A pan-cancer analysis of the glutaminase and their association with prognosis, tumor microenvironment, and therapeutic targets

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Research

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Abstract

Background

Glutamine metabolism plays a key role in various biological processes of tumor. Glutaminase (GLS) is involved in Glutamine metabolism and plays a conserved regulatory role in the process. Nevertheless, there is no comprehensively analysis of GLS in pan-cancer.

Materials

Comprehensive bioinformatics analysis was adopted to investigate the expression level, prognostic values, and association between expression of GLS and TME, immune cells' infiltration, immune checkpoint genes, TMB, MSI, drug sensitivity in pan-cancer. Bioinformatics tools including CCLE, immunophenoscore (IPS), Tumor Immune Dysfunction and Exclusion (TIDE), GSEA, and TIMER databases were used.

Results

Differently expressed GLS between tumor and normal tissues were analyzed, and the clinical prognoses, MMR, MSI, and TMB in multiple types of cancer were associated with GLS expression. Furthermore, GLS closely correlated with tumor immunity and drug sensitivity, and found GLS were predicted to be involved in cancer metabolism and immunity pathways, through gene set enrichment analysis (GSEA).

Conclusion

The GLS expression could be used as a prognostic biomarker for determining prognosis and provide further insights into anti-glutamine metabolism for cancer.

Introduction

Glutamine serves as an important nutrient with many cancer types displaying glutamine dependence[1]. Glutamine provides the major source of acetyl-CoA for lipid synthesis via reductive carboxylation, which significantly reduces the need for acetyl-CoA derived from glucose[2, 3]. Besides, it is reported that the immunosuppressive microenvironment relieved by blocking glutamine, and at the same time contributes the activity of T cells[4]. Hence, targeting glutamine metabolism has proposed new targeting opportunities for cancer treatment. Such as allosteric inhibitors of GLS have shown promise for the treatment of cancer, and one highly potent compound in this class. CB-839, GLS inhibitors, has been proven effective against hematological malignancies and showed strong efficacy in triple negative breast cancer in preclinical studies[5, 6]. However, despite recent advances in target the glutamine metabolism treatment for malignant tumors, only a few tumors have affects. Therefore, a comprehensively analysis the relationship between GLS and prognosis, potential mechanisms in multiple tumors is of great significance to improve the prognosis of patients.

In the current study, we have comprehensively analyzed the prognostic value of GLS expression within different cancer subtypes present in series of public databases. In addition, the relationship between GLS

expression and MMR, MSI, and TMB. Furthermore, we confirmed that GLS expression level were associated with tumor immune and drug sensitivity.

Materials And Methods

Sample Information

Gene expression quantification data, corresponding clinical characteristics, TMB data, mutation data, and MSI data of pan-cancers were downloaded from the online database UCSC Xenin, which originated from TCGA database (<https://xena.ucsc.edu/>)[7]. For the TCGA analysis of pan-cancers, GLS expression level was extracted and integrated by Perl software. The tumor cell line expression data were downloaded from the CCLE database (<https://portals.broadinstitute.org/>)[8]. In total, 5 eligible Gene-Expression Omnibus (GEO) cohorts (GSE109211, GSE104580, GSE140077, GSE58386, and GSE59357) were gathered in this study for further analysis[9-12]. The method “Wilcox.test” was applied to analyze GLS expression in different cancer types.

Survival analyses of GLS in human cancer

The Kaplan-Meier (KM) survival curve and Cox proportional hazards models were used to assess the overall survival (OS) and disease-free survival (DFS) of the 33 cancer types. Finally, the figures were generated using the R package “survival” and “forestplot”. Moreover, pan-cancer analysis of GLS was verified by the online database Kaplan-Meier Plotter (<https://kmplot.com/analysis/>) and PrognoScan (<http://dna00.bio.kyutech.ac.jp/PrognoScan/index.html>)[13].

