Original Article

CPS1 expression and its prognostic significance in lung adenocarcinoma

Geting Wu[1#], Zijin Zhao[2#], Yuanliang Yan[3,4], Yangying Zhou[5], Jie Wei[3,4], Xi Chen[3,4], Wei Lin[1], Chunlin Ou[1], Jia Li[1], Xiang Wang[3,4], Kun Xiong[6], Jianhua Zhou[1], Zhijie Xu[1,4]

[1]Department of Pathology, Xiangya Hospital, Central South University, Changsha 410008, China; [2]Department of Neurosurgery, Xiangya Hospital, Central South University, Institute of Skull Base Surgery and Neuro-oncology at Hunan, Changsha 410008, China; [3]Department of Pharmacy, Xiangya Hospital, Central South University, Changsha 410008, China; [4]National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha 410008, China; [5]Department of Oncology, Xiangya Hospital, Central South University, Changsha 410008, China; [6]Department of Anatomy and Neurobiology, School of Basic Medical Sciences, Central South University, Changsha 410013, China

Contributions: (I) Conception and design: J Zhou, Z Xu; (II) Administrative support: X Wang, J Li; (III) Provision of study materials or patients: Y Yan, Y Zhou; (IV) Collection and assembly of data: J Wei, X Chen, W Lin, C Ou, K Xiong; (V) Data analysis and interpretation: G Wu, Z Zhao, Z Xu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Zhijie Xu. Department of Pathology, Xiangya Hospital, Central South University, Changsha 410008, China; National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha 410008, China. Email: xzj1322007@csu.edu.cn; Jianhua Zhou. Department of Pathology, Xiangya Hospital, Central South University, Changsha 410008, China. Email: zhoujh15@163.com.

Background: Studies have increasingly shown that carbamoyl phosphate synthetase 1 (*CPS1*) plays a vital role in the occurrence and development of human malignant disease. Unfortunately, the detailed function of *CPS1* in the development and prognosis of lung cancer, especially lung adenocarcinoma (LADC), is still not fully understood. In this research, we performed a comprehensive bioinformatics analysis with respect to the function of *CPS1* in human LADC.

Methods: Several biological databases including UALCAN, GEPIA and Oncomine were used to analyze the expression of *CPS1* in LADC. Meanwhile, TCGA and GEO databases were utilized to analyze relevant clinical data. In addition, databases including Methsurv, etc., were used to analyze *CPS1* methylation levels in LADC.

Results: The Oncomine platform, UALCAN and gene expression profiling interactive analysis (GEPIA) were used and revealed that the expression levels of *CPS1* were significantly increased in LADC tissues. Furthermore, we analyzed the methylation level of *CPS1* in LADC and found that cases with high levels of *CPS1* showed hypomethylated *CPS1*. The clinical data from the Wanderer database, which is linked to The Cancer Genome Atlas (TCGA) database, demonstrated that the expression and methylation values of *CPS1* were both significantly related to the clinical characteristics and prognosis of LADC. Through analysis of the dataset from the Gene Expression Omnibus (GEO) database, we found that the expression level of *CPS1* was markedly downregulated in human A549 lung cancer cells treated with the chemotherapeutic drug motexafin gadolinium (MGd) in a time-dependent manner.

Conclusions: Our work indicated that *CPS1* is upregulated in LADC samples and that *CPS1* might be used as a potential biomarker for the diagnostic and prognostic evaluation of LADC. Determining the detailed biological function of *CPS1* in LADC tissues will provide promising and insightful information for our further study.

Keywords: Carbamoyl phosphate synthetase 1 (*CPS1*); lung adenocarcinoma; expression; diagnosis; therapeutic target

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Introduction

Malignant lung cancer tumors are the most common cause of cancer-related deaths worldwide. Every year, countless patients worldwide die from this disease (1). Among them, the incidence of lung adenocarcinoma (LADC), the most common histological subtype of lung cancer, is increasing year by year (2,3). Due to the delay in diagnosis, however, the treatment effect for LADC is strongly unsatisfactory (4,5). Despite the emerging advances in diagnostic and therapeutic techniques, it remains a serious global public health concern that is characterized by a lack of effectual progress in advanced diagnosis and treatment (6). Therefore