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Article

SLC38A3 Promotes the Proliferation and Migration of Tumor Cells and Predicts Poor Prognosis in Colorectal Cancer

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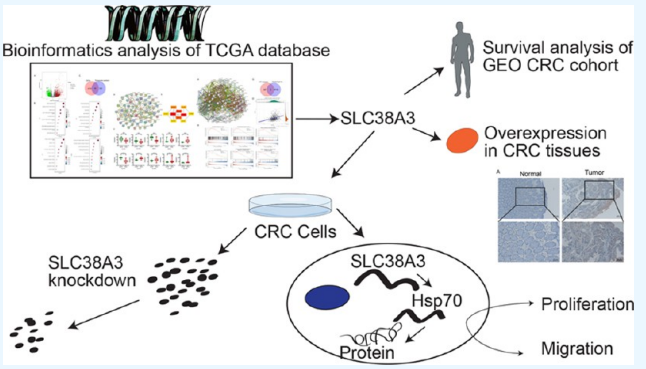
ABSTRACT: Previous studies have revealed that abnormal expressions of membrane transporters were associated with colorectal cancer (CRC). We herein performed a comprehensive bioinformatics analysis to identify the key transporter protein-related genes involved in CRC and potential mechanisms. Differentially expressed transporter protein-related genes (DE-TPRGs) were identified from CRC and normal samples using The Cancer Genome Atlas database. SLC38A3 expression was validated by immunohistochemistry and RT-qPCR, and the potential mechanism was explored. A total of 63 DE-TPRGs (29 up-regulated and 34 down-regulated) were screened. Inside, *ABCC2*, *ABCG2*, *SLC4A4*, *SLC9A3*, *SLC15A1*, and *SLC38A3* were identified as hub genes. SLC38A3 is indeed upregulated in colorectal cancer patients. Furthermore, we found that knockdown of *SLC38A3* inhibited the proliferation and migration of HCT116 cells, and Hsp70 ATPase activator could rescue it. Overall, *SLC38A3* is a novel potential biomarker involved in CRC progression and promotes the proliferation and migration of tumor cells by positively regulating the function of Hsp70.



INTRODUCTION

Colorectal cancer (CRC) poses a significant public health challenge in modern medicine, ranking as the third most common cancer and the fourth leading cause of cancer-related deaths globally.[1] The risk of developing CRC is influenced by various factors, such as lifestyle, behavior, and genetic predisposition.[2−4] The process of CRC development is intricate and involves gene mutations, cellular contexts, and environmental factors. Despite the availability of current treatment methods such as radiotherapy, chemotherapy, and surgical resection, their effectiveness remains suboptimal.[5] We need to develop more innovative and effective diagnostic and therapeutic interventions. Therefore, it is of great significance to deeply understand the complex molecular mechanisms underlying cellular proliferation, and invasion in CRC.[6]

Previous studies have indicated that abnormal expression of membrane transporters can lead to cancer, such as ATP-binding cassette transporters (ABCTs) and solute carriers (SLCs).[7] The ABCT superfamily contains 48 members, categorized into seven subfamilies ranging from *ABCA* to *ABCG*.[8] While they are typically located in the plasma membrane of cells, ABCTs have also been observed within the Golgi, mitochondria, and endoplasmic reticulum.[9] These crucial transporters are responsible for facilitating the movement of diverse substrates such as steroids, glycolipids, phospholipids, and xenobiotics across membranes.[10] ABCTs play a pivotal role in numerous physiological processes, including cell detoxification, lipid trafficking, membrane homeostasis, etc. Their involvement in these processes has underscored their importance.[11] SLCs are recognized mainly for their ability to regulate the influx/efflux of exogenous/endogenous substances.[12,13] Therefore, SLCs may participate in the occurrence and development of tumors by mediating the substance metabolism to regulate cellular functions. Additionally, studies have demonstrated that SLCs can independently contribute to the activation of various signaling network cascades, potentially promoting the formation of metastatic tumors.[14−16] Therefore, membrane transporters represent a broad range of potential therapeutic targets for cancer treatment.

For CRC, a study showed that *SLCO4A1* expression was upregulated in CRC tissues and was significantly associated with poor prognosis. In cellular experiments, *SLCO4A1* knockdown resulted in significantly reduced viability, invasion, and migration compared with control cells. Thus, *SLCO4A1* may be a valuable marker of poor prognostic and a new target

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for CRC treatment.[17] In addition, the role of *SLC7A5*, *SLC9A3*, *ABCC2*, *ABCG2*, etc. in the invasion and progression of CRC has been reported.[18−20] Nevertheless, current research on the molecular mechanisms by which these transporters impact CRC, as well as their potential application as drug targets in clinical treatment, remains insufficient. Here, we performed a comprehensive bioinformatics analysis to identify differentially expressed transporter protein-related genes (DE-TPRGs) in CRC and determine their potential functions involved in CRC progression. Additionally, we conducted a more extensive investigation into the role of *SLC38A3* in the CRC.

RESULTS

Enrichment Analysis Results and DE-TPRGs. We screened DEGs between CRC and normal samples from The Cancer Genome Atlas (TCGA) database. The volcano plot indicated that 4796 DEGs were identified, of which 1479 were downregulated and 3317 were upregulated (Figure 1A). All

