ORIGINAL RESEARCH

SLC12A3: A Novel Prognostic Biomarker of Clear Cell Renal Cell Carcinoma

Qian Gao, MM; Xia Ye, MM; Fei Li, MM

ABSTRACT

Objective • Clear renal cell carcinoma (ccRCC) is a common and deadly urinary system tumor. The TNM system determines treatment and prognosis based on cancer advancement. While nephron-sparing surgery is an option for localized ccRCC, advanced cases are challenging, and molecular-targeted therapy is crucial.

Methods • Here, we implemented microarray datasets to identify a total of 119 differentially expressed genes (DEGs) and ten hub genes by a protein-protein interaction network (PPI) and performed module analysis through STRING and Cytoscape.

Results • Data from this analysis shed light on a positive correlation between SLC12A3 (solute carrier family 12 member 3) and tumor-correlated cells. SLC12A3 can predict prognosis and immune infiltration levels in KIRC patients.

Conclusion • Our findings demonstrated that SLC12A3 expression accounts for favorable prognosis and increased immune infiltration of various cell types. This could lead to potential therapeutic aims and biomarkers for KIRC (kidney renal clear cell carcinoma). (*Altern Ther Health Med.* [E-pub ahead of print.])

Qian Gao, MM, Resident doctor; **Fei Li, MM,** Attending doctor; Department of Radiation Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing, China; **Xia Ye, MM,** Resident doctor, Department of Radiation Oncology, The First People's Hospital of Nantong, Nantong, China.

Corresponding author: Fei Li, MM
E-mail: happylife.i@163.com

INTRODUCTION

Renal cell carcinoma (RCC) is the most common type of kidney cancer, accounting for approximately 90% of all cases.[1-6] ccRCC is an aggressive type of kidney cancer, comprising 80% of all RCC cases.[7,8] Surgical resection, chemotherapy, and radiotherapy are not effective against this aggressive tumor.[9-11] Despite recent advancements, metastatic ccRCC remains an incurable cancer, with a survival rate of less than 20% over five years.[12,13] The most promising integrative therapies for advanced and metastatic diseases are antiangiogenic therapy and immune checkpoint inhibition therapy. Numerous exploratory studies have conclusively identified markers from epigenetics that effectively regulate gene expression in ccRCC through histone modification, methylation of DNA, and ncRNA expression.[14] Despite the contribution of relevant studies to identifying new therapeutic targets for tumorigenesis and ccRCC development, a complete understanding of the molecular mechanism of ccRCC is yet to be achieved.[15] Identifying effective biomarkers related to the emergence and progression of ccRCC holds great importance.[16]

During the last decade, gene expression profiling has displayed promising promise in the diagnosis and targeted therapy of cancers. In this study, bioinformatics and microarray technology are used to explore the molecular processes that drive tumor growth, we will analyze the expression patterns of DEGs in ccRCC and adjacent normal tissues using data from the Gene Expression Omnibus (GEO). Using PPI, we conducted functional annotation and pathway analysis of these DEGs with DAVID and identified hub genes closely associated with ccRCC. Through survival analysis on the Kaplan-Meier plotter bioinformatics platform, we further explored the significance of these genes. Additionally, we examined the role of SLC12A3 in ccRCC, a potential biomarker, and its connection to immune inhibitors and infiltrating immune cells. The findings of this present study exhibited that SLC12A3 could serve as a valuable biomarker for ccRCC.

METHODS

Data extraction

The GEO database available at https://www.ncbi.nlm.nih.gov offers extensive gene expression data collected by research institutions worldwide. NCBI has compiled multiple GEO datasets to create a comprehensive gene expression

database. Here, we selected three GEO gene expression profiles (GSE53757, GSE40435, and GSE17895). The GSE53757 dataset comprised 72 ccRCC and 72 noncancerous samples, while the GSE40435 dataset comprised 101 ccRCC and 101 noncancerous samples. The GSE17895 dataset contained 138 ccRCC samples and 22 noncancerous samples.

Data management of DEGs

To accurately identify genes expressed differently in ccRCC samples compared to standard models, We used the online analysis tool GEO2R, available on the National Center for Biotechnology Information website (https://www.ncbi.nlm.nih.gov/geo/geo2r/), to conduct our analysis. We established the threshold for DEGs at an adjusted P-value of <0.01 and |logFC| of ≥2.0, with calculated values for both the adjusted P-value and absolute log fold change (|logFC|). We identified the overlapping genes in the three datasets by the Venn diagram web tool (http://bioinformatics.psb.ugent.be/webtools/Venn/).

