

Metabolomic profiling of oral squamous cell carcinoma reveals glutaminolysis-related proteins: GLS1 and GLUD1 are potential diagnostic and prognostic factors

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Abstract

It is essential to explore potential molecular involved in oral squamous cell carcinoma (OSCC) malignant transformation and progression. The purpose of this study was to delineate the metabolic characteristics of OSCC patients. We firstly report GC-MS based metabolic profiling of OSCC tissues from 8 patients, including tumor tissues and its matched normal tissues, then another 81 OSCC patients with prognostic information were used to verify metabolomics results by exam key enzymes expression. A panel including ten metabolite biomarker were identified, which can distinguish OSCC tumor from normal controls. On the base of metabolites with significantly difference, we applied KEGG for further pathway analysis and found OSCC have highly active glutaminolysis metabolism. Therefore, glutaminolysis-related key enzymes GLS1 and GLUD1 were detected by immunohistochemical and results demonstrated that GLS1 and GLUD1 were highly expressed in OSCC. Moreover, Kaplan-Meier survival analysis also showed and GLS1 and GLUD1 expressions were correlated with poor prognosis and tumor progression in OSCC patients. In conclusion, our results indicated that OSCC have highly active glutaminolysis metabolism, and GLS1 and GLUD1 enzymes are potential diagnostic and prognostic factors for OSCC.

Background

Oral squamous cell carcinoma (OSCC) is one of the most common cancers in head and neck region. There are 6 million deaths worldwide every year due to OSCC (1) and tobacco use (smoked or chewed), alcohol consumption and human papillomavirus infection are regarded as the most important risk factors for OSCC (2). The diagnostic methods of OSCC including physical examination, endoscopy, radiography, computed tomography, magnetic resonance imaging, serum and urine analyses, and histopathological examination of tissue biopsies. Although these managements for OSCC have been greatly improved, survival rate also remains poor due to regional and distant metastases (3).

Changes in metabolic pathways have been observed in almost all tumors, leading to dependence on specific nutrients or enzymes. The most striking feature of cancer cells is that they alter their metabolic pathways to meet cancer cell energy need. Changes in cell metabolism can contribute to transformation and tumor progression. Metabolic phenotypes can also be exploited to image tumors, provide diagnostic and prognostic information (4). Therefore, better understanding of the molecular and related key pathways that lead to the progression of OSCC is essential for improving diagnostic and prognostic predictors.

Lactate fermentation and aerobic respiration provide energy for cancer cell progression. Warburg Effect indicated that cancer cells can obtain energy from lactic acid fermentation even oxygen is enough, namely aerobic glycolysis (5, 6). In addition, cancer cells can also obtain energy from other metabolism pathway, for example amino acid and lipid metabolism, pentose phosphate pathway, glutaminolysis, and mitochondrial biogenesis (7–9). Glutaminolysis, deposing glutamine into glutamate, further alpha ketoglutarate for maintaining tricarboxylic acid cycle, [replenish](#) glucose metabolism and provide energy for cells, then leads to a glutamine addiction in some cancer cell. It has been reported that the c-myc can regulate genes which is necessary for glutamine catabolism (5). Thus, cancer cells metabolic reprogramming may be determined by their genetic profile. Conclusively, it is very important to discover reliable biomarkers for OSCC early diagnosis or predict prognosis (10, 11).

Metabolomic studies is a useful tool for identifying cancer therapeutic targets, tumor tissue and [biological fluids](#) samples were widely used in metabolomics research. However, metabolomics studies of oral squamous cell carcinoma tissues are very limited up to date, most studies have focused on distinguishing OSCC from normal groups use plasma or saliva samples (12). Our purpose was to explore tissue metabolite biomarkers of OSCC, which not only discriminate tumor from normal control, but also investigate a panel of potential prognostic and therapeutic marker of OSCC.

Methods

Tumor Tissue Specimens

Our study was reviewed and approved by the medical ethics committee of Stomatological hospital, Medical School of Nanjing University, and carried out according to the recommendations of the Declaration of Helsinki. Matched pairs of tumor and normal samples (8 pairs, n = 16) were collected from Stomatological hospital, Medical School of Nanjing University from 2018, another batch of independent fresh tissues samples (n = 20) were collected for verify metabolomics results. All fresh tissues were snap-frozen in liquid nitrogen within 30 min after surgery and preserved at -80°C till for GC-MS analysis. All tumor tissues and normal tissues were confirmed by HE staining of frozen section (*Supplementary Figure 1*). In key enzyme validated group, another 81 patients with oral squamous cell carcinoma and matched normal tissues were recruited, all patients underwent radical resection at Stomatological Hospital, Medical School of Nanjing University from 2013.

Chemicals and reagents

Reagents required for sample handling were of analytical grade, such as ammonium formate and formic acid (ROE Scientific Inc, Newark, USA), isopropyl alcohol and methyl tert-butyl ether (MTBE) (TEDIA Inc, Fairfield, USA), acetonitrile Methanol (Merck KGaA Inc, Darmstadt, Germany). Milli-Q water was used throughout the course of the experiment (Millipore, Billerica, MA, USA). Methanol, chloroform and pyridine were purchased from Tedia (Tedia way, Fairfield, USA), and myristic-d 27 acid was obtained from Sigma-Aldrich (St. Louis, MO, USA, respectively). Rabbit polyclonal GLUD1 and GLS1 antibody were purchase from Proteintech (Wuhan, China). Enzy Chrom™ Glutamine Assay Kit were purchased from BioAssay Systems (EGLN-100, USA), Glutamate Colorimetric Assay Kit were purchased from Biovision (Catalog #K629-100, USA).

Sample Preparation.