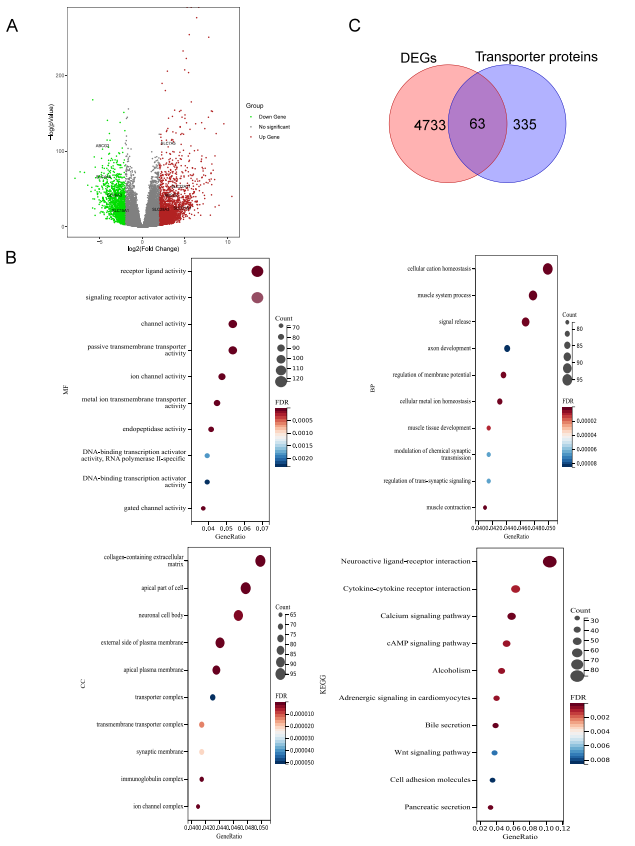

Figure 1. Transporter protein-related differentially expressed genes in the TCGA COAD-READ data set. (A) Volcano plot of DEGs. Red dots represent DEGs up-regulated in CRC tissue, and green dots represent DEGs down-regulated in CRC tissue. (B) GO and KEGG enrichment results of DEGs. (C) Acquisition of transporter protein-related differentially expressed genes.

DEGs are listed in Table S1. Figure 1B shows the enrichment analysis results. All results are presented in Tables S2 and S3. For GO-MF, significant enrichments were observed for "channel activity" and "passive transmembrane transporter activity". For GO-BP, the major enriched terms were "cellular cation homeostasis" and "muscle system process". For GO-CC, the "apical part of cell" and "external side of plasma membrane" were significantly enriched. Kyoto Encyclopedia of Genes and Genomes (KEGG) indicated that DEGs were mainly enriched in "neuroactive ligand−receptor interaction", "calcium signaling pathway", and "bile secretion", among others. Gene set enrichment analysis (GSEA) showed similar results (Figure S1). Interestingly, the enrichment results showed that transporter protein-related genes were involved in CRC pathogenesis. Therefore, the intersecting genes between DEGs and transporter protein-related genes were further identified by the Venn diagram. As a result, 63 DE-TPRGs were ultimately identified (Figure 1C).

Acquisition of the Hub Gene. A protein−protein interaction (PPI) network of DE-TPRGs including 61 nodes and 144 edges was constructed through the STRING online platform (Figure 2A). Using the CytoHubba plug-in of Cytoscape software, the top 10 genes with the highest degree scores (*SLC9A3*, *SLC15A1*, *ABCG2*, *ABCC2*, *SLC22A11*, *SLC4A4*, *SLC7A5*, *SLC28A3*, *SLC38A3*, and *SLCO1B1*) were obtained (Figure 2B). The expression patterns of these genes in CRC and normal samples were presented in box plots (Figure 2C). Survival analysis showed that six hub genes were significantly associated with CRC prognosis in the external GEO data set: *ABCC2* (P = 0.041), *ABCG2* (P = 0.011), *SLC4A4* (P = 0.0098), *SLC9A3* (P = 0.031), *SLC15A1* (P = 0.017), and *SLC38A3* (P = 0.031) (Figure 2 D).

Landscape of Immune Infiltration. To better understand immune infiltration in the tumor environment, an immune infiltration analysis was performed using CIBERSORT. Figure 3A shows the proportion of 22 immune cell infiltrators in all samples. All results are presented in Table S4. The first 647 represented the CRC group, and the last 51 represented the normal group. As shown in Figure 3B, 18 types of immune cell markers exhibited significantly diverse expression. Notably, eight types of immune cells (T cells CD4 memory resting, macrophages M0, NK cells resting, macrophages M1, T cells CD4 memory activated, mast cells activated, T cells follicular helper, and neutrophils) exhibited significantly higher expression in the CRC group. Conversely, a reduced level of infiltration was observed for 10 types of immune cells (macrophages M2, Plasma cells, T cells CD8, B cells naive, monocytes, B cells memory, dendritic cells resting, eosinophils, NK cells activated, and mast cells resting) in comparison to the normal group. In addition, immune cell correlation analysis showed hub genes are highly associated with macrophages M2, T cells CD4 memory activated, NK cells activated, and macrophages M2, among others. (Figure 3 C).

Potential Mechanism of SLC38A3. For next-level investigation, we selected SLC38A3 which was rarely reported in colorectal cancer among hub genes. We performed a PPI network analysis of *SLC38A3* using the STRING online database, resulting in a network of 100 nodes (refer to Figure 4A). The full list is displayed in Table 1. GSEA results showed that *SLC38A3* was positively associated with (i) "Endoplasmic reticulum lumen", (ii) "Endopeptidase regulator activity", and (iii) "Protein containing complex". In contrast, a significant negative correlation was noted between *SLC38A3* and "NK T cell activation", "intrinsic apoptotic signaling pathway in response to oxidative stress", and "negative regulation of I kappab kinase NF kappab signaling" (Figure 4B). All results are presented in Table S5. In addition, 622 genes highly related to *SLC38A3* were identified in the CRC cohort by correlation analysis (Table S6). Subsequently, three intersecting genes

