	136	0.7(0.41–1.19)	0.1846
	III	24	0.93(0.3–2.9)	0.9055
	IV	4	-	-
SLC1A3	I	370	0.7(0.47–1.03)	0.0684
	II	136	0.69(0.42–1.12)	0.1277
	III	24	1.2(0.44–3.23)	0.7217
	IV	4	-	-
SLC1A4	I	370	0.54(0.36–0.8)	0.0021**
	II	136	0.51(0.31–0.84)	0.0068**
	III	24	0.88(0.32–2.45)	0.8101
	IV	4		
SLC1A5	I	370	1.77(1.19–2.63)	0.0042**
	II	136	1.45(0.89–2.36)	0.1353
	III	24	1.7(0.62–4.7)	0.2969
	IV	4		
SLC1A6	I	370	0.93(0.63–1.38)	0.723
	II	136	2.19(1.33–3.61)	0.0017**
	III	24	0.63(0.23–1.72)	0.3637
	IV	4	-	-
SLC1A7	I	370	0.52(0.34–0.78)	0.0015**
	II	136	0.82(0.49–1.38)	0.4582
	III	24	0.14(0.04–0.54)	0.0012**
	IV	4	-	-

Notes: **p<0.01, ***p<0.001.

Abbreviations: LUAD, lung adenocarcinoma; OS, overall survival; HR, hazard ratio; CI, confidence interval.

Genetic Alteration Differences of SLC1A Family Genes in LUAD Patients

We analyzed the SLC1A family genes alterations and correlations from TCGA-LUAD dataset using c-BioPortal. The chance genes genetic alteration of SLC1A1, SLC1A2, SLC1A3, SLC1A4, SLC1A5, SLC1A6 and SLC1A7 were 6%, 5%, 13%, 5%, 5%, 5% and 7%, respectively (Figure 8A). The form of SLC1A family in LUAD includes missense

Table 4 The Relationship Between SLC1A Family Genes and OS in Different Smoking History of LUAD Patients

Genes	Smoking History	Cases	HR (95% CI)	P-value
SLC1A1	YES	246	0.52(0.32–0.84)	0.0063**
	NO	143	0.48(0.2–1.11)	0.0804
SLC1A2	Yes	246	0.58(0.35–0.95)	0.0301*
	No	143	0.61(0.26–1.41)	0.2459
SLC1A3	Yes	246	0.86(0.54–1.37)	0.5269
	No	143	0.78(0.35–1.74)	0.5425
SLC1A4	Yes	246	0.82(0.51–1.3)	0.3943
	No	143	0.68(0.3–1.54)	0.3578
SLC1A5	Yes	246	1.3(0.82–2.09)	0.2649
	No	143	0.79(0.35–1.78)	0.5662
SLC1A6	Yes	246	0.96(0.6–1.53)	0.8621
	No	143	1.79(0.8–4.02)	0.1539
SLC1A7	Yes	246	0.55(0.33–0.91)	0.0174*
	No	143	0.59(0.25–1.4)	0.2295

Notes: *p<0.05, **p<0.01.

Abbreviations: LUAD, lung adenocarcinoma; OS, overall survival; HR, hazard ratio; CI, confidence interval.

Table 5 The Relationship Between SLC1A Family Genes and OS in Surgery Methods of LUAD Patients

Genes	Surgery Success	Cases	HR (95% CI)	P-value
SLC1A1	YES	204	0.79(0.38–1.63)	0.5261
SLC1A2	Only surgical margins negative	204	0.36(0.26–1.54)	0.4243
SLC1A3	Only surgical margins negative	204	2.11(0.99–4.51)	0.0482*
SLC1A4	Only surgical margins negative	204	1.18(0.57–2.41)	0.6562
SLC1A5	Only surgical margins negative	204	1.63(0.78–3.38)	0.1862
SLC1A6	Only surgical margins negative	204	0.99(0.48–2.05)	0.985
SLC1A7	Only surgical margins negative	204	0.59(0.28–1.24)	0.1593

Notes: *p<0.05.

