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Original Article

Elucidating cuproptosis-related gene SLC31A1 diagnostic and prognostic values in cancer

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Abstract: Objectives: Cancer remains a global health challenge, necessitating the identification of novel biomarkers and therapeutic targets. Cuproptosis, a recently recognized form of cell death linked to copper metabolism, presents a promising avenue for anticancer strategies. We investigated the clinical significance of SLC31A1, a key regulator of cuproptosis, in multiple cancer types, aiming to elucidate its potential as a diagnostic biomarker, prognostic, indicator and therapeutic target. Methods: We conducted a pan-cancer analysis through TIMER2.0, evaluating SLC31A1 expression across multiple cancer types. Survival analysis was performed using KM plotter. Expression validation was carried out using UALCAN and Human Protein Atlas (HPA) databases. Methylation analysis was conducted with the help of ULACAN and OncoDB. Mutational analysis was performed using cBioPortal database. Immune infiltration analysis via the TIMER2.0 and gene enrichment analysis via the Metascape were performed to gain insights into the potential mechanisms underlying SLC31A1's role in cancer. Finally, Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was employed to confirm SLC31A1 expression in clinical samples. Results: Out of analyzed cancer, SLC31A1 exhibited significant up-regulation and correlation with worse overall survival (OS) across Breast Cancer (BRCA), Cervical Squamous Cell Carcinoma (CESC), Head and Neck Squamous Cell Carcinoma (HNSC), and Esophageal Carcinoma (ESCA). Mutational and promoter methylation analyses further revealed that hypomethylation is the major cause of SLC31A1 overexpression among BRCA, CESC, HNSC, and ESCA. Immune infiltration analysis showed significant associations between SLC31A1 expression and the presence of CD8+ T cells, CD4+ T cells, and macrophages in the tumor microenvironment. Gene enrichment analysis provided valuable insights into potential molecular pathways in context to BRCA, CESC, HNSC, and ESCA. Furthermore, when SLC31A1 was analyzed using clinical samples through RT-qPCR, this gene showed promising diagnostic potential, reflected by high Area Under the Curve (AUC) values. Conclusion: Our pan-cancer study highlights the up-regulation of SLC31A1 and its correlation with worse OS in BRCA, CESC, HNSC, and ESCA. In sum, outcomes of this study showed that SLC31A1 could be a potential biomarker and novel therapeutic target of BRCA, CESC, HNSC, and ESCA.

Keywords: SLC31A1, pan-cancer analysis, biomarker

Introduction

Cancer stands as the foremost global cause of mortality, inflicting significant healthcare and socio-economic challenges [1-6]. Current approaches to cancer treatment encompass surgical interventions, chemotherapy, radiotherapy, targeted therapy, and immunotherapy [7]. Despite encountering challenges such as drug resistance, adverse effects, and persisting uncertainties, the prognosis and survival rates continue to fall short of expectations [8-10]. Therefore, it is imperative to improve prognosis prediction and identify novel treatment targets for cancer patients.

The SLC31A1 gene, encoding the copper transporter protein Ctr1, is a fundamental player in

maintaining copper homeostasis within the human body [11]. Copper, an essential trace element, serves as a cofactor for a myriad of enzymes critical for various physiological processes, including energy production, antioxidant defense, and connective tissue formation [12]. As such, the regulation of copper uptake and distribution is meticulously controlled, as deviations from the normal can result in cytotoxicity and severe health consequences [13]. Recent advancements in our understanding of copper metabolism have unveiled a new layer of complexity in the context of human diseases, particularly in cancer [14]. Dysregulation of copper homeostasis has been implicated in various aspects of tumor progression, including angiogenesis, metastasis, and drug resistance [15]. Furthermore, the emergence of a novel cell death mechanism known as cuproptosis, initially proposed by Tsvetkov and colleagues, has shed light on the intricate relationship between copper and cancer [16].

The SLC31A1 gene, responsible for mediating the cellular uptake of dietary copper, has garnered significant attention in the field of cancer research. Studies have begun to uncover the pivotal roles of SLC31A1 in the context of different cancer types, such as lung, stomach, kidney, and colorectal cancers [17, 18]. Its intricate involvement in copper transport and its potential impact on tumor development make SLC31A1 a compelling candidate for in-depth investigation.

In response to the increasing recognition of SLC31A1's significance in cancer biology, we embarked on a comprehensive pan-cancer analysis. Leveraging state-of-the-art bioinformatics and wet laboratory methodologies, we aim to unravel the molecular intricacies of SLC31A1 across diverse cancer types. Our study encompasses an in-depth exploration of SLC31A1 expression patterns, clinical prognostic associations, gene enrichment, and immune infiltration, providing a holistic view of its impact on cancer biology.

In summary, this research endeavors to elucidate the multifaceted roles of the SLC31A1 gene in the context of various cancers. By dissecting its molecular functions, clinical implications, and potential therapeutic relevance, we aim to contribute valuable insights that may ultimately inform novel diagnostic approaches and therapeutic strategies for cancer patients.

Methods

Expression analysis of SLC31A1 in pan-cancer view

TIMER2.0 stands as a pivotal database, revolutionizing pan-cancer analysis by providing invaluable insights into the intricate interplay between tumor biology and the immune system [19]. Building upon its predecessor, TIMER (Tumor Immune Estimation Resource), this enhanced platform offers an expansive suite of tools and resources for researchers. In the present study, GEPIA2 platform was utilized with default settings for the expression analysis of the SLC31A1 in pan-cancer view.

Survival analysis of SLC31A1

The Kaplan Meier (KM) plotter is a vital tool in the realm of survival analysis [20]. This web-based platform harnesses extensive clinical data to assess the impact of specific genes on patient survival across various cancer types. Researchers can easily explore gene expression's prognostic value, identifying potential prognostic biomarkers. KM Plotter's intuitive interface offers Kaplan-Meier survival curves, providing insights into how gene expression correlates with patient outcomes. In this study, KM plotter tool was used to analyze the effect of SLC31A1 dysregulation on the overall survival (OS) of cancer patients.

Expression analysis of SLC31A1 across different stages of the specified cancers

The UALCAN database is a powerful and user-friendly resource for cancer researchers [21]. It harnesses extensive data from The Cancer Genome Atlas (TCGA) to facilitate in-depth investigations into gene expression, protein abundance, and patient survival in various cancer types. UALCAN offers a user-friendly interface, allowing researchers to explore and visualize gene expression patterns across different tumor stages, molecular subtypes, and patient demographics. In the present study, this database was used for the expression analysis of SLC31A1 across different stages of the specified cancers, in which this gene shows signifi-

cant dysregulation as well as significant correlation with worse OS.