50 mg of frozen tumor and normal tissues were transferred into 2 mL Eppendorf tube and 400 μ L ice-cold 75% methanol, 10 μ L tinidazole (internal standard, 50 μ g/mL) as an internal standard was added and mixed well with an oscillating mill (Retsch MM 400, 25 Hz, 5 min) and 5 min of vortexing, add 1 mL ice-cold MTBE and 200 μ L water. The mixture centrifuged at 15,000g for 10 min at 4 °C resultant clear supernatant was collected and dried in vacuum 45 °C for 3 h. The dried lower phase aliquots were derivatized subsequently by adding 30 μ L methoxylamine hydrochloride in pyridine (10 mg/L) and 1 μ L 1,2-13C 2 - myristic acid (internal standard, 5 mg/mL), vortexed and left for 90 min at 30 °C with the Thermo Mixer C (Eppendorf). Then 30mL BSTFA was added with 1% TCMS and shaken at 37 °C for 30 min. The derivatized samples were centrifuged and analyzed within 24 hours.

Gas chromatography-mass spectrometry

Derivatized samples were analyzed by A Trace 1310 Gas Chromatograph equipped with an AS 1310 auto sampler connected to a TSQ 8000 triple quadrupole mass spectrometer (Thermo Scientific, Waltham, MA) was used to perform GC-MS analysis. TG-5MS GC column was used to separate 1 μ L of derivatized of tumor and normal tissues samples for GC-MS analysis (Thermo, 0.25 mm x 30 m, 0.25 μ m) in split mode with a 20:1 ratio of above samples. The carrier gas (such as helium) maintained at a constant flow of 1.2 mL/min. Initial temperature of GC oven was 60 °C and kept for 1 min, gradually increase the temperature to 320°C and keep it for 5 minutes. The transfer line temperature was set to 250 °C, and the EI ion source temperature was 280 °C, the voltage was 70 eV. A scan range of mass spectra was 50–500m/z and a time range of 3.5–19 min.

Real-time PCR

TRIzol reagent was used to isolate total RNA (Invitrogen, USA) according to the manufacturer's instructions. Real-time PCR was performed with Thermal Cycler Dice Real Time System (TaKaRa, Japan). The primer sequences of GLS1 were: 5'-AGGGTCTGTTACCTAGCTTGG-3' and 5'-ACGTTTCGCAATCCTGTAGATTT-3', GLUD1

were: 5'-CTCCAGACATGAGCACAGGTGA-3' and 5'-CCAGTAGCAGAGATGCGTCCAT-3'. The primer sequences of β -actin were: 5'-CCTGGCACCCAGCACAAAT-3' and 5'-GGGCCGGAAGTCGTCATACT-3'. The relative quantity of GLS1 and GLUD1 mRNA level was calculated based on the standard $\Delta\Delta$ CT methods.

Immunohistochemistry

Immunohistochemistry was used to detect Rabbit polyclonal GLUD1 and GLS1 antibody (Proteintech, China) in 67 patients with early OSCC and matched normal tissues per the company' s protocol, PBS was used as negative control. The staining result was determined by counting 1000 tumor cells in three 100x magnification fields by two independent pathologists and further classified as low expression (the percentage of positive rate, $<25\%$) and high expression (the percentage of positive rate $\geq 25\%$).

Metabolomics statistical analysis

After removed the artificial peaks due to derivatization, raw data files of GC-MS analysis determined by NIST 2014 standard mass spectral databases built-in Xcalibur 2.2 software (Thermo Scientific, Waltham, MA). Sandardized using internal parameters, the peak area of metabolites were calculated with Xcalibur 2.2 software. We use Metaboanalyst1 3.0 perform statistical analyses, which contain the R package of statistical computing software. Total spectral intensity and additionally Pareto scaled

were used to normalized initial experimental data.

Univariate analysis was by ANOVA, such as fold change, T test, Volcano Plot. Multivariate analysis was via unsupervised Principal Component Analysis (PCA) followed by Partial Least Squares Discriminant Analysis (PLS-DA). Hierarchical cluster analysis was carried out using Dendrogram and Heatmap. We also applied KEGG for further metabolite pathway analysis. Immunohistochemical results were analyzed by SPSS 17.0 software package. The relationships between GLS1 protein expression and the clinicopathological parameters were determined by Chi-square tests. We estimated survival curves using the Kaplan-Meier method and compared them using a two-sided log-rank test p.

Results

Metabolomic Profiling Reveals numbers of changed metabolites and biomarker in OSCC and Matched Normal Tissues

Global metabolic profiling from 8 matched pairs (n = 16) of OSCC cancer tissues and adjacent normal tissues revealed that 83 metabolites changed with **significant** difference (Fold change >1.5 or ≥ 0.667) from 244 named features (Supplementary Table1,2). Among the 83 metabolites, 51 metabolites were up-regulated and 34 were down-regulated, then 10 metabolites with important features were further **distinguished** by Volcano Plot (*Figure1A, Table1*).. Univariate biomarker analysis reveal 45 of 83 metabolites can be the biomarkers which can distinguish tumor tissue from normal tissue (AUC ≥ 0.75 , *Table2*). Furtherly, classical ROC curve analysis of an individual biomarker can effectively distinguish tumor tissue from normal tissue (*Figure1 B, C, D*).

Partial Least Squares-Discriminant Analysis (PLS-DA) of Metabolic data Show Separation of OSCC Tumors from Normal Tissues

Firstly, an unsupervised **principal component analysis** (PCA) based on all metabolome data was performed, but it showed no clearly separation (*Supplementary Figure 2*). PLS-DA is a supervised method that uses multivariate regression techniques to extract via linear combination of original variables (X) the information that can predict the class membership (Y). Using Partial

Least Squares-Discriminant Analysis (PLS-DA) to transform the large number of metabolic variables into a smaller number of orthogonal variables in order to analyze variation between tumor and normal tissues, and to provide a high-level overview of the dataset, we found that samples derived from tumor samples showed good separation from normal tissue (*Figure 2 A, B*). In the hierarchical clustering analysis (HCA) of 10 typical differential metabolites, control samples tended to cluster separately from the tumor samples (*Figure 2 C, D*).