Abbreviations: LUAD, lung adenocarcinoma; OS, overall survival; HR, hazard ratio; CI, confidence interval.

mutation, amplification and so on. However, the genetic alteration of SLC1A family genes had no correlation with the clinical prognosis of LUAD patients. We also used c-BioPortal to calculate the correlations of SLC1A family genes by analyzing their mRNA expression (RNA Seq V2 RSEM) and using Pearson's correction. The results indicated significant and positive correlations in the following: SLC1A1 with SLC1A2, SLC1A3, SLC1A4 and SLC1A7; SLC1A2 with SLC1A3, SLC1A4 and SLC1A7; SLC1A3 with SLC1A4, SLC1A6 and SLC1A7; SLC1A4 with SLC1A5 and SLC1A6; SLC1A5 with SLC1A6. The results indicated significant and negative correlations in the following: SLC1A1 and SLC1A2 with SLC1A5 and SLC1A6, respectively; SLC1A3 with SLC1A5; SLC1A4, SLC1A5 and SLC1A6 with SLC1A7, respectively (Figure 8B).

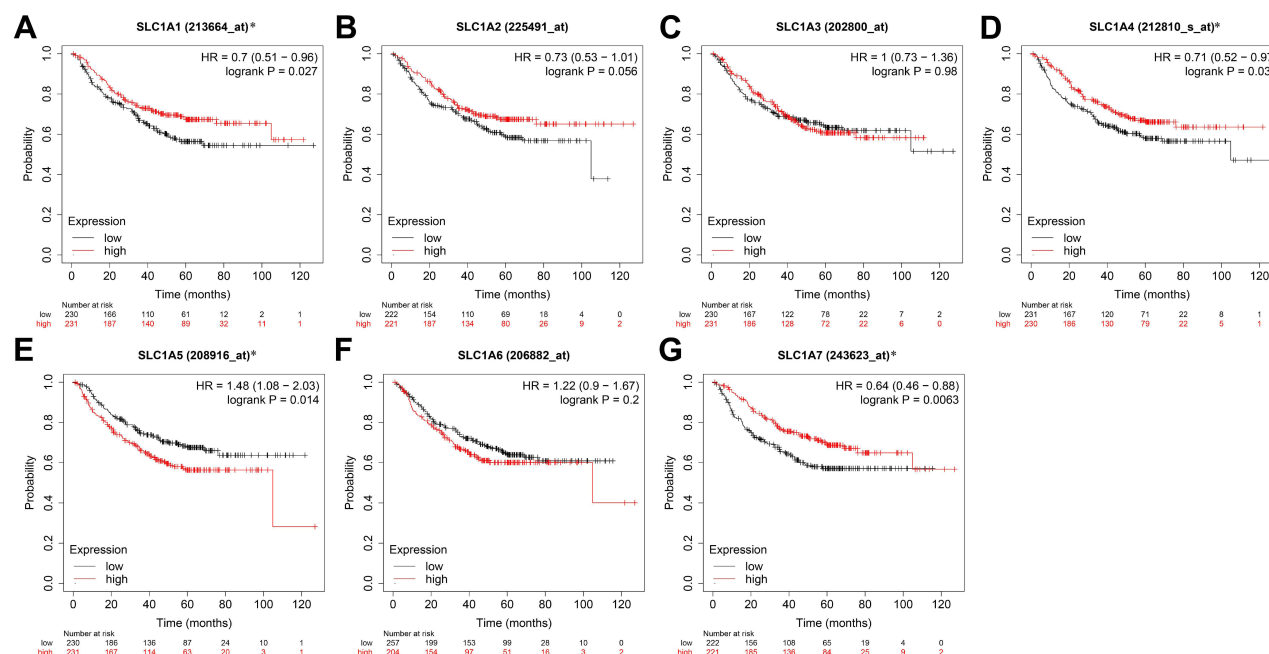


Figure 5 The correlation between expression level of SLC1A family genes and first progression survival in LUAD patients. First Progression Survival curves for SLC1A1 (A), SLC1A2 (B), SLC1A3 (C), SLC1A4 (D), SLC1A5 (E), SLC1A6 (F) and SLC1A7 (G) in ESCA patients. *P<0.05 was considered statistically significant. The red line indicates high expression, and the black line indicates low expression.

Abbreviation: LUAD, lung adenocarcinoma.