GO and KEGG pathway analyses of DEGs

Functional enrichment analysis is crucial to identifying and connecting genes with similar functions to biological phenotypes. Gene Ontology (GO) annotations are neatly categorized into Molecular Function (MF), Biological Process (BP), and Cellular Components (CC). KEGG is a highly esteemed database that stores information on biological pathways, diseases, genomics, chemicals, and drugs. In this research, we utilized DAVID tools (https://david-d.ncifcrf.gov) to conduct GO and KEGG pathway enrichment analysis of DEGs. We established genes with a count of ≥2 and $P < .01$ as the minimum threshold to ensure statistical significance.

PPI network establishment and hub genes digging

The PPI network of DEGs was thoroughly analyzed using the STRING database from https://string-db.org/, with a combined score > 0.4 as the cutoff criterion. We used Cytoscape (version 3.9.1) to visualize the PPI network and to identify modules in the network. We employed Cytoscape's Molecular Complex Detection (MCODE) algorithm with a degree cut-off of 2, node score cut-off equal to 0.2, max depth at 100, and k-score = 2. The degree of each protein node was calculated using Cytoscape, and the top ten genes were identified as the hub genes through our rigorous analysis.

Transcriptional expression levels of hub genes on ccRCC

After analyzing the mRNA expression levels, we utilized the reliable GEPIA online database to compare the hub genes in ccRCC tissues versus normal tissues. (http://gepia.cancer-pku.cn/).

Hub genes Survival analysis

The prognostic effect and the significance of the ten potential hub genes were assessed using the Kaplan-Meier plotter tool, which can be accessed from http://kmplot.com/analysis/. A total of 530 ccRCC patients were included in the overall survival analysis.

TCGA datasets analysis

Transcriptomic and relevant clinical data of KIRC were obtained from the TCGA database, and 248 KIRC samples were further extracted by Excel screening and integration (Screening criteria: Samples with time 0, Gx (G-stage unknown), Nx (T-stage unknown), Mx (M-stage unknown), unknown, and duplicate were excluded).

Assay of linked omics database

We analyzed 32 types of cancer in depth using the LinkedOmics database, which is publicly accessible (http://www.linkedomics.org/login.php). To study the co-expression of SLC12A3, we used Pearson's test and visually represented the results using a volcano plot and heatmap. Furthermore, we performed gene set enrichment analysis (GSEA) to identify enriched GO terms and KEGG pathways among the genes with SLC12A3.

Immuno-inhibitor evaluation

We utilized TISIDB (http://cis.hku.hk/TISIDB/index.php), a web portal that combines various data types to study the connections between SLC12A3 and immunoinhibitory in tumor and immune system interactions.[17]

Immune infiltration analysis

While conducting our analysis, we employed TIMER at https://cistrome.shinyapps.io/timer/, specifically designed to analyze tumor-infiltrating immune cells comprehensively. Using this tool, we investigated the potential relationship between the SLC12A3 gene and immune cell infiltration in tumors. This provided valuable insights into the role of SLC12A3 in the immune response to cancer.[18]

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). The χ^2 test was forcefully employed to investigate the possible link between clinicopathological traits and SLC12A3 expression. $P < .05$ was considered statistically significant, and data were presented as mean ± standard deviation.

RESULTS

DEGs Identification

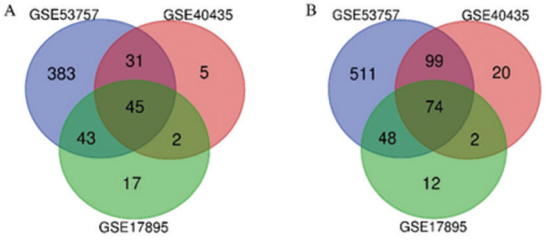
We have found three gene expression patterns: GSE53757, GSE40435, and GSE178959. The first pattern had 144 samples (72 ccRCC and 72 standards), the second had 202 illustrations (101 ccRCC and 101 regulars), and the third had 160 pieces (138 ccRCC and 22 measures) (Table 1). The application of the cutoff criteria revealed 1234 differentially expressed genes (DEGs) in the gene expression profile of GSE53757, with 502 upregulated and 732 downregulated. Similarly, GSE40435 produced 278 DEGs, including 83 upregulated and 195 downregulated. We also identified 243 DEGs, with 107 enhanced and 136 decreased genes. As shown in the Venn diagram, all three groups shared 119 DEGs, with 45 significantly upregulated and 74 downregulated genes (Figure 1).

Table 1. Summary of the three microarray databases derived from the GEO database.

