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Bioinformatic Analysis of The Prognostic Value of A Panel of Six Amino Acid Transporters in Human Cancers

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Abstract

Objective: Solid tumor cells utilize amino acid transporters (AATs) to increase amino acid uptake in response to nutrient-insufficiency. The upregulation of AATs is therefore critical for tumor development and progression. This study identifies the upregulated AATs under amino acid deprived conditions, and further determines the clinicopathological importance of these AATs in evaluating the prognosis of patients with cancers.

Materials and Methods: In this experimental study, the Gene Expression Omnibus (GEO) datasets (GSE62673, GSE26370, GSE125782 and GSE150874) were downloaded from the NCBI website and utilized for integrated differential expression and pathway analysis v0.96, Gene Set Enrichment Analysis (GSEA), and REACTOME analyses to identify the AATs upregulated in response to amino acid deprivation. In addition, The Cancer Genome Atlas (TCGA) datasets with prognostic information were assessed and employed to evaluate the association of identified AATs with patients' prognoses using SurvExpress analysis.

Results: Using analysis of NCBI GEO data, this study shows that amino acid deprivation leads to the upregulation of six AAT genes; SLC3A2, SLC7A5, SLC7A1, SLC1A4, SLC7A11 and SLC1A5. GSEA and REACTOME analyses identified altered signaling in cells exposed to amino acid deprivation, such as pathways related to stress responses, the cell cycle and apoptosis. In addition, Principal Component Analysis showed these six AAT genes to be well divided into two distinct clusters in relation to TCGA tumor tissues versus normal counterparts. Finally, Log-Rank analysis confirmed the upregulation of this panel of six AAT genes is correlated with poor prognosis in patients with colorectal, esophageal, kidney and lung cancers.

Conclusion: The upregulation of a panel of six AATs is common in several human cancers and may provide a valuable diagnostic tool to evaluate the prognosis of patients with colorectal, esophageal, kidney and lung cancers.

Keywords: Amino Acid Transporters, Glutamine, Prognosis, Tumorigenesis

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Introduction

Tumor development and progression includes a series of interconnected steps such as the activation of oncogenes and/or the loss of tumor suppressors leading to replicative immortality and constitutive cell proliferation (1). As solid tumors grow larger and larger, the angiogenic switch is turned on to induce blood vessel formation (2) which provides both nutrients and a route for tumor metastasis. The processes outlined above are defined as the hallmarks of human cancers. Recently, two emerging hallmarks and two enabling characteristics have been illustrated and characterized in human cancers. An example is dysregulated cellular metabolism which has been widely investigated and considered as one of the emerging hallmarks (3, 4). The Warburg effect and active glutaminolysis represent the most common characteristics of tumor metabolism (5, 6). Glutaminolysis is mainly the conversion of glutamine to glutamate. Generally, tumor cells are addicted to glutamine, thus glutamine is considered as a conditionally essential amino acid. Besides glutamine, there are twenty other proteinogenic amino acids in eukaryotes. Biologically, amino acids participate in a variety of cellular processes that contribute to tumor development and progression. Amino acids are precursors in energy production, biosynthetic and reductive processes. They also participate in epigenetic regulation and ammonia detoxification processes (7-12). Accordingly there is a high demand for amino acids in tumor cells, with increased proliferation leading to overexpression of amino acid transporters (AATs) (13). In normal cells, the expression of AATs is finely adjusted to control the uptake and distribution of amino acids. In

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contrast, tumor cells acquire a large quantity of amino acids, mainly through the upregulation of AATs; the expression of AATs being reprogrammed to promote the absorption of amino acids in accordance with the elevated level of cellular proliferation.

The AATs are a group of membrane-bound proteins that facilitate the uptake or excretion of amino acids at cellular or organelle levels (14). They belong to the solute carrier (SLC) superfamily and are grouped into different categories based on their substrate specificity and different working mechanisms (15). Biologically, AATs regulate a variety of processes, such as, energy production, biosynthesis, redox homeostasis, gene transcription and translation, signaling pathways, and cell proliferation. Due to their critical roles in controlling various biological functions, the dysregulation of AATs leads to the development of pathologies, such as, tumors, neurodegenerative diseases, metabolic diseases, etc. In human tumors the dysregulation of AATs facilitates the metabolic reprogramming that controls autophagy and cell proliferation through ATP generation, protein & nucleotide synthesis, and NADPH production.

Normally, solid tumors have defective blood vessel systems that lead to insufficient nutrient supplies, such as low amino acid or glucose levels, and hypoxia (12). A recent paper identified that SLC7A5 can be induced when cells are exposed to media low in histidine, tyrosine, and methionine levels (16). Under stress conditions, tumor cells resort to conserved signaling to upregulate AATs. For example, amino acid deprivation can activate eukaryotic translation initiation factor 2α (eIF2α)-activating transcription factor 4 (ATF4) signaling through general control nondepressible 2 (GCN2). Therefore, it is common that AAT promotion includes amino acid response elements (AAREs) that can be bound by ATF4 transcription factor leading to increased transcription of AATs (17-20). This study identifies the upregulated AATs under amino acid deprived conditions, and further determines the clinicopathological importance of these AATs in evaluating the prognosis of patients with cancers.

Materials and Methods

Collection of published and open-access datasets

In this experimental study, mRNA expression and RNA-Seq data were obtained from the Gene Expression Omnibus (GEO) on the NCBI website (https://www.ncbi.nlm.nih.gov/geo/). MCF7 breast cancer cells in the GSE62673 dataset were first exposed to complete medium or medium without one/all amino acid(s) for 24 hours. mRNAs were then extracted and analyzed using the Affymetrix Human Genome U133A 2.0 Array. MDA-MB-231 breast cancer cells from the GSE26370 dataset were cultured in medium with/without glutamine for 24 hours and the mRNA levels were also analyzed using the Affymetrix Human Genome U133A 2.0 Array. Wild-type 3T3 cells from the GSE125782 dataset were maintained in the presence or absence of glutamine for 18 hours and the RNAs sequenced using the Illumina HiSeq 2500 platform. KPC pancreatic ductal adenocarcinoma cells from the GSE150874 dataset were cultured in regular or glutamine-free medium for 24 hours, and the RNA sequencing was conducted using the Illumina NextSeq 500 platform.

Analysis of the microarray or RNA-Seq data using the iDEP v0.96 online tool

The microarray or RNA-Seq datasets were normalized and analyzed using the iDEP v0.96 online tool (http://bioinformatics.sdstate.edu/idep/) (21). iDEP (integrated differential expression and pathway analysis) is an online tool that facilitates analyzing transcriptomic profiling like microarray or RNA-Seq data. iDEP can be applied for exploratory analysis, identification of differentially expressed genes, and pathway analysis.