Correlation Analysis

The correlation of neoantigens, five MMRs genes (MLH1, MSH2, MSH6, PMS2, EPCAM), Tumor mutational burden (TMB), Microsatellite instability (MSI), with GLS expression was assessed using Spearman's rank correlation coefficient. Data Visualization was done using ggplot package. The significant correlations were declared at $P < 0.05$, $R > 0.20$.

Genetic alteration analysis

The cBioPortal web (<https://www.cbioportal.org/>) was used to queries the genetic alteration features of GLS. The option of "Quick select" and "TCGA Pan-Cancer Atlas Studies" was selected. The alteration frequency, copy number alteration (CNA), and mutation type were depicted in the "Cancer Types Summary" module overall TCGA database.

Correlation analysis of GLS expression with Immune Infiltration Cells

Tumor IMMune Estimation Resource (TIMER) was used to determine the potential correlation between GLS expression and tumor-infiltrating immune cells, including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells of 31 human cancers[14], and visualized by the R-package “corrplot.”

Immunophenoscore and TIDE analysis

The immunophenoscore score was computed based on the gene expression values of immune-related genes to describe four classes of immune cells: (1) effector cells (2) immunosuppressive cells, (3) MHC molecules and (4) selected immunomodulator[15], through which machine learning can derived the Immunophenoscore of a patient without bias. The Immunophenoscores of GC patients were obtained from TCIA (<https://tcia.at/>). The Tumor Immune Dysfunction and Exclusion (TIDE) (<http://tide.dfci.harvard.edu/>) was utilized to model distinct tumor immune evasion mechanisms[16], including dysfunction of tumor infiltration cytotoxic T lymphocytes (CTLs) and exclusion of CTLs by immunosuppressive factors. A higher TIDE score represented the potential of tumor immune escape.

Drug sensitivity correlation analysis of glutamine metabolism genes

We used the CellMiner database (<https://discover.nci.nih.gov/cellminer/home.do>) to download the same sample of gene expression and drug sensitivity[17]. R package “limma”, and “ggplot2” were used for data processing and visualization.

Gene Set Enrichment Analysis (GSEA)

GSEA is a computational method used to analyze gene pathways and functions (<https://www.gsea-msigdb.org/gsea/index.jsp>)[18]. Samples were separated into a low and high group based on the cut-off value of GLS median expression, and the gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway were analyzed via the GSEA software.

Statistical analysis

Statistical analyses were conducted using R version 4.0.4, SPSS 24.0 (IBM, NY, USA). Comparisons between groups were performed using the t-test or rank-sum test. The survival curves were constructed by K-M method. For linear correlation, the Pearson correlation coefficient was evaluated. P-values <0.05 were considered statistically significant.

Results

GLS expression level in pan-cancers

We found that GLS was highly expressed in multiple cancer types, including CHOL, COAD, ESCA, HNSC, LIHC, READ, STAD, and THCA. A lower level of GLS expression is detectable in BRCA, GBM, KICH, KIRC, KIRP, LUAD, LUSC, and UCEC at the same time (Fig.1a). Additionally, GLS was generally enhanced in the tumor cell lines of 31 tumors (Fig. 1b). In our results indicated that the expression of GLS is generally differently expressed in solid tumors.

GLS is associated with prognosis and pathological stages in pan-cancer

To evaluate the prognostic values of GLS in various cancers, we performed pan-cancer analysis in different databases. As shown in the Figure, GLS was a protective factor for PCPG and SKCM (HR<1, P<0.05, Fig. 2a). GLS was a significant unfavorable prognostic factor in UCEC, KIRP, LIHC, and HNSC (HR>1, P<0.05, Fig. 2a). Highly expressed GLS correlated negatively with DSF in LIHC, UCEC, PRAD, KIRC, and KIRP(HR>1, P<0.05, Fig.

2b) and positively with DSF in KIRC, SKCM ($HR < 1$, $P < 0.05$, Fig. 2b). Patients were stratified based on the expression of GLS, and survival curves were constructed for overall survival and progression-free survival (Fig. 2c, d).