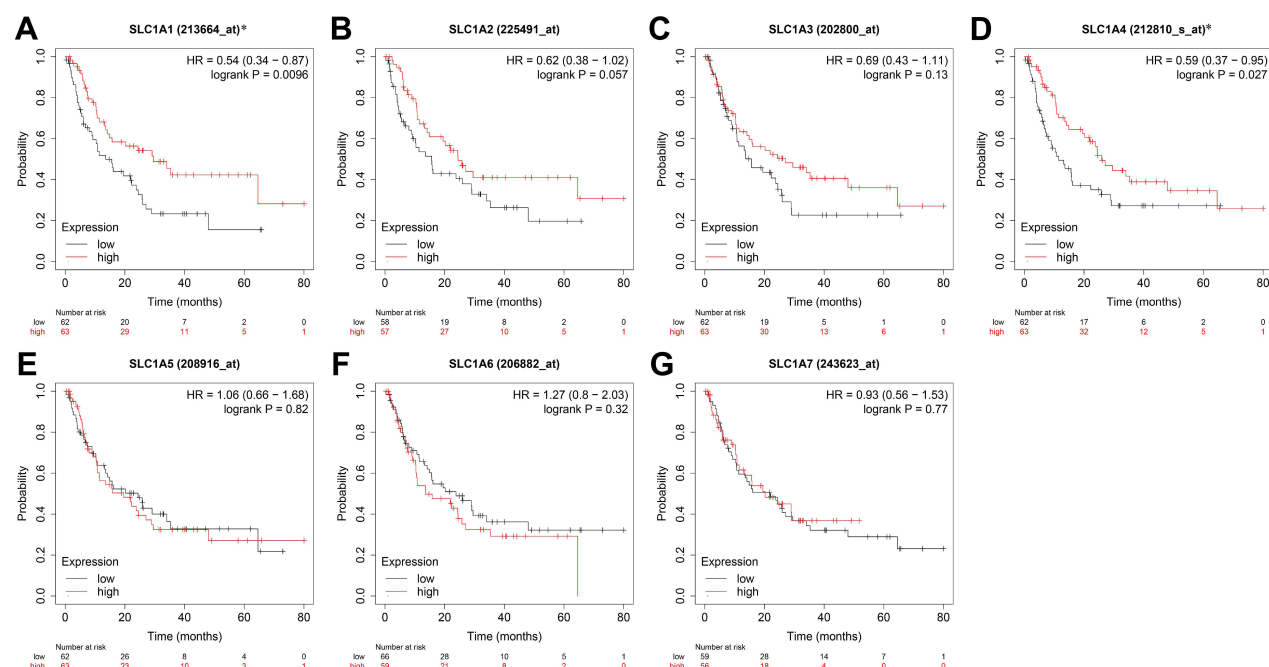


Figure 6 The correlation between expression level of SLC1A family genes and PPS in LUAD patients. Post progression survival curves for SLC1A1 (A), SLC1A2 (B), SLC1A3 (C), SLC1A4 (D), SLC1A5 (E), SLC1A6 (F) and SLC1A7 (G) in ESCA patients. *P<0.05 was considered statistically significant. The red line indicates high expression, and the black line indicates low expression.

Abbreviation: LUAD, lung adenocarcinoma.

Immune Infiltrates in Correlation with SLC1A Family Genes in LUAD

Consequently, we evaluated the correlation between the expression of SLC1A family genes and the level of immune infiltration. As shown in Figure 9, the expression of SLC1A1, SLC1A3, SLC1A4, SLC1A6 and SLC1A7 were negatively