Normalization of microarray data and Gene Set Enrichment Analysis

GEO datasets (GSE62673, GSE26370, GSE125782 and GSE150874) were uploaded to the R-Project Bioconductor and standardized using the Robust Multiarray Average (RMA) method. The signal intensities were shown on a Log^2 scale and the normalization of gene expression was evaluated using the LIMMA package from the R Bioconductor. In this study, R-Project Bioconductor (ver. 4.1.0, 09/10/2021) was performed to normalize the Affymetrix data. The detailed codes are the same as those in our previous paper (4).

The normalized datasets were processed according to the instructions on the GSEA website (http://software.broadinstitute.org/gsea/index.jsp). Thereafter, GSEA was applied to analyze gene signatures using the Hallmark gene sets. NES was applied to rank the gene-set enrichment. The FDR q-value was calculated to estimate the probability of a false positive finding. In addition, the FWER P value was utilized to estimate the probability of a false positive finding for NES.

REACTOME analysis

REACTOME (https://reactome.org/) is an open-access, manually curated pathway database that provides interpretation and visualization of relevant pathways based on the microarray or RNA-Seq data (22, 23). The data were prepared following the requirements published on the website: https://reactome.org/userguide/analysis. The normalized microarray dataset GSE62673 was uploaded to the online system and underwent PADOG analysis, which down-weighs the genes existing in different pathways.

Pathway analysis using ShinyGO v0.741

ShinyGO (http://bioinformatics.sdstate.edu/go/)

was developed using R-Bioconductor packages that facilitate data analysis and the visualization of results in a graphical way (24). For ShinyGO analysis, the six AAT genes ENSG00000168003, ENSG00000103257, ENSG00000139514, ENSG00000115902, ENSG00000151012 and ENSG00000105281 were listed and applied to build the pathway networks.

DepMap analysis

DepMap analysis was conducted using the online portal (https://depmap.org). The Cancer Dependency Map project provides all the data available to the public under a Creative Commons license in order to create open science. The generated datasets are posted on the DepMap portal prior to publication every three months.

Identification of the alteration in glutamine-deprivation related signaling pathways

Glutamine was selected to validate the repeatability of the response of cells to amino acid deprivation because a plethora of studies have focused on investigating how cells reprogram gene expression to adapt to glutamine-deprivation. For this analysis, the most affected genes were listed to generate and compare the enrichment plots.

GEPIA2 analysis

GEPIA2 is an online tool that helps to explore the large TCGA and GTEx datasets (http://gepia2.cancer-pku.cn/#index) (25). The expression of one single gene can be compared via Boxplots in multiple cancers and their normal counterparts, and the expression of multiple genes can be compared using a matrix plot. GEPIA2 can also be applied to Principal Component Analysis (PCA) of multiple genes in different cancer types through presenting the results in either 2D or 3D plots.

Analysis of gene expression in human samples using cBioportal

The six AAT genes were analyzed according to gene mutations, copy number variations and mRNA levels on the cBioportal website: http://www.cbioportal.org/.

Establishing the clinical significance of the six AAT genes using SurvExpress

The SurvExpress database (http://bioinformatica.mty.itesm.mx:8080/Biomatec/Surviva X.jsp) was applied to investigate the correlation between the expression of six AAT genes with the prognosis of patients. The six AAT genes included SLC3A2, SLC7A5, SLC7A1, SLC1A4, SLC7A11 and SLC1A5.

Dissection of prognostic importance using ENCORI Pan-Cancer survival analysis

This analysis was performed using the ENCORI Pan-Cancer survival analysis online tool: http://starbase.sysu.edu.cn/panGeneSurvivalExp.php# by searching on the six AAT genes. The p values were presented as Dot plots.

Results

Comparison of gene expression under conditions of amino acid-deprivation

Dataset # GSE62673 was identified by searching the NCBI GEO website. This study was performed to dissect unique transcriptional responses to the withdrawal of one amino acid or all amino acids at one time while the control cells were maintained in regular complete medium. The dataset also provides useful information on how tumor cells respond to amino acid-deprivation. In particular, it will help identify factors that can evaluate patients' prognoses. The iDEP v0.96 online tool was employed to compare the range of log ratios associated with replicate spots (Fig. S1A, See Supplementary Online Information at www.celljournal.org), especially for the group with minor variation in data distribution, an indicator of the good quality of the dataset. Next, a heatmap was generated using the 1,000 most variable genes in all groups. The heatmap demonstrates glycine-deprivation leads to almost no alteration in gene expression relative to the control group while methionine-deprivation causes the most changes relative to the control group compared to other amino acid(s) deprivation (Fig.1A). These results were also validated by the PCA analysis (Fig.S1B, See Supplementary Online Information at www.celljournal.org) and the number of differentially expressed genes in each group (Fig.1B).

k-Means clustering enrichment analysis

k-Means enrichment analysis was performed using the 2,000 most variable genes through comparing a total of five pathways on the iDEP v0.96 website. These include Gene Ontology (GO) Cellular Component, GO Biological Process, GO Molecular Function, Curated Reactome and Kyoto Encyclopedia of Genes, and Genomes (KEGG) Metabolic Pathways. The exported results of the k-Means analysis were used to generate waterfall plots based on the number of changed genes (Fig.2, Fig.S2, See Supplementary Online Information at www.celljournal.org). Amino acid deprivation activates the conserved signaling pathways, for example the GCN2-mediated eIF2α phosphorylation that drives ATF4 expression to maintain cellular homeostasis (26). Accordingly, k-Means clustering enrichment detected activation of CHOP-ATF4 and CHOP-C/EBP complexes in the absence of amino acid(s) (Fig.2A). Under amino acid-depleted conditions, cells can activate the signaling related to apoptotic processes (Fig.2B, Fig.S2B, See Supplementary Online Information at www.celljournal.org) and the binding of transcription or

translation factors to relevant DNA or RNA targets (Fig.2C). Importantly, the restriction of amino acid availability also leads to the alteration of cell cycle related pathways like Mitotic G_1-G_1/S phases, Mitotic G_2-G_2/M phases and Mitotic prometaphase (Fig.S2B, See Supplementary Online Information at www.celljournal.org). Taken together, these results indicate that cells will first activate the evolutionally conserved signaling to effectively alleviate apoptosis when cells are exposed to short-term and acute nutrient-depletion. However, with long-term treatment, the cells will undergo apoptosis even with induction of the protective signaling due to the irreversible damage to the cells.