Expression confirmation of SLC31A1 at the protein level

The Human Protein Atlas (HPA) database is a comprehensive resource providing insights into the human proteome [22]. It offers a wealth of information on the expression, localization, and function of human proteins across various tissues and cell types. Researchers and clinicians rely on HPA to explore protein expression patterns in health and disease, aiding in the identification of potential biomarkers and therapeutic targets. In this study, we employed the HPA to confirm the expression of SLC31A1 at the protein level.

Mutational analysis of SLC31A1

cBioPortal is a pivotal database for cancer genomics research [23]. It offers an intuitive platform to explore large-scale cancer genomic datasets, enabling researchers to delve into genetic alterations, pathways, and clinical relevance across various cancer types. With user-friendly visualization tools, it simplifies the analysis of complex genomic data, making it accessible to a wide range of scientists. This database was used in the present study to perform mutational analysis of SLC31A1 across different cancers.

Promoter methylation analysis of SLC31A1

OncoDB database serves as comprehensive platform housing information on cancer-related genes, mutations, expression, promoter methylation level, and associated functional data [24]. Researchers can access curated and up-to-date genomic data, enhancing their ability to investigate the molecular underpinnings of cancer. In our study, we used UALCAN and OncoDB databases for promoter methylation analysis of SLC31A1 in different cancers.

Assessment of association between SLC31A1 and immune cell infiltration

TIMER2.0 also offers a platform to explore the complex interplay between tumors and the immune system [19]. In the current work, TIMER2.0 was used to explore associations between SLC31A1 expression and infiltration level of CD8+ T cells, CD4+ T cells, and macrophages immune cells.

PPI development and gene enrichment analysis of SLC31A1

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database is an essential resource for exploring protein-protein interactions [25]. It compiles a wealth of data to help researchers unravel the complex networks of protein relationships. We used STRING in our study for the construction of SLC31A1 protein.

Metascape is another valuable database for functional enrichment analysis [26]. It streamlines the interpretation of large-scale omics data by identifying enriched biological processes, pathways, and molecular functions. Metascape's user-friendly interface and comprehensive gene annotation help researchers gain insights into the biological significance of their datasets, facilitating discoveries in diverse fields, from genomics to proteomics. In this study, we used this resource for the gene enrichment analysis of the SCL31A1-assocated genes.

Sample collection, RNA preparation, and quantitative real-time PCR (qRT-PCR)

In Multan's Nishtar Hospital, we gathered a total of 18 BRCA tissue samples; each paired with corresponding adjacent control samples. Ethical approval was obtained from the Pakistan Agriculture Research Institute (PARC) after securing informed consent from all patients involved in the study. RNA extraction from the collected cells was performed using the TRIzol reagent from Invitrogen, based in MA, United States. The isolated RNA was subsequently utilized for reverse transcription, employing the HiScript Q RT SuperMix for qPCR from Vazyme in Jiangsu, China. Quantitative RT-PCR assays were carried out using the SYBR Green PCR Master Mix, also from Vazyme, and the ABI Prism 7,900 Sequence detection system, provided by Applied Biosystems in Canada. GAPDH served as the internal control, and the results for each sample were normalized to GAPDH expression levels. The emitted fluorescence signals during RT-qPCR were captured

using the Applied Biosystems Sequence Detection Software (SDS v1.3.1). Subsequently, calculations were performed to determine Cq/CT (cycle quantification/cycle threshold), ΔCT, and ΔΔCT values, following the established 2-ΔΔCT method [21]. A student t-test was applied to find differences in the expression levels of DEGs between OP and normal control samples. A *p*-value < 0.05 was considered a significant difference. The following primers of GAPDH and SCL31A1 were used for RT-qPCR analysis.

GAPDH-F 5'-ACCCACTCCTCCACCTTTGAC-3', GAPDH-R 5'-CTGTTGCTGTAGCCAAATTCG-3'; SLC31A1-F: 5'-GGGGATGAGCTATATGGACTCC-3', SLC31A1-R: 5'-TCACCAAACCGGAAAACAGTAG-3'.

Receiver operating characteristic (ROC) curve

The ROC curve serves as a comprehensive metric that encapsulates the continuous variables of sensitivity and specificity. The Area under the ROC curve (AUC) serves as an indicator of the diagnostic performance of the test. Typically, an AUC exceeding 0.7 is indicative of an accurate diagnostic test. The ROC curve analysis was executed utilizing the SLC31A1 RT-qPCR expression data.

Statistics details

For enrichment analysis, we used Fisher's Exact test for computing statistical difference. Correlational analyses were carried out using the Pearson method. For comparisons, a student t-test was adopted in the current study. All the analyses were carried out in R version 3.6.3 software.

Results

Expression analysis of SLC31A1 in pan-cancer view

Utilizing TIMER2, our initial exploration focused on SLC31A1 expression across both normal and malignant pan-cancer tissues (**Figure 1A**). Our findings unveiled substantial variations in SLC31A1 expression patterns. Notably, certain tumors, such as cholangiocarcinoma (CHOL), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and thyroid carcinoma (THCA), exhibited significantly lower SLC31A1 expression compared to adjacent normal tissues (**Figure 1A**). Conversely, SLC31A1 expression showed marked up-regulation in tumors like bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), and uterine corpus endometrial carcinoma (UCEC) (**Figure 1A**). However, SLC31A1 expression variations in other cancers, including colon adenocarcinoma (COAD), kidney chromophobe (KICH), pancreatic adenocarcinoma (PAAD), rectum adenocarcinoma (READ), remained insignificant (**Figure 1A**).

Survival analysis of SLC31A1

Subsequently, for a more comprehensive exploration of the clinical significance associated with the cuproptosis-related SLC31A1 gene, we employed the KM plotter tool to assess patients' OS. As depicted in **Figure 1B**, **1C**, our analysis revealed that in BRCA (P = 0.0086), CESC (P = 0.05), HNSC (P = 0.028), and ESCA (P = 0.044), individuals exhibiting high SLC31A1 expression experienced significantly shorter OS compared to those with low SLC31A1 expression. These findings underscore the pivotal role of SLC31A1 in patient survival, particularly within the contexts of BRCA, CESC, HNSC, and ESCA.