OSCC have Highly Active Glutaminolysis metabolism Anaplerosis Tricarboxylic Acid Cycle

Altered cell metabolism enables tumors to sustain their increased energetic and biosynthetic needs. We found OSCC have highly active glutaminolysis metabolism by [metabolic pathway](#) analysis (*Figure 3A*). In order to elucidate the important role of energetics alteration in OSCC tissues, additional experiments were performed to validate the role of glutaminolysis in OSCC tumorigenesis. We found the intermediate product of glutaminolysis such as glutamine, glutamate and α -ketoglutarate were differentially elevated in OSCC tissues compared to normal adjacent tissues by metabolomic profiling analysis (*Table 3*), glutamine, glutamate and α -ketoglutarate concentration in tumor tissues were significantly higher than that in normal tissues (*Figure 3B-D*), suggesting glutaminolysis may be involved in the development of OSCC. Then we explored two key enzymes of glutaminolysis-GLS1 and GLUD1 and figured out their higher expression in OSCC tissues than normal tissues in mRNA level (*Figure 4 A, B*). Meanwhile, at protein level, we discovered the same result as mRNA level by examining the expression of GLS1 and GLUD1 protein in 81 OSCC tissues using [immunohistochemical](#) (*Figure 4C*). All these results demonstrated OSCC have highly active glutamine catabolism, and glutaminolysis was likely to play a remarkable role in OSCC tumorigenesis. But its mechanism of glutaminolysis promoting tumorigenesis was still unveiled. We hypothesized glutamine converted into glutamate and further α -ketoglutarate in order to capturing nutrients for TCA cycle to support cancer cell metabolic needs. Detailed mechanism [hypothesis](#) of glutamine supplying for Tricarboxylic Acid Cycle to product ATP in tumor showed in *Figure 5*.

Glutaminolysis-related proteins GLS1 and GLUD1 were predictor of poor prognosis in OSCC tissues

Next, we further illuminated role of glutaminolysis-related proteins on OSCC progression. We detected the expression of GLS1 and GLUD1 protein in 81 OSCC tissues, the relationship between GLS1 and GLUD1 protein expression and clinicopathological parameters and prognosis were evaluated. GLS1 expression were also related with TNM, LNM and invasive depth of tumor, but not correlated with age, sex, grade, smoking, WPOI, GLUD1 expression only correlated with invasive depth of tumor (*Table 4*). Our result validated GLS1 and GLUD1 expression correlated with poor prognosis of patients (*Figure 6*). The result further confirmed that glutaminolysis may promote OSCC tumorigenesis and clinical progress, the specific mechanism needs to be further studied.

Discussion

Cell metabolic pathway was a complex network which consists of key enzyme, [regulatory](#) genes and metabolite. Cancer cells undergo metabolic reprogramming, which regulatory networks were altered to adapt to the metabolic pressures and provide energy for cancer cell growth, metabolic reprogramming was one of the hallmarks of cancer (13–15). Compared to other 'omics' (such as proteome/genome/transcriptome), most metabolites are small molecular compounds, highly conservative and stable performance. The statistical analysis of metabolomics data is more convenient, the results are easier to understand and more accurate.

The metabolomic profile of OSCC is an untapped resource for a cancer not only with recently increasing incidence but also that would highly benefit from advances for OSCC early diagnosis. Most of the samples for the metabolomics study of OSCC were biofluids (eg. plasma, urine, saliva) and cell lines (16–19), tissue samples were rarely reported (20), the most important

reason is that biofluids are readily available. Most studies are based on biofluids metabolomics to distinguish from cancer patients to healthy control people, we need to use tissue samples to further study the metabolomics of OSCC, which can not only distinguish the cancer tissue from the normal paired tissue, but also study the metabolic pathway leading to **carcinogenesis**, so as to find therapeutic targets for cancer in the field of metabolomics.

In this pilot study, we used GC-MS to analyze the metabolomics of OSCC tumor tissue and matched normal tissue samples, a total of 244 metabolites were identified, there were 85 known metabolites significant differences between the tumor and normal tissues (Fold change >1.5 or ≤ 0.667), 10 metabolites with important features were further distinguished by Volcano Plot. In our study, most amino acids, such as ornithine, L-cysteine, gamma-aminobutyric acid, lysine, aspartic acid, tyrosine, serine, alanine, glycine, N-methyl alanine, proline, ornithine significantly increase in OSCC tumor tissues. Our results are consistent with the previous findings in which oral squamous cell carcinoma tissues had higher amino acid levels than normal tissues (21–22). However, there were reports revealed lower relative concentration of amino acids as compared to healthy groups in some cancers, such as breast, pancreatic, oral, and colorectal cancers)(23–26). This anomaly suggests that cancer cells build a second metabolic pathway to generate energy for rapid growth, which need more glucogenic amino acids. It can also revealed during the proliferation of cancer cells, the continuous use of amino acids leads to an increase in the concentration of amino acids at the time of biosynthesis, and a decrease in the concentration of amino acids at the end of biosynthesis. Altered cell metabolism enables tumors to sustain their increased energetic and biosynthetic needs.

We also found the intermediate product of glutaminolysis such as glutamine, glutamate and α -ketoglutarate were differentially elevated in OSCC tissues, demonstrated OSCC have highly active glutamine catabolism. We further validated that glutaminolysis-related proteins GLS1 and GLUD1 correlated with recurrence and poor prognosis in OSCC. Therefore, it can be inferred that glutaminolysis was likely to play an important role in OSCC tumorigenesis, its mechanism of promoting tumorigenesis may be due to glutamine which converted into glutamate and α -ketoglutarate, and then into the tricarboxylic acid cycle produce energy for cancer cell (Fig 5). **Regulatory molecular mechanism** of GLS1 and GLUD1 promote OSCC tumorigenesis need to be further study. With the deepening of the research on the interaction mechanism between Cell metabolism and cancer development, we believe that GLS1 and GLUD1 may be a new target for cancer treatment.

Overall, the results from this based on GS-MS global metabolomic analysis between tumor tissues and matched normal tissues revealed a number of metabolic readouts, including changes in metabolites related to energetics. In PLS-DA and hierarchical clustering analysis (HCA), cancer tissues were well-separated from normal tissues. Glutaminolysis-related key enzymes GLS1 and GLUD1 correlated with clinical progress and overall survival in OSCC, suggesting altered metabolites may be useful prognostic biomarkers of OSCC.