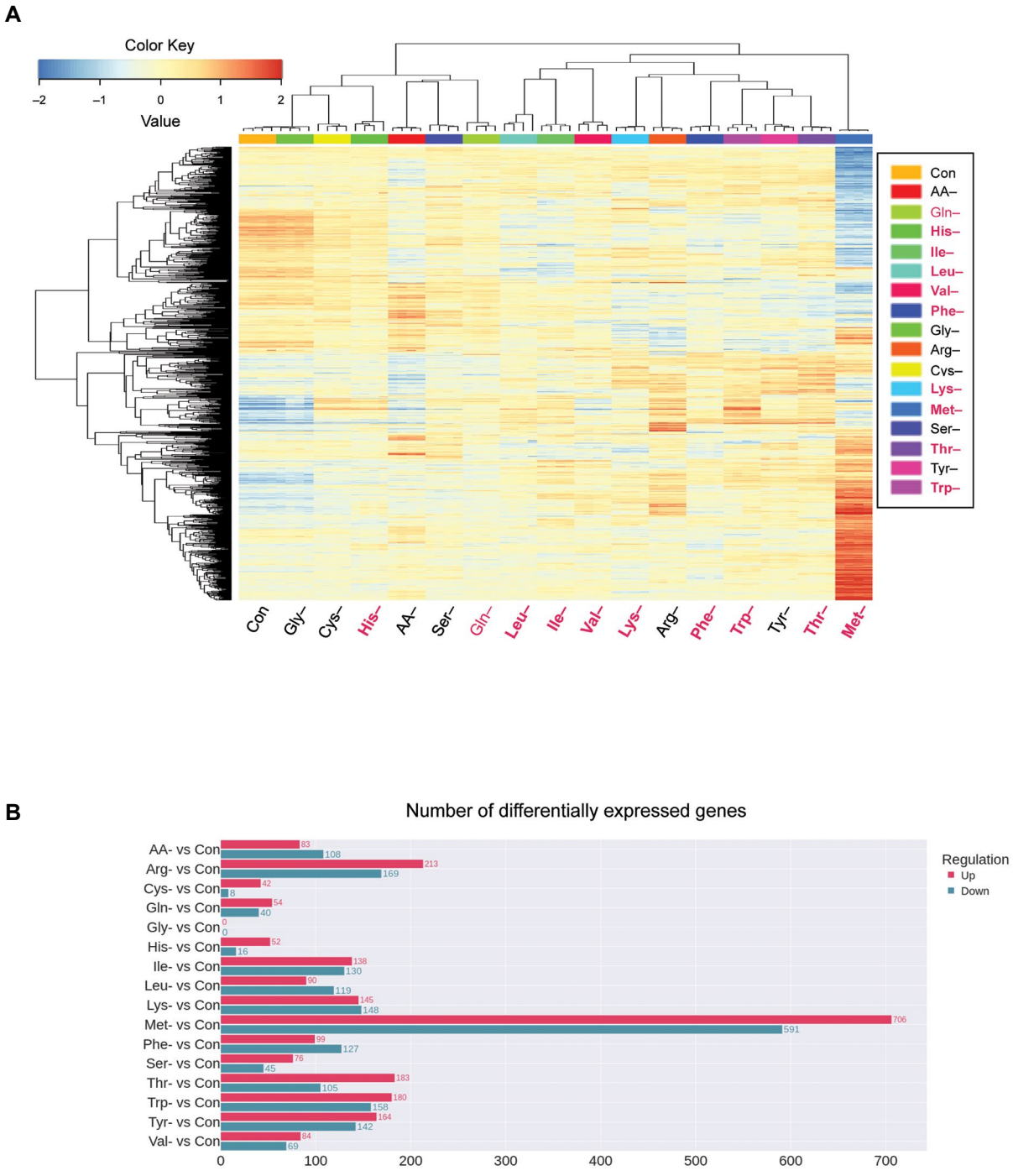

Fig.1: Comparison of the most variable genes between amino acid(s)-deprived groups and control group. **A.** The heatmap, generated using the iDEP v0.96 online tool, shows the gene expression pattern in different groups. Red font indicates the essential amino acids and conditionally essential amino acids. "Con" stands for "Control" group with cells maintained in regular culture medium. **B.** The numbers of differentially expressed genes (either up- or down-regulated) among different groups without one or all amino acid(s) versus that in control group.

