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Research article

# Pan-cancer analysis reveals copper transporters as promising potential targets

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ABSTRACT

*Background:* Copper transport proteins (*SLC31A1, ATP7A, ATP7B*) regulate copper levels in the body and may be involved in tumor development. However, their comprehensive expression and function across various cancers remain unclear.

*Methods:* The expressions of copper transporters in 33 tumors and normal tissues were analyzed using TCGA, GTEx, CCLE, ULCAN, and HPA databases. Cox regression assessed their impact on patient survival. Gene alterations were explored using cBioPortal. Spearman correlation tests were performed to investigate the associations between copper transporters and tumor mutation burden (TMB), microsatellite instability (MSI), and infiltration of immune cells. Gene functions were analyzed using STRING and GeneMANIA databases. Drug sensitivity was assessed using GSCALite database. *ATP7B* expression in lung squamous cell carcinoma (LUSC) was validated by immunohistochemical staining.

*Results:* Copper transporters exhibited variable expression patterns across various cancer types, indicating their potential dual role as either oncogenes or tumor suppressor genes, depending on the cancer type. Significant associations were found between these transporters and tumor stage, as well as prognosis in most tumors studied. Pathway analysis identified links between copper transporters and tumor-related pathways like apoptosis and RAS/MAPK. Copy number variation (CNV) analysis revealed varying degrees of gene amplification and deletion of copper transporters in most tumors. Copper transporters exhibited strong correlations with immune features, including TMB, MSI, and immune-infiltrating cells, suggesting their potential role in guiding immunotherapy. They were also associated with sensitivity to various chemotherapeutic and immunotherapeutic drugs. Immunohistochemical tests validated the correlation between elevated *ATP7B* level and worse progression-free survival (PFS) in LUSC.

*Conclusion:* Copper transporters may serve as potential tumor markers and therapeutic targets.

## 1. Introduction

Globally, cancer is now the primary reason for mortality [1]. The main reason for the high death rate is the lack of effective

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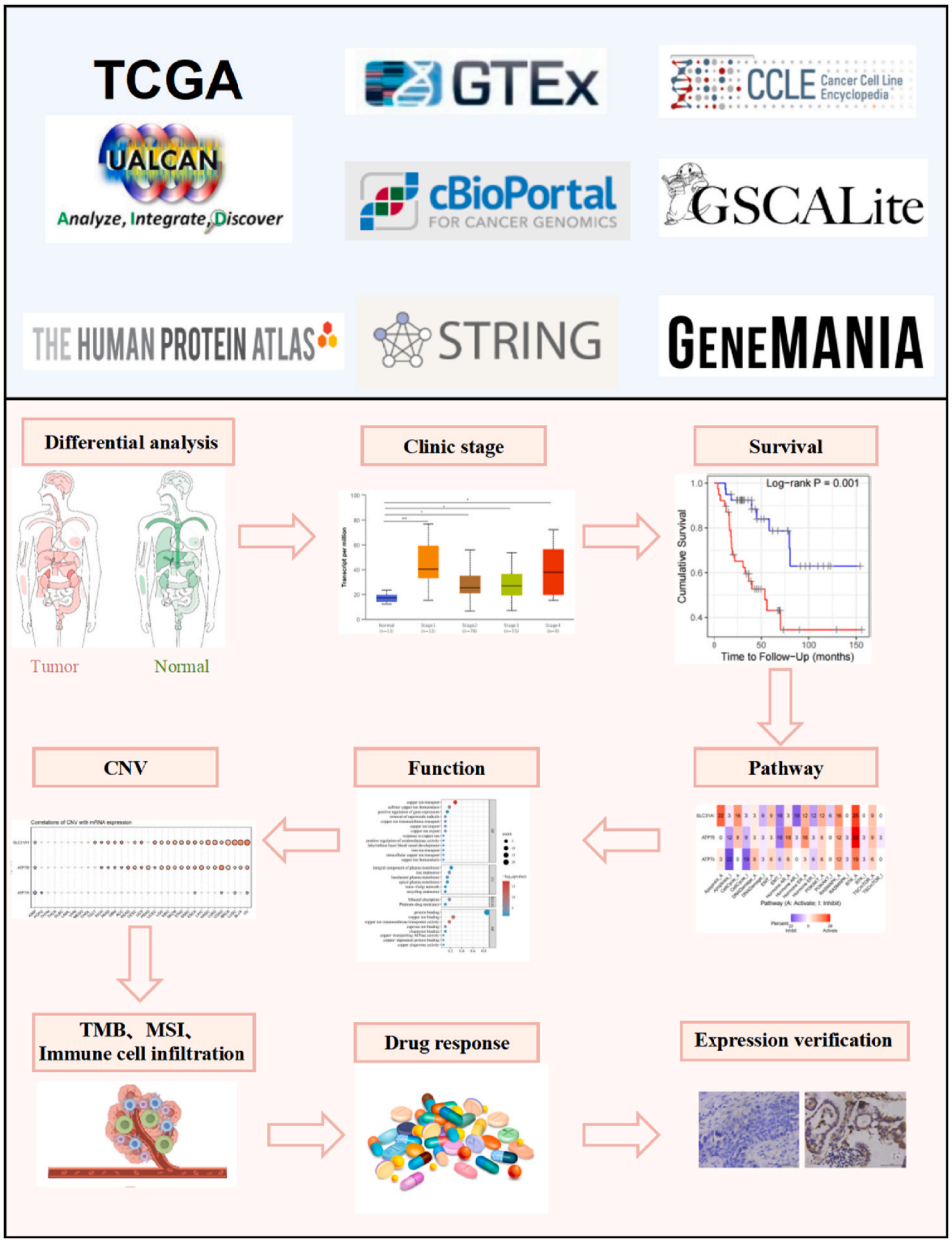


**Fig. 1. Flowchart of the study design.** By comprehensive use of datasets from TCGA, CCLE, GTEx, UALCAN, cBioPortal, GSCALite, HPA, STRING, GeneMANIA, we explored the association of copper transporters with tumor stages, prognosis, cancer-related signaling pathways, gene mutation, immune cell infiltration, and drug sensitivity in 33 tumors.

treatment methods and unclear understanding of how the disease spreads [2]. Hence, it is crucial to discover novel therapeutic targets and create innovative approaches for treating cancer.

Copper, an essential trace element, plays a key role in multiple biological functions including protecting against oxidative stress, supporting cellular energy production, and enhancing the immune system [3]. Intracellular copper concentration is typically maintained within a homeostatic range. Deviations, either through overload or deficiency, can lead to cytotoxic effects and potential cell death [4]. Organisms have evolved intricate systems to maintain copper balance to reduce the harmful impacts [5]. Solute carrier family 31 member 1 (*SLC31A1*), a high-affinity copper transporter, helps bring copper into cells, while ATPase Copper Transporting Alpha (*ATP7A*) and ATPase Copper Transporting Beta (*ATP7B*) work to remove extra copper. Together, these three copper transporters maintain cellular copper balance [6].

Recent research has emphasized the importance of copper transporters (*SLC31A1*, *ATP7A*, *ATP7B*) in the field of cancer biology [7]. For instance, increased expression of *SLC31A1* was linked to unfavorable outcomes in breast cancer [8], bladder cancer [9], and glioma

[10], while leading to improved survival in non-small-cell lung cancer [11]. Compared with normal tissues, *ATP7A* expression was significantly elevated in hepatocellular carcinoma [12] and esophageal squamous cell carcinoma [13], while decreased in ovarian carcinoma [14]. Patients with overexpression of *ATP7B* had higher survival rates in gliomas [15], but unfavorable clinical outcomes in hepatocellular carcinoma [16]. These results underscore the dual role of copper transporters and highlight the need for further research to elucidate their specific functions in each cancer type.

This research involved a comprehensive analysis of the mRNA and protein expression patterns of copper transporters in 33 kinds of cancer and normal tissue samples. Information from multiple databases was integrated to investigate the associations between these transporters and different variables such as tumor stages, prognosis, cancer-related pathways, genetic mutations, immune cell infiltration, and drug response. Preliminary verification was conducted through immunohistochemical experiments. The research design overview is illustrated in Fig. 1.