Dataset ID	Gastric cancer	Normal	Total number
GSE53757	72	72	144
GSE40435	101	101	202
GSE17895	138	22	160

Abbreviations: GEO, Gene Expression Omnibus

Figure 1. Screening of DEGs in GEO datasets. (A) The Venn diagrams of upregulated DEGs in the GSE53757, GSE40435, and GSE178959 datasets. (B) The Venn diagrams of downregulated DEGs in the GSE53757, GSE40435, and GSE178959 datasets.



DEGs Functional enrichment analyses

We performed evaluations to enhance the capabilities of DEGs by DAVID. According to the GO analysis results, the DEGs showed a significant amount in the hydrogen peroxide catabolic process, positive manipulation of gene expression, monocyte chemotaxis, chemotaxis, Organisms eliminate cells and protect against fungus, Gram-negative bacteria, and other dangers through various immune responses including humoral and innate (Figure 2A). DEGs were concentrated in the cytoplasm's perinuclear region within the cell component, secretory granule, cell surface, hemoglobin complex, and haptoglobin-hemoglobin complex (Figure 2B). DEGs were concentrated in xenobiotic transporter, protease binding, oxygen transporter, protein phosphatase binding, and organic acid-binding (Figure 2C). Pathways analysis showed that the differentially expressed genes were enriched in critical routes such as cancer, drug resistance, and signaling pathways (Figure 2D).

The foundation of the PPI network hub gene

The STRING database was employed to predict protein activities among the DEGs. We hired the Cytoscape software to generate a PPI network for DEGs, which included 95 nodes and 222 edges (Figure 3A). As shown in Figure 3B and Table 2, the most important genes in the PPI were identified based on the number of connections they had with other genes, it turned out that EGF was the most excellent gene with a degree of connectivity of 17, followed by plasminogen (PLG; degree=16), lysyl oxidase (LOX; degree=14), integrin subunit beta 2 (ITGB2; degree=13), solute carrier family 12 member 3 (SLC12A3; degree=13), complement component 3 (C3; degree=12), caveolin 1 (CAV1; degree=11), CXCR4; degree=11), aquaporin 2 (AQP2 degree=11), and uromodulin (UMOD; degree=11). Five hub genes were enhanced, and five were decreased in

Figure 2. GO and KEGG pathway enrichment analysis of DEGs on (A) Biological processes. (B) Cellular components. (C) Molecular functions. (D) KEGG pathway.

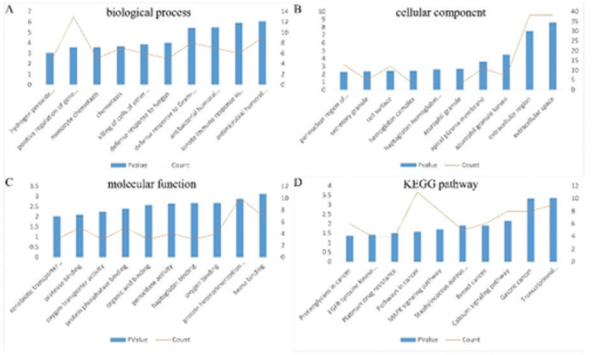


Figure 3. PPI network of DEGs for screening of the above hub genes. (A) Protein–protein interaction network of DEGs was performed via Cytoscape. Red nodes denote the upregulated DEGs, while the downregulated DEGs are marked with green nodes. (B) The hub gene subnetwork. The colors range from orange to red, indicating a higher degree score. Five of the ten nodes are upregulated genes, while the other five are downregulated. (C) and (D) The modules chosen from the PPI network are the most crucial, with a score = 4.5. (E) The module with a score = 4. (F) with a score = 3.6.

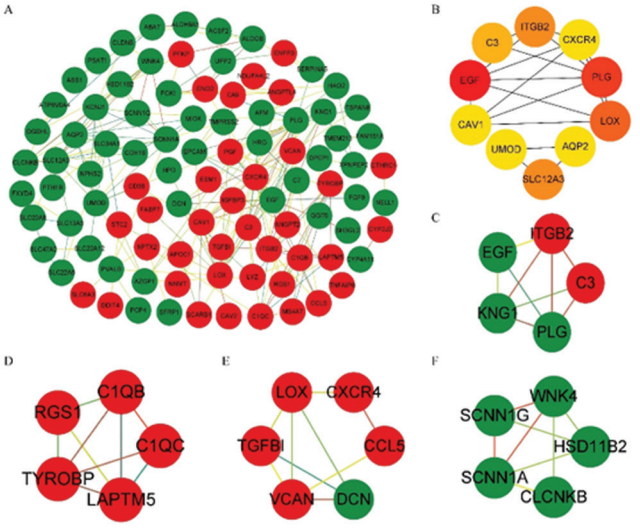
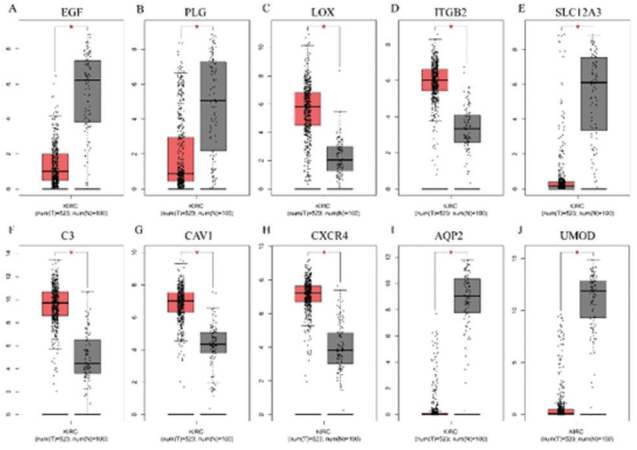