To further verify above results, the Kaplan-Meier plotter and PrognoScan database were analyzed. The results indicated that GLS was a significant unfavorable prognostic factor in Esophageal Adenocarcinoma (Fig. 3a), Head-neck squamous cell carcinoma (Fig. 3b), Kidney renal papillary cell carcinoma (Fig. 3d), Liver hepatocellular carcinoma (Fig. 3e), Testicular Germ Cell Tumor (Fig. 3g), and Uterine corpus endometrial carcinoma (Fig. 3h). GLS was a protective factor for Kidney renal clear cell carcinoma (Fig. 3c) and Lung adenocarcinoma (Fig. 3f). The clinical significance of GLS in Head-neck squamous cell carcinoma, Kidney renal papillary cell carcinoma, and Liver hepatocellular carcinoma was consistent with TCGA results. In PrognoScan database, high GLS expression levels were associated with poorer OS in ovarian cancer, blood cancer, and breast cancer. Conversely, high expression of GLS was correlated with better OS in colorectal cancer, lung cancer, and brain cancer (Table 1). In order to assess GLS expression levels at multiple cancers stage, we extracted pathological stage from 33 tumors. Fig. 4 shows that stage-specific expressional changes in GLS expression in case of a few tumor types, such as READ, SKCM, KIRP, LIHC, and THCA. Taken together, our findings indicate that the expression of GLS is of prognostic relevance for several human cancers, although their correlation may vary depending on the cancer type.

GLS expression is related to immune checkpoint (ICP) Genes, TMB, and MSI in pan-cancer

TMB, MSI, and neoantigens plays a critical role in tumor's response to antitumor immunity and predict immunotherapy[19-21]. The relationship of GLS expression and the immune checkpoint genes were displayed as Fig. 5a. Our data showed that the expression of most ICP genes were positively correlated with GLS expression in multiple cancers, such as BLCA, BRCA, LIHC, and LUAD, suggesting that GLS might coordinate the activity of these ICP genes in different tumors, and potentially serve as an ideal immunotherapy target.

TMB, defined as the sum of somatic nonsynonymous mutations[22]. MSI represents the random changes in the length of microsatellites in tumor cells due to the insertion or deletion of repeating units, compared to normal cells, and the emergence of new microsatellite alleles[23]. Here we examined the correlation between GLS expression and several MMR genes (MLH1, MSH2, MSH6, PMS2 and EPCAM) indicated almost all the MMRs genes were positively correlated with the expression level of GLS (Fig. 5b). In addition, expression of GLS was significantly and negatively correlated with TMB in DLBC and KICH, and significantly and positively in LGG, BRCA, THCA, UCEC, and READ (Fig. 5c; Supplementary Table S1). For MSI, there were positive correlations with GLS expression in LGG, BRCA, THCA, UCEC, and READ and negative correlations with in DLBC and KICH (Fig. 5d; Supplementary Table S1).

Analysis of genetic alterations

The genetic alterations of GLS in distinct tumors among the TCGA cohorts were further studied. The highest alteration frequency of GLS ($> 1.2\%$) was found for patients with prostate tumors and was associated with "amplification" (Fig. 6a). The "amplification" type of copy number alteration (CNA) was the major type not only in prostate cancer, but also in ovarian cancer, breast cancer, bladder cancer, pheochromocytoma and

paraganglioma (PCPG), esophageal cancer, stomach cancer, KIRP, and lung. Due to our team has been engaged in the research of LICH and PAAD for a long time[24, 25]. Therefore, we further analyzed the relationship between GLS and LICH, and PAAD. We further performed significantly mutated gene (SMG) analysis for LICH, and PAAD samples in the low GLS expression group versus the high GLS expression group. The SMG mutational landscapes in LICH showed that TP53 (33% vs. 19%) had higher somatic mutation rates in the low GLS expression group, whereas CTNNB1 (8% vs. 37%) and TNN (25% vs. 16%) had higher somatic mutation rates in the high GLS expression group (Fig. 6c; Supplementary Fig.1a). Similarly, The SMG mutational landscapes in PAAD showed that TP53 (64% vs. 60%) had higher somatic mutation rates in the low GLS expression group, whereas KRAS (74% vs. 83%) and SMAD4 (15% vs. 33%) had higher somatic mutation rates in the high GLS expression group (Fig. 6d; Supplementary Fig.1b).