## 2. Methods

### 2.1. Transcriptome analysis of copper transporters

Copper transporters mRNA levels in healthy tissues were sourced from the GTEx database (https://commonfund.nih.gov/GTEx), with tumor tissue levels obtained from the TCGA (https://www.cancer.gov/aboutnci/organization/ccg/research/structural-genomics/tcga). Furthermore, details on mRNA levels in cancer cells were acquired from the Cancer Cell Line Encyclopedia (CCLE) (https://sites.broadinstitute.org/ccle). Transcriptome information from 33 tumors was processed with the 'RMA' software to eliminate NA values and duplicates, then converted to log2(TPM + 1). Finally, the expressions of copper transporters between normal individuals and tumor patients were compared using the R package "limma"and "ggplot2".

### 2.2. Analysis of copper transporters protein levels in pan-cancer tissues

Immunohistochemistry pictures from the Human Protein Atlas (HPA) (https://www.proteinatlas.org/) were utilized to evaluate the varying expressions of copper transporters. Protein expression data differences were acquired through the 'CPTAC' tool within the UALCAN databas(http://ualcan.path.uab.edu/index.html), based on the methodology outlined in a prior research paper [17]. Briefly, Log2 spectral count ratio values from CPTAC were first normalized within each sample profile, and then normalized across all samples. A comparison between normal and cancer tissues was conducted using two sets of t-tests.

### 2.3. Pathological stage analysis

The 'Expression Analysis' module of UALCAN database was employed to investigate the mRNA levels of copper transporters across various cancer stages, using the approach described by Darshan et al. [17]. Gene expression levels were normalized to Transcripts Per Million (TPM). *t*-test was used to explore the correlation between protein expression and pathological stage.

### 2.4. Survival analysis

Data on survival and clinical characteristics were obtained from TCGA for each individual sample. The software packages 'survival' and 'forestplot' were employed to examine the impact of copper transporters on survival metrics including progression-free survival (PFS) and overall survival (OS) [18]. A Cox model was used to estimate the risk of survival (Hazard Ratio, HR) associated with each gene.

### 2.5. Analysis of cancer pathway

Using GSCALite (https://guolab.wchscu.cn/GSCA/#/), this research examined how copper transporters impact the activation of cancer pathways [19]. The pathway activity score (PAS) differences between these subsets were analyzed using the Student's T-test. P-values were corrected for false discovery rate (FDR), with a threshold of FDR $\leq$0.05 indicating statistical significance. A higher PAS for a gene's high expression indicates potential pathway activation, while a lower PAS suggests an inhibitory effect.

### 2.6. Constructing protein-protein interaction (PPI) and gene-gene interaction (GGI) networks

The STRING database (www.string-db.org), a prediction server utilized for analyzing protein-protein interaction (PPI) data, was used to acquire a list of proteins interacting with the copper transporters family [20]. We utilized GeneMANIA (http//genemania.org/) to explore genes that interact with our specified target genes [21]. The gene lists from both sources were combined and analyzed for enrichment using the R package 'ClusterProfiler'.

### 2.7. Mutation analysis

The frequency of changes in copper transporters in different types of cancers was analyzed using the CBioPortal database (https://www.cbioportal.org/) [22]. The percentage of Copy Number Variation (CNV) and its correlation with mRNA expression in each cancer

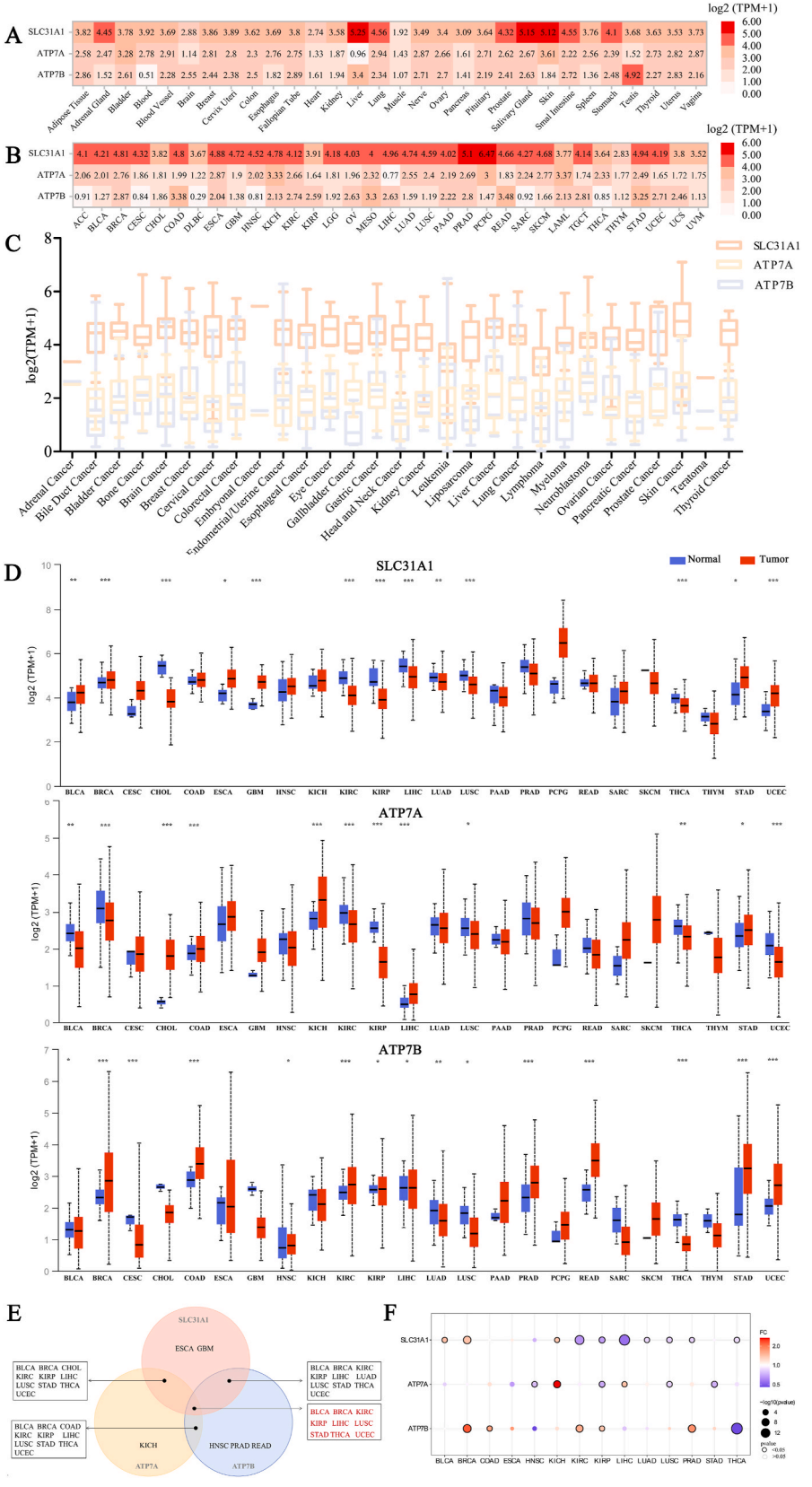


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**Fig. 2.** mRNA expression of copper transporters in tumor and normal samples.
(A) Heatmap of the mRNA expressions of copper transporters in normal tissues. Information was acquired from the GTEx.
(B) Heatmap of the mRNA expression profiles of copper transporters in 33 cancers. Data were sourced from the TCGA.
(C) mRNA expression of copper transporters in tumor cells. Data were downloaded from the CCLE.
(D) Comparison of mRNA expressions of copper transporters between normal individuals and tumor patients. Normal samples were depicted by blue box plots, whereas tumor samples were depicted by red box plots. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.
(E) Venn diagram identifying cancers with differential expression in all three copper transporters. The Venn diagram illustrated the overlap of cancer types where the expressions of all three copper transporters consistently changed.
(F) mRNA differences of copper transporters in tumor and adjacent normal tissues. The fold change (FC) was determined by dividing the average expression in tumor samples by the average expression in normal samples. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

type were calculated. The single nucleotide variation (SNV) percentage was computed using dividing the Number of Mutated Samples by the Number of Cancer Samples.

### *2.8. Correlation of copper transporters expression with tumor mutation burden (TMB) and tumor microsatellite instability (MSI)*

TMB and MSI scores were assessed using mutation data retrieved from TCGA following the method described by Cheng et al. [23]. Briefly, Spearman's method was utilized to examine the relationship between the expression of copper transporters and levels of TMB and MSI.

### *2.9. Tumor immunology analysis*

TCGA was utilized to assess the immune infiltration, utilizing the analysis techniques outlined by Wang and colleagues [24]. Spearman correlation analysis heat map was created to show how immune scores and copper transporter gene expression are related in various types of cancer.