Confirmation of SLC31A1 protein expression and examination of variations across different cancer stages in BRCA, CESC, HNSC, and ESCA

In the meantime, we conducted an examination of SLC31A1 expression in patients with various cancer stages in BRCA, CESC, HNSC, and ESCA using UALCAN. Our analysis revealed a notable and statistically significant overexpression of SLC31A1 in individuals at different cancer stages within these specific cancer types when compared to control groups (**Figure 2A**).

Next, we conducted a comprehensive analysis of SLC31A1 expression at the protein level using HPA database. Our examination involved immunohistochemical staining evaluation of SLC31A1 in primary tumor tissues of BRCA,

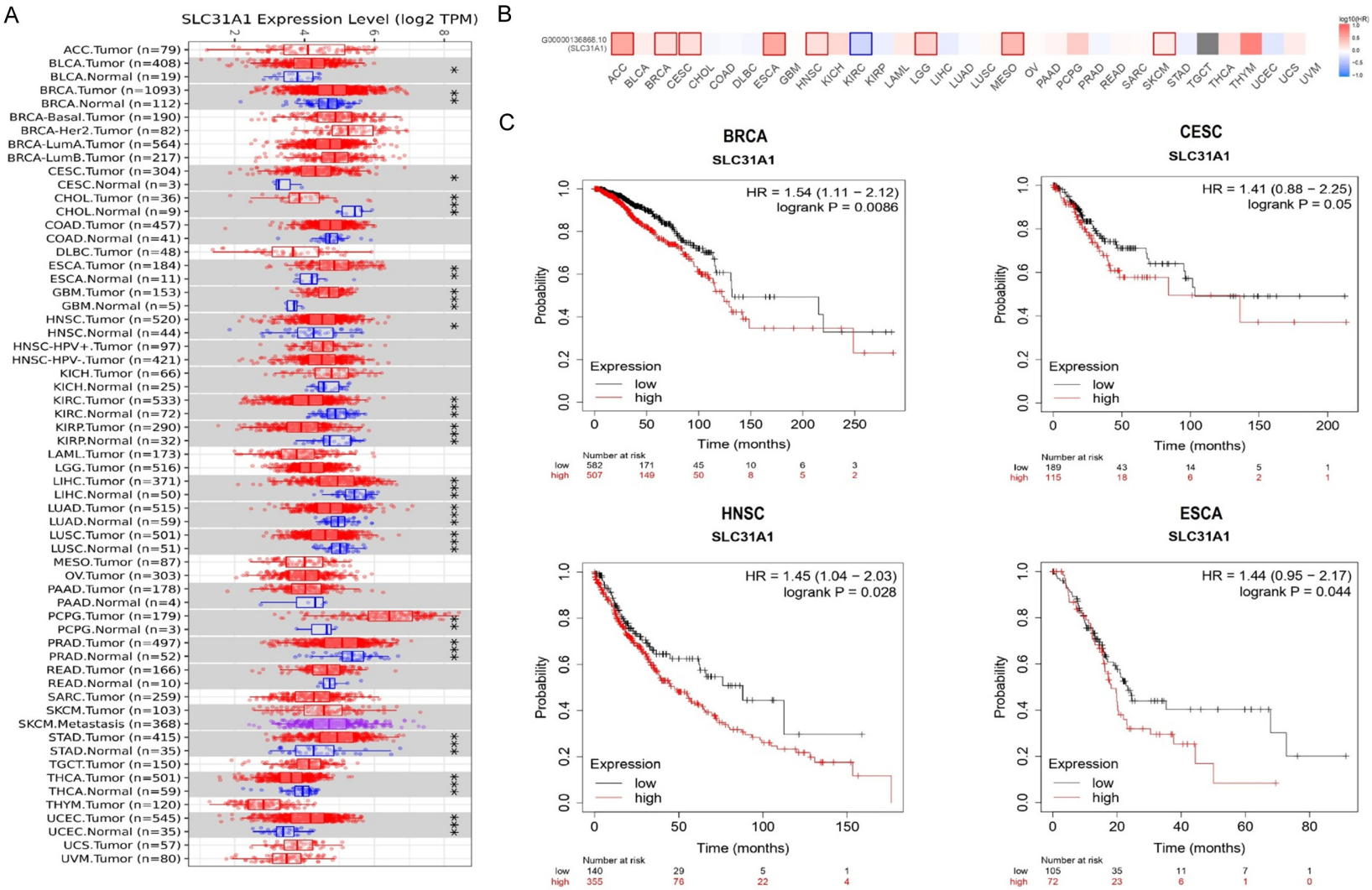

Figure 1. Expression and survival analysis of SLC31A1 across various cancers. (A) SLC31A1 expression in various cancer types compared to normal controls assessed using TIMER2.0 and (B, C) Kaplan-Meier plots depicting overall survival (OS) based on SLC31A1 expression. Significance ($P < 0.05$) indicates its impact on patient survival.