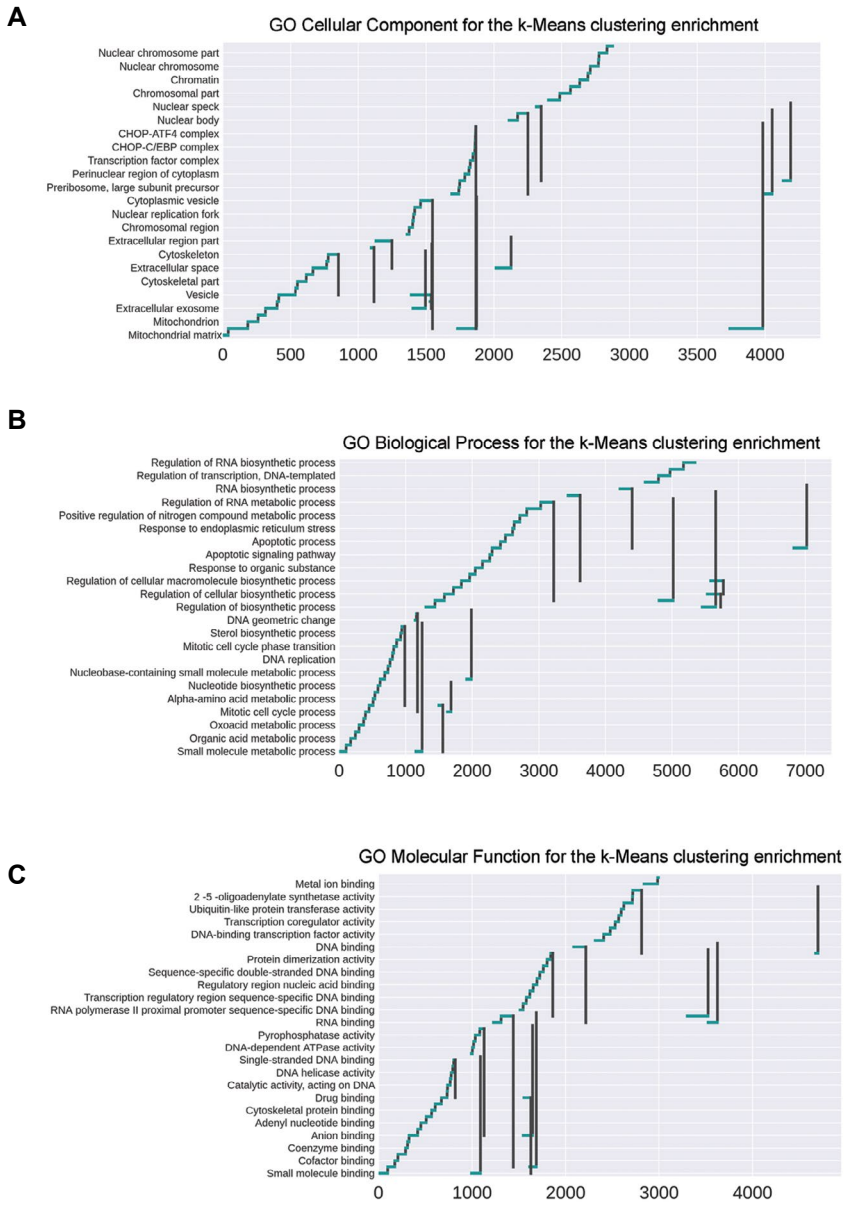

Fig.2: The waterfall plots demonstrate the k-Means clustering enrichment. **A.** The number of genes for pathway alteration in GO Cellular Component for the k-Means clustering enrichment. **B.** The number of genes for pathway alteration in GO Biological Process for the k-Means clustering enrichment. **C.** The number of genes for pathway alteration in GO Molecular Function for the k-Means clustering enrichment.

REACTOME analysis of different responses under conditions of amino acid-deprivation

REACTOME provides a platform for interpreting signaling, metabolic molecules and their relations with biological pathways and processes. For example, it facilitates establishing reaction networks through dissecting the metabolism of proteins, nucleic acids, complexes, vaccines, anti-cancer therapeutics and small molecules. The withdrawal of amino acids leads to the alteration of pathways like metabolism, the cell cycle, DNA Replication, DNA Repair, metabolism of proteins, programmed cell death, cellular responses to stimuli and so forth. Generally, there is a clear reduction in pathways related to the cell cycle, DNA Replication and DNA Repair, suggesting the amino acid deprivations affect cell cycle progression by interfering with the supply of molecules for DNA replication and reducing the building blocks for cell proliferation. Accordingly, reduction in amino acid levels can activate cellular responses to stimuli. For example, GSEA analysis showed the activation of unfolded protein response as one of the hallmark pathways generated by the deprivation of one/all amino acid(s) (Fig.S3, See Supplementary Online Information at www.celljournal.org). The REACTOME analysis also identified the upregulation of apoptosis-related signaling on deprivation of Arginine, Cystine, Glutamine, Isoleucine, Leucine, Lysine, Phenylalanine, Threonine, Tyrosine or Valine (Fig.S3, See Supplementary Online Information at www.celljournal.org), including both essential and non-essential amino acids.

Expression of AAT genes under amino acid-deprived conditions

Mammalian cells have a family of SLC membrane transporters, such as amino acids or glucose, to uptake organic molecules, inorganic ions and ammonia. It remains unknown what kinds of AATs will be altered in tumor cells on amino acid withdrawal. To address this question, SLC family member genes were listed from the GSE62673 dataset and analyzed using the iDEP v0.96 online tool. In accordance with the whole genome alterations, the glycine-deprived group had the lowest number of differentially expressed genes, while methionine-deprived group had the most alterations when compared with the control group (Fig.3A). PCA analysis also showed a pretty similar pattern of genes in the glycine-deprived group and the most changes in the methionine-deprived group (Fig.3B). Methionine initiates protein synthesis in eukaryotes, as seen in the PCA analysis, restriction of methionine demonstrates the greatest effects on global gene translation. For this reason, the expression of SLC family membrane transporters were analyzed and compared excluding the all-amino-acid-deprived group and the methionine-deprived group. Normally, solid tumors have insufficient blood supply, leading to low amino acid levels. Under this condition, the upregulated genes are supposed to play important roles in maintaining cell survival and proliferation. Based on this hypothesis, upregulated genes were analyzed under the depletion of different amino acids. Following this process, six AAT genes were identified as consistently upregulated; SLC3A2, SLC7A5, SLC7A1, SLC1A4, SLC7A11 and SLC1A5, when comparing the amino acid-deprived group to the control group (Fig.3C, Fig.S4, See Supplementary Online Information at www.celljournal.org). According to the biological functions of the six transporters, the pathway network was generated using ShinyGO (Fig.3D), highlighting that these AAT genes regulate various aspects of cellular functions that contribute to tumor development and progression.