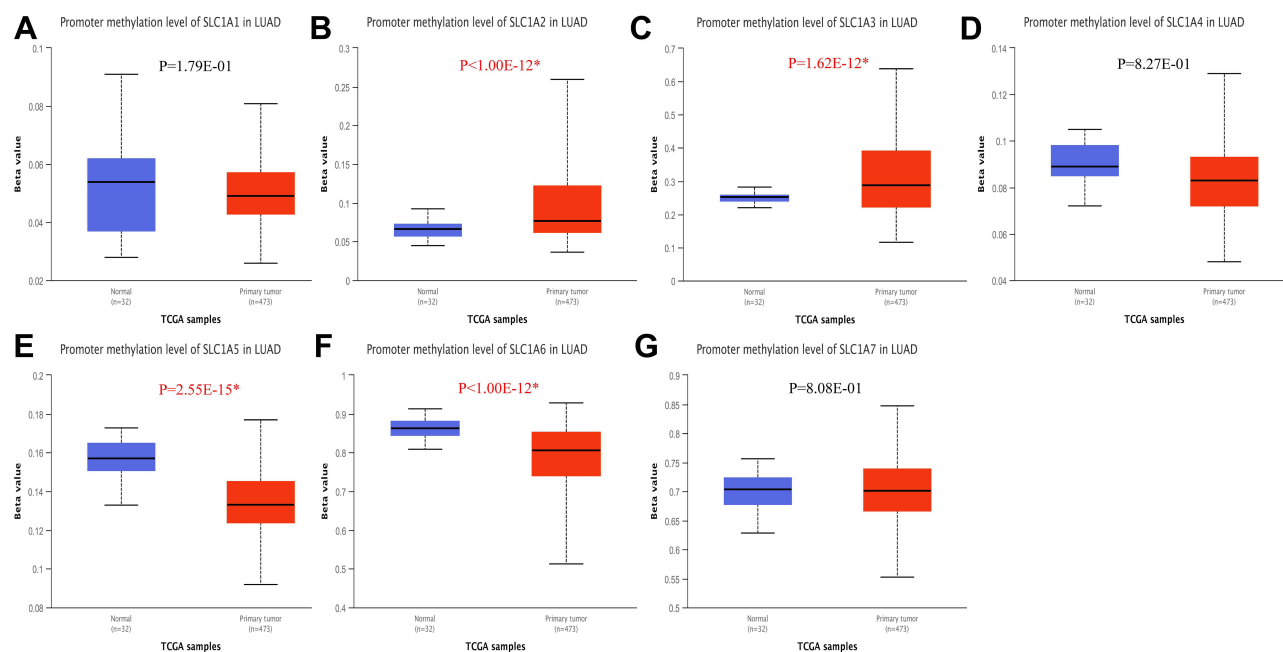


Figure 7 The promoter methylation level of SLC1A family members between the normal and primary LUAD. (A) SLC1A1, (B) SLC1A2, (C) SLC1A3, (D) SLC1A4, (E) SLC1A5, (F) SLC1A6 and (G) SLC1A7. * $P < 0.05$ was considered statistically significant.

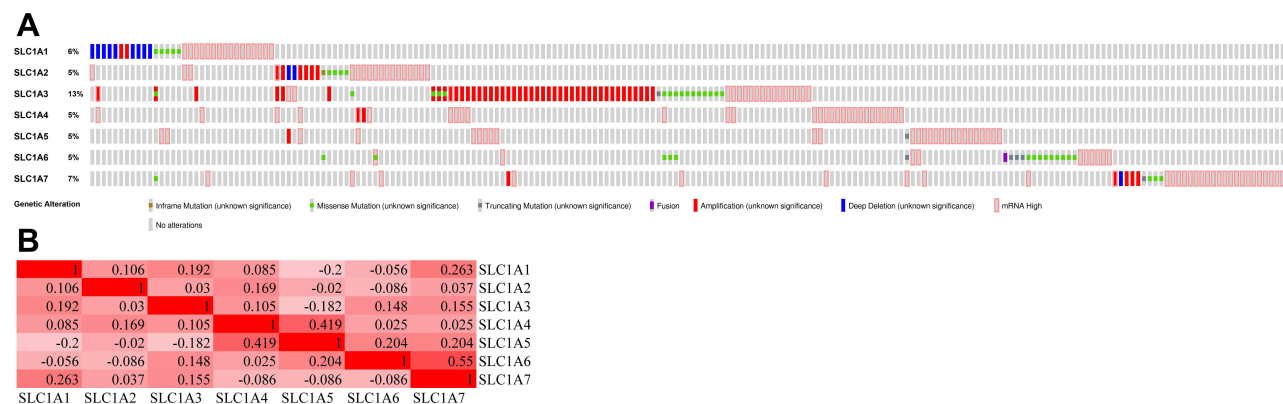


Figure 8 Genetic alteration of SLC1A family members in LUAD. (A) Oncoprint in c-BioPortal showed the distribution and proportion of samples with alterations in SLC1A family genes. (B) Correlation between different SLC1A family genes in LUAD. Color depth represents Spearman correlation coefficients calculated by c-BioPortal. **Abbreviation:** LUAD, lung adenocarcinoma.