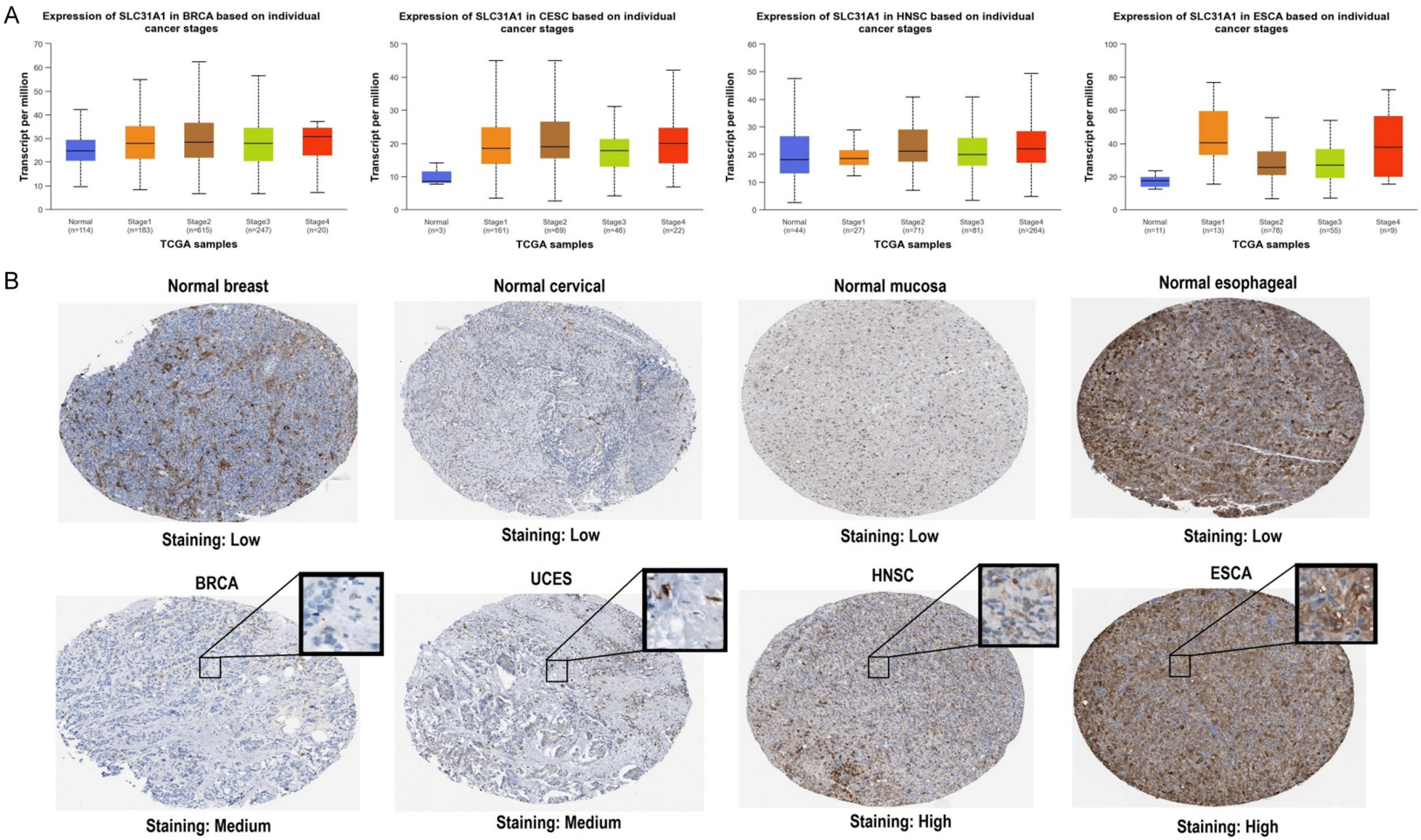

Figure 2. Expression analysis outcomes of SLC31A1 across BRCA, CESC, HNSC, and ESCA. (A) UALCAN-based results of SLC31A1 expression analysis within BRCA, CESC, HNSC, and ESCA patients belonging to different cancer stages and (B) This subfigure represents immunohistochemical (IHC) staining images of SLC31A1, comparing normal tissue samples with those of BRCA, CESC, HNSC, and ESCA. A *p*-value < 0.05 was considered significant. BRCA = Breast Cancer, CESC = Cervical Squamous Cell Carcinoma, HNSC = Head and Neck Squamous Cell Carcinoma, ESCA = Esophageal Carcinoma.

CESC, HNSC, and ESCA, in comparison to normal tissues. The results depicted in **Figure 2B** showcased notably stronger SLC31A1 staining in the primary tumor tissues of these cancer types than in their corresponding normal tissues. This compelling evidence led us to infer a pivotal role for SLC31A1 in driving the progression of BRCA, CESC, HNSC, and ESCA.

Promoter methylation analysis of SLC31A1 in BRCA, CESC, HNSC, and ESCA

Numerous investigations have highlighted the critical role of promoter methylation in shaping gene expression dynamics, emphasizing its pivotal involvement in the oncogenic pathways implicated in tumorigenesis [27, 28]. Therefore, we analyzed the disparity in promoter methylation of SLC31A1 between BRCA, CESC, HNSC, and ESCA tissues and normal controls using UALCAN and OncoDB databases. In BRCA, CESC, HNSC, and ESCA, the levels of SLC31A1 promoter methylation were found to be significantly decreased as compare to control samples (**Figure 3**). These results indicated that SLC31A1 promoter methylation may result in the transcription overexpression of SLC31A1 in BRCA, CESC, HNSC, and ESCA.

Mutational analysis of SLC31A1 in BRCA, CESC, HNSC, and ESCA

To explore the genomic alterations of SLC31A1 in BRCA, CESC, HNSC, and ESCA, we employed the cBioPortal platform to assess TCGA datasets for mutations detection. As depicted in **Figure 4A**, the mutation rates in BRCA, CESC, HNSC, and ESCA were notably low, hovering around 0.9%, 0.78%, 0.76%, and 0%, respectively. Furthermore, the predominant type of SLC31A1 alteration observed in these cancer patients was gene amplification, as depicted in **Figure 4A**. Conversely, only a limited number of mutations were identified within the SLC31A1 gene, specifically affecting the amino acids in the encoded protein (**Figure 4B**, **4C**). In summary, our analysis unveiled genetic variations with minimal impact on the dysregulation of SLC31A1 in BRCA, CESC, HNSC, and ESCA. Nevertheless, further validation through additional clinical data is imperative to substantiate this apparent relationship.

Assessment of association between SLC31A1 and immune cell infiltration

Recent research has illuminated the pivotal contributions of immune cells, specifically T cells and tumor-associated macrophages, in driving tumor advancement [29, 30]. In this study, we harnessed the TIMER algorithm to explore the association between SLC31A1 and the infiltration of immune cell subtypes, including CD8+ T cells, CD4+ T cells, and macrophages within BRCA, CESC, HNSC, and ESCA. In **Figure 5**, it becomes evident that SLC31A1 exhibits a noteworthy positive correlation with the presence of CD8+ T cells, CD4+ T cells, and macrophages across BRCA, CESC, HNSC, and ESCA (**Figure 5**). This crucial finding underscores the involvement of SLC31A1 in tumor development within the context of immune cell activity, potentially positioning it as a promising novel biomarker for immune cell infiltration.

PPI network and enrichment analysis of SLC31A1

Firstly, the STRING database unveiled an intricate network encompassing a total of 10 genes associated with SLC31A1 (**Figure 6A**). Subsequently, in our quest to decipher the molecular mechanisms underpinning SLC31A1's oncogenic functions in conjunction with these 10 genes, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses on this gene set employing the Metascape tool. Via the GO analysis, it was noted that SCL31A1 enriched genes were associated with a variety of GO terms (**Figure 6B-E**). For example, with "melanosome membrane, chitosome, pigment and granule membrane" etc., Cellular components (CC) terms (**Figure 6B**), "copper-dependent protein binding, copper ion transmembrane transporter activity, and copper chaperon activity" etc., Molecular Function (MF) terms (**Figure 6C**), and "copper ion export, copper ion transmembrane transport, and copper ion import" etc., Biological Process (BP) terms (**Figure 6D**). Moreover, as shown in **Figure 6E**, KEGG analysis results further revealed the notable involvement of these genes in the dysregulation of different diverse pathways, including "mineral absorption, platinum drug resistance, and ferroptosis".