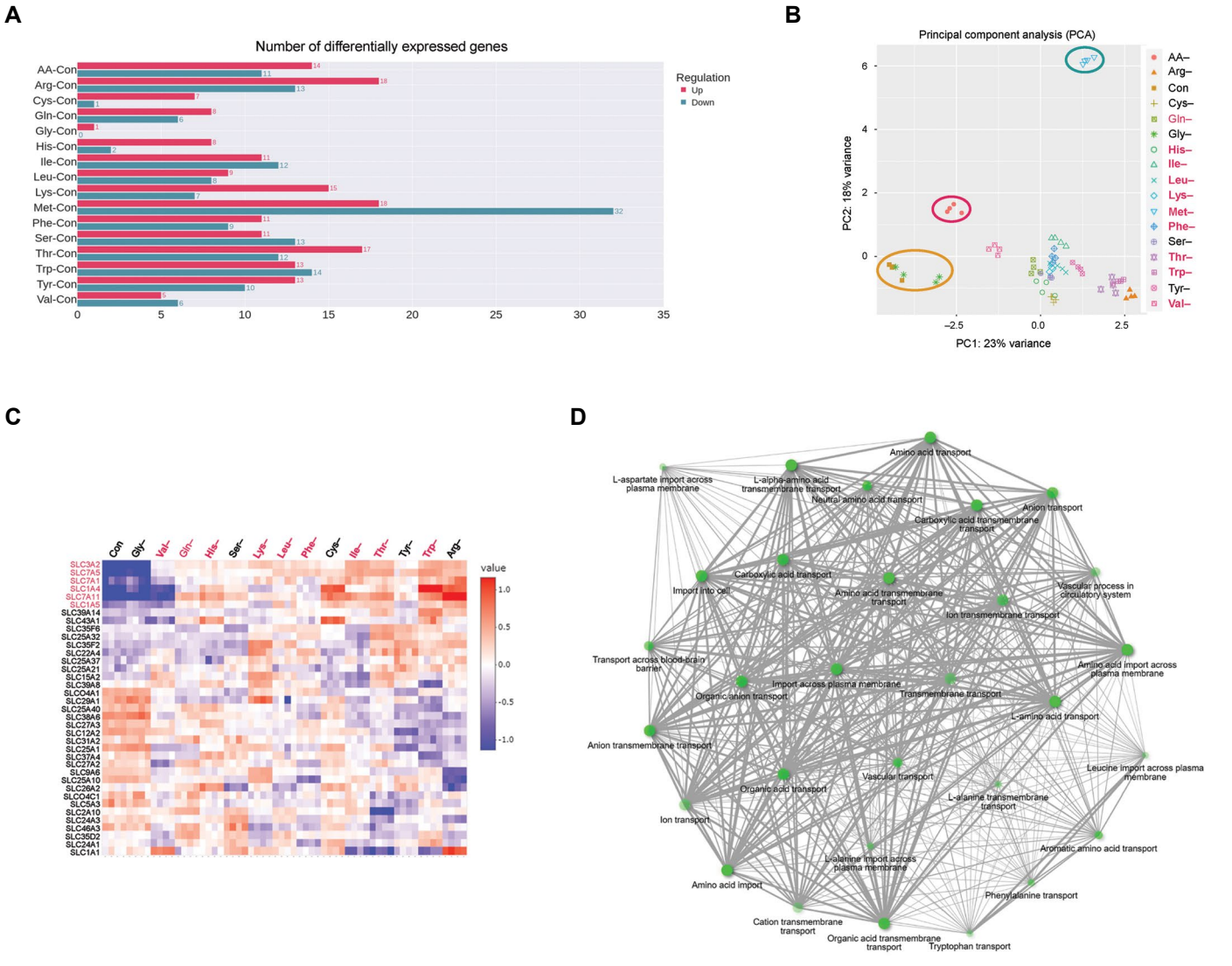

Fig.3: Upregulation of the six AAT genes in amino acid-deprivation. **A.** Comparison of the number of differentially expressed transporter genes among different groups. **B.** The PCA analysis indicates the alteration of AAT genes under different amino acid-depleted conditions. **C.** The heatmap demonstrates the most-altered AATs in amino acid-deprived conditions except that for methionine- and all amino acid- depletion. **D.** The ShinyGO online tool establishes the pathway network based on the six AAT genes.

Comparison of gene expression under glutamine-deprivation in independent studies

Glutamine is a widely investigated amino acid since it functions as a conditionally essential amino acid in tumor cells. Glutamine-deprivation leads to the upregulation of genes like ASNS, DDIT3, DDIT4, TRIB3, ATF3 and ATF4 (Fig.4A), which are well-known for their involvement in the integrated stress response. Importantly, glutamine-deprivation enhances the expression of genes related to cellular response to stress and apoptosis while reducing those related to DNA replication and cell cycle progression (Fig.4B, C). To further validate the findings from MCF7 cells in the GSE62673 dataset, three other datasets that included glutamine-deprivation were collected and analyzed to examine the alteration of genes related to the stress response and cell cycle progression (Fig.S5, See Supplementary Online Information at www.celljournal.org). These analyses showed that glutamine-deprivation induces the expression of the six AAT genes in both human and mouse cells, except for SLC1A4 and SLC1A5 in mouse pancreatic ductal adenocarcinoma KPC cells (Fig.4D), highlighting that upregulation of these six AAT genes function as conserved factors to mediate cellular response to the withdrawal of amino acids.