Correlations of GLS expression with immune cell infiltration and immunotherapy response.

Previous evidence has demonstrated an association between the tumor genome somatic mutations and responsiveness to immunotherapy and immune cell infiltration. Consequently, we investigated whether GLS play a role in responsiveness to immunotherapy and immune cell infiltration. Our results showed that GLS expression had a strong relationship with dendritic cell in 16 cancer types, macrophage in 18 cancer types, neutrophil in 23 cancer types, CD8+ T cells in 18 cancer types, B cells in 14 cancer types and CD4+ T cells in 19 cancer types (Fig. 7a). It suggested that GLS may serve a role in immune infiltration. Furthermore, GLS is significantly associated with the immune infiltration of LICH and PAAD (Fig. 7b, c). Immunotherapy using anti-PD-1/PD-L1 and anti-CTLA4 has become a breakthrough in cancer treatment. In addition to well-known predictors, such as TIDE and IPS, are strongly recommended to evaluate the immune response[15]. To further research the roles of GLS expression in LICH and PAAD, the relationship between GLS expression and immunotherapy response, we utilized the TIDE and IPS. Our analysis revealed that the TIDE was significantly decreased in the high expression patients. The significant therapeutic benefits and immune response to ICIs treatment were confirmed in patients with high GLS expression compared to those with low GLS expression (response rate cohort: 89% vs. 81%, Fig. 7d). Meanwhile, GLS expression were not associated with TIDE in PAAD patients (Fig. 7e). The IPS-PD1(-)/CTLA4(-) and IPS-PD1(-)/CTLA4(+) were significantly higher in the low-expression group ($P < 0.05$; Fig. 7f, g). Meanwhile, IPS-PD1(+)/CTLA4(-) and IPS-PD1(+)/CTLA4(+) were no significant difference in two groups ($P > 0.05$; Fig. 7f, g). The results suggested that GLS low expression may have a better opportunity for ICIs application in PD1(-)/CTLA4(-) or IPS-PD1(-)/CTLA4(+) patients.

Drug sensitivity analysis of GLS expression

The relationship between drug sensitivity and the expression levels of GLS was explored using data available from the CellMiner database, which is a database and query tool specially designed for cancer research groups, can help integrate and study molecular and pharmacological data of the National Cancer Institute (NCI-60)[17]. The result indicated that GLS expression was negatively related to drug sensitivity of Tyrothricin ($r=-0.486$ $P<0.001$; Fig. 8a), Vinorelbine ($r=-0.418$ $P=0.001$; Fig. 8d), Vinblastine ($r=-0.384$ $P=0.002$; Fig. 8f), Paclitaxel ($r=-0.395$ $P=0.002$; Fig. 8g), Acetalax ($r=-0.391$ $P=0.002$; Fig. 8h), and SR16157 ($r=-0.379$ $P=0.003$; Fig. 8i). GLS expression was positively related to drug sensitivity of Ibrutinib ($r=0.421$ $P<0.001$; Fig. 8b) and Afatinib ($r=0.387$ $P=0.002$; Fig. 8c), and Staurosporine ($r=0.414$ $P=0.001$; Fig. 8e). The GEO cohorts were analyzed to further verify above results. The significant therapeutic benefits and response to sorafenib and

transarterial chemoembolization (TACE) treatment were confirmed in LICH patients with low GLS expression patients compared to those with high GLS expression patients (response rate of sorafenib cohort: 47% vs. 15%, Fig. 9a; response rate of TACE cohort: 63% vs. 47%, Fig. 9b). In addition, GLS were significantly higher in gemcitabine and fluorouracil (5-FU) resistant pancreatic cancer cells than sensitive cells (Fig. 9c). However, we have got the opposite result in dasatinib resistant cells. Above results indicated that GLS expression was obviously related to drug sensitivity in tumor.