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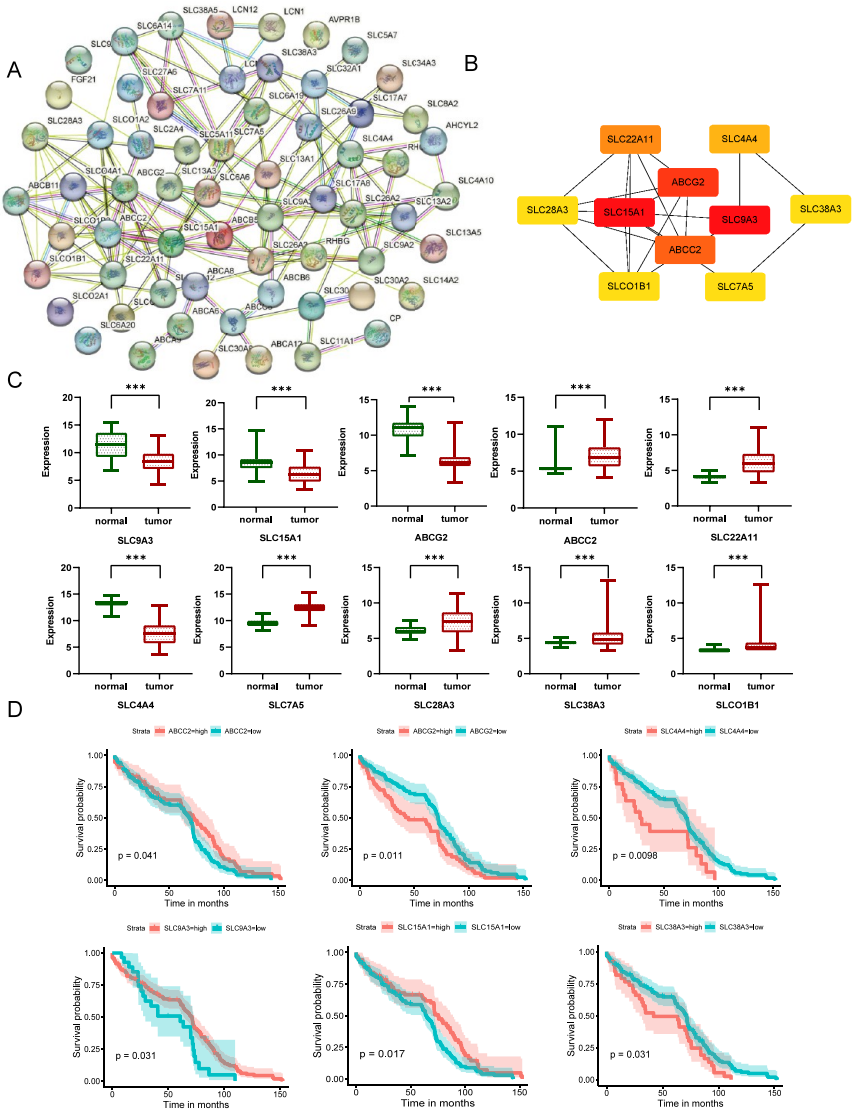

Figure 2. Acquisition of Hub Genes. (A) PPI network of transporter protein-related differentially expressed genes. (B) Subnetwork of hub genes. (C) Expression boxplots of the 10 highest-scoring genes from tumor tissues and paracarcinoma tissues. (D) Kaplan−Meier plots of hub genes.

were identified among the PPI network and highly correlated genes, including *SLC15A1*, *SLC3A2*, and *DNAJC5* (Figure 4C). Among them, *DNAJC5* was an innovative gene in the intersecting genes besides the transporters' family. We observed a notable positive correlation between *DNAJC5* and *SLC38A3* (Figure 4D).

SLC38A3 was Overexpressed in CRC Specimens. Immunohistochemistry demonstrated that *SLC38A3* expression was significantly upregulated in CRC tissues compared to paracarcinoma tissues (Figure 5A). In addition, RT-qPCR showed that the expression level of *SLC38A3* was significantly elevated in CRC tissues compared to paracarcinoma tissues (Figure 5B). These results were consistent with the findings from the bioinformatics analysis.

Knockdown of *SLC38A3* Inhibits Proliferation and Migration of CRC Cells. To knock down the expression of *SLC38A3* in HCT116 human CRC cell lines, a siRNA-mediated gene silencing approach was employed. Results revealed that *SLC38A3* expression was significantly lower in siRNA-interfered cells compared to the control group (Figure 6A). This indicated that cells with stable, low expression of *SLC38A3* were successfully constructed. For cellular proliferation, HCT116 cells transfected with *SLC38A3* siRNA resulted in a significant reduction in cellular growth proliferation compared to the NC group (Figure 6B). Similarly, significant cellular colony reduction was observed in HCT116 with *SLC38A3* siRNA in the colony formation assay. In contrast, transfected cells cultured with the addition of 115-7c (an ATPase agonist of Hsp70) showed a restored ability to proliferate compared to knockdown cells. In our cloning formation and scratch assay experiments, we also observed similar results. Interfering with *SLC38A3* significantly reduced the cloning formation ability and migration ability of HCT116 cells. Pretreatment with 115-7C partially reversed the decrease in cell cloning formation ability and migration ability caused by *SLC38A3* interference (Figure 6C and D).

DISCUSSION

Searching for mechanisms to target transporters has great potential for the clinical treatment of CRC and improving chemotherapy resistance. In this study, we performed a comprehensive bioinformatics analysis to identify the key