Conclusions

Our study has shown that a GC-MS based metabolite analysis was able to identify biomarker metabolites which can significantly differentiate OSCC tissues from normal control tissues. In this study, we found OSCC have highly active glutaminolysis metabolism and key enzymes GLS1 and GLUD1 expression predict poor prognosis. All of these results propose that amino acid metabolism reprogramming may represent new potential for the treatment of OSCC. However, further studies are needed to elucidate the potential role of glutaminolysis in the carcinogenesis of OSCC.

Abbreviations

OSCC (Oral squamous cell carcinoma); GC-MS (Gas chromatography-mass spectrometry); MTBE (Methyl tert-butyl ethe); PCA (Principal Component Analysis); PLS-DA (Partial Least Squares Discriminant Analysis); TCA (tricarboxylic acid cycle).

Declarations

Ethics approval and consent to participate

Our study was reviewed and approved by the medical ethics committee of Stomatological hospital, Medical School of Nanjing University, all participants have signed informed consent forms.

Consent for publication

If the paper is accepted, we agree to be published in BMC cancer journal.

Availability of data and material

All data and material are available online.

Competing interests

The authors have declared that no competing interests exist.

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Not applicable

Authors' contributions

All authors have read and approved the manuscript

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Tables

Table1 Differential metabolites identified by volcano plot between Tumor tissues and Normal tissues

Metabolites	FC	log2(FC)	T-test	-log10(p)
lactic acid	0.026484	-5.2387	0.010187	1.992
glutamate	1.8472	0.88538	0.010699	1.9707
cholesterol	1.5545	0.63648	0.012175	1.9145
L-kynurenine	4.1458	2.0517	0.01704	1.7685
maltose	0.43077	-1.215	0.017056	1.7681
glyceric acid	0.40825	-1.2925	0.020264	1.6933
muconic acid	0.45291	-1.1427	0.026927	1.5698
thymine	2.1013	1.0713	0.034229	1.4656
uridine	2.1739	1.1203	0.039587	1.4024
gamma-aminobutyric acid	2.1963	1.1351	0.048703	1.3124

Table2: AUC, Log2FC, T-test and K-Means Cluster for univariate biomarker analysis between Tumor vs Normal

Metabolite	AUC	T-test	FC	clusters
glyceric acid	0.90625	0.020263583	1.559758051	4
L-kynurenine	0.890625	0.01703969	-2.384660019	4
lactic acid	0.859375	0.010186657	3.961683805	4
3-phenyllactic acid	0.859375	0.150295122	-1.38418393	4
uridine	0.859375	0.03958714	-1.033201307	4
DL-dihydrosphingosine	0.84375	0.246178302	-0.758356323	4
maltose	0.84375	0.017056452	1.108871082	4
cholesterol	0.84375	0.012174503	-0.697491019	3
erythrose	0.828125	0.218302006	-1.269236856	4
shikimic acid	0.828125	0.083961183	1.580229129	4
glutamate	0.828125	0.010698648	-1.138591391	1
lysine	0.828125	0.16327267	-2.005991618	3
tyrosine	0.828125	0.28972172	-1.283733246	3
guanane minor	0.828125	0.077329268	-1.989413281	4
isoleucine	0.8125	0.369122716	-1.040642878	3
alanine	0.8125	0.062507179	-1.064175873	3
threonine	0.8125	0.166605649	-1.012271996	3
gamma-aminobutyric acid	0.8125	0.048702548	-1.312175177	4
2-monopalmitin	0.8125	0.206634602	-1.217606154	4
2-butyne-1-4-diol	0.796875	0.123377924	-1.508763844	4
proline	0.796875	0.100611034	-1.305627947	1
serine	0.796875	0.068531884	-1.095540417	1
thymine	0.796875	0.034228601	-1.310557445	4
aspartic acid	0.796875	0.11211079	-1.260295546	1
trans- trans - muconic acid	0.796875	0.026926829	1.116963309	4
3-phosphoglycerate	0.796875	0.091735922	1.516835695	4
ornithine	0.796875	0.084090828	-1.40080693	4
N-NG-dimethylarginine	0.796875	0.629332052	-0.47900185	4
myo-inositol	0.796875	0.17348918	-0.901023216	4
5-6-dihydrouacil	0.78125	0.074136388	-1.249525575	4
ornithine	0.78125	0.297464546	-1.246547326	4
glutamine	0.78125	0.052844562	-2.513999997	5
methyl O-D-galactopyranoside	0.78125	0.554285841	-0.557800681	4
xanthine	0.78125	0.064304611	-1.268376146	4
guanine	0.78125	0.111292837	-3.836919148	4
maltotriose	0.78125	0.058617443	3.059741816	4
methanolphosphate	0.765625	0.060319259	-0.598770557	4
valine	0.765625	0.210513753	-0.820960529	1
leucine	0.765625	0.184554351	-0.859372484	1
threonine minor	0.765625	0.59765254	-0.586025306	3
lyxono-1-4-lactone	0.765625	0.037757986	-0.360097774	4
histidine	0.765625	0.748314461	-6.679822061	4
talose	0.765625	0.08125211	0.552059053	5
cortisone	0.765625	0.516437121	-0.929686162	4
1-monopalmitin	0.765625	0.365944753	-1.10190128	4

Table3 The key intermediate product of glutaminolysis fold change

Metabolite name	Tumor/Normal Fold Change	log2(FC)
glutamine	5.2738	2.3988
glutamate	1.8472	0.88538
α -ketoglutarate	1.5717	0.6523

Table4 GLS1 and GLUD1 expression in OSCC tissues(n=81)

Characteristics	GLS1			GLUD1		
	Positive(n=47)	Negative(n=34)	P	Positive(n=43)	Negative(n=38)	P
Age						
□ 60	24	20	0.18	23	21	0.507
≥ 60	23	14		20	17	
Sex						
Male	27	19	0.888	28	18	0.122
Female	20	15		15	20	
Smoking history						
Yes	11	10	0.802	12	9	0.999
No		24		31	29	
Histological grade						
Well differentiated	40	31	0.633	36	35	0.42
Poorly differentiated	7	3		7	3	
TNM						
□-□	25	29	0.004	25	29	0.102
□-□	22	5		18	9	
LNM						
Yes	20	5	0.014	16	9	0.232
No	27	29		27	29	
WPOI						
1-3	15	13	0.638	14	14	0.816
4-5	32	21		29	24	
Invasive depth			0.006			0.001
>4mm	34	13		32	15	
<4mm	13	21		11	23	