Predicted functions and pathways of GLS in LICH and PAAD

To observe the functions and pathways of GLS expression in LICH and PAAD, the LICH and PAAD samples were divided into two groups by GSEA, as shown in Tables 2 and 3. The top 10 most enriched signaling pathways or biological processes listed. As shown in Figure 10, gene silencing, regulation of cellular amide metabolic process, epidermis development, and mRNA Binding in LICH (Fig. 10a; Table2). Plasma membrane receptors, immunoglobulin production, and ribosomal subunit were most significantly enriched in PAAD (Fig. 10b; Table2). In addition, the KEGG pathways in focal adhesion, mismatch repair, nucleotide excision repair, regulation of actin cytoskeleton, pathways in cancer, and cytokine-cytokine receptor interaction in LICH (Fig. 10c; Table3). The KEGG pathways in valine, leucine and isoleucine biosynthesis, Citrate cycle (TCA cycle), and olfactory transduction in PAAD (Fig. 10d; Table3). These results indicate that GLS is widely involved in tumor metabolism and immunity.

Discussion

Glutaminase plays a critical role in regulating the tumor cell growth[26]. Recent study found that the expression of GLS is upregulated, and significantly correlated with advanced clinical stage in leukemia [27], intrahepatic cholangiocarcinoma [24], and breast cancers [28]. It plays a vital role in up-regulating cell metabolism for tumor growth and is a potential therapeutic target for cancer treatment[29, 30]. Despite the important roles of GLS in tumor, GLS has not been well-studied in tumor immunity and drug sensitivity.

Here, pan-cancer analysis was conducted to clarify the correlation between clinicopathological characteristics and prognostic significance of GLS expression. Firstly, we analyzed GLS expression levels in pan-cancers using TCGA and CELL databases. The results showed that GLS had significantly different expression in different types of cancer. Furthermore, high GLS expression patients had a worse prognosis in HNSC, KIRP, UCEC, and LIHC, which is consistent with previous studies[31]. However, GLS is an indicator of a better prognosis in PCPG and SKCM. The discrepancies in GLS levels and prognosis in different cancer types in different databases might reflect different data collection approaches and underlying mechanisms pertinent to different biological properties.

Tumor-infiltrating lymphocytes (TILs) have been shown to profoundly affect tumor prognosis and immunotherapeutic efficacy[32]. The results of our studies indicated that GLS had a strong association with TILs. For example, CD8+T cells, macrophages and dendritic cells was correlated with the GLS expression. As a universal component of the TME, macrophages have multiple functions, including tumorigenesis, immune evasion, metastasis, and chemoresistance[33]. The correlation between GLS and tumor mutational burden (TMB), microsatellite instability (MSI), and neoantigens also proved. MSI is associated with a higher risk of

cancer, such as increased TMB and higher quality of tumor-infiltrating lymphocytes[34]. TMB can be used as the independent predictor of the outcome of immune checkpoint inhibitor therapy[35]. In LICH and PAAD, we found that GLS expression has a close association with TILs in the TME. Moreover, we observed that GLS expression is related to TIDE and IPS, which is play a vital role in predict the potential response of ICIs[22], implying that GLS expression could influence the therapeutic efficacy of immunotherapy in LICH. Furthermore, GLS expression was obviously associated with drug sensitivity. We identified the robust prediction ability of the GLS expression in the drug sensitivity via GEO cohorts, including TACE, sorafenib, gemcitabine, 5-FU, and dasatinib treatments. TACE and sorafenib treatments were widely used in the treatment of patients with LICH. Furthermore, sorafenib was the only systemic therapy option for patients with advanced HCC[36]. In addition, gemcitabine and 5-FU as first-line therapy for patients with advanced pancreas cancer[37]. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) afatinib are standard-of-care for first-line treatment of EGFR-mutant advanced non-small cell lung cancer (NSCLC)[38]. Bruton's tyrosine kinase (Btk) inhibitor ibrutinib is recommended that chronic lymphocytic leukaemia patients with TP53 deletion/mutation in front line[39]. Our results provided a theoretical basis for the use of immune checkpoint inhibitor combined with target glutamine metabolism treatment for the future. However, further experimental research to prove its function.