Table 2. Top ten hub genes of high-level connectivity

Symbol	Description	Degree
EGF	Epidermal growth factor	17
PLG	Plasminogen	16
LOX	Lysyl oxidase	14
ITGB2	Integrin subunit beta 2	13
SLC12A3	Solute carrier family 12 members 3	13
C3	Complement component 3	12
CAV1	Caveolin 1	11
CXCR4	C-X-C motif chemokine receptor 4	11
AQP2	Aquaporin 2	11
UMOD	Uromodulin	11

Figure 4. Hub gene differential expression validated via GEPIA. Expression levels of (A) EGF, (B) PLG, (C) LOX, (D) ITGB2, (E) SLC12A3, (F) C3, (G) CAV1, (H) CXCR4, (I) AQP2 and (J) UMOD in ccRCC tissues and normal tissues.



*P < .05 vs. normal tissues. T for tumor and N for normal tissue on the horizontal axis, while the vertical axis displays the number of transcripts per million reads.

Figure 5. Kaplan–Meier overall analysis. Prognostic values of (A) EGF, (B) PLG, (C) LOX, (D) ITGB2, (E) SLC12A3, (F) C3, (G) CAV1, (H) CXCR4, (I) AQP2 and (J) UMOD in ccRCC patients.

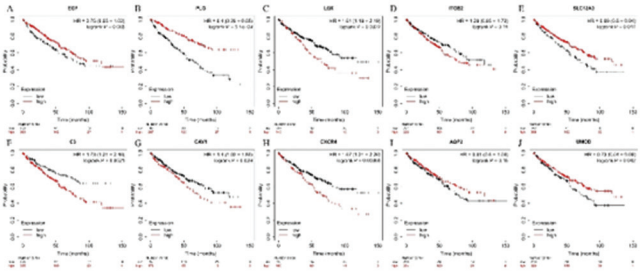


Table 3. The relationship of SLC12A3 expression with KIRC clinicopathological features

Variables	Low SLC12A3 expression	High SLC12A3 expression	*P* value
n	124	124	
T staging			.698
T1 to 2	75	72	
T3 to 4	49	52	
N staging			.013
N0	122	112	
N1	2	12	
M stage			.864
M0	103	104	
M1	21	20	
Pathologic stage			.933
I	53	50	
II	15	17	
III	34	37	
IV	22	20	
Gender			.434
Female	45	51	
Male	79	73	
Histological grade			.898
G1-2	55	56	
G3-4	69	68	
Age (years)			.307
≤60	52	60	
>60	72	64	

ccRCC. Four modules were selected to analyze the PPI network using MCODE (Figure 3C, 3D, 3E, 3F).

Hub genes mRNA expression validation

Using the GEPIA database, we have unequivocally confirmed the mRNA expressions of ten central genes. Our rigorous analysis has revealed that five genes exhibit increased expression in ccRCC while the remaining five exhibit decreased expression. (Figure 4).

Hub genes in survival analysis

Our analysis using the Kaplan-Meier plotter unequivocally demonstrates the prognostic significance of ten hub genes. The data clearly shows that elevated levels of LOX (P = .0022) (Figure 5C), C3 (P = .0021) (Figure 5F), CAV1 (P = .029) (Figure 5G), and CXCR4 (P = .00068) (Figure 5H) are strongly associated with lower survival rates in patients with KIRC. These findings are of utmost importance and should be noticed. On the contrary, high expressions of PLG (P = 5.1e-09) (Figure 5B), SLC12A3 (P = .017) (Figure 5E), and our analysis also showed that the expression of the UMOD gene was significantly associated with better overall survival in patients with kidney renal clear cell carcinoma (KIRC), as indicated by a P = .042 (see Figure 5J) However, EGF, ITGB2, and AQP2 were not significant markers of survival prognosis (P =0.065, P = .11, and P =0.16) (Figure 5A, 5D and 5I).