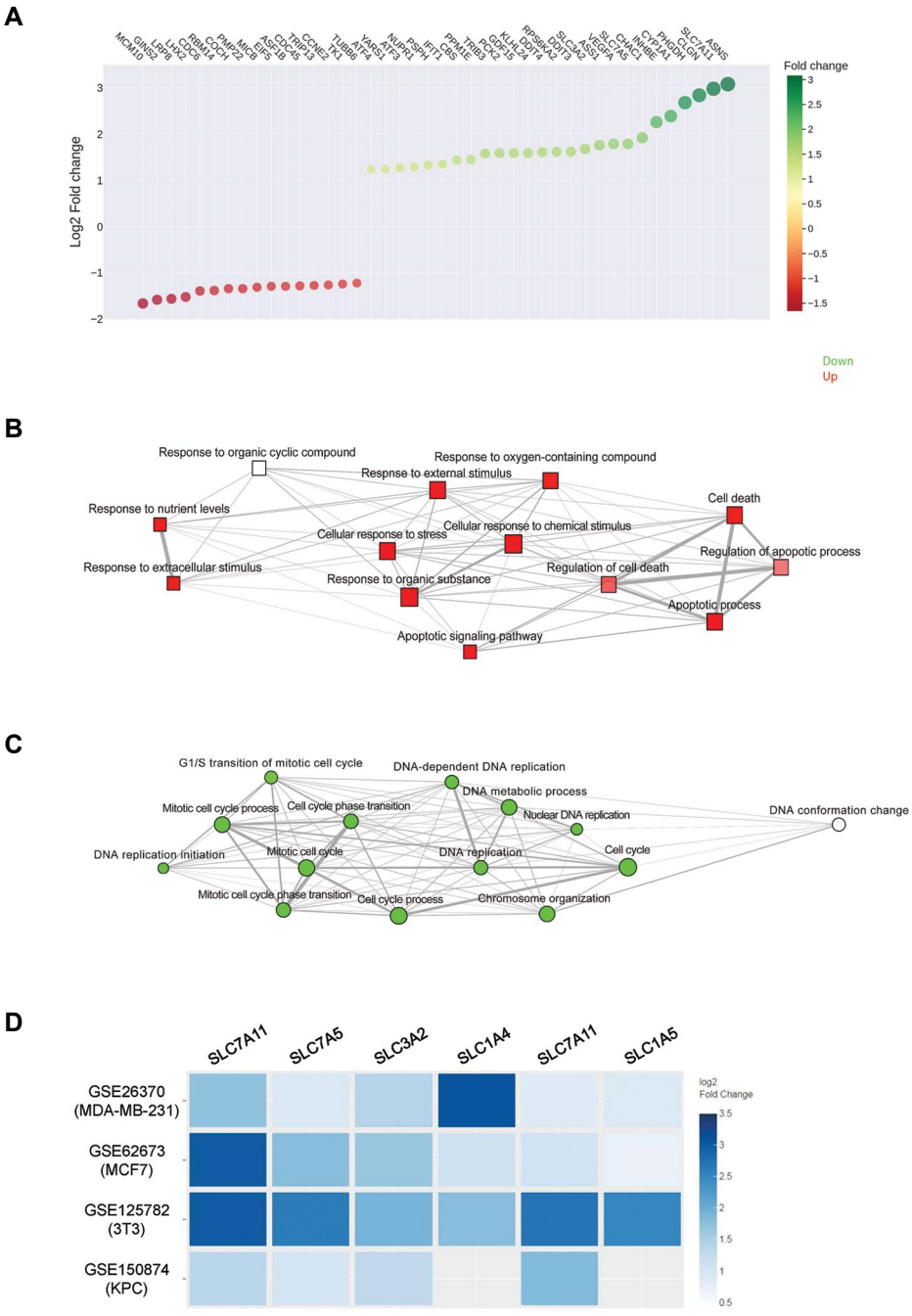

Fig.4: Comparison of gene expression pattern in glutamine-deprivation in independent studies. **A.** The most altered genes under glutamine-deprivation in the GSE62673 dataset. **B**, **C.** The activated or inactivated signaling pathways under glutamine-deprivation. **D.** The matrix plot indicates the log2 fold change of listed AATs in these datasets: GSE26370 (MDA-MB-231), GSE62673 (MCF7), GSE125782 (3T3) and GSE150874 (KPC).

Distinct expression of the six AATs in human tumor and normal tissues

Expression of the six AAT genes was compared using the GEPIA2 online tool regarding to the TCGA tumor tissues and their normal counterparts. Interestingly, there is an obvious upregulation of six AAT genes in different tumors relative to their normal counterparts (Fig.5A, Fig.S6, See Supplementary Online Information at www.celljournal.org). To further demonstrate the importance of these six AAT genes, PCA analysis was performed to study their enrichment in TCGA tumor samples versus normal tissues. Out of the twenty-three tumor types, there are six types of tumors that demonstrate a distinct separation of signals between tumor and normal tissue on both 2D and 3D plots (Fig.5, Fig.S7, See Supplementary Online Information at www.celljournal.org). These tumors include colon adenocarcinoma (COAD), esophageal cancer (ESCA), kidney renal clear cell carcinoma (KIRC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC) and rectal adenocarcinoma (READ). In accordance, cBioportal analysis also suggests there is a pattern of co-expression of these six AAT genes in human tumors like COAD, KIRC, LUAD, LUSC and esophageal adenocarcinoma (Fig.S8, See Supplementary Online Information at www.celljournal.org).

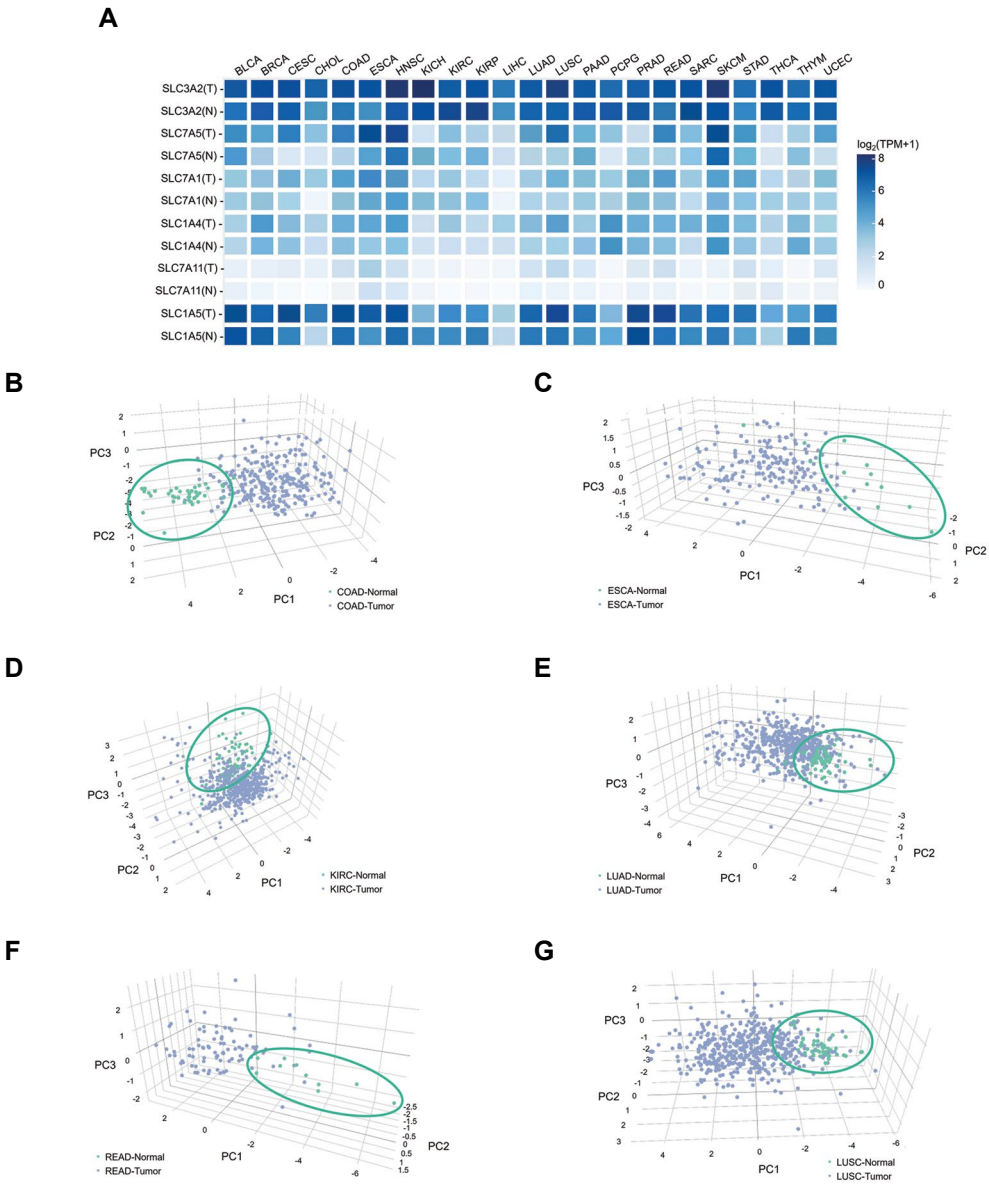

Fig.5: Expression of the six AAT genes in human tumors. **A.** The matrix plot compares expression of the indicated AAT genes in TCGA tumor tissues and in normal counterparts. This matrix is produced using the Multiple Gene Comparison function of the online tool GEPIA2. Generally, the gene list was uploaded first, and the TCGA tumor and normal data were chosen for further analysis. The comparison was performed using log2(TPM+1) for the log scale. **B**-**G.** The PCA analysis demonstrates this panel of six AAT genes is well separated into two distinct clusters when comparing normal and corresponding tumors including **B.** COAD, **C.** ESCA, **D.** KIRC, **E.** LUAD, **F.** READ, and **G.** LUSC. TPM; Transcripts per million, COAD; Colon adenocarcinoma, ESCA; Esophageal cancer, KIRC; Kidney clear cell carcinoma, LUAD; Lung adenocarcinoma, LUSC; Lung squamous cell carcinoma, and READ; Rectal adenocarcinoma.