Finally, our single gene set enrichment analysis performed in LICH and PAAD showed that GLS can participate in a wide range of metabolic and immune pathways, including focal adhesion, mismatch repair, cytokine-cytokine receptor interaction, leucine and isoleucine biosynthesis, Citrate cycle (TCA cycle), and olfactory transduction. TCA cycle-associated intermediate, itaconate, have been demonstrated to have a transcriptional, epigenetic, and post-translational impact on the regulation of gene expression, which drives immune responses to pathogens and antigens stimulation as well[40]. This suggests that GLS expression may involve in the regulation of these signaling pathways and affected immune cell infiltration and drug sensitivity. However, there are still many limitations and puzzles to be solved. We intend to perform in-depth studies to validate our current conclusions via experiments in future research.

Conclusions

Our study demonstrated the expression profile of GLS and prognostic value in pan-cancer. Also, TMB, MSI, and MMRs might contribute to GLS dysregulation in cancers, and GLS is closely linked to tumor immunity and may be a potential therapeutic method for immunotherapy combined with target glutamine metabolism treatment.

Abbreviations

ACC
Adrenocortical carcinoma
BLCA
Bladder Urothelial Carcinoma
BRCA
Breast invasive carcinoma

CESC
Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL
Cholangiocarcinoma
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
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
DFS
Disease-Free Survival
GEO
Gene Expression Omnibus
GSEA
Gene set enrichment analysis
KEGG
Kyoto Encyclopedia of Genes and Genomes
K-M
Kaplan-Meier curves
MMRs
Mismatch repair system
MSI
Microsatellite instability
OS
Overall Survival
TMB
Tumor mutation burden
TCGA
The Cancer Genome Atlas

Declarations

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Authors' contributions

SJ and LK designed the study; SJ collected data and wrote the manuscript. SJ performed the data analysis and drew the figures as well as tables; SJ and LK reviewed and revised the manuscript. Besides, the organization, revision, and submission of this manuscript were completed by LK. The final manuscript draft was read and approved by all authors.

Availability of data and materials

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

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Ethics approval and consent to participate

This was not applicable to this manuscript.

Consent for publication

Consent for publication was obtained from all participants.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 GLS expression was related to the prognosis of different cancers in PrognoScan.