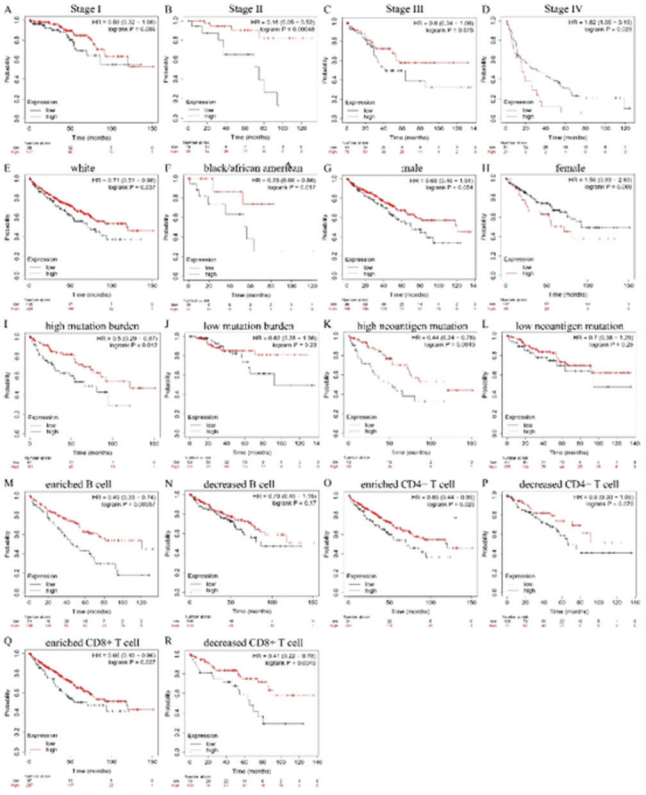
The connection between the levels of SLC12A3 and clinicopathologic pattern

Our thorough analysis of 248 KIRC samples revealed a significant correlation between high expression levels of SLC12A3 and the N stage (P = .013) in KIRC patients. We also examined various clinicopathological characteristics, such as pathologic stage, T stage, M stage, sex, histologic grade, and age labeling index. Still, we found no significant differences in comparison of the groups with bidirectional SLC12A3 expression. Our findings, presented in Table 3, demonstrate the importance of monitoring SLC12A3 expression levels in KIRC patients, particularly those with advanced N stage.

Sub-analysis of the link between SLC12A3 expression and overall survival in KIRC patients

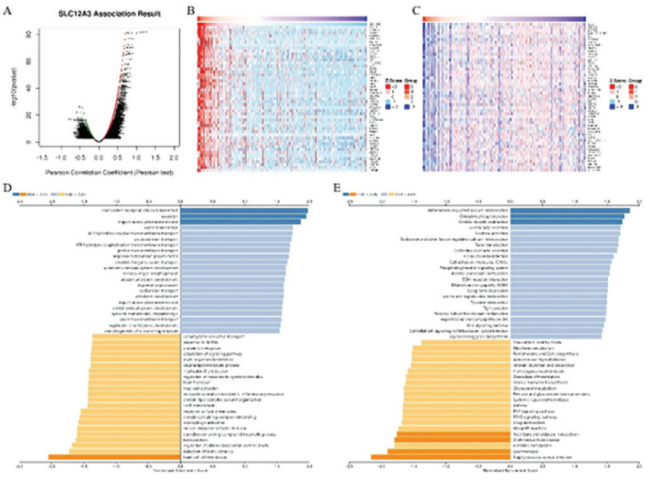
The current study delved into the prognostic significance of SLC12A3 in various subtypes, as previous research has shown that increased SLC12A3 expression is linked to more prolonged overall survival among KIRC patients, such as stage, gender, race, grade, mutation burden, neoantigen load, and cellular content. According to the expression of SLC12A3, Samples were divided into low and high-expression groups. High expression of SLC12A3 had a significant correlation with overall survival, especially in stage II (HR=0.16; P = .00048; Figure 6B) and stage IV (HR=1.85; P =0.029; Figure 6D) but not in stage I (HR=0.59; P = .086; Figure 6A) and stage III (HR=0.6; P = .075; Figure 6C). Patients with higher

Figure 6. The ccRCC patients were scrutinized in this investigation, with their SLC12A3 expression levels being the basis for their grouping. The patients with low and high SLC12A3 expression are differentiated by the black and red lines in the Kaplan-Meier overall survival sub-analyses. Comprehensive survival analysis of SLC12A3 in ccRCC in stages (A) I, (B) II, (C) III, (D) IV, (E) white, (F) black/African American, (G) male, (H) female, (I) high mutation burden, (J) low mutation burden, (K) high neoantigen load, (L) low neoantigen load, (M) enriched B cell, (N) decreased B cell, (O) enriched CD4+ T cells, (P) decreased CD4+ T cells, (Q) enriched CD8+ T cells, (R) decreased CD8+ T cells.