Verification of SLC31A1 expression through RT-qPCR

For the confirmation of SLC31A1 expression, we employed 18 clinical tissue samples from BRCA patients, each paired with adjacent control samples, utilizing the RT-qPCR technique.

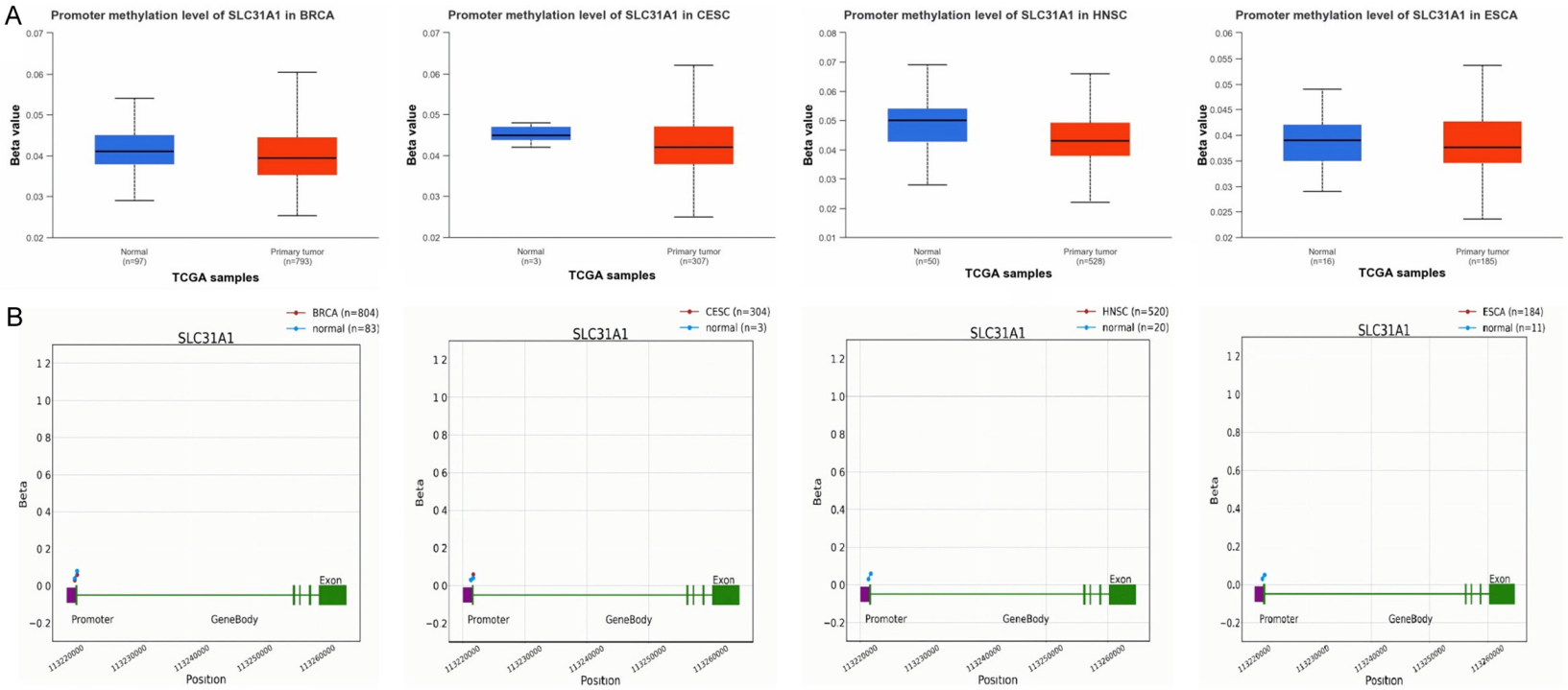

Figure 3. Promoter methylation analysis of SLC31A1 in BRCA, CESC, HNSC, and ESCA using UALCAN and OncoDB databases. (A) This subfigure illustrates the outcomes of promoter methylation analysis conducted on the SLC31A1 gene across BRCA, CESC, HNSC, and ESCA via the UALCAN database and (B) This subfigure illustrates the outcomes of promoter methylation analysis conducted on the SLC31A1 gene across BRCA, CESC, HNSC, and ESCA via the OncoDB database. A *p*-value < 0.05 was considered significant. BRCA = Breast Cancer, CESC = Cervical Squamous Cell Carcinoma, HNSC = Head and Neck Squamous Cell Carcinoma, ESCA = Esophageal Carcinoma.