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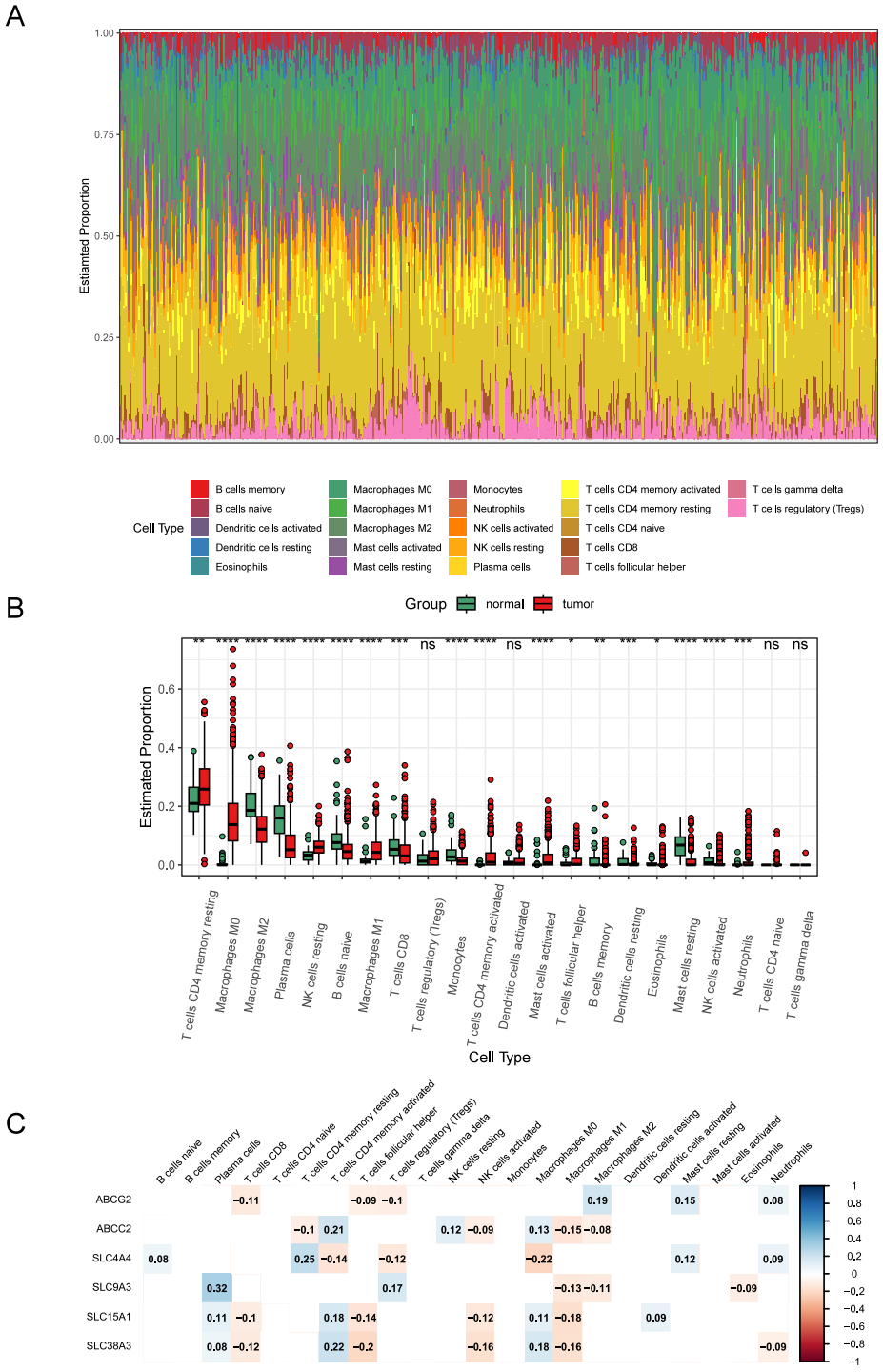

Figure 3. Landscape of Immune Infiltration. (A) Histogram of the percentage of immune infiltration. (B) Box diagram of each immune cell grouping. (C) Correlation between prognostic genes and immune cells.

transporter protein-related genes involved in CRC, including *SLC9A3*, *SLC15A1*, *ABCG2*, *ABCC2*, *SLC4A4*, and *SLC38A3*. There are many members of the transporter protein family, and they play different roles in CRC. Abnormal expression of *ABCC2* and *ABCG2* in CRC has been demonstrated, and their roles in transporting lipids and leading to drug resistance have been reported. *SLC4A4* and *SLC9A3* can transport H^+ and HCO_3^-, respectively, thus affecting tumor cell PH regulation. *SLC15A1* has a significant effect on intestinal peptide transport affects intestinal diseases.[19,21−23] *SLC38A3* is known for its involvement in amino acid transport and is extensively documented in neurological disorders.[24−26] Previous studies have explored the role of *SLC38A3* in glutamine metabolism and its effect on tumor progression. Our study identified *SLC38A3* as a novel prognostic marker gene in CRC, and the knockdown of *SLC38A3* in HCT cells inhibited CRC cell proliferation and migration.

Immune infiltration analysis showed that the CRC group had a significantly higher percentage of infiltration by M2 macrophages compared to M1 macrophages. M2 macrophages

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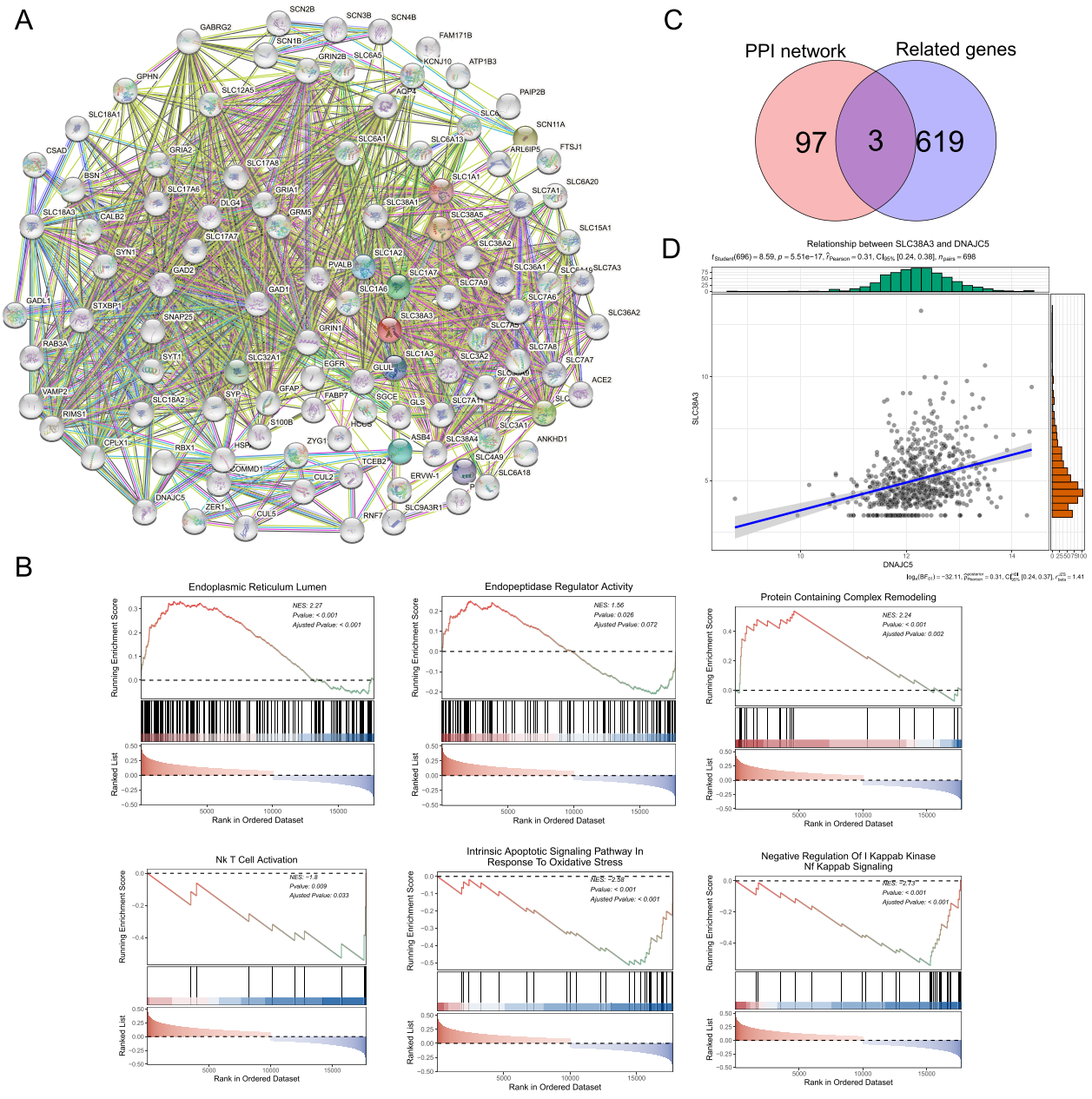