correlated with tumor purity. SLC1A1 was positively correlated with CD8⁺ T cells, CD4⁺ T cells, macrophages, neutrophils and dendritic cells. There is also has a positive correlation between SLC1A2 with B cells, CD4⁺ T cells and dendritic cells. For SLC1A3, the results showed a significant positive correlation with B cells, CD8⁺ T cells, CD4⁺ T cells, macrophages, neutrophils and dendritic cells. SLC1A4 was positively correlated with the infiltrating levels of B cells, CD4⁺ T cells and dendritic cells. Conversely, SLC1A5 was negatively correlated with macrophages, neutrophils and dendritic cells. We observed a weak correlation between SLC1A6 and neutrophils. Furthermore, SLC1A7 has significant positive correlations with B cells, CD4⁺ T cells, macrophages and dendritic cells. All the above results showed that SLC1A family genes could affect immune infiltration in LUAD.

Correlation and Functional Enrichment Analysis of SLC1A Family Genes

In this study, we used GeneMANIA to construct a network map of SLC1As and their 20 related genes. Different colored lines in the network map represent different modes of action, including physical interactions, co-expression, predicted,

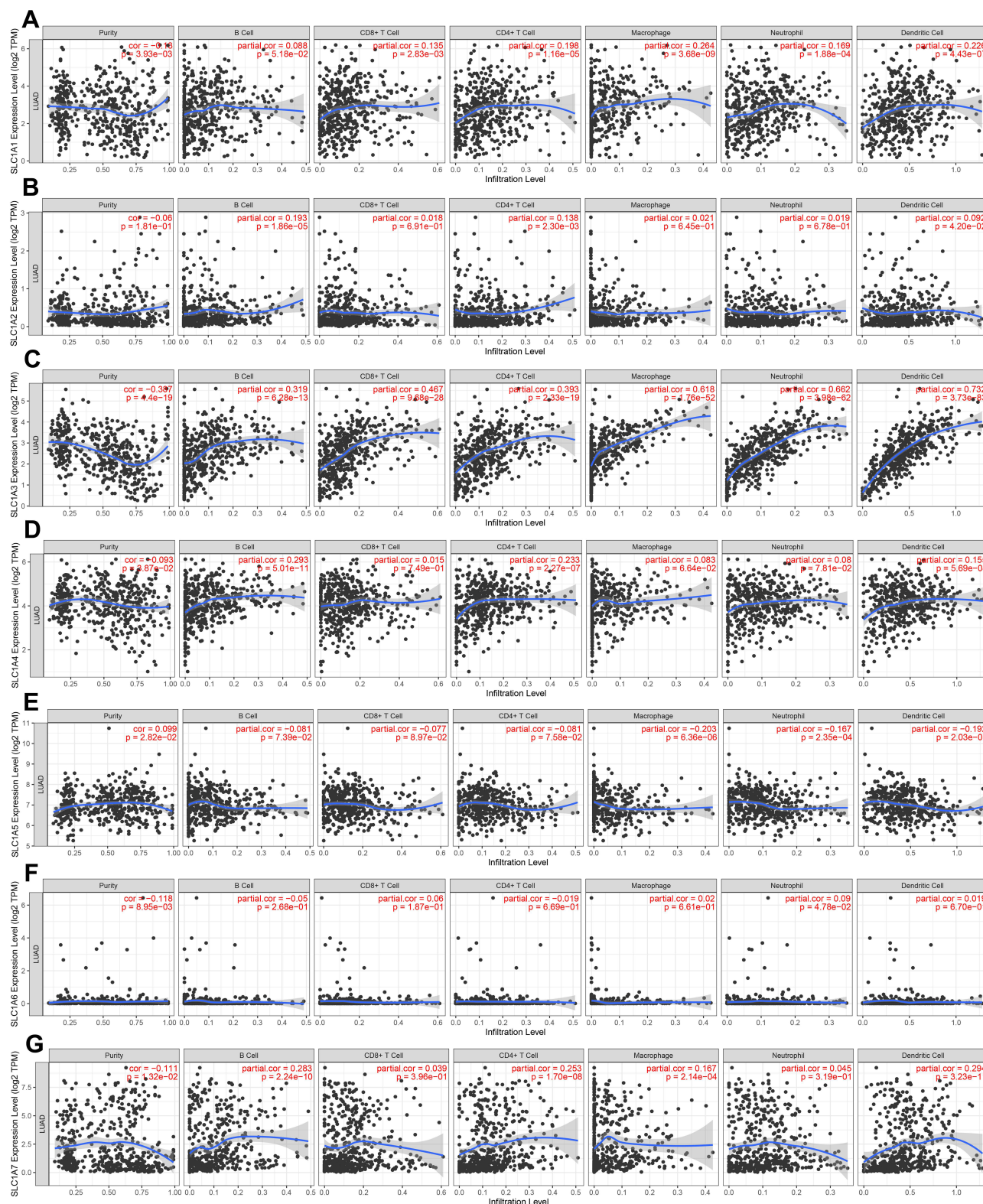


Figure 9 The correlation between SLCIA family members and immune infiltration. The expression level of SLCIA1 (A), SLCIA2 (B), SLCIA3 (C), SLCIA4 (D), SLCIA5 (E), SLCIA6 (F) and SLCIA7 (G) was associated with immune cell infiltration.

co-location, genetic interactions, pathway, and shared protein domains (Figure 10A). SLC1As and their related genes were introduced into DAVID for enrichment analysis. These genes were found to be involved in 9 different cell components, including integral component of membrane, plasma membrane, membrane, extracellular exosome, integral component of plasma membrane, endoplasmic reticulum membrane, golgi apparatus, cell surface, melanosome, were significantly associated with SLC1As (Figure 10B). Biological processes, including ion transport, L-glutamate transport, glutamine transport, glutamate secretion, amino acid transport, chemical synaptic transmission, D-aspartate import, were remarkably regulated by the SLC1As in cancers (Figure 10C). These genes mainly participated in the regulation of 8 molecular functions, such as amino acid transmembrane transporter activity, glutamate sodium symporter activity, and glutamate binding (Figure 10D). KEGG pathway enrichment analysis showed that these genes were mainly enriched in 2 pathways, Glutamatergic synapse, and Protein digestion and absorption (Figure 10E).

Discussion

Lung cancer is the most common cancer, with about 1,700,000 new cases every year.²⁸ LUAD is continuously growing in the proportion of lung cancer, which is presently the top diagnosed histological type in both males and females.²⁹ The overall outcomes for advanced stage LUAD are still poor.³⁰ As a result, it's worth looking into new prognostic biomarkers and therapeutic targets for LUAD lesions.

Abnormal intracellular energy metabolism is one of the characteristic changes of tumor cells.^{31,32} Glutamine is one of the important energy sources of malignant tumor cells, which provides carbon and nitrogen sources for the rapid proliferation of tumors.³³ A series of amino acid transporters are required to transport hydrophilic glutamine from extracellular to intracellular and different members of the solute carrier transporter family can recognize and transport it. SLC1A family genes are belong to the transporter family, which were abnormally expressed and involved in carcinoma-associated molecular mechanisms in various tumors. Nevertheless, the expression and role of each SLC1A family genes have not yet been comprehensively elucidated in LUAD. In this study, we systemically analyzed the expression,