SLC12A3 mRNA expression levels demonstrated improved overall survival, particularly among those of Caucasian ethnicity (HR=0.71; P = .037; Figure 6E) and black/African American (HR=0.23; P = .017; Figure 6F), however, sample number was too low for meaningful analysis in Asian. In addition, the study found that higher levels of SLC12A3 were linked to increased overall survival rates in patients with a high mutation burden (HR=0.5; P = .012; Figure 6I) and a high neo-antigen load (HR=0.44; P = .0043; Figure 6K). However, there were no differences observed among patients with a low mutation burden (HR=0.62; P = .23; Figure 6J), low neo-antigen load (HR=0.7; P = .25; Figure 6L), male (HR=0.68; P = .054; Figure 6G) and female (HR=1.56; P = .088; Figure 6H). High SLC12A3 expression is strongly associated with improved OS in KIRC patients with enriched B cells, CD4+ T cells, and CD8+ T cells, as well as decreased CD8+ T cells. This information is important for interpreting the disease and potential treatments, while no correlation between reduced B cells (HR=0.73; P = .17; Figure 6N) and decreased CD4+ T cells (HR=0.6; P = .076; Figure 6P). These data suggest that high SLC12A3 expression benefits prognosis in KIRC patients with high mutation burden and neoantigen load. With a good correlation of high SLC12A3 expression with OS in different subtypes, an independent biomarker that shows promising results for predicting the prognosis of KIRC has been identified.

Figure 7. Co-expression genes linked to SLC12A3. (A) The co-expression genes of SLC12A3 in ccRCC. (B) Heat map of top 50 genes connected to SLC12A3. (C) Same as B, but in SLC12A3. (D) Enrichment analysis of co-expression genes involved in the biological process of ccRCC. (E) Same as D, but in the KEGG pathway of ccRCC.



Co-expression network and enrichment analysis of SLC12A3 in ccRCC

Our analysis found that over 10 000 genes positively correlate with SLC12A3, while almost 10 000 genes show a negative correlation (P < .05) (as shown in Figure 7A). To further investigate, the heatmap displays the highest 50 genes that exhibit a relation with SLC12A3. (Figures 7B and 7C). Additionally, we conducted GO and KEGG enrichment analyses of the SLC12A3-related genes in ccRCC cohorts. Data revealed that SLC12A3-related genes inhibited the activities of foam cell differentiation in biological processes (BP). However, monovalent inorganic cation homeostasis, excretion, and import across plasma membrane were promoted. (Figure 7D). It was found that genes related to SLC12A3 negatively correlated with staphylococcus aureus infection, ascorbate and alternate metabolism leishmaniasis, and graft-versus-host disease. However, these genes were positively associated with aldosterone-regulated sodium reabsorption, oxidative phosphorylation, and cardiac muscle contraction (Figure 7E).

There is a connection between the expression of SLC12A3 and immune inhibition.

Advancements in immune checkpoint inhibitors have improved our knowledge of the human immune system.[19]

Figure 8. Correlations of SLC12A3 expression and immune inhibitors in KIRC. SLC12A3 expression with (A) BTLA, (B) CD96, (C) CD244, (D) CD274, (E) CSF1R, (F) CTLA4, (G) LAG3, (H) LGALS9, (I) PDCD1, (J) PDCD1LG2, (K) TGFBR1, (L) TIGIT, and (M) VTCN1 and negatively correlated with (N) KDR.

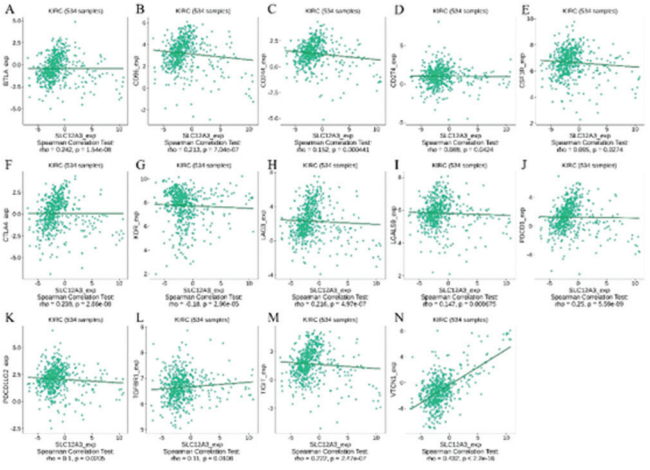
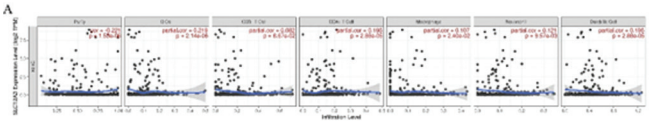