Figure 4. Analysis of SLC38A3. (A) PPI network of SLC38A3. (B) GSEA results. (C) Genes significantly correlated with SLC38A3. (D) Correlation between SLC38A3 and DNAJC5.

can produce various immunosuppressive factors and chemokines to reduce antigen presentation and hinder T cell function, allowing tumor cells to evade immune surveillance. Previous studies have suggested that M1 macrophages may be associated with a better patient prognosis. Blocking glutamine metabolism may promote the conversion of M2 macrophages to the more desirable M1 phenotype.[27,28] Immune cell correlation results showed that expression of *SLC38A3* was negatively correlated with the M1 macrophage, thus targeted inhibition of *SLC38A3* might be beneficial for macrophage M1 polarization.

Furthermore, we adopted a novel approach of single gene correlation analysis and the construction of a single gene PPI network to investigate the role of *SLC38A3* in CRC progression. First, we observed elevated expression of *SLC38A3* in CRC tissues, consistent with the findings of He Yu et al.[29] In addition, our research revealed that *SLC38A3* has a significant impact on both the proliferation and migration of CRC cells. Through correlation analysis and the PPI network, we identified a positive association between *SLC38A3* and *DNAJC5*, which is a chaperone protein of the heat shock protein Hsp70. These Hsp70s are essential molecular chaperones that play a crucial role in various biological processes by regulating polypeptide folding, translocation across membranes, and protein−protein interactions.[30] The J protein family has also been proven to increase the ATPase activity of Hsp70, which contributes to its binding ability with client proteins.[31] Inhibition of Hsp70 has been identified as a

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Table 1. 100 Nodes of the SLC38A3 PPI Network

SLC3A1	GAD1	AQP4	GLS	SLC6A11	RBX1	DNAJC5	SLC6A18
SLC1A3	KCNJ10	CPLX1	SLC6A13	SNAP25	TCEB2	GADL1	SLC7A6
SLC6A19	FABP7	STXBP1	SLC18A1	SYT1	RNF7	CALB2	SLC7A7
SLC6A20	GRIN1	GPHN	SLC7A5	SLC17A6	ZER1	SLC9A3R1	SLC7A3
SLC15A1	GAD2	VAMP2	SLC38A1	SYP	ZYG11B	SLC7A11	SLC36A2
ERVW-1	GRIN2B	RIMS1	SLC1A6	SLC1A2	COMMD1	SLC3A2	SLC38A9
SLC1A7	GRIA1	SLC18A3	SLC38A2	SLC6A1	SLC38A4	SLC1A5	SLC7A8
SLC4A9	SLC12A5	SLC17A8	ATP1B3	S100B	SGCE	EGFR	SLC7A1
PVALB	DLG4	ASB4	SCN11A	SYN1	CUL2	ACE2	HCCS
SLC32A1	GFAP	SLC18A2	SLC7A9	BSN	CUL5	SLC38A5	ARL6IP5
SLC17A7	SLC1A1	HSPA8	SLC36A1	GRIA2	PAIP2B	SCN3B	FAM171B
SCN4B	GABRG2	CSAD	ANKHD1	GRM5	RAB3A	SCN1B	PDZK1
GLUL	SCN2B	SLC6A5	FTSJ1				