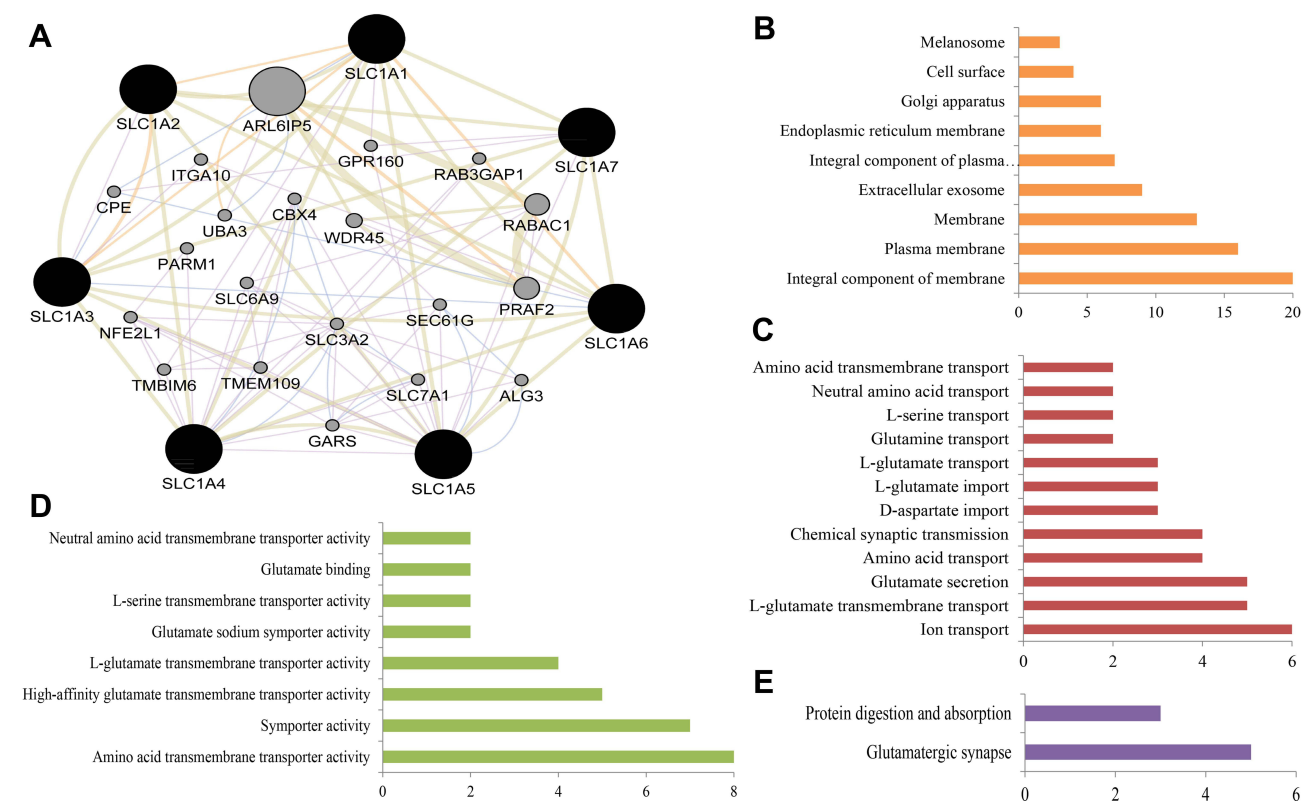


Figure 10 Functional enrichment analysis of SLC1A family related genes. (A) Network of SLC1A family members and its 20 related genes was analyzed by GeneMANIA. (B) Cellular component; (C) Biological processes; (D) Molecular functions; (E) KEGG pathway analysis. $P < 0.01$ is regarded as statistically significant.

prognostic role, DNA methylation, genetic alteration, immune infiltrates, and biological function of SLC1A family genes in LUAD through a variety of bioinformatics tools.

The studies of SLC1A1 have widely participated in neuropsychiatric disorders.^{34,35} The article showed that the expression of SLC1A1 in clear cell renal cell carcinoma tissues significantly increased than normal kidney tissues, and was higher in males than females.³⁶ Bianchi et al, reported that high-expressed SLC1A1 was associated with the differentiation of glioma cells.³⁷ Fan's study suggests that SLC1A1 may be involved in the occurrence and development of osteosarcoma by bioinformatics analysis.³⁸ The expression and glutamate transporter activity of SLC1A1 were altered in SN38-resistant colorectal cancer cells.³⁹ A recent report believed that lower expression of SLC1A1 was significantly positively correlated with infiltrating levels of CD8⁺T cells and dendritic cells. Meanwhile, SLC1A1 has clinical diagnostic value for colorectal cancer.⁴⁰ In our results, lower-expressed SLC1A1 in LUAD cancer tissues was correlated with tumor stages, race, gender, smoking, tumor histological subtypes, nodal metastasis status and showed poorer overall survival in LUAD. SLC1A2 is important for the nervous system function.⁴¹ Few studies explored the relation between SLC1A2 and cancer. SLC1A3 is primarily expressed in the cerebellum and cerebral neocortex, and its abnormal expression has been linked to neurological disorders like Alzheimer's disease,⁴² and episodic ataxia.⁴³ However, research revealed that SLC1A3 has also related to tumor metabolism and progression.⁴⁴ Recent research suggests that SLC1A3 promotes gastric cancer progression via the PI3K/AKT signaling pathway.