Figure 9. Correlation of SLC12A3 expression with immune infiltration levels in KIRC. (A) SLC12A3 expression was positively associated with B cells, CD4+ T cells, CD8+ T cells, macrophage, neutrophil, and dendritic cell infiltration levels.



Our study confirms a clear link between SLC12A3 expression and immune checkpoint inhibitors. Our analysis of the TISIDB database indicated that the genes in Figure 8 are unequivocally associated with SLC12A3 (Figure 8).

The connection of SLC12A3 expression and immune infiltration

In addition, our analysis also revealed a positive correlation between the expression of the SLC12A3 gene and the presence of various types of immune cells within tumors in patients with kidney renal clear cell carcinoma (KIRC). These immune cells included B cells, T cells, macrophages, neutrophils, and dendritic cells (Figure 9).

DISCUSSION

ccRCC is a complex and uncommon form of cancer.[20,21] Its development can be attributed to genetic or acquired factors.[20] The most prevalent acquired risk factors include hypertension, smoking, chronic analgesic use, obesity, and diabetes.[20] Regarding genetics, ccRCC tumors show prominent mutation heterogeneity.[22] Many patients with ccRCC experience the loss of 3p, which refers to the deletion of the short arm of chromosome 3.[23] The two most prevalent genetic abnormalities in ccRCC are PBRM1 (protein polybromo1) and VHL (von Hippel–Lindau tumor suppressor).[20,22] Other genomic alterations include KDM5C, SETD2, or BAP1.[24,25] During the initial phase of metastatic RCC, increased mortality rate and resistance to treatment have triggered a substantial economic burden due to the lack of adequate diagnosis methods.[26] Hence, it is crucial to identify novel biomarkers that can aid in detecting and evaluating metastasis in ccRCC.[27] Over the years, biotechnology has advanced, and as a result, researchers have delved into examining the genetic changes at the gene level of ccRCC. This has led to identifying candidate genes that can aid diagnosis and treatment.

We found 119 genes that differed in expression between ccRCC and standard samples, with 45 upregulated and 74 downregulated. These genes involve various biological processes, including immune response and chemotaxis. Chemotaxis is essential for physiological processes, but in tumors, it can facilitate the spread of cancer cells.[28] For a carcinoma cell to successfully metastasize, it must invade, infiltrate, extravasate, and grow in a distant location. Chemotaxis is crucial in these essential stages of tumor cell dissemination.[29] Chemokines' activities in tumor immunity could be complex. It varies due to the dynamic and diverse regulation of chemokine ligands and receptors by tumor cells, immune cells, and stromal cells.[30] During tumor development, chemokines regulate various aspects of immune cell biology, such as activation, phenotype, function, and recruitment.[30] KEGG pathway analysis focuses on several key areas, including Proteoglycans in cancer, EGFR tyrosine kinase inhibitor resistance, Platinum drug resistance, Pathways in cancer, MAPK signaling pathway, Staphylococcus aureus infection, Breast cancer, Calcium signaling pathway, Gastric cancer, and Transcriptional misregulation in cancer. Is the MAPK pathway critical for signal transduction and phosphorylation events related to tumorigenesis?[31] Activated kinases send signals outside the cell, affecting growth, differentiation, and other functions.[31] Calcium-mediated signaling pathways are related to metabolism control and cancer metastasis.[32,33] DEGs' biological process and signaling pathway are significant in tumor growth and can be potential candidates for ccRCC diagnosis and treatment.

A network called PPI screened DEG interactions and identified ten hub genes, with five upregulated (LOX, ITGB2, C3, CAV1, CXCR4) and five downregulated (EGF, PLG, SLC12A3, AQP2, UMOD). Verification using GEPIA showed that mRNA levels in cancer tissue matched earlier findings. Finally, the survival analysis showed that increased LOX, C3, CAV1, and CXCR4 expressions significantly correlated with worse survival probability for KIRC patients. On the contrary, increased terms of PLG, SLC12A3, and UMOD were predominantly related to improved overall survival for KIRC patients. However, EGF, ITGB2, and AQP2 were not significant markers of survival prognosis. Therefore, the valuable genes are LOX, C3, PLG, CAV1, CXCR4, SLC12A3, and UMOD.