Analysis of the prognostic importance of the six AAT genes in human cancers

For most TCGA tumors, overexpression of any one of the six AAT genes is significantly correlated with a poor prognosis (Fig.S9, See Supplementary Online Information at www.celljournal.org). SurvExpress analysis was applied to investigate correlation between the six AAT genes as a panel and the patients' prognoses. Particular attention was paid to those tumors characterized by a distinct expression pattern for the six AAT genes compared to that for their normal counterparts in the PCA analysis (Fig.5). Dysregulated expression, particularly overexpression, of the six AAT genes as a panel was significantly correlated with a poor prognosis in patients with COAD, ESCA, KIRC, LUAD and COADREAD. Although there is no significance of dysregulated expression of those six AAT genes in evaluating prognosis of patients with LUSC when setting the p value at 0.05, the general pattern remains consistent with that of COAD, ESCA, KIRC, LUAD and COADREAD (Fig.6). Taken together, the survival analyses identified the clinicopathological importance of these six AAT genes in assessing patients' prognoses.

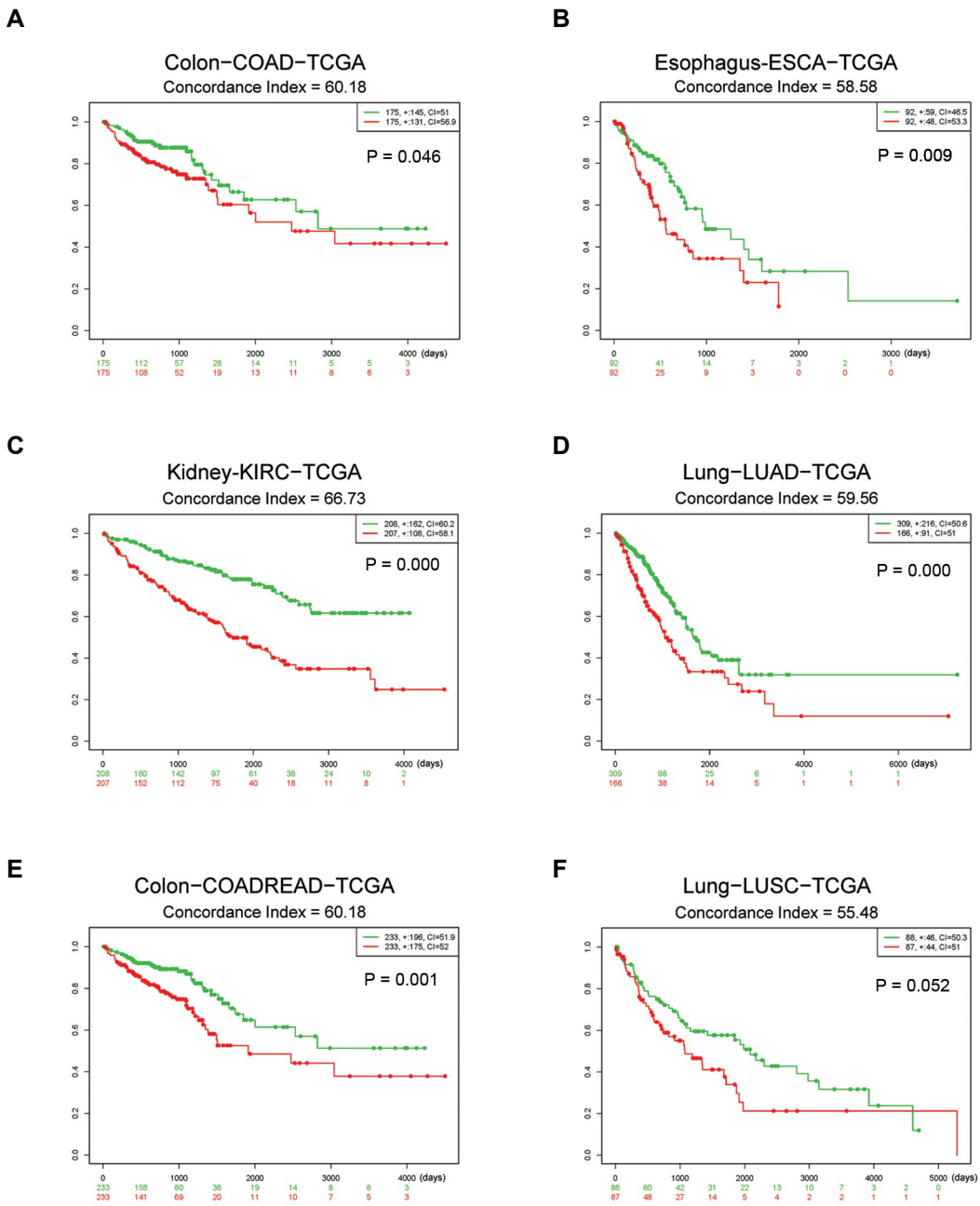

Fig.6: Survival analysis based on the expression of six AAT genes in human tumors. The Log-Rank analysis shows that expression of the panel of six AAT genes (SLC3A2, SLC7A5, SLC7A1, SLC1A4, SLC7A11 and SLC1A5) can be applied to evaluate the prognosis of patients with **A.** COAD, **B.** ESCA, **C.** KIRC, **D.** LUAD, **E.** COADREAD, and **F.** LUSC. The green lines indicate tumors with reduced expression while the red lines are for tumors with overexpression of the panel of six AAT genes. Statistical analyses were performed using the Log Rank test.