### *2.10. Drug sensitivity analysis*

We examined the associations between gene expression and drug response by using the Genomics of Drug Sensitivity in Cancer (GDSC) database available at www.cancerrxgene.org, following a methodology outlined in a prior research [25].

### *2.11. Immunohistochemistry*

Twenty-three LUSC samples were gathered from Xiangya Hospital, Central South University. All patients signed informed consent. Approval for the study was given by the Xiangya Hospital Medical Ethics Committee(No.CTXY-110008-2). Tissue sections were prepared and stained following established protocols [26]. Expression levels were classified as either high or low depending on the median value.

## 3. Results

### *3.1. Transcriptomic expression analysis of copper transporters*

The expressions of copper transporters in normal tissues were depicted in Fig. 2A. The analysis revealed that *SLC31A1* expression was predominant in liver tissue and minimal in muscle tissue. Skin tissue exhibited the highest levels of *ATP7A*, whereas liver tissue displayed the lowest. Notably, *ATP7B* expression was prominent in testicular tissue but reduced in most other normal tissues.

Subsequently, the expressions of copper transporters across various cancers were investigated through the TCGA database. *SLC31A1* was expressed in all cancers, with PCPG showing the highest levels and THYM the lowest, as illustrated in Fig. 2B. *ATP7A* showed minimal expression in LIHC and was most expressed in LAML, whereas *ATP7B* expression was minimal in DLBC and maximal in READ. Fig. 2C displayed the varying expression levels of these transporters in diverse cancer cell lines, showcasing the fluctuating expression in tumor cells.

Furthermore, a comparison of copper transporter expression between normal individuals and tumor patients was conducted using TCGA data, as illustrated in Fig. 2D. Significant disparities in *SLC31A1* expression were observed in 13 cancer types, except in cancers lacking normal tissue data. *ATP7A* and *ATP7B* exhibited significant differential expression in 12 and 15 cancer types, respectively. The Venn diagram showed that the three copper transporters had similar differential expression in BLCA, BRCA, KIRC, KIRP, LIHC, LUSC, STAD, THCA, and UCEC (Fig. 2E).

Additionally, paired samples of tumors and adjacent normal tissues in the TCGA database were used for paired comparison analyses (Fig. 2F). Only 14 kinds of cancers have over 10 pairs of such tissues. The findings showed varying expression of *SLC31A1* in BLCA, BRCA, KIRC, KICH, KIRP, LIHC, LUSC, LUAD, PRAD, THCA. At the same time, *ATP7A* was differently expressed in KICH, KIRP, LIHC, LUSC, HNSC, STAD. Whereas *ATP7B* was differently expressed in BRCA, COAD, KIRC, KIRP, PRAD, THCA.

In summary, the expression patterns of copper transporter genes varied across different cancer types, suggesting their potential

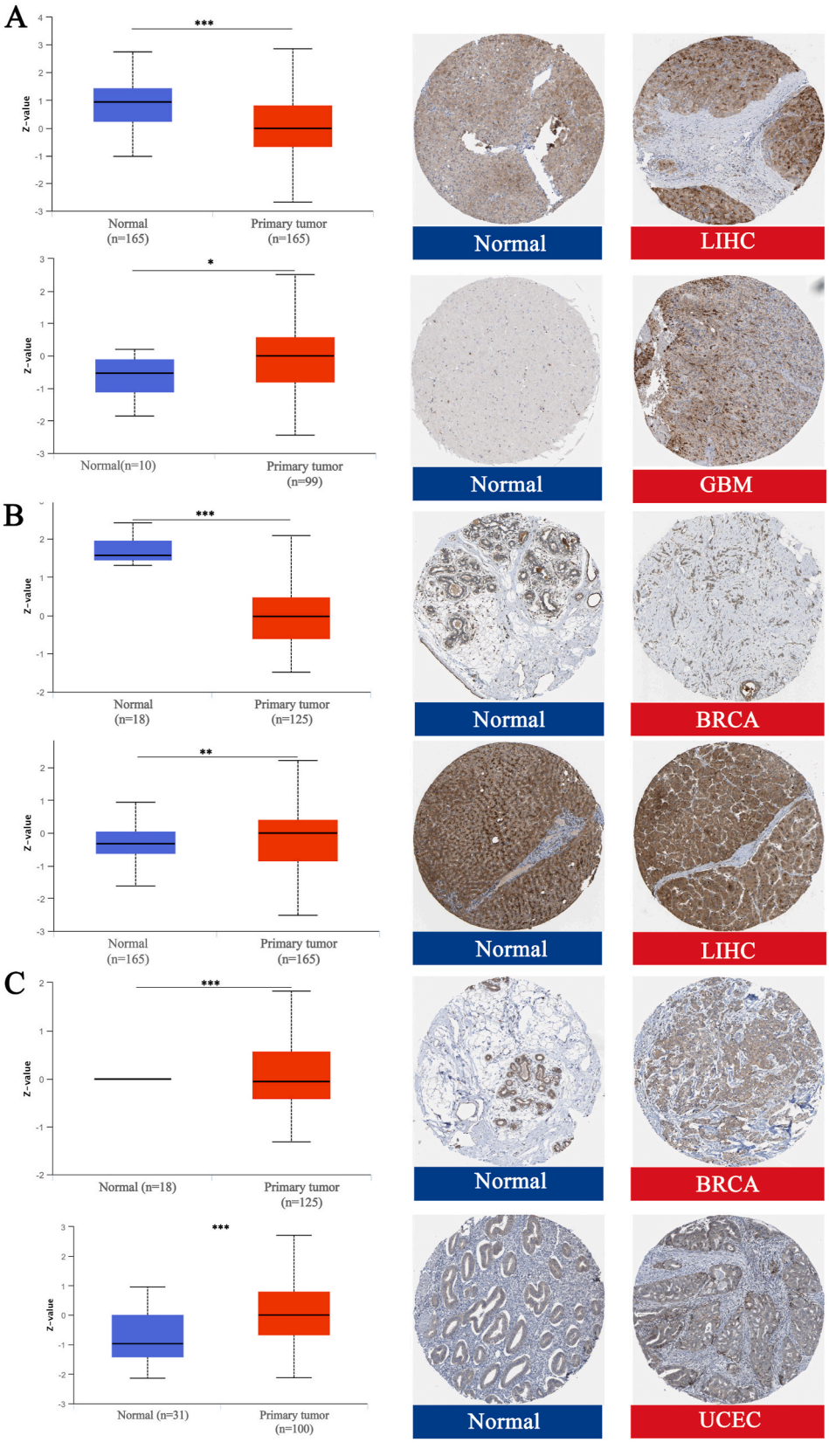


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**Fig. 3.** Comparison of copper transporter protein levels in normal tissue and tumor tissue.
(A) Comparing the levels of *SLC31A1* levels between normal and tumor tissues with representative immunohistochemistry images.
(B) Comparison of *ATP7A* levels between normal and tumor tissues with representative immunohistochemistry images.
(C) Comparison of *ATP7B* levels between normal and tumor tissues with representative immunohistochemistry images.
*p < 0.05; **p < 0.01; ***p < 0.001.

roles as oncogenes or tumor suppressor genes, contingent on the type of cancer.

### 3.2. Comparison of copper transporters protein expressions in normal and tumor tissues

The protein levels of copper transporters were evaluated by analyzing immunohistochemistry (IHC) from the HPA database and comparing them with protein expression data from the ULCAN database. As demonstrated in Fig. 3, findings from both databases aligned. In comparison with normal tissues, *SLC31A1* expression was markedly decreased in LIHC and increased in GBM. *ATP7A* was significantly upregulated in LIHC but downregulated in BRCA. Furthermore, *ATP7B* showed enhanced expression in BRCA and UCEC.

### 3.3. Correlations between copper transporters expressions and clinicopathology

A thorough examination was performed to assess the levels of copper transporters *SLC31A1*, *ATP7A*, and *ATP7B* in different cancer stages. In comparison with normal tissues, *SLC31A1* expression was notably elevated in tumor tissues at pathological stages in BLCA, BRCA, ESCA, and UCEC. However, its expression was reduced in the clinical stages of CHOL, KIRC, KIRP, LIHC, LUAD, LUSC, and THCA (Fig. 4A). *ATP7A* expression was elevated in the clinical stages of CHOL, KICH, LIHC, and UCEC, while it was reduced in the pathological stages of BRCA, KIRC, KIRP, LUSC, and THCA (Fig. 4B). *ATP7B* expression was reduced in the clinical stages of LUSC and THCA, while it was raised in the disease stages of BRCA, COAD, KIRC, READ, STAD and UCEC (Fig. 4C). These findings suggested that copper transporters may hold substantial clinical relevance for the early diagnosis of these malignancies.