After reading a large number of literature on these seven genes, we found that studies relating to SLC12A3 in KIRC needed to be more detailed.

The encoding of electroneutral cation-chloride cotransporters is the responsibility of the SLC12 family, crucial in maintaining liquid metabolism.[34] However, more research is needed on the SCL12 subfamily than other SLC families. Some genes in this family are linked to tumor growth and invasiveness in numerous tumors.[35-37] The SLC12A3 gene is unequivocally situated on chromosome 16q13 and comprises precisely 26 exons. It is accountable for encoding a sodium-chloride cotransporter (NCCT), which is remarkably susceptible to thiazide.[38] SLC12A3 mutations have been linked to Gitelman Syndrome (GS), an inherited condition that follows an autosomal recessive pattern.[39] It's worth noting that SLC12A3 expression is strongly linked to increased levels and SLC12A9 and lower survival rates in UVM.[40] According to our research, the downregulation of SLC12A3 in ccRCC is linked to an unfavorable prognosis. SLC12A3 could be a valuable biomarker for ccRCC depending on the correspondence with immune infiltration levels.

Our study's analysis of clinicopathologic features has firmly established a robust correlation between the N stage and the expression levels of SLC12A3. SLC12A3 expression was not significantly related to other clinicopathologic features. Co-expression analysis showed genes related to excretion, import, foam cell differentiation, and cation homeostasis. Pathway analysis revealed associations with infection, metabolism, immune response, and energy homeostasis. High SLC12A3 expression was favorable for KIRC patients with high mutation burden and neoantigen load, leading to a clear immune checkpoint blockade therapy response.[41,42] Significant improvements in the survival rates of KIRC patients have been observed as a direct result of clinical studies that have specifically targeted immune checkpoints.[43,44] Several factors are positively associated with SLC12A3. However, it is negatively correlated with KDR. It's good to note that VEGFR-2 is a transmembrane receptor kinase protein.[45] VEGFR-2 is a vital protein that aids in tumor angiogenesis, encoded by the KDR gene on chromosome 4.[46] Blocking VEGF/VEGFR-2 signaling can treat cancer by stopping angiogenesis.[47] Lower expression of SLC12A3 correlates with higher KDR, indicating that SLC12A3 may hinder KIRC progression by interfering with VEGF/VEGFR-2 signaling. Pathway antibodies show promise in treating various cancers.[48,49] Our study found a positive correlation between SLC12A3 expression and favorable prognosis in KIRC.[50-53] More focus is needed to study the connection between SLC12A3 and its immune inhibitors in ccRCC and comprehend their interaction mechanism.

Scientists are studying immune cells linked to tumors, which are vital components of the tumor microenvironment (TME).[54,55] Immune cells are the double-edged sword that promotes or restrains tumor development and is crucial in fighting against cancer.[56-58] Immune cells are vital in combatting cancer by detecting and destroying tumor cells in the body.[59] Cancer cells possess numerous mechanisms to avoid detection by immune cells.[60-63] It is a well-established fact that TILs have the individual capability to forecast the outcome of cancer patients,[64] as essential members of TME are involved in specific antitumor immune responses.[65] Neutrophils in mouse transplantation models are known to promote angiogenesis, tumor progression, and metastasis by secreting MMP9 into the TME.[66] Moreover, it has been shown that macrophages, the immune cells responsible for safeguarding the body against tumors, are culpable for promoting the growth and metastasis of these cancerous cells rather than directly attacking them.[67,68] This study unequivocally demonstrates the significant impact of TILs on tumor development. Our study postulated that the SLC12A3 gene is important in the immune response to tumors in patients with KIRC. We found that the expression of SLC12A3 is associated with the presence of six different types of immune cells within tumors. By influencing the status of these TILs, SLC12A3 may either promote or inhibit tumor growth. Our results provide valuable insights into the role of SLC12A3 in immune infiltration.

CONCLUSION

Our findings demonstrated that SLC12A3 expression accounts for favorable prognosis and increased immune infiltration of various cell types. This could lead to potential therapeutic aims and biomarkers for KIRC.

ETHICAL COMPLIANCE

Not applicable.

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CONFLICT OF INTEREST

The authors assert that no conflict of interest exists.

AUTHORS CONTRIBUTIONS

Qian Gao and Xia Ye contributed equally to this work. QG, XY, and FL designed the study and performed the experiments, QG and XY collected the data, FL analyzed the data, and QG, XY, and FL prepared the manuscript. All authors read and approved the final manuscript.

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