Figures

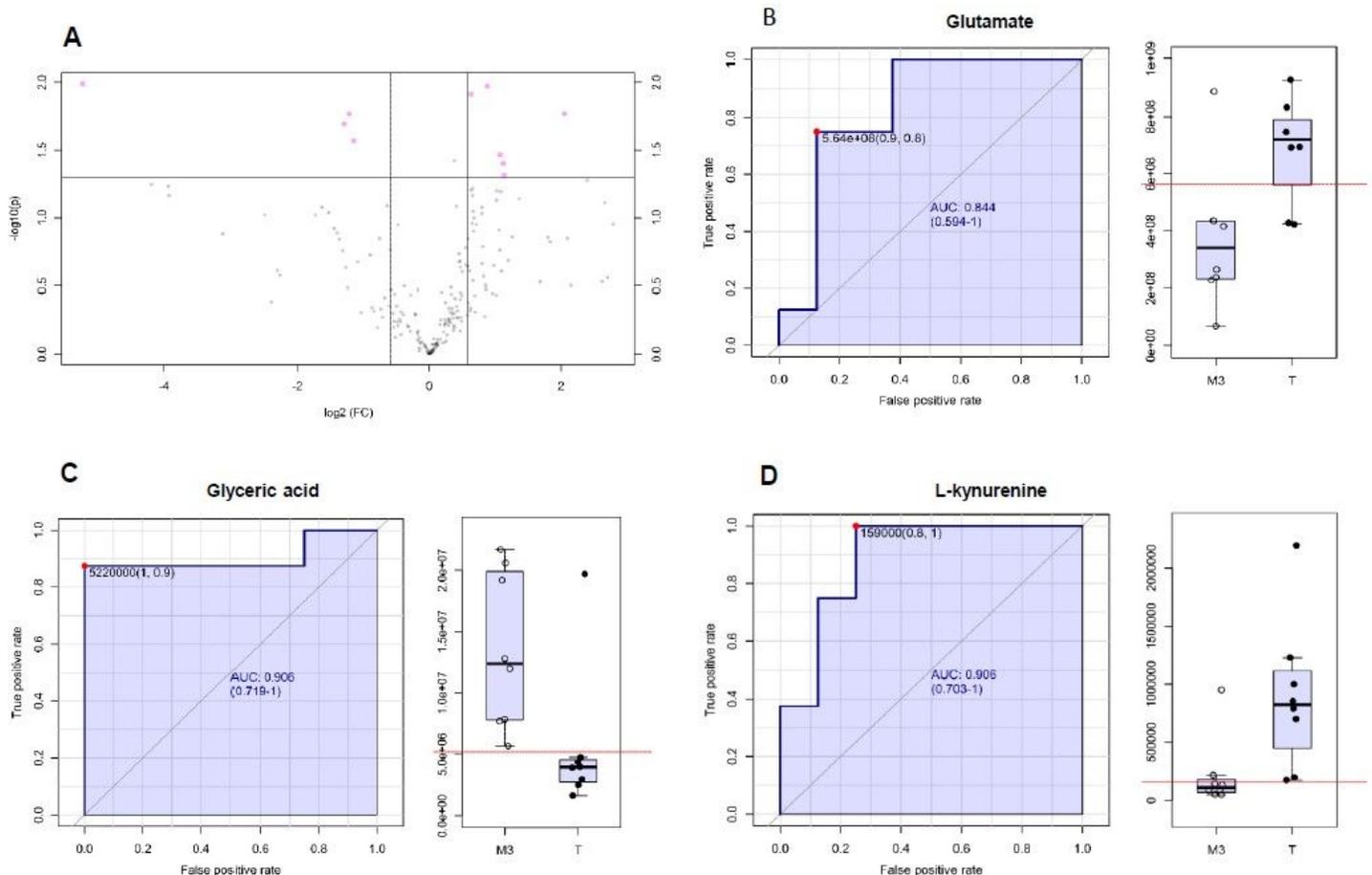


Figure 1

Univariate analysis of metabolomics profile. A, Important features selected by volcano plot with fold change threshold (x) 1.5 and t-tests threshold (y) 0.05. The red circles represent features above the threshold. B, C, D Classical ROC curve analysis of an individual biomarker such as glutamate, glyceric acid, L-kynurenine (AUC ≥ 0.75 , and t-test $p \leq 0.05$).