Gene	Dataset	Cancer type	Endpoint	Number	Cox P-value	HR 95% CI (low-high)	HR
GLS	DUKE-OC	Ovarian cancer	OS	133	0.021	14.39 [1.50 - 138.05]	2.666
	GSE11121	Breast cancer	DMFS	200	0.000	0.40 [0.25 - 0.62]	-0.926
	GSE12093	Breast cancer	DMFS	136	0.049	0.56 [0.32 - 1.00]	-0.578
	GSE12276	Breast cancer	RFS	204	0.032	1.24 [1.02 - 1.50]	0.212
	GSE12417-GPL97	Blood cancer	OS	163	0.022	1.56 [1.07 - 2.29]	0.446
	GSE17537	Colorectal cancer	DFS	55	0.033	2.77 [1.09 - 7.05]	1.018
	GSE17537	Colorectal cancer	DSS	49	0.021	0.03 [0.00 - 0.57]	-3.679
	GSE17537	Colorectal cancer	OS	55	0.018	0.12 [0.02 - 0.69]	-2.144
	GSE2034	Breast cancer	DMFS	286	0.021	0.75 [0.58 - 0.96]	-0.294
	GSE22138	Eye cancer	DMFS	63	0.002	0.62 [0.45 - 0.84]	-0.485
	GSE26712	Ovarian cancer	DFS	185	0.007	1.46 [1.11 - 1.92]	0.379
	GSE26712	Ovarian cancer	OS	185	0.005	1.52 [1.13 - 2.04]	0.420
	GSE30929	Soft tissue cancer	EFS	140	0.024	2.35 [1.12 - 4.93]	0.855
	GSE31210	Lung cancer	OS	204	0.000	0.37 [0.22 - 0.64]	-0.981
	GSE31210	Lung cancer	RFS	204	0.009	0.57 [0.38 - 0.87]	-0.556
	GSE3494-GPL97	Breast cancer	DSS	236	0.035	0.73 [0.54 - 0.98]	-0.318
	GSE4271-GPL96	Brain cancer	OS	77	0.007	0.47 [0.27 - 0.81]	-0.758
	GSE4412-GPL97	Brain cancer	OS	74	0.034	0.50 [0.27 - 0.95]	-0.684
	GSE4573	Lung cancer	OS	129	0.020	0.50 [0.28 - 0.90]	-0.688
	GSE8894	Lung cancer	RFS	138	0.039	0.00 [0.00 -	-10.867

					0.59]	
GSE9891	Ovarian cancer	OS	278	0.033	1.28 [1.02 - 1.61]	0.248
GSE9893	Breast cancer	OS	155	0.001	1.53 [1.18 - 1.99]	0.428
jacob-00182-CANDF	Lung cancer	OS	82	0.050	0.44 [0.20 - 1.00]	-0.811

Table2. The information of GO terms from top 10 GSEA enrichment analysis of PAAD and LICH.

Tumor	GO	setSize	NES	pvalue
PAAD	GO_PLASMA_MEMBRANE_SIGNALING_RECEPTOR_COMPLEX	293	1.509	0.012
	GO_IMMUNOGLOBULIN_PRODUCTION	202	1.354	0.024
	GO_RIBOSOMAL_SUBUNIT	186	-1.492	0.045
	GO_POSITIVE_REGULATION_OF_MITOTIC_CELL_CYCLE	162	-1.470	0.040
	GO_STRUCTURAL_CONSTITUENT_OF_RIBOSOME	162	-1.614	0.040
	GO_INNER_MITOCHONDRIAL_MEMBRANE_PROTEIN_COMPLEX	135	-1.347	0.042
	GO_T_CELL_RECEPTOR_COMPLEX	125	1.697	0.013
	GO_ANTIMICROBIAL_HUMORAL_RESPONSE	122	-1.330	0.043
	GO_LARGE_RIBOSOMAL_SUBUNIT	117	-1.513	0.040
	GO_CELLULAR_TRANSITION_METAL_ION_HOMEOSTASIS	109	-1.251	0.038
LICH	GO_GENE_SILENCING	467	1.344	0.010
	GO_REGULATION_OF_CELLULAR_AMIDE_METABOLIC_PROCESS	464	1.262	0.010
	GO_EPIDERMIS_DEVELOPMENT	462	1.191	0.050
	GO_MRNA_BINDING	449	1.401	0.010
	GO_SENSORY_PERCEPTION_OF_SMELL	444	1.231	0.010
	GO_OLFACTORY_RECEPTOR_ACTIVITY	418	1.240	0.010
	GO_SKIN_DEVELOPMENT	415	1.209	0.020
	GO_GENE_SILENCING_BY_RNA	379	1.400	0.010
	GO_EPIDERMAL_CELL_DIFFERENTIATION	355	1.245	0.020
	GO_KERATINOCYTE_DIFFERENTIATION	300	1.273	0.040

Table3. The information of KEGG terms from top 10 GSEA enrichment analysis of PAAD and LICH.