### 3.4. The prognostic value of copper transporters

Survival association analyses were performed for each type of cancer to evaluate the prognostic importance of copper transporters, including OS (Fig. 5A) and PFS (Fig. 5B). Increased levels of *SLC31A1* were linked to worse OS in patients with ACC, BLCA, BRCA, LGG, MESO, and SKCM. The hazard ratios (HR) for these types of cancer were found to be significantly higher than 1, suggesting an increased risk of death from the disease ($p < 0.05$). In contrast, higher *SLC31A1* expression was associated with improved OS in KIRC, suggesting a protective effect ($HR < 1$, $p < 0.05$). Furthermore, patients exhibiting high *ATP7A* expression demonstrated reduced survival rates in LGG, whereas those with elevated *ATP7A* expression showed enhanced survival in ACC, KIRC, MESO. Elevated *ATP7B* expression correlated with diminished survival in UCEC, while correlating with improved survival in KIRC and LGG.

Elevated levels of *SLC31A1* were linked to worse survival rates in ACC, BLCA, BRCA, CESC, LGG, MESO, and UVM for PFS, while showing a positive correlation with better survival in KIRC and READ. Furthermore, increased *ATP7A* expression was linked to worse survival in BLCA, LGG, and LIHC, while exhibiting improved survival in MESO and KIRC. Conversely, elevated *ATP7B* expression was associated with reduced survival rates in COAD and LUSC, yet enhanced survival rates in KIRC, LGG, and THCA. These findings highlighted the complex role of copper transporters in cancer prognosis, demonstrating variable effects across different cancer types.

### 3.5. Cancer-related pathways analysis

In the majority cancers, the expressions of copper transporters exhibited significant correlations with both tumor stage and prognosis. This discovery prompted us to speculate that these transporters could be involved in the advancement of tumors by affecting signaling pathways within the tumor. The analysis of pathways indicated a notable participation of copper transporters in different signaling pathways related to cancer (Fig. 6). *SLC31A1* primarily participated in the stimulation of RTK (25 % activation compared to 0 % inhibition), apoptosis (22 % activation compared to 3 % inhibition), and RAS/MAPK (16 % activation compared to 0 % inhibition). For *ATP7A*, the main pathways were inactivation of cell cycle (0 % activation vs. 19 % suppression) and apoptosis (3 % activation vs. 22 % suppression). *ATP7B* primarily activated the RTK pathways, with a 28 % activation rate compared to a 3 % inhibition rate. The findings suggested that copper transporters may have a significant impact on regulating pathways associated with cancer.

The red boxes showed the percentage of 33 types of tumors in which the pathway was activated, while the blue boxes showed the percentage of tumor types in which the pathway was suppressed.

### 3.6. Molecular interaction network and enrichment analysis

We performed molecular interaction network analysis and enrichment analysis to further understand the potential functions of copper transporters. A total of 8 proteins that interact with them, were obtained from the STRING database (Fig. 7A). Additionally, utilizing the GeneMANIA website, we identified 20 genes that interact with the copper transporter family (Fig. 7B). We conducted Gene Ontology (GO) enrichment analysis by merging these two gene lists. In the biological process category, these molecules were predominantly involved in copper transport and copper homeostasis. Analysis of cellular component enrichment revealed that the

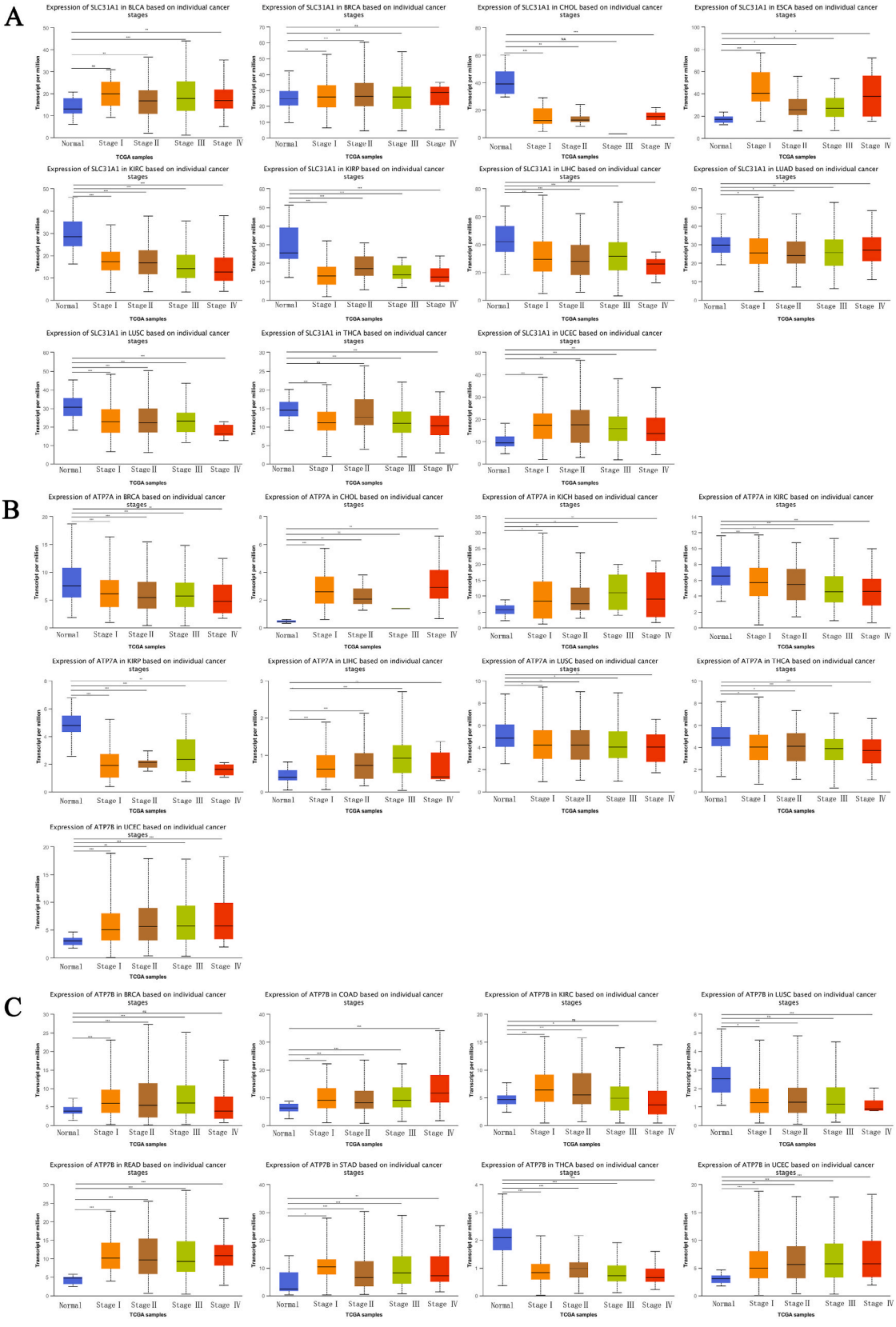


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**Fig. 4.** Expression level of copper transporters in different pathological stages.
(A) *SLC31A1* expression levels across various pathological stages.
(B) *ATP7A* expression levels across various pathological stages.
(C) *ATP7B* expression levels across various pathological stages.
*p < 0.05, **p < 0.01, and ***p < 0.001.

majority of compounds were concentrated in late endosomes, basolateral and apical plasma membranes, recycling endosomes, trans-Golgi network, and integral components of the plasma membrane. Analysis of molecular function enrichment revealed that the majority of molecules were involved in the activity of transporting copper ions across membranes and binding copper ions (Fig. 7C). Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis highlighted their involvement in mineral absorption and platinum drug resistance.

### 3.7. Analysis of genetic alteration

To explore the gene alteration status of copper transporters across pan-cancer, we utilized data from the CBioPortal database. Fig. 8A displays the primary gene modifications identified, including mutation, amplification, and deep deletion. The mutation rate of *SLC31A1* was highest among patients with pancreatic, esophageal, and bone cancer. Likewise, patients with melanoma, bladder, and non-small cell lung cancer exhibited the highest mutation rate of *ATP7A*. Furthermore, the mutation rate of *ATP7B* was highest in colorectal, esophageal, and lung cancer.

An examination was carried out on genetic variations in copper transporters. Surprisingly, the distribution of the CNV displayed that the main types of CNV were amplification and deletion in the heterozygous state(Fig. 8B). Moreover, CNV profiles varied across different cancer types.