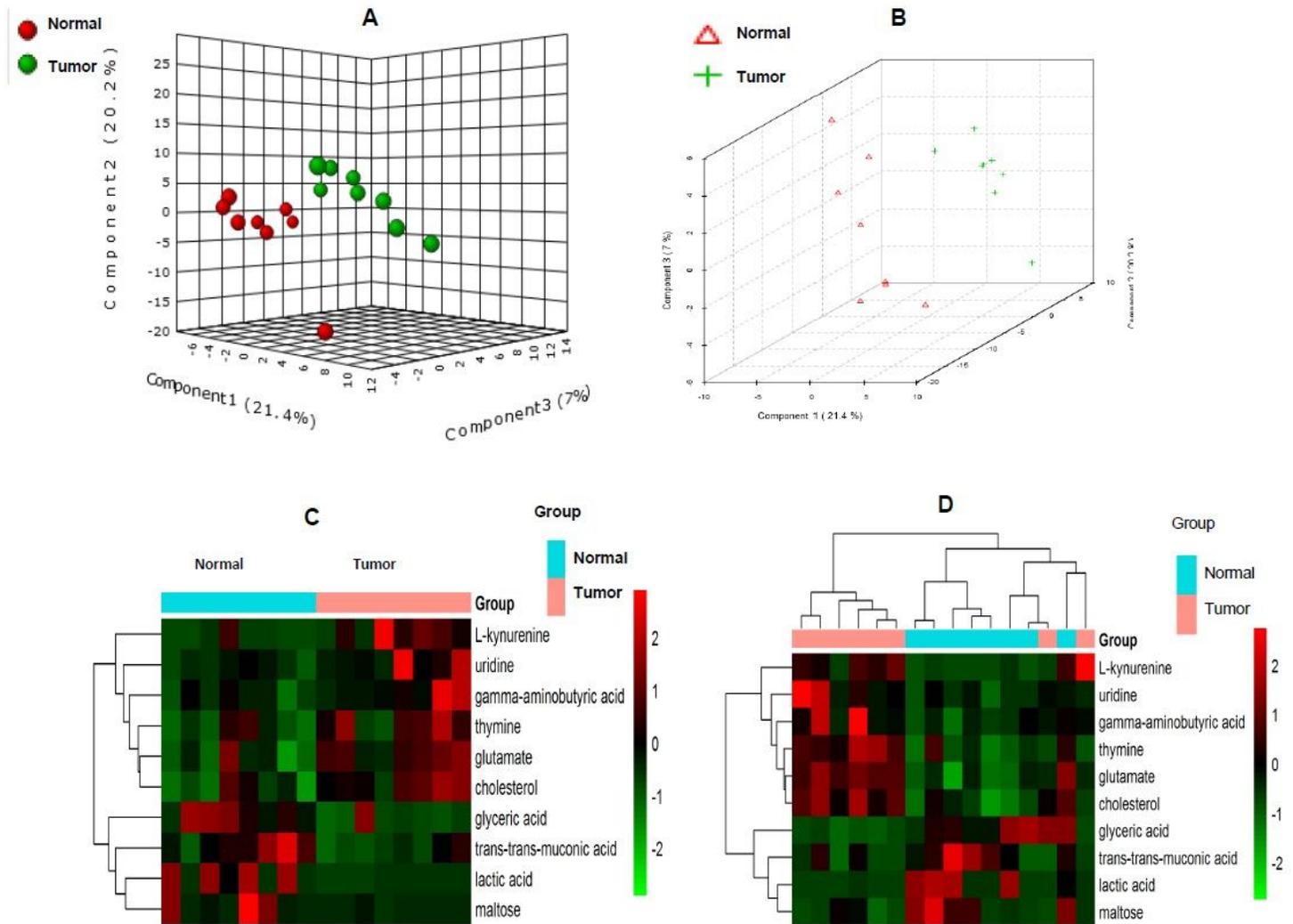


Figure 2

Multivariate analysis, A, B Partial Least Squares-Discriminant Analysis (PLS-DA), C, Heat map of samples showing normalized intensities of ten statistically significant metabolites in tumor and normal group. D, Comparison of two groups using individual normalized intensities of ten significance metabolites.

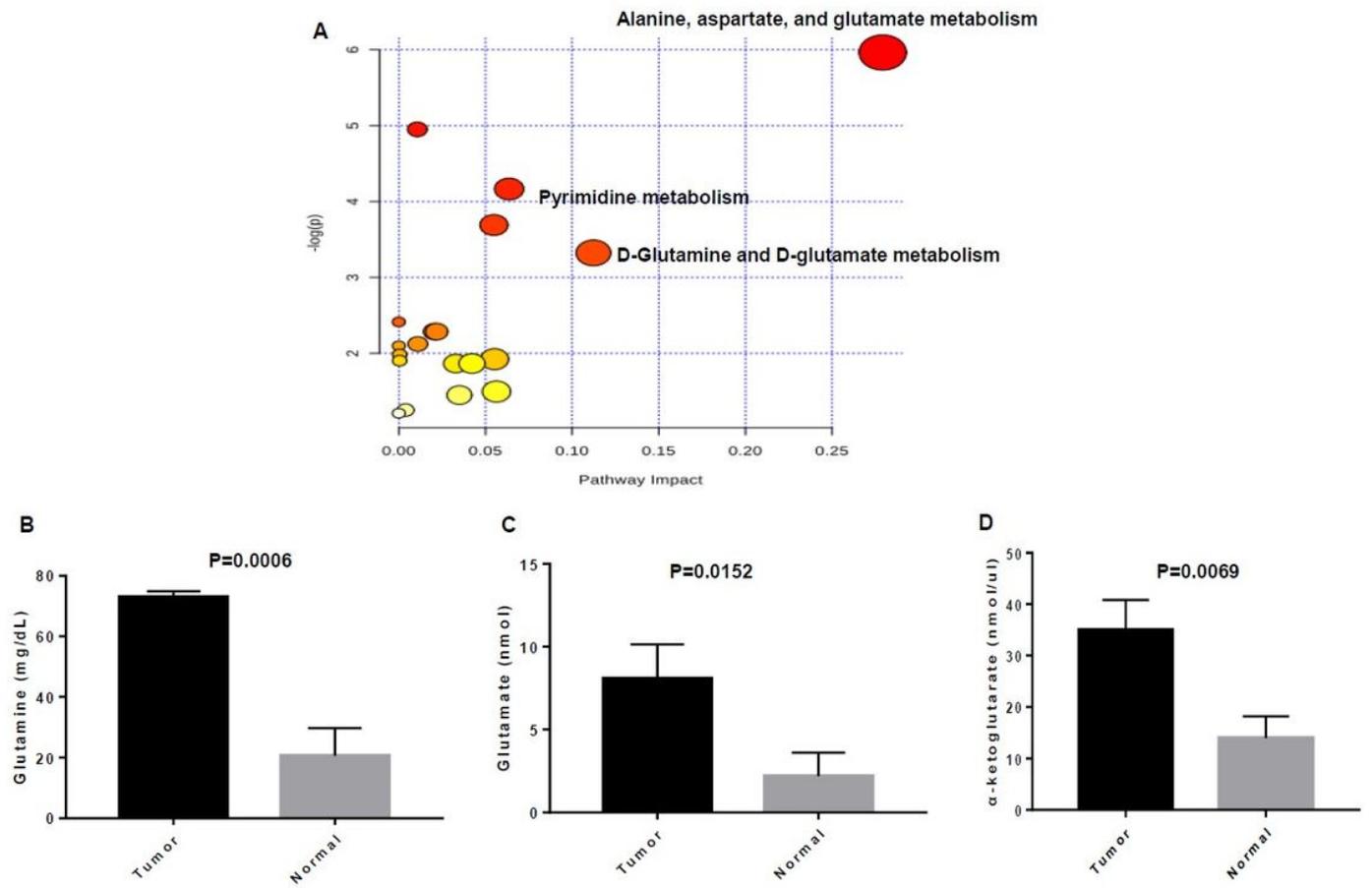


Figure 3

A, Metabolism pathway analysis of OSCC. B-D, quantitative analysis. glutamine, glutamate and α -ketoglutarate quantitative analysis by Assay Kit.