⁸ Sun et al, believed that over-expressed SLC1A3 contributed to L-asparaginase resistance in solid tumors.⁴⁵ Previous studies demonstrated that high expression of SLC1A3 in glioblastoma,⁴⁶ chondrosarcoma,⁴⁷ thyroid cancer.⁴⁸ Nevertheless, there was no significant difference in the expression of SLC1A3 between LUAD tissues and control normal tissues in our study. As a Na-dependent amino acid transporter, SLC1A4 has been characterized in early neuronal development and diseases. However, more and more studies focused on its role in cancer in recent years. Morozova et al, found that SLC1A4 was overexpressed in neuroblastoma tumor-initiating cells, and might constitute a therapeutic target for this cancer.⁴⁹ In Hepatocellular Carcinoma (HCC), the mRNA and protein expression of SLC1A4 were upregulated, and that could be a novel prognostic marker and promising immunotherapeutic targets of HCC.⁹ White et al, demonstrated that SLC1A4 and SLC1A5 might be regulated by androgen receptor (AR), Myc and mechanistic target of rapamycin complex 1 (mTOR) and was high-expressed in prostate cancer. Our results showed the mRNA and protein levels of SLC1A4 were also significantly elevated in LUAD, and were associated with a poor prognosis. SLC1A5 is known to be critical for tumor growth and proliferation across many cancer subtypes.⁵⁰ Numerous studies showed that SLC1A5 is involved in the genesis and progress of various malignancies including lung cancer. SLC1A5 was upregulated in non-small cell lung cancer and associated with lymph node metastasis,⁵¹ and could promote cancer cell proliferation via activating mTOR signaling,⁵² and impair apoptotic cell death.⁵³ Consistent with these studies, our results demonstrated that higher expression of SLC1A5 was correlated with tumor stages, race, gender, smoking, tumor histological subtypes, nodal metastasis status in LUAD. The reports found that SLC1A6 expression was upregulated in nasopharyngeal carcinoma cells and decreased cisplatin sensitivity.¹⁵ The potential roles of SLC1A7 in tumor are not well documented so far. Our results elucidated that the expression of SLC1A6 and SLC1A7 were down-regulated and up-regulated in LUAD, respectively.