Positive correlation between mRNA expression and CNV was identified through correlation analysis, with *SLC31A1* in OV, *ATP7A* in LUSC, and *ATP7B* in COAD showing notable associations(Fig. 8C). Conversely, a negative correlation was observed for *ATP7A* in KIRP and PCPG. These findings suggested that CNV alterations of copper transporters may contribute to their dysregulated expressions, potentially exerting a significant role in tumor progression.

### 3.8. The relationship between copper transporters and tumor mutation burden (TMB), tumor microsatellite instability (MSI)

TMB is a measurable indicator that shows the amount of mutations in cancer cells and can provide insight into the immune system's reaction. *SLC31A1* expression exhibited a strong positive correlation with TMB in various cancer types including LUAD, LGG, PAAD, BLCA, UCEC, BRCA, COAD, STAD, SARC, ACC and THYM, but had a notable negative correlation with UVM and THCA (Fig. 9A). Regarding *ATP7A*, positive correlations were observed in LGG and THYM, indicating higher mutation burdens associated with increased *ATP7A* expression in these tumors, while negative correlations were found in UVM, THCA, LUAD, KIRP, KIRC, COAD, and BRCA. *ATP7B* expression showed notably positive correlations with TMB in several tumors, including ESCA and THYM, while exhibiting significant negative correlations with DLBC, CESC, BRCA, LGG, COAD, THCA, and SKCM.

Following this, an examination was implemented to determine the relationship between expression and MSI (Fig. 9B). The expression of *SLC31A1* showed strong positive correlations with COAD, UCEC, READ and STAD, but had significant negative correlations with DLBC, PRAD, LUSC, and LUAD. *ATP7A* showed significant positive associations with COAD, LUSC, UCEC, READ and ACC. Conversely, *ATP7A* was negatively associated with DLBC and PRAD. Furthermore, *ATP7B* exhibited significant positive correlations with LUAD, LUSC and CHOL, while showing negative associations with DLBC, PCPG, and COAD.

Overall, these results highlighted the diverse roles of copper transporters in influencing TMB and MSI across different cancers, suggesting their potential as biomarkers for cancer prognosis and therapy.

### 3.9. Tumor immune cell infiltration

We collected infiltration data for six types of immune cells by utilizing the TIMER database. Fig. 10 showed that the three copper transporters were associated with immune cell infiltration in most tumors. Specifically, *SLC31A1* level showed a positive correlation with immune cell infiltration in various cancers, including BRCA, BLCA, COAD, KIRC, LGG, LUAD, MESO, OV, PRAD, READ, SARC, THCA, UCEC, and UCS. *ATP7A* expression exhibited strong positive correlations with the infiltration of all six types of immune cells in KIRC, LGG, LIHC, and READ. Conversely, *ATP7B* expression showed negative associations with the infiltration of these immune cells in SKCM and STAD. These results highlighted the intricate relationship between copper transporter levels and the infiltration of immune cells in tumor microenvironment.

### 3.10. Drug sensitivity analysis

Genomic aberrations and immune infiltration can significantly influence drug responses. Our analysis revealed several significant correlations between copper transporter expression and drug sensitivity(Fig. 11). Higher level of *ATP7B* was associated with decreased sensitivity to Nilotinib, suggesting that *ATP7B* expression may lead to resistance to this medication. Likewise, the expression of *ATP7A* showed an inverse relationship with the susceptibility to SB52334, indicating a possible involvement in conferring resistance to this

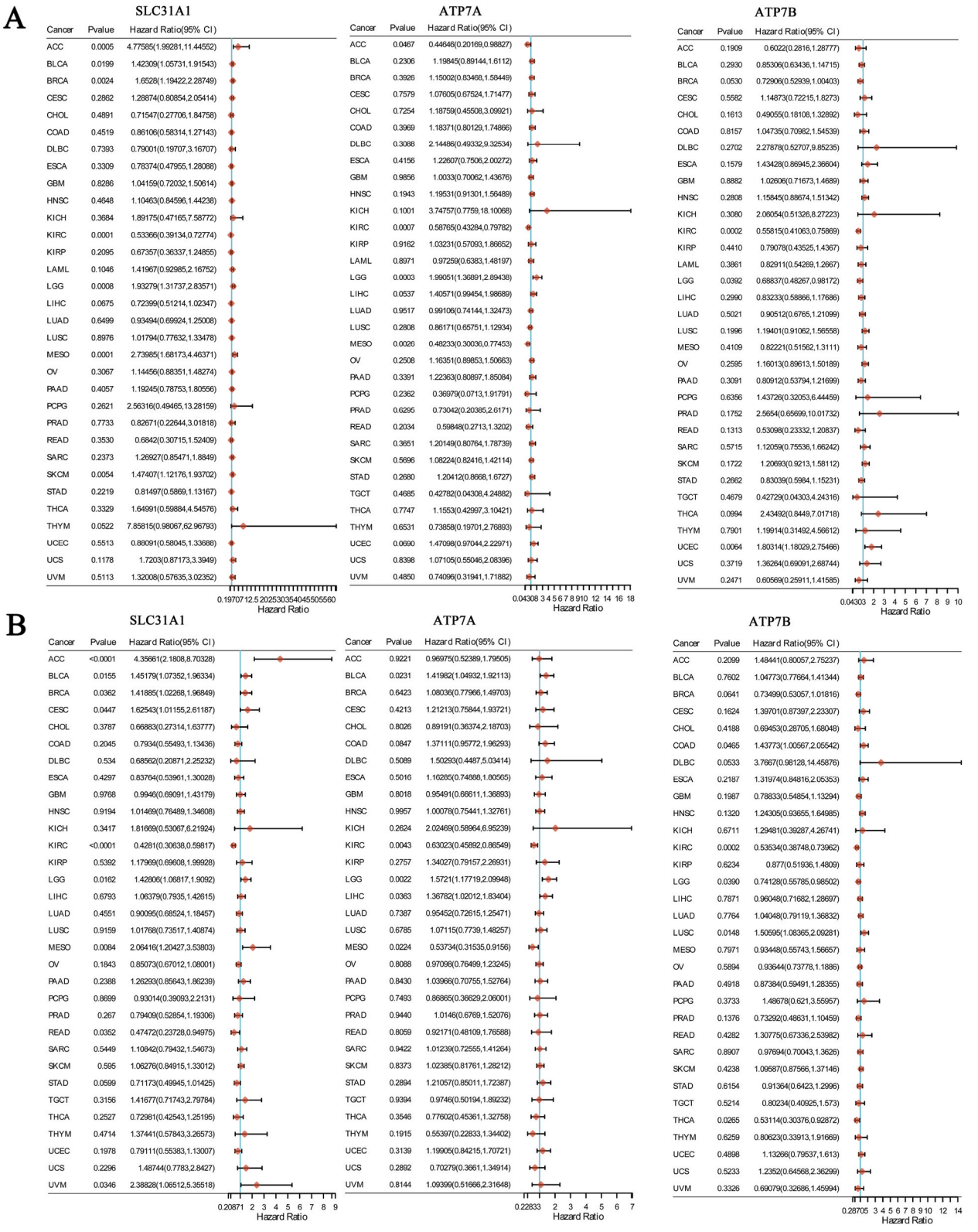


**Fig. 5. The prognostic value of copper transporters.**
(A) Overall survival (OS) .
(B) Progression-free survival (PFS).

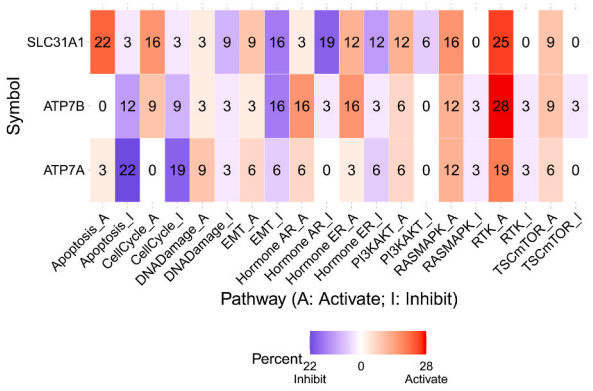


**Fig. 6.** Effects of copper transporters on pathway activity.

treatment. *SLC31A1* expression was notably linked to the responsiveness of various chemotherapy medications and targeted treatments, such as methotrexate, vorinostat, temozolomide, docetaxel, trametinib, and more. These findings highlighted the potential impact of dysregulated copper transporters' expression on chemotherapy and targeted drug therapy resistance. Comprehending these connections may guide the creation of improved treatment plans and individualized treatments for individuals with cancer.