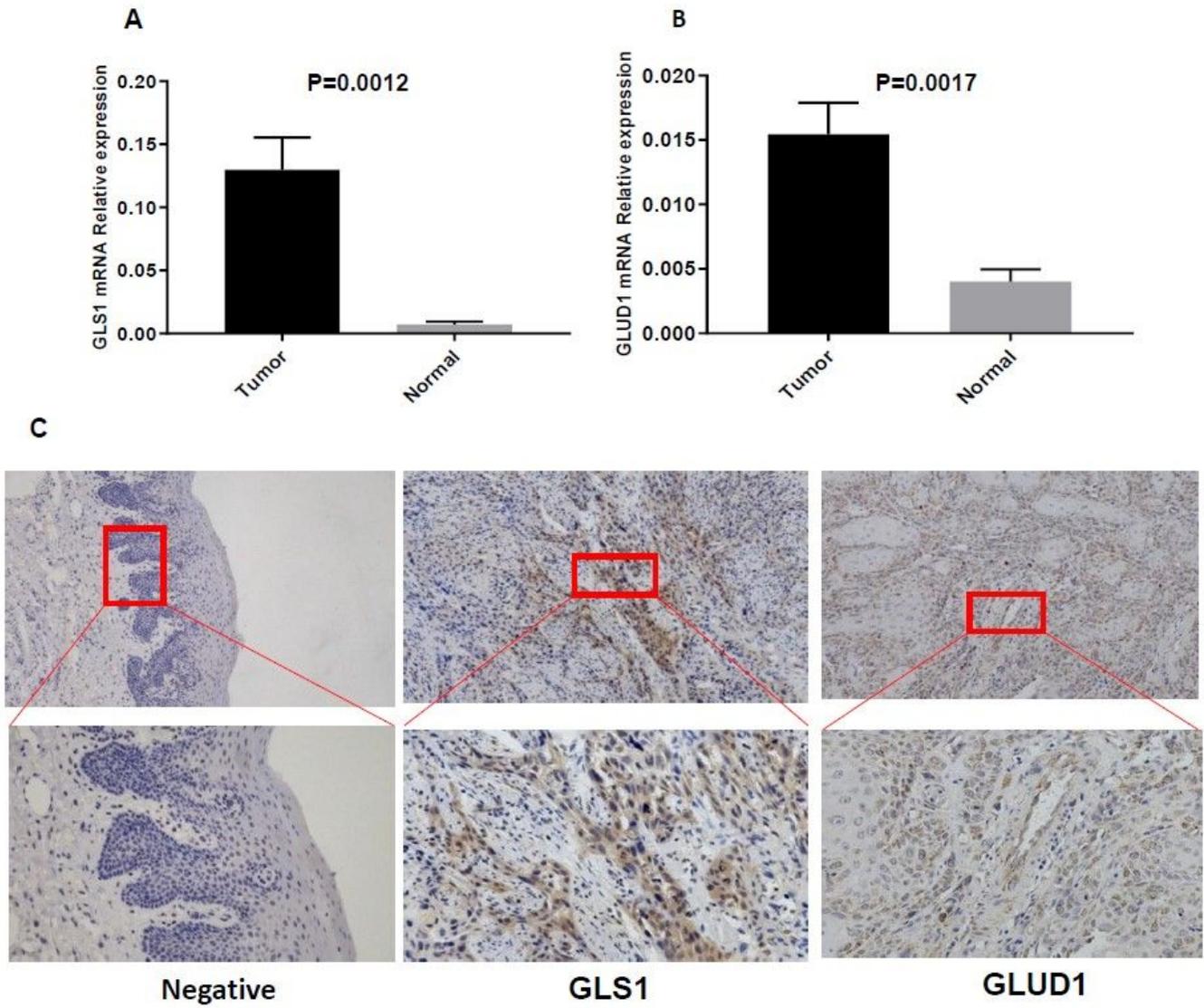


Figure 4

A, B GLS1 and GLUD1 mRNA level in OSCC tissues and normal tissues. C, GLS1 and GLUD1 protein expression in OSCC tissues and normal tissues D GLUD1 high expression predict poor OS and DFS.