SLC1A family is thought to involve in tumor progression via regulating microenvironments and multiple pathways.^{3–8,39,45,54} To explore the potential mechanism of SLC1A family members in LUAD, we used various bioinformatics software for analysis. We deduced missense mutation and amplification is the frequent form in SLC1A family genes genetic alteration, and the genetic alteration frequency was not higher than 7%, which suggested that might be associated with the pathogenesis of LUAD. Clinically relevant genomic alterations were identified in LUAD patients.⁵⁵ The landscape of genetic alterations found in squamous cell lung cancer, the difficulties in identifying and treating these alterations could facilitate targeted therapy.⁵⁶ It showed that the occurrence and pathogenesis of lung cancer are closely linked to the issues of genetic mutation. Previous studies have also demonstrated the important role of SLC1A1, SLC1A4 and SLC1A5 in tumor microenvironment and immune response. SLC1A1 regulates impaired T-cell immunity and beneficial for immunotherapy of natural-killer T-cell lymphoma.⁵⁷ SLC1A4 was identified as lymphocyte phosphatase-associated phosphoprotein partners that were involved in human lymphocyte activation.⁵⁸ SLC1A5 attenuated inflammatory T cell responses through activate mTORC1 signaling.⁵⁹ SLC1A5 expression

correlated highly with tumor immune-cell infiltration in hepatocellular carcinoma.⁶⁰ SLC1A5 reflected the status of the tumor immune microenvironment in gastric cancer.⁶¹ These findings revealed that SLC1A family genes had an impact on immune infiltration, and varying levels of genetic alteration in LUAD. This study was a pioneer work to a comprehensive analysis of the expression and major role of SLC1A family genes in LUAD based on bioinformatics analysis, but the main limitations of our research should be noted. More sample sizes, protein expression, clinical and basic experimental data are needed to verify the biological significance of SLC1A family genes in LUAD in the future study.

Conclusion

The expression of SLC1A family genes was associated with the clinicopathological features. Besides, SLC1A family genes have prognostic value for LUAD patients and are associated with the immune infiltrates. The findings suggested that members of the SLC1A family could be a promising target for the development of LUAD therapeutics as well as a reliable predictor of LUAD prognosis.

Ethics Approval and Consent to Participate

Because all data sources were from public databases, Ethics Committee of Affiliated Hospital of North Sichuan Medical College waived the requirement for an ethics statement.

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Disclosure

The authors declare no conflicts of interest, financial or otherwise.

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