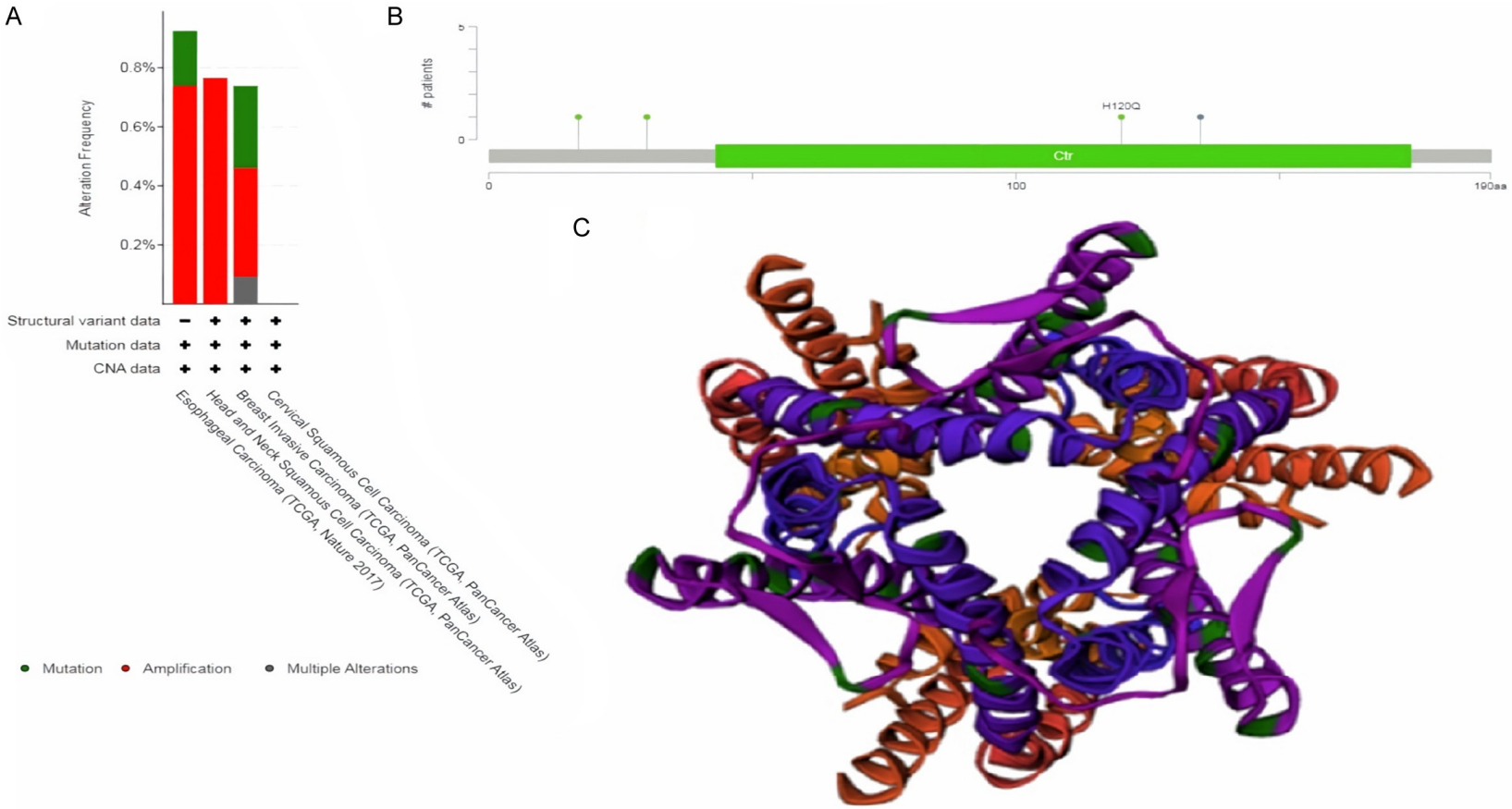

Figure 4. Mutational analysis outcomes of SLC31A1 across BRCA, CESC, HNSC, and ESCA using cBioPortal database. (A, B) Diverse mutation patterns of SLC31A1 observed in BRCA, CESC, HNSC, and ESCA within the cBioPortal database, along with specific protein-level mutation sites and (C) The 3D protein structure of SLC31A1. BRCA = Breast Cancer, CESC = Cervical Squamous Cell Carcinoma, HNSC = Head and Neck Squamous Cell Carcinoma, ESCA = Esophageal Carcinoma, 3D = 3 dimensional.

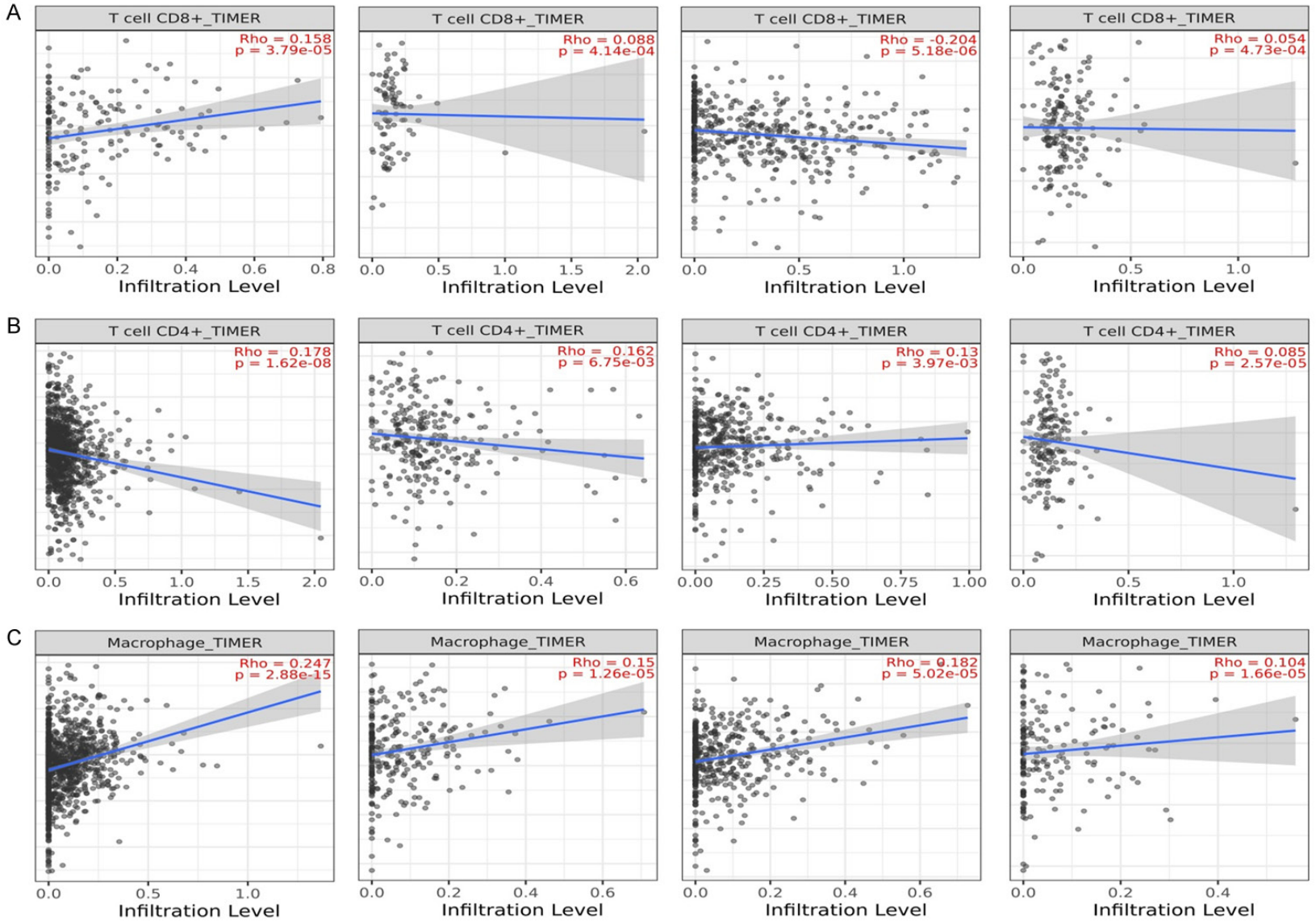

Figure 5. Immune cell infiltration analyses of SLC31A1 in BRCA, CESC, HNSC, and ESCA samples. A-C. TIMER-based correlation analysis of SLC31A1 genes expression and infiltration levels of CD8+ T cells, CD4+ T cells, and Macrophages across BRCA, CESC, HNSC, and ESCA samples. A *p*-value < 0.05 was considered significant. BRCA = Breast Cancer, CESC = Cervical Squamous Cell Carcinoma, HNSC = Head and Neck Squamous Cell Carcinoma, ESCA = Esophageal Carcinoma.