### *3.11. The verification of the expression of ATP7B by IHC in LUSC*

Due to the differential expression of *ATP7B* in LUSC and its relationship with survival observed in the bioinformatics analysis, we further validated these findings by using IHC. Representative IHC pictures were shown in Fig. 12A. The results suggested that *ATP7B* expression did not exhibit significant differences across various tumor stages (Fig. 12B). Following this, we examined the relationship between *ATP7B* expression and patient prognosis, finding that while *ATP7B* expression was not correlated with OS, it was significantly correlated with PFS (Fig. 12C and D).

## 4. Discussion

In this current study, we performed an extensive analysis across various types of cancer to examine how copper transporters (*SLC31A1*, *ATP7A*, and *ATP7B*) were expressed and their relationships with tumor stages, prognosis, cancer-related pathways, gene mutations, immune cell presence, and drug response. Moreover, we validated the role of *ATP7B* in LUSC using IHC. Our research offers new perspectives on the possible functions of these transporters in cancer biology and treatment.

The role of copper metabolism-related genes in tumors has garnered widespread attention [27]. Liu H. and colleagues examined the cuproptosis gene group, which consists of 7 genes that promote cuproptosis, 3 genes that inhibit cuproptosis, and 2 copper transporters, in relation to SNV, CNV, methylation, mRNA levels, interactions between pathways, and miRNA controls in 33 different tumor types [28]. This study provides new insights into the genetics of cuproptosis genes, yet the protein expression profiles of these genes across various cancer types remain to be elucidated. Later, Wu et al. conducted a comprehensive study on cuproptosis regulators across various types of cancer using TCGA, serving as a valuable resource for upcoming research on cuproptosis [29]. However, they did not investigate copper transporters. There are several differences between our study and previous studies. Firstly, our study focuses on copper transporters (*SLC31A1*, *ATP7A*, *ATP7B*) by integrating multi-omics data, including mRNA expression (from TCGA, GTEx and CCLE), protein expression (from HPA and UALCAN). These comprehensive datasets provide a thorough understanding of copper transporters. Secondly, clinical tissue samples were collected for immunohistochemical test to validate the results of ccbioinformatics analysis, thereby ensuring their reliability. Thirdly, we studied the relationship between copper transporters and immune infiltration, as well as drug sensitivity. Understanding these connections may guide the development of improved treatment plans and personalized therapies for cancer patients. In summary, our study enhanced the understanding of copper transporters in cancer biology and distinguish our work from existing literature.

Our current research revealed variations in copper transporters' expression among different cancer types, indicating their possible functions as either oncogenes or tumor suppressor genes, determined by the specific cancer type. Likewise, earlier research has indicated that elevated level of *SLC31A1* was linked to unfavorable outcomes in breast cancer [8], bladder cancer [9], glioma [10], while leading to improved survival in non-small-cell lung cancer [11]. Additionally, previous pan-cancer analysis indicated that SLC31A1 was highly expressed in most tumors and lowly expressed in a few compared to healthy tissues [30,31]. These findings are consistent with our results. Compared with normal tissues, *ATP7A* expression was significantly elevated in hepatocellular carcinoma [12] and esophageal squamous cell carcinoma [13], while decreased in ovarian carcinoma [14]. As for ATP7B, patients with overexpression of *ATP7B* had higher survival rates in gliomas [15], but unfavorable clinical outcomes in hepatocellular carcinoma [16]. Previous pan-cancer results have also confirmed the dual role of ATP7B in various tumors [32]. Collectively, these results underscore the dual role of copper transporters in cancer biology and highlight the necessity for further research to elucidate their specific mechanisms in each cancer type.

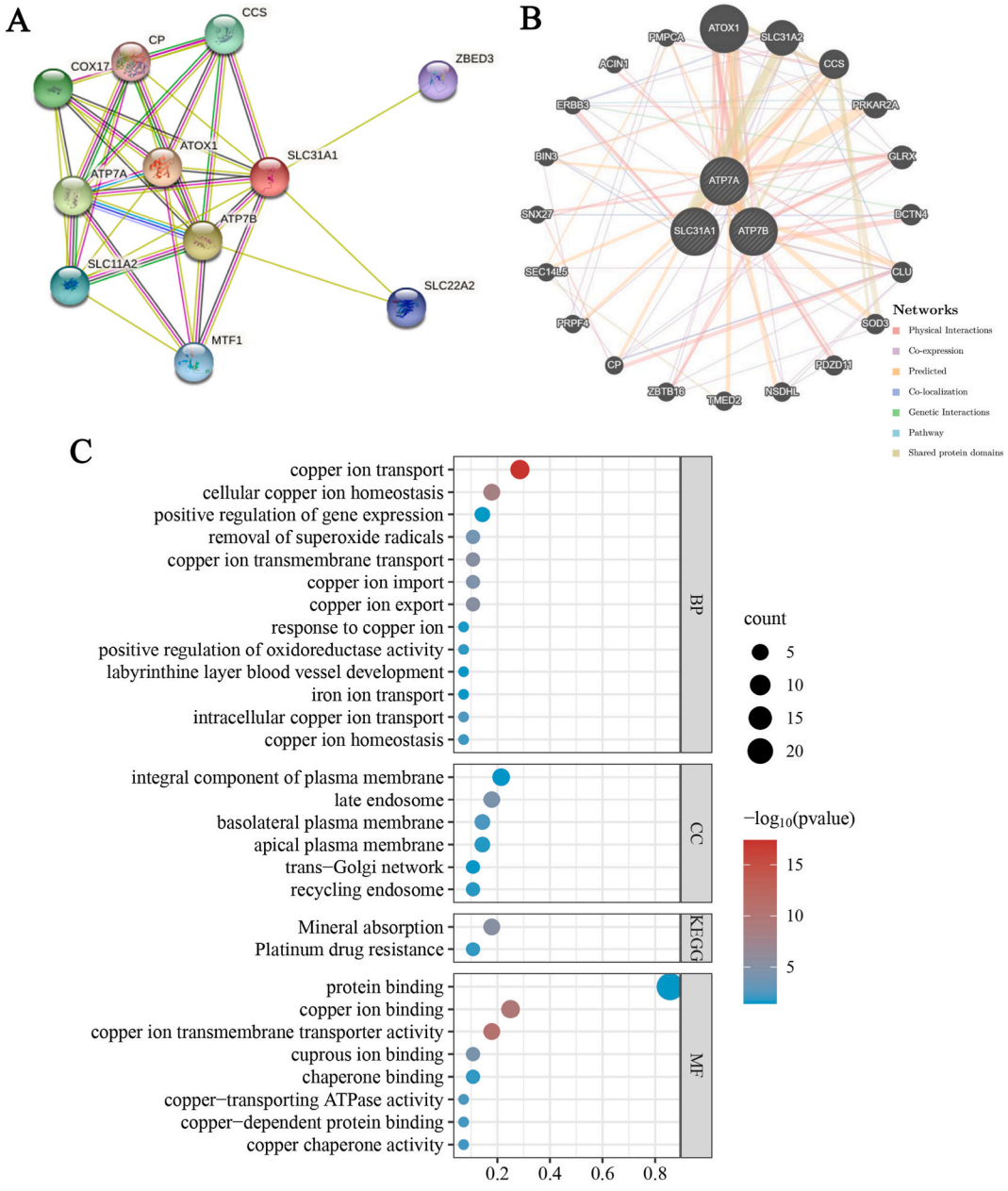


**Fig. 7.** Potential functions of copper transporters.
(A) The PPI network of copper transporters and their interacting proteins
(B) The GGI network between genes associated with copper transporters.
(C) Functional enrichment analysis on molecules that interact with copper transporters.

In our immune infiltration analysis, we have observed that *SLC31A1* and *ATP7A* facilitate immune cell infiltration, whereas ATP7B impedes immune cell infiltration in most tumors. These distinct effects of the three copper transporters on immune infiltration may contribute to the dual role of copper ions in immune responses. For instance, Voli et al. proposed that copper may upregulate PD-L1 expression, thereby promoting immune evasion [33]. Conversely, copper can promote M1 macrophage polarization and trigger immunogenic cell death [6,34]. However, the intricate mechanisms underlying the interplay between copper and immunity remain elusive and warrant further investigation in future studies.