DepMap analysis indicates the biological importance of these six AAT genes in human cancers

The biological importance of these six AAT genes was evaluated using DepMap analysis. DepMap determines the genes required for cell growth by performing genome-wide RNAi or CRISPR loss-of-function screening analyses in more than 1,000 cancer cell lines. The RNAi screening employs DEMETER2, a method based on large-scale RNAi data, to demonstrate the effect of knockdown of the six AAT genes on cell growth (27). For all cell lines tested, transient knockdown of SLC3A2, SLC7A5, SLC1A5 and SLC7A1 demonstrated stronger effects than knockdown of SLC1A4 and SLC7A11 (Fig.S10, See Supplementary Online Information at www.celljournal.org). In accordance with previous reports, SLC3A2, SLC7A5 and SLC1A5 were related to uptake of glutamine, a well-known amino acid contributing to cell survival and proliferation.

Discussion

Recent studies have focused on glutamine instead of other amino acids because tumor cells undergo more apoptosis in the absence of glutamine than other amino acids. However, this doesn't mean that other amino acids are not important for tumor cells. To culture mammalian cells, formulated medium is utilized that makes it easier to test the response of tumor cells to the deprivation of a single amino acid or all amino acids at one time. In the GSE62673 dataset, amino acids are removed to investigate whether there is a conserved response in tumor cells under single amino acid- or all amino acid- deprived conditions. iDEP v0.96 analysis was applied this dataset to show glycine-deprivation results in minor changes in gene expression relative to the control group while methionine-deprivation leads to the most dramatic alterations, suggesting the effects of methionine-deprivation on gene transcription are exaggerated because it is coded by the start codon that contributes to the synthesis of most polypeptides. The k-Means clustering enrichment, REACTOME and GSEA analyses showed activation of the integrated stress response. This response enhances the adaptivity of cells to acute stresses but is lost under chronic stress conditions, finally inducing apoptotic signaling. In addition, amino acid withdrawal attenuates cell proliferation through blocking cell cycle progression due to reduced DNA replication and elongated DNA repair time. After validating the reliability of this dataset (GSE62673), the expression of AATs was further analyzed and a panel of six AAT genes was identified to be upregulated; SLC3A2, SLC7A5, SLC7A1, SLC1A4, SLC7A11 and SLC1A5. The analyses also demonstrated that a distinctive enrichment pattern exists for these six AAT genes in tumor tissues relative to normal counterparts.

LAT1/SLC7A5 is an obligatory amino acid exchanger that can transport leucine, isoleucine, valine, phenylalanine, methionine, tyrosine, histidine and tryptophan into cells with the efflux of glutamine (14). In order to perform its biological function, LAT1 forms a heterodimer with 4F2 cell-surface antigen heavy chain (4F2hc, also termed CD98hc/SLC3A2) (28). LAT1 activates mTORC1 signaling through enhancing leucine uptake. The expression of LAT1/SLC7A5 is elevated and associated with poor prognosis in colorectal cancer, esophageal cancer, renal cell carcinoma, lung cancer, breast cancer and pancreatic cancer, etc. (29). xCT/SLC7A11, linked by a covalent disulfide bond to CD98hc/SLC3A2, functions as a bidirectional AAT that mediates cysteine uptake with the excretion of glutamate (30). xCT thus provides cysteine for the synthesis of glutathione, which maintains the intracellular oxidative/reductive balance. There is an obvious upregulation of xCT/SLC7A11 in human cancers including colorectal cancer, esophageal cancer, lung cancer, pancreatic cancer, head and neck cancer among others (31, 32). CD98hc/SLC3A2 controls amino acid transport and integrin signaling, which play critical roles in driving tumor development and progression (33). CD98hc forms a heterodimer with LAT1, LAT2 or xCT to promote the uptake of relevant amino acids. Besides contributing to amino acid uptake, CD98hc can also bind to β1 or β3 integrin to mediate cell survival, proliferation, and migration. CD98hc is highly expressed in human tumors like renal cell carcinoma, lung cancer, breast cancer, sarcoma, head and neck cancer, among others (34).

ASCT1/SLC1A4 is specifically expressed in the cell plasma membrane. As a serine and cysteine transporter ASCT1 is linked with brain homeostasis (35). The full-length form of ASCT2/SLC1A5 is predominantly localized in the plasma membrane while the short variant is localized in the inner membrane of mitochondria (36). ASCT2 is a Na^+-dependent transmembrane transporter that works to uptake neutral amino acids, such as glutamine, particularly in cancer cells. ASTC2 expression is elevated in colorectal cancer, gastric cancer, head and neck cancer and leukemia (37). CAT1/SLC7A1 is a plasma membrane transporter that is glycosylated but not associated with an ancillary protein to perform its functions. CAT1 facilitates the uptake of proteinogenic amino acids like arginine and lysine and non-proteinogenic ones such as ornithine. CAT1 is mainly overexpressed in colorectal and breast cancers (38). Like indicated above, the six AAT genes are overexpressed at both mRNA and protein levels in various human cancers. Consistently, the six AATs can be considered as a panel to evaluate patients' prognoses. However, a lot of unknown questions remain to be further investigated, for example, how these six AAT genes are induced when deprived of amino acids, whether there is functional redundancy in these AAT genes at cellular levels, and what is the therapeutic potential of the dysregulation of the six AAT genes.

As already mentioned, any of the six single AATs can be involved in regulating a variety of processes including cell cycle progression, proliferation, migration, invasion, survival and the production of factors in response to stress conditions. Importantly, although the six AATs are upregulated together when cells are exposed to amino acid deprivation, their clinical importance as a panel

in evaluating patients' prognoses remains unclear. Our current study showed their importance in determining the prognosis of patients with colorectal, esophageal, kidney and lung cancers. The next step will be to further validate these bioinformatic findings in real world human specimens using IHC staining. In the event of good validation, this panel of AATs could be used as diagnostic markers. Moreover, we anticipate that the expression of these six AATs can be applied to assess patients' response to treatments like anti-angiogenic treatment, which generally leads to an insufficient supply of amino acids in solid tumors.

Conclusion

To sum up, solid tumor cells employ conserved signaling to upregulate the expression of a panel of six AATs in order to enhance the uptake of amino acids to maintain homeostasis. This study identifies these AATs include SLC3A2, SLC7A5, SLC7A1, SLC1A4, SLC7A11 and SLC1A5. Clinically, the upregulation of these AATs is significantly correlated with poor prognosis in patients with colorectal, esophageal, kidney and lung cancers. Future studies are required to further determine their biological roles and their potential as therapeutic targets.

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Authors' Contributions

S.Q.; Conception, Data analysis, Interpretation, Preparation, Revision of manuscript, and supervision. W.C.; Data analysis and Interpretation. Y.L., H.X., C.Y., Y.W.; Data analysis, Interpretation, Preparation, and Revision of manuscript. All authors read and approved the final manuscript.

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