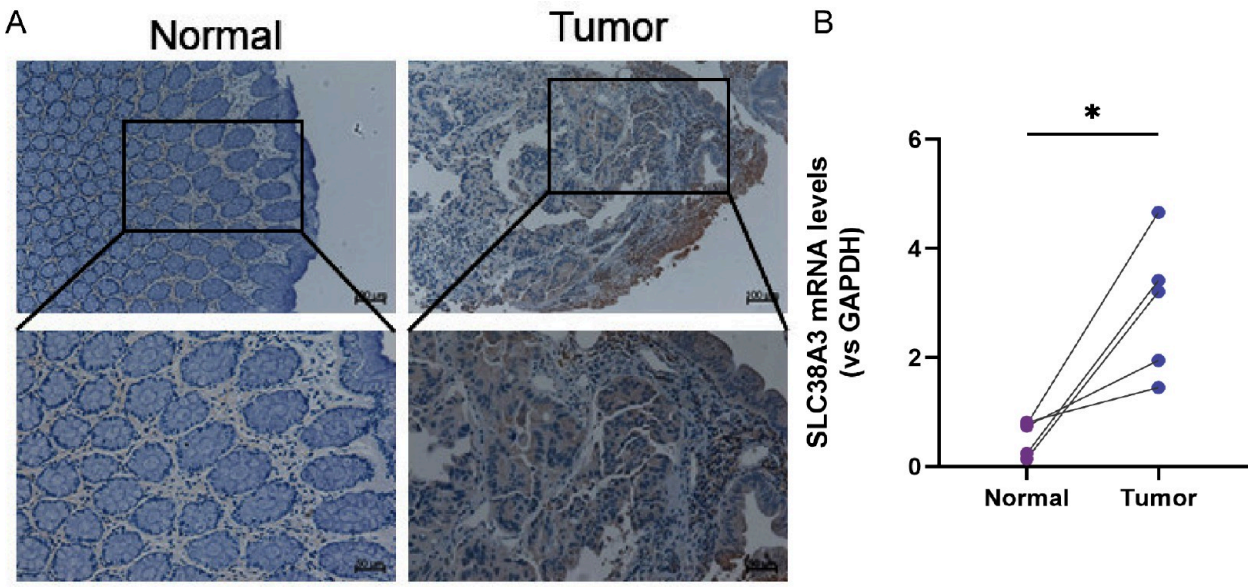

Figure 5. Expression of *SLC38A3* in tumor tissues and normal tissues. (A) The comparison of the protein expressions of SLC38A3 between tumor tissues and adjacent tissues by IHC. $n = 5$ biological replicates. (B) The mRNA expression of SCL38A3 between tumor tissues and adjacent tissues was measured by RT-qPCR. *$P < 0.05$ vs normal. $n = 5$ biological replicates. Statistics were determined using an independent samples t test.

promising chemotherapeutic approach for CRC treatment.[32] GSEA results showed *SLC38A3* was positively associated with endopeptidase and the endoplasmic reticulum, which are responsible for processing and modification of proteins. There are two important factors related to the development of colorectal cancer. First, dysregulation of translational regulatory mechanisms of proteins is commonly observed in solid tumors, including colorectal cancer. Second, the abnormal folding of proteins in the endoplasmic reticulum is closely associated with the development of colorectal cancer.[33,34] In contrast, a significant negative correlation was noted between *SLC38A3* and some pathways that are detrimental to tumor growth.[35,36] For example, in a study of normal versus colorectal cancer patients, a better prognosis was observed in patients with higher Valpha24+ NKT cell infiltration.[37]

To further investigate the effect of *SLC38A3* on tumor tissue and the mechanism, a cell model of *SLC38A3* knockdown by siRNA transfection was constructed. The proliferation and migration of HCT116 cells knocked down with *SLC38A3* were significantly slower than those of the NC group, indicating that *SLC38A3* promotes the proliferation and migration of tumor cells. The proliferation and migration ability of transfected cells with the addition of HSP70 ATPase agonist 115-7c was significantly restored compared to the knockdown group. Thus, combined with the results of bioinformatics analysis, we hypothesize that *SLC38A3* may affect tumor cell progression by positively regulating *DNAJC5* to promote biological processes such as protein folding in which Hsp70 is involved.

In conclusion, these results highlight the potential of *SLC38A3* as a therapeutic target in the treatment of CRC. Further research needs to be done to explore the potential therapeutic applications of targeting this protein. However, the current study is somewhat limited. Our data are limited and from publicly available databases, and a larger number of samples are needed for validation. In addition, we have studied only the complex molecular mechanisms of *SLC38A3* at the cellular level, which needs further validation in animal models.

CONCLUSIONS

SLC38A3, a new oncogenic gene, plays an important role in promoting proliferation and migration of tumor cells by positively regulating the protein folding function of *DNAJC5* affecting Hsp70, and it may be used as a new biomarker for the diagnosis and treatment of CRC.

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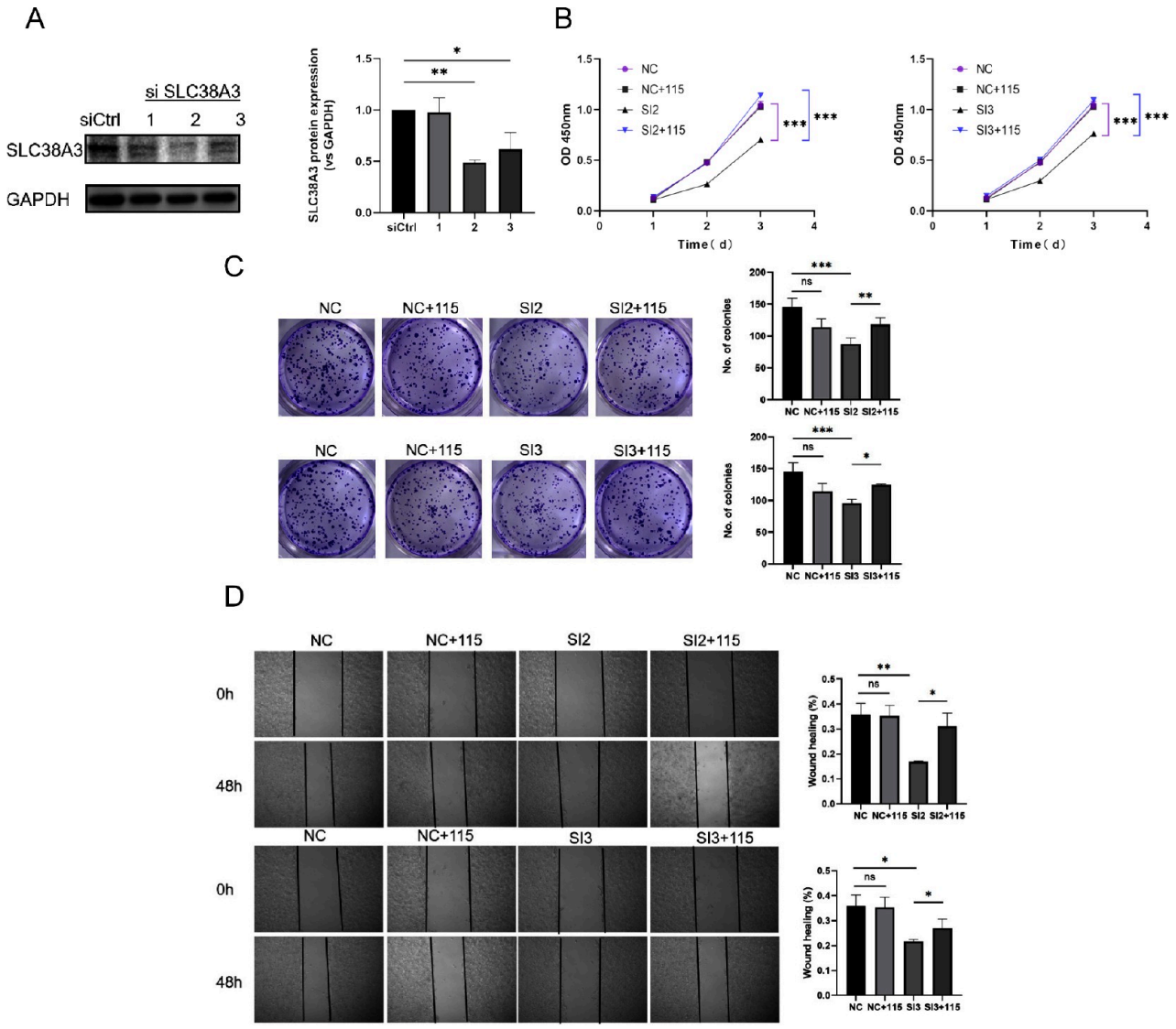