Considering the differential expression of *ATP7B* in LUSC and its relationship with survival in the bioinformatics analysis, we collected 23 cases of LUSC to validated these findings. Consistent with the bioinformatics analysis, patients with high *ATP7B* expression had worse PFS. However, *ATP7B* expression was not show significant differences across various tumor stages. The small sample size could be the reason for this.

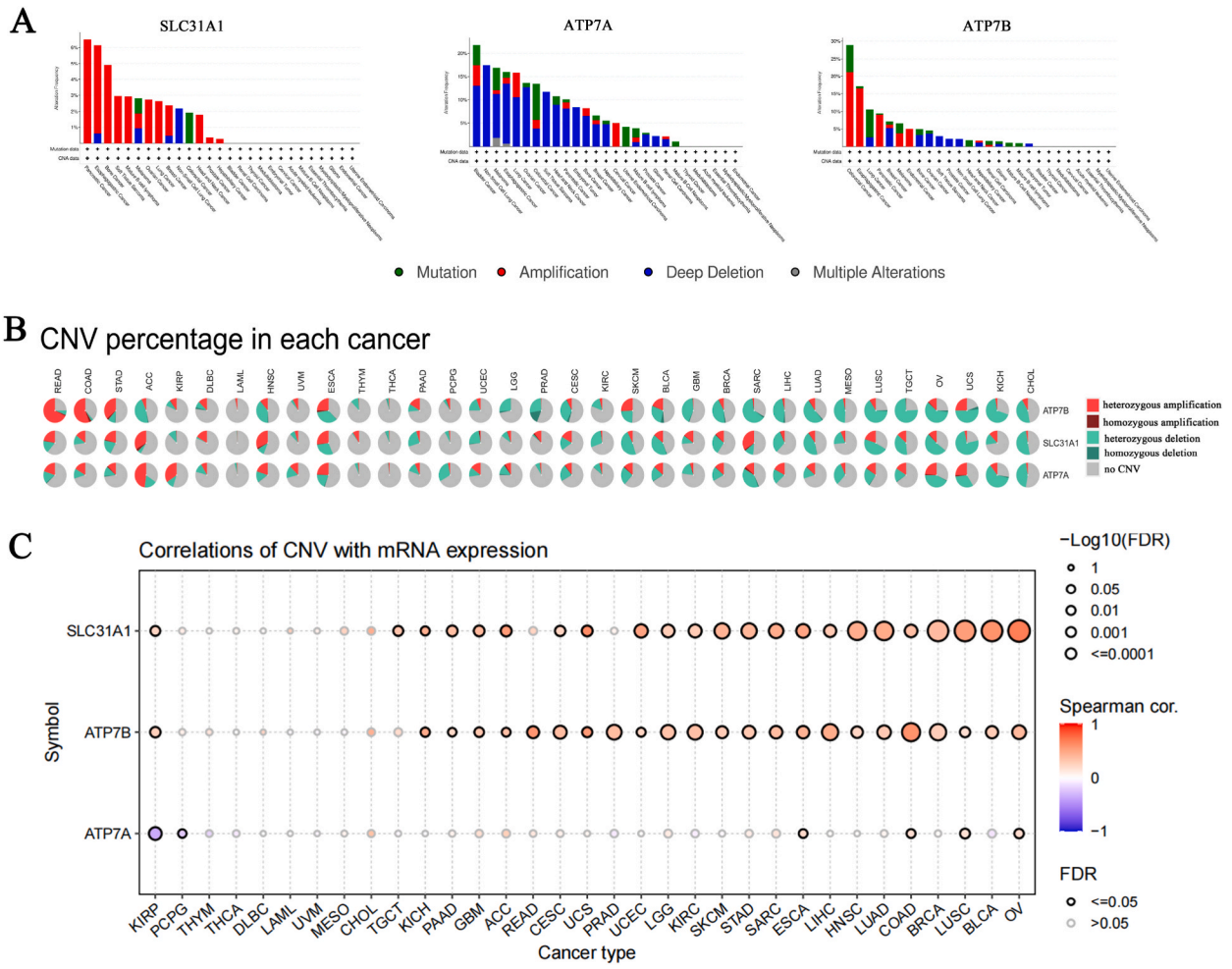


**Fig. 8.** The alteration of copper transporters in pan-cancers.
(A) The frequency of alterations categorized by different mutation types. (B)Distribution of copy number variations (CNV) across 33 cancers. (C) Relationship between copy number variations and messenger RNA levels. The statistical significance was depicted by the size of the data points, with bigger dots indicating greater significance. FDR denoted false discovery rate.

Although our research offers valuable perspectives, it is crucial to recognize specific constraints. The main constraint is the dependence on bioinformatics and clinical confirmation of limited sample sizes, lacking direct experimental validation. Future research should include clinical studies with large samples, as well as animal and in vitro molecular experiments.

## 5. Conclusion

Overall, our research has revealed important connections between the levels of copper transporters and different aspects of cancer biology, such as tumor stage, clinical outcome, immune cell presence, and cancer-related signaling pathways in various types of tumors. These observations present copper transporters as potential targets for personalized therapy in the future.

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Abbreviation

| | Detail |
|---|---|
| ACC | Adrenocortical carcinoma |

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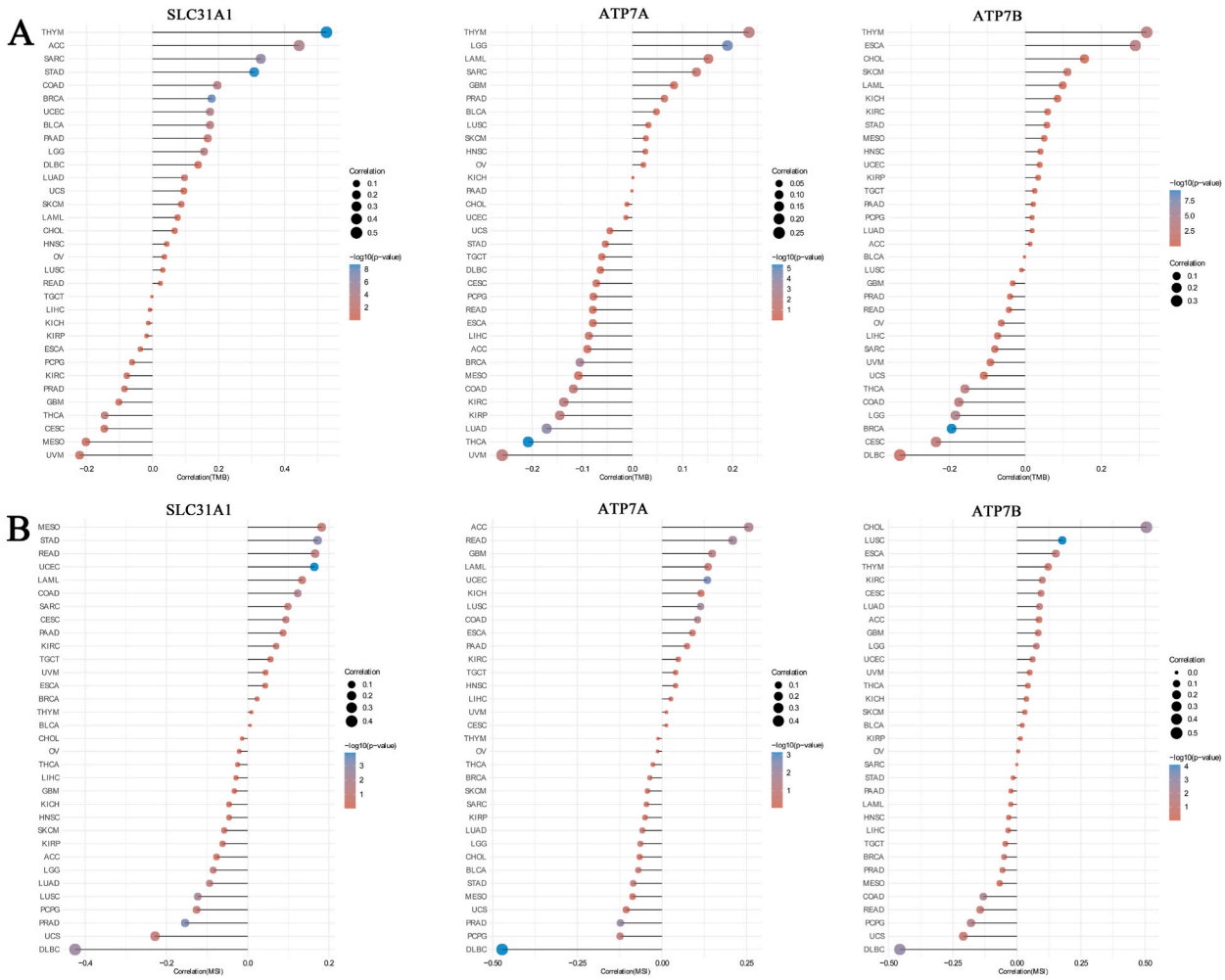


**Fig. 9.** Correlation between the gene expression and TMB and MSI in pan-cancer.
(A) The relationship between the expression of copper transporters and TMB across various tumors.
(B) The association between the expression of copper transporters and MSI across diverse tumors.