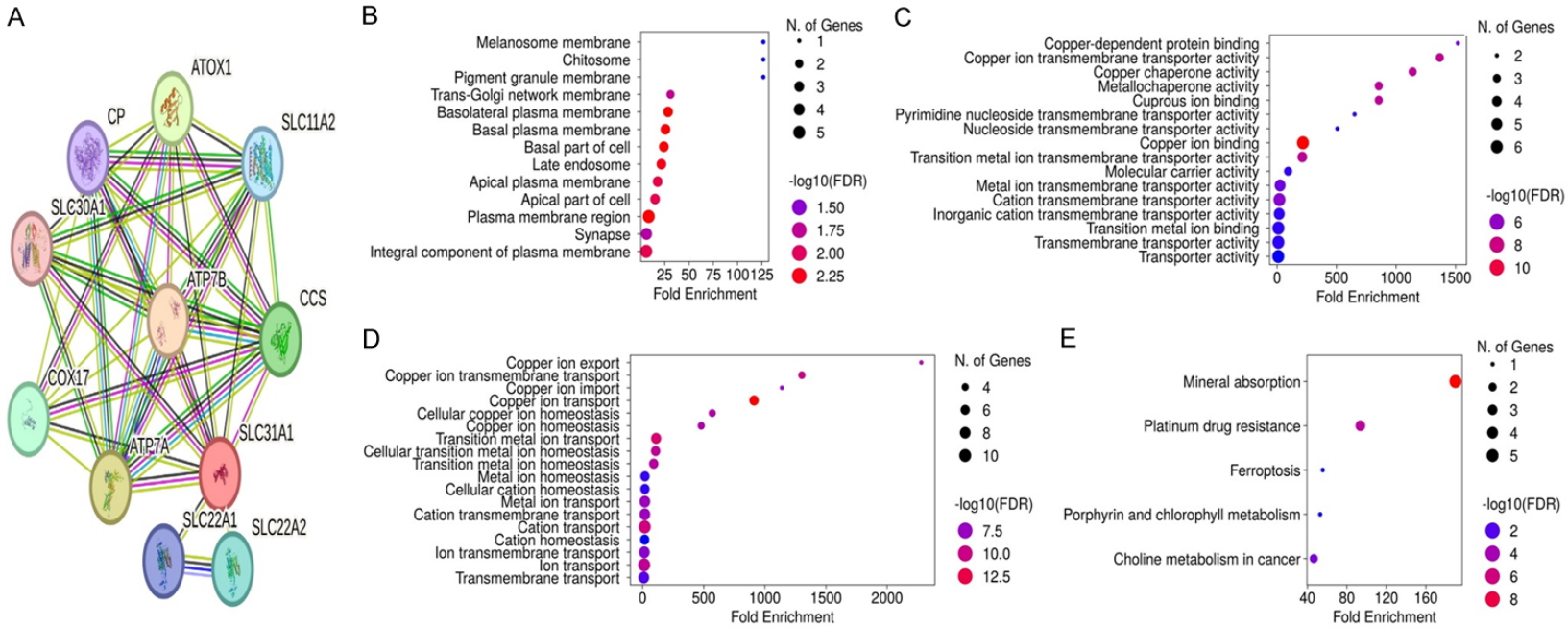

Figure 6. Gene enrichment analysis of SLC31A1-associated genes via the Metascape. (A) This panel present PPI of SLC30A1-enriched genes, (B) This panel highlights CC terms, (C) This panel highlights BP terms, (D) This panel highlights MF terms, and (E) This panel highlights KEGG terms. A *p*-value < 0.05 was considered significant. CC = Cellular Component, BP = Biological Process, MF = Molecular Function, KEGG = Kyoto Encyclopedia of Genes and Genomes.

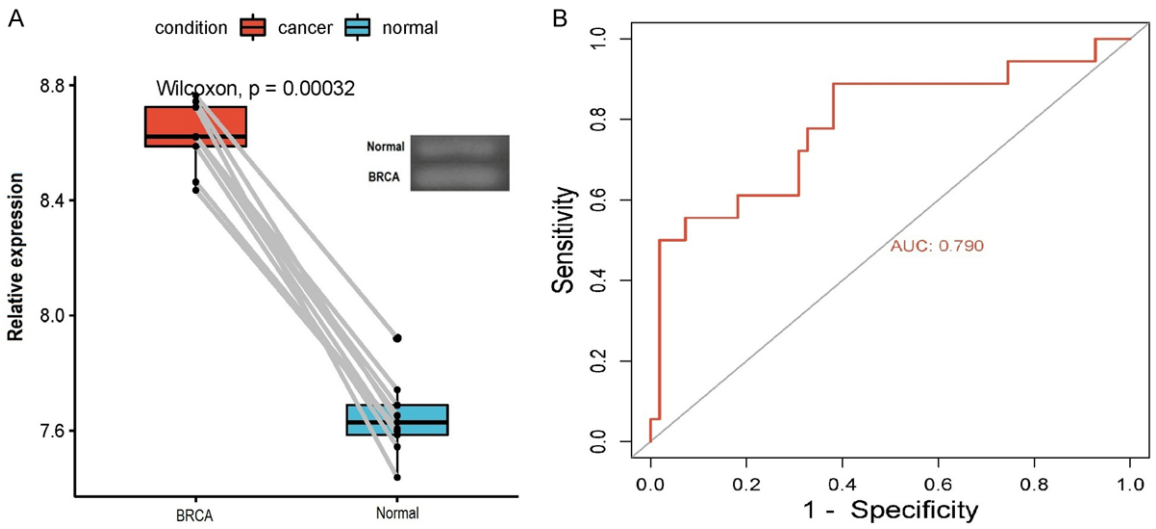

Figure 7. RT-qPCR-based validation of mRNA expression level of SLC31A1 in clinical BRCA samples paired with controls. (A) RT-qPCR analysis outcomes of SLC31A1 across BRCA samples paired with controls and (B) RT-qPCR expression-based ROC analysis outcomes of SCL31A1. A *p*-value < 0.05 was considered significant. ROC = Receiver operating characteristic curve, RT-qPCR = Reverse transcription-quantitative polymerase chain reaction.