Figure 6. *SLC38A3* promotes cell proliferation, migration and invasion of CRC. (A) The transfection efficiency of siSLC38A3 was demonstrated by Western blotting. (B) Silencing *SLC38A3* decreased the cell viability of HCT116 cells, and 115-7c demonstrated the reverse. CRC cells were transfected with siSLC38A3 or negative control siRNAs for 48 h before serum deprivation. Cell viability was detected by CCK8. (C) Colony formation assays with siCtrl, siSLC38A3 transfected cells, and siSLC38A3 transfected cells with 115-7c for 14 d. Data represent $\bar{x} \pm s\bar{x}$, $n = 3$ independent experiments. (D) Wound healing assay of siCtrl, siSLC38A3 transfected cells, and siSLC38A3 transfected cells with 115-7c for 48 h. Scale bars represent 50 μm. All data were statistically analyzed using an independent samples t test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

MATERIALS AND METHODS

Identification of Differentially Expressed Genes. The gene expression profile and corresponding clinical information were downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/).[38] The TCGA COADREAD data set consisted of 647 CRC tissues and 51 normal tissues. The Dseq2 R package[39] was utilized to identify differentially expressed genes (DEGs) between CRC and normal tissues. The criteria for identifying DEGs was set at $|\log_2\text{foldchangel} > 2$ and an adjusted P-value of <0.05. Subsequently, DEGs were visualized with a volcano map using the ggplot2 R package.[40]

Enrichment Analysis of DEGs and Identification of DE-TPRGs. To further examine the potential functions of DEGs, gene ontology (GO)[41] enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG)[42] pathway enrichment analysis of DEGs were performed using the clusterProfiler R package.[43] GO enrichment analysis consisted of three parts: biological process (BP), cellular component (CC), and molecular function (MF). Terms with $P < 0.05$ were considered to be significantly enriched. The enrichment results were visualized using dot plots via the Sangerbox online platform.[44]

Transporters-related gene lists were collected in Reactome (https://reactome.org/) and the KEGG database (https://www.kegg.jp/kegg/). The search keywords were "transporter proteins", "ATP-binding cassette transporter", and "Solute carrier transporter" with the following searching strategies: "Homo Sapiens" (Species) and "Pathway" (Type). Transporter-related genes are displayed in Table S7. The intersection of DEGs and transporter protein-related genes was visualized by a Venn plot.

Acquisition of Hub Genes. The STRING (https://string-db.org/) is an online database that was designed to collect and evaluate protein−protein interaction (PPI) information of the

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DE-TPRGs. Genes were selected to construct the PPI network with a combined score ≥0.4. We then imported the results of STRING into Cytoscape software (version 3.8.2), and the key subnetworks were built through the cytoHubba plugin. The 10 highest-scoring genes (maximum correlation criterion, Degree algorithm) were acquired. Survival analysis was subsequently conducted on CRC samples from the external data set GSE87211 obtained from the Gene Expression Omnibus (GEO) database.[45] Specifically, CRC samples were categorized into high- and low-expression groups based on the expression level of each gene. Genes with meaningful results were identified as hub genes. Kaplan−Meier survival curves and log-rank tests were utilized for survival analysis.

Evaluation of Immune Infiltration. The gene expression data normalized by the DESeq2 R package were uploaded to the CIBERSORT online platform (http://cibersort.stanford.edu/) to obtain the relative proportions of 22 types of infiltrating immune cells. CIBERSORT is an analytical tool developed by the Alizadeh Lab and Newman Lab to impute gene expression profiles and provide an estimation of the abundances of member cell types in a mixed cell population, using gene expression data.[46] In addition, correlations between hub genes and immune cells were estimated by a Spearman correlation analysis.