(*continued*)

| | |
|---|---|
| BLCA | Bladder Urothelial Carcinoma |
| BRCA | Breast invasive carcinoma |
| CESC | Cervical squamous cell carcinoma and endocervical adenocarcinoma |
| CHOL | Cholangio carcinoma |
| COAD | Colon adenocarcinoma |
| DLBC | Lymphoid Neoplasm Diffuse Large B-cell Lymphoma |
| ESCA | Esophageal carcinoma |
| GBM | Glioblastoma multiforme |
| HNSC | Head and Neck squamous cell carcinoma |
| KICH | Kidney Chromophobe |
| KIRC | Kidney renal clear cell carcinoma |
| KIRP | Kidney renal papillary cell carcinoma |
| LAML | Acute Myeloid Leukemia |
| LGG | Brain Lower Grade Glioma |
| LIHC | Liver hepatocellular carcinoma |
| LUAD | Lung adenocarcinoma |
| LUSC | Lung squamous cell carcinoma |
| MESO | Mesothelioma |
| OV | Ovarian serous cystadenocarcinoma |
| PAAD | Pancreatic adenocarcinoma |
| PCPG | Pheochromocytoma and Paraganglioma |
| PRAD | Prostate adenocarcinoma |

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**Fig. 10. Correlation between copper transporters' expression and immune infiltration.** (A) *SLC31A1*. (B) *ATP7A*. (C) *ATP7B*. *p < 0.05, **p < 0.01, and ***p < 0.001.

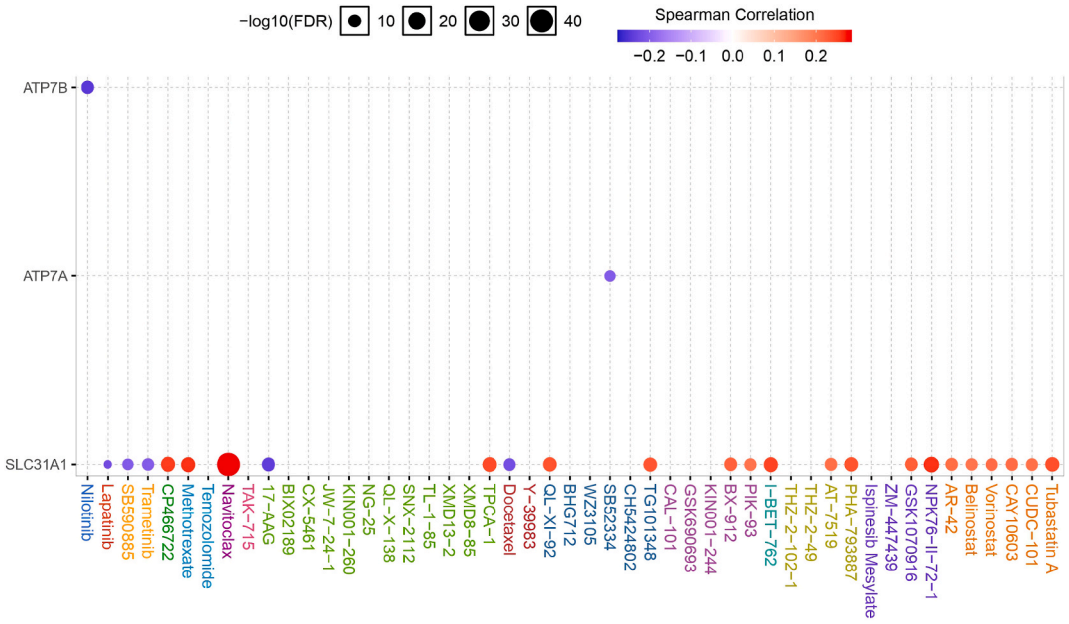


**Fig. 11. Drug sensitivity analysis of copper transporters.** FDR, false discovery rate.

(*continued*)

| | |
|---|---|
| READ | Rectum adenocarcinoma |
| SARC | Sarcoma |
| SKCM | Skin Cutaneous Melanoma |
| STAD | Stomach adenocarcinoma |
| TGCT | Testicular Germ Cell Tumors |
| THCA | Thyroid carcinoma |
| THYM | Thymoma |
| UCEC | Uterine Corpus Endometrial Carcinoma |
| UCS | Uterine Carcinosarcoma |
| UVM | Uveal Melanoma |
| TCGA | The Cancer Genome Atlas |

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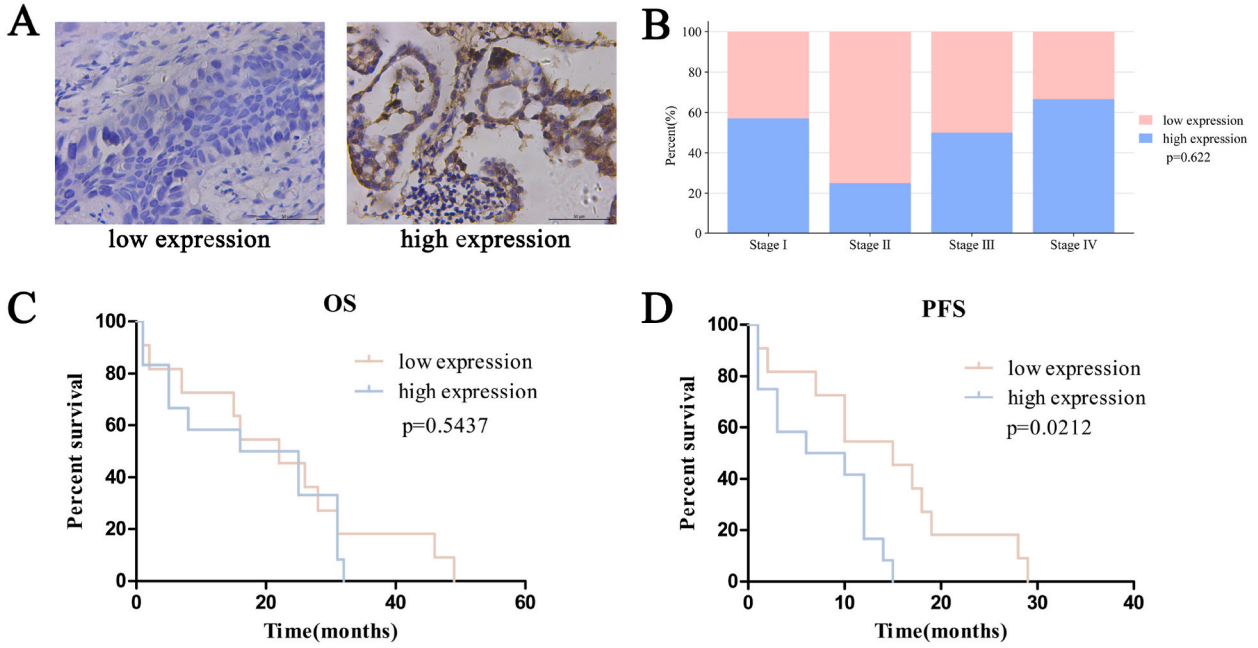


**Fig. 12.** Verification of *ATP7B* expression in LUSC.
(A) Representative IHC images of *ATP7B* expression.
(B) The relationship between *ATP7B* expression and tumor stages.
(C) The relationship between *ATP7B* expression and OS.
(D)The relationship between *ATP7B* expression and PFS.

(*continued*)

| | |
|---|---|
| CCLE | Cancer Cell Line Encyclopedia |
| GTEx | Genotype-Tissue Expression |
| HPA | Human Protein Atlas |
| FC | fold change |
| PPI | protein–protein interaction |
| GGI | gene-gene interaction |
| PFS | progression free survival |
| DSS | disease-specific survival |
| CNV | copy number variation |
| TMB | tumor mutation burden |
| MSI | tumor microsatellite instability |
| TPM | Transcripts Per Million |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| GO | Gene Ontology |

## CRediT authorship contribution statement

**Yueqin Li:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing. **Zhen Yu:** Methodology, Resources.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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