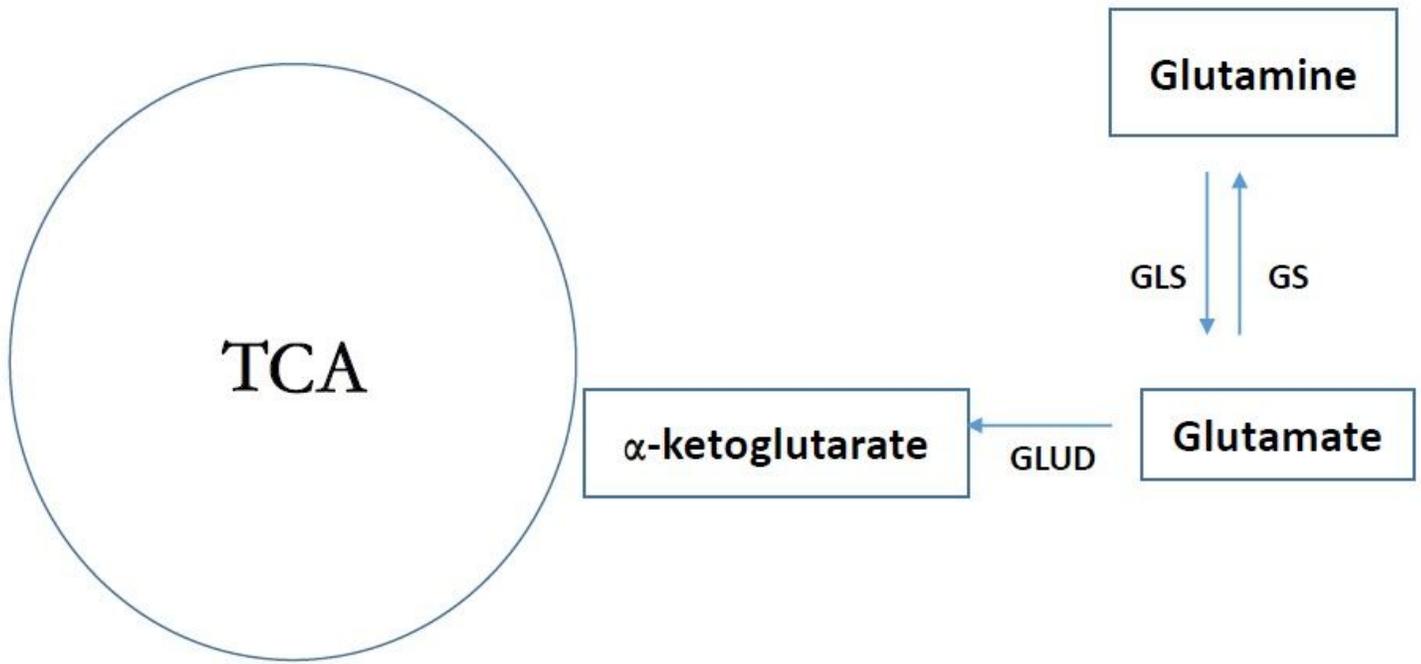


Figure 5

Illustration of Glutamine Anaplerosis Tricarboxylic Acid Cycle in OSCC.

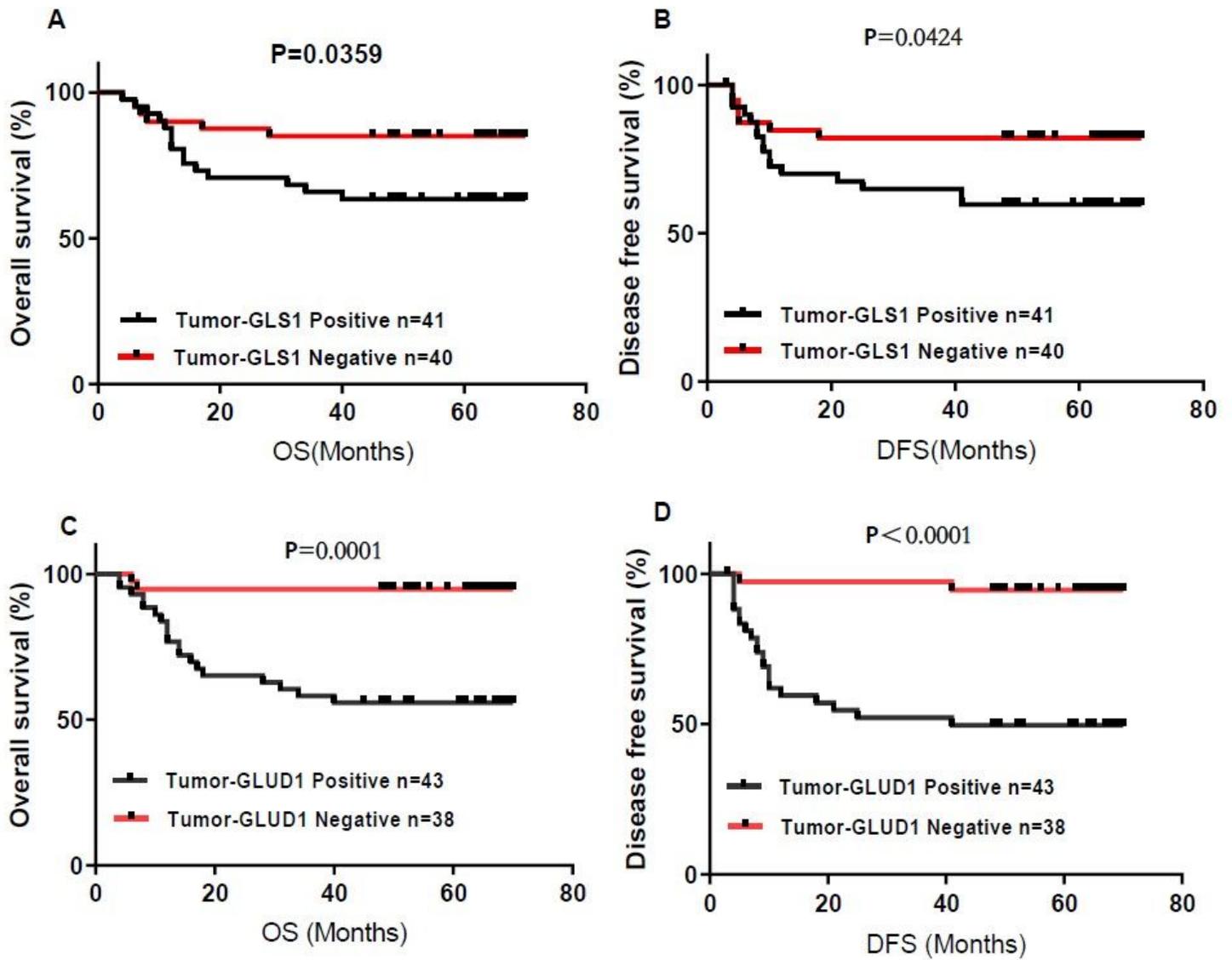


Figure 6

A, B GLS1 high expression predict poor OS and DFS. C, D GLUD1 high expression predict poor OS and DFS.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [renamed19bbb.xlsx](#)
- [Supplementarytable1Metabolomeanalysisofrawdata.xlsx](#)
- [Supplementaryfigures.pdf](#)