Potential role of SLC38A3 in CRC. To further identify the proteins that *SLC38A3* potentially directly modulates in CRC progression, we first identified 100 genes interacting with *SLC38A3* using the STRING database. Subsequently, correlations of other genes with *SLC38A3* were calculated in CRC samples using Spearman correlation analysis. A gene list for gene set enrichment analysis (GSEA) was constructed and ranked according to correlation coefficients from high to low. Then GSEA analysis was performed based on the gene list. Genes with $P < 0.05$ and correlation coefficients ≥0.3 from correlation analysis were identified as *SLC38A3*-related genes. Finally, Venn diagrams were used to identify the intersection between the PPI network genes and *SLC38A3*-related genes. The intersecting genes might be involved in mediating the function with *SLC38A3* in CRC progression

Immunohistochemistry. The CRC tissue and para-carcinoma tissues were collected and routinely embedded into paraffin. Colon rectal sections were stained with hematoxylin. For immunochemistry staining, after the endogenous peroxidases were quenched with 3% H_2O_2 and unspecific binding was blocked with 2% bovine serum albumin (Amresco, Solon, OH), sections were stained with F4/80, Ly-6G, CD3, 3-nitrotyrosine, 8-oxoG, or Nrf2 overnight at 4 °C. After extensive washing, sections were incubated with respective biotinylated secondary antibodies. Positive staining was visualized using DAB Substrate (Cwbiotech, Beijing, China) following the ABC Kit (Vector Laboratories, Burlingame, CA).

RNA Extraction and RT-qPCR. Clinical samples were obtained from patients with CRC who were surgically treated at The Third Xiangya Hospital from February 2022 to March 2023. The study has been approved by the Ethics Committee of Third Xiangya Hospital (R20021). Total RNA was isolated from cells using the TRIzOn reagent (CWBIO, Beijing, China). A HiFiScript cDNA Synthesis Kit (CWBIO, Beijing, China) was usedfor reverse transcription, and then ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) was used for RT-qPCR. *SLC38A3* primer sequences were as follows: forward primer CTCCAACCTGTCCATCGCTGTC and reverse primer AAACGGGTCCACCTTGCTGTAG.

Cell Culture and Silencing of *SLC38A3*. Human CRC cell line HCT116 was obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were maintained in DMEM high glucose medium (Hyclone, Logan, UT, USA) supplemented with 10% FBS (Gibco, USA) and 1% antibiotic (penicillin-streptomycin, Gibco, USA). Cells were transfected with Specific small interfering RNA (siRNA) against *SLC38A3*. SiRNAs were custom designed and provided by Guangzhou RiboBio Co., Ltd. (RiboBio). The three siRNAs targeting *SLC38A3* include *SLC38A3*_1, *SLC38A3*_2 and *SLC38A3*_3. Cells were seeded on 60 mm culture plates and transfected with oligonucleotides using lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Transfected cells were incubated at 37 °C in 5% CO_2. After 48 h, cells were harvested for Western blotting.

Cell Proliferation Assay. In order to examine the involvement of *SLC38A3* expression with cellular proliferation, control cells, transfected HCT116 cells, and transfected cells cultured with 115-7c (an ATPase agonist of Hsp70) were spread in five 96-well plates at a density of 2500 cells per well, and incubated at 37 °C in a 5% CO_2 incubator. 115-7c was formulated into solution at a concentration of 20 nm to examine whether reduced expression of *DNAJC5* due to *SLC38A3* knockdown affects the ATPase activity of Hsp70 and inhibits tumor cell proliferation. One plate was removed every 24 h for 3 consecutive days, 100 μL of CCK8 solution (90 μL of complete medium plus 10 μL of CCK8 stock solution) was added to each well, and the plates were incubated in the incubator for 1 h. The absorbance at 450 nm was measured. For the colony formation assay, control cells, transfected HCT116 cells, and transfected HCT116 cells cultured with 115-7c were seeded into 12-well plates with a cell count of 1000 cells per well and repeated three times. The cells were incubated for 14 days until the appearance of a naked eye cell cluster terminated. Samples were washed three times with PBS, PBS was discarded, cells were fixed with 1 mL of paraformaldehyde for 10 min, and paraformaldehyde was discarded. Then, 1 mL of 0.5% crystalline violet was added to each well, and cells were stained for 20 min avoiding light. The samples were washed three times with PBS, dried, and photographed.

Wound Healing Assay. Control cells, transfected HCT116 cells, and transfected HCT116 cells cultured with 115-7c were seeded in 6-well plates, and monolayers were gently scratched with a sterile 200 mL pipet tip when they had grown to 90% confluence. After washing with PBS three times to remove floating cells, the remaining adherent cells with a thin "wound" were incubated at 37 C in 5% CO_2. The photos were photographed at 0, 12, 24, 36, and 48 h using an inverted microscope. All independent experiments were performed in triplicate.

Statistical Analysis. Histochemistry and RT-qPCR were performed using five pairs of biological samples. Cell experiments were repeated at least three times. All data were expressed as the mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism 8.0 for Windows. The Spearman correlation coefficient test was used to analyze the association between *SLC38A3* and other genes in the TCGA cohort. The survival curves were plotted by Kaplan−Meier analysis and compared using a log-rank test.

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Differences were considered statistically significant when the *P*-value was <0.05.

■ ASSOCIATED CONTENT

Data Availability Statement

The data sets used or analyzed during the current study are available from the corresponding author on reasonable request. The complete results of the authors' analyses are provided in the Supporting Information. Publicly available data sets can be downloaded from the TCGA (https://www.cancer.gov/tcga) and GEO (https://www.ncbi.nlm.nih.gov/gds) databases.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c00901.

All differential genes, KEGG results, GO results, results of immune filtration analysis, GSEA results of SLC38A3-related genes, genes with a correlation of 0.3 or more with SLC38A3, and list genes related to transporters (XLSX)

GSEA results of DEGs (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CRC: colorectal cancer
DE-TPRGs: differentially expressed transporter protein-related genes
TCGA: The Cancer Genome Atlas
PPI: protein−protein interaction
SLCs: solute carriers
ABCTs: ATP-binding cassette transporters
EMT: epithelial−mesenchymal transition
DEGs: differentially expressed genes
GO: gene ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes
BP: biological process
CC: cellular component
MF: molecular function
GEO: Gene Expression Omnibus
GSEA: gene set enrichment analysis
ESCC: esophageal squamous cell carcinoma

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