Tumor	KEGG	setSize	NES	pvalue
PAAD	KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_BIOSYNTHESIS	11	1.650	0.014
	KEGG_CITRATE_CYCLE_TCA_CYCLE	31	1.427	0.015
	KEGG_OLFACTORY_TRANSDUCTION	385	-1.189	0.048
LICH	KEGG_PATHWAYS_IN_CANCER	232	1.657	0.011
	KEGG_FOCAL_ADHESION	153	1.964	0.011
	KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	145	1.818	0.011
	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	125	1.965	0.011
	KEGG_CHEMOKINE_SIGNALING_PATHWAY	124	1.650	0.023
	KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	85	1.712	0.012
	KEGG_RIBOSOME	85	-2.479	0.048
	KEGG_CELL_ADHESION_MOLECULES_CAMS	81	1.638	0.037
	KEGG_JAK_STAT_SIGNALING_PATHWAY	79	1.724	0.025
	KEGG_PEROXISOME	73	-2.623	0.038

Figures

Figure 1

GLS expression levels in pan-cancer. a The mRNA expression of GLS in different cancers in the TCGA database. b The expression of GLS in different tumor cells from CELL database (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Figure 2

mRNA Expression Levels Prognostic Significance of GLS in different Cancers. a Correlation of GLS expression with OS for pan-cancer in TCGA. b The effect of GLS on DFS in different cancer types in TCGA. c GLS expression for OS in 5 types of cancers. d GLS expression for DSF in 2 types of cancers (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Figure 3

Kaplan-Meier survival curves of GLS generated from Kaplan-Meier plotter database. a Esophageal Adenocarcinoma; b Head-neck squamous cell carcinoma; c Kidney renal clear cell carcinoma; d Kidney renal papillary cell carcinoma; e Liver hepatocellular carcinoma; f Lung adenocarcinoma; g Testicular Germ Cell Tumor; h Uterine corpus endometrial carcinoma.

Figure 4

The correlations between GLS expression and pathological stages in pan-cancer. a ACC; b KIRP; c LICH; d READ; e SKCM; f THCA.

Figure 5

Correlations between GLS and immune checkpoints, as well as other variables of interest. a The correlations between GLS and confirmed immune checkpoints in multiple cancers. b Co-expression of GLS with DNA methyltransferases. c, d The radar graphs of correlation of GLS with TMB and MSL (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Figure 6

Mutation feature of GLS in pan-cancer of TCGA and the differences of SNVs between low and high expression groups in LICH and PAAD patients. a, b The alteration frequency with mutation type and mutation site are displayed. c, d Waterfall plot for the GLS low expression and high expression groups in LICH and PAAD patients.

Figure 7

GLS expression associated with immune infiltration and predicts immunotherapeutic benefits. a Correlation analysis between immune infiltration and GLS expression in pan-cancers. b, c Correlations between immune infiltration and GLS expression in LIHC and PAAD. d, e Differences in GLS expression among TIDE scores and distinct immunotherapy clinical response in LIHC and PAAD patients. f, g The difference analysis of IPS between low and high- GLS expression groups in LIHC and PAAD patients.

Figure 8

Drug sensitivity analysis of GLS expression. a Tyrothricin; b Ibrutinib; c Afatinib; d Vinorelbine; e Staurosporine; f Vinblastine; g Paclitaxel; h Acetalax; i SR16157.

Figure 9

GLS expression associated with chemoresistance. a, b The correlation of GLS expression with clinical response to sorafenib and transarterial chemoembolization (TACE) in LICH patients. c GLS expression associated with gemcitabine, 5-FU, and dasatinib resistant in pancreatic cancer cells.

Figure 10

GSEA in high GLS group and low GLS group. a, c Results of GLS ranked in the top 5 for its correlation with GO and KEGG in LICH; b, d Results of GLS ranked in the top 5 for its correlation with GO and KEGG in PAAD.

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