This analysis revealed a notably elevated mRNA expression level of SLC31A1 in BRCA tissues in comparison to the corresponding normal control tissues (**Figure 7A**). Additionally, an ROC curve was constructed for SLC31A1, revealing an impressive AUC of 0.790 with a *p*-value below 0.05, based on its expression level (**Figure 7B**). These ROC curves highlighted substantial diagnostic potential indicative of exceptional discriminatory capability.

Discussion

In this study, we assessed the clinical relevance of SCL32A1, a pivotal gene involved in regulating cuproptosis, across diverse cancer types. Our findings suggest a potentially significant involvement of cuproptosis in the context of cancer. The recent insights into the pathophysiological implications of cuproptosis have opened up new avenues for understanding anticancer treatments. SLC31A1, a fundamental regulator of copper (Cu) uptake, is ubiquitously expressed in most cell types [31, 32]. Cuproptosis represents an emerging mechanism of cell death intricately tied to the tricarboxylic acid cycle and reliant on mitochondrial respiration [33]. Notably, SLC31A1 has been proposed as a potential biomarker in the context of cancer therapy and has been implicated in chemoresistance across specific cancer types [34, 35]. High-affinity copper uptake protein 1 (CTR1), encoded by SLC31A1, serves as the primary conduit for cellular Cu uptake [36, 37]. In a recent study, CTR1 was revealed to potentially function as a redox sensor capable of driving neovascularization [38]. This discovery, coupled with a robust correlation between CTR1 and Programmed death-ligand 1 (PD-L1), has catalyzed clinical trials assessing Cu chelators as promising candidates for immune checkpoint inhibitors [39]. In the context of the present study, our comprehensive exploration aimed to elucidate the potential role of SLC31A1 in the multifaceted landscape of multiple tumor types.

Our investigation uncovered substantial upregulation of SLC31A1 at both the mRNA and protein levels in the tumor tissues of BRCA, CESC, HNSC, and ESCA when compared to their respective normal controls. Also, this study revealed that higher expression of SLC31A1 was associated with the poor OS of the BRCA, CESC, HNSC, and ESCA patients. The overexpression of SLC31A1 in cancer cells is intriguing, considering its primary role as a copper transporter. Copper is an essential micronutrient that participates in critical cellular processes, including angiogenesis, energy production, and oxidative stress response [40, 41]. Therefore, the overexpression of SLC31A1 in BRCA, CESC, HNSC, and ESCA cells may signify a reprogramming of copper homeostasis to sup-

port the energy demands and growth of malignant cells. Moreover, elevated copper levels have been linked to increased tumor angiogenesis, which is consistent with the role of SLC31A1 in facilitating copper uptake [42]. Hence, the up-regulation of SLC31A1 might be a crucial factor in promoting tumor progression. To the best of our knowledge, this study is the first to report SLC31A1 as the pan-cancer biomarker of BRCA, CESC, HNSC, and ESCA.

Furthermore, to comprehensively understand the genetic landscape of SLC31A1 in BRCA, CESC, HNSC, and ESCA, we explored its mutational profile using the cBioPortal platform. Surprisingly, we observed a relatively low mutation rate in SLC31A1, with gene amplification being the predominant genetic alteration. These findings suggest that, in these cancers, SLC31A1's oncogenic effects might primarily be driven by changes in gene copy numbers rather than frequent mutations directly affecting the protein's structure or function.

Amplifications in SLC31A1 may lead to increased copper uptake by cancer cells, potentially fueling the pro-tumorigenic processes that copper supports. Copper is involved in the activation of key enzymes in cellular respiration, such as cytochrome c oxidase, which plays a pivotal role in oxidative phosphorylation [43, 44]. Consequently, amplifications in SLC31A1 could enhance copper delivery to mitochondria, boosting cellular energy production and promoting cancer cell survival.

The interplay between SLC31A1 and the tumor microenvironment, particularly immune cell infiltration, emerged as a significant aspect of our study. Our analysis using the TIMER algorithm unveiled a striking positive correlation between SLC31A1 expression and immune cell infiltration, with a focus on CD8+ T cells, CD4+ T cells, and macrophages across the selected cancer types. This observation suggests that SLC31A1 may influence the immune landscape within the tumor microenvironment.

The tumor microenvironment is characterized by a complex interplay between cancer cells and various immune cell populations. Tumor-infiltrating lymphocytes, such as CD8+ T cells and CD4+ T cells, play pivotal roles in the anti-tumor immune response by recognizing and targeting cancer cells [45, 46]. Macrophages, on the other hand, exhibit diverse polarization states and can either promote or inhibit tumor progression, depending on their functional orientation [47].

The positive correlation between SLC31A1 expression and immune cell infiltration raises intriguing questions about its potential immunomodulatory effects. It is conceivable that SLC31A1, through its role in copper transport and its impact on oxidative stress, might shape the immune microenvironment. Copper ions, for instance, are known to influence immune cell function, affecting the proliferation and activation of T cells [48, 49]. The observed correlation could signify that elevated SLC31A1 expression creates an immune-permissive microenvironment, potentially promoting tumor immune evasion. Alternatively, it might reflect an ongoing immune response against the tumor, eliciting increased SLC31A1 expression as a compensatory mechanism.

Conclusion

This comprehensive pan-cancer investigation reveals the up-regulation of the cuproptosis-regulatory gene SLC31A1, which is associated with poorer OS in patients with BRCA, CESC, HNSC, and ESCA. Furthermore, our analysis of immune infiltration and gene enrichment provides new insight into potential mechanisms related to SLC31A1 in cancers. In sum, these findings underscore the need for additional experimental and clinical research to unravel the functional role of SLC31A1 and explore its potential practical utility in cancer therapy, diagnosis, and prognostic predictions.

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Disclosure of conflict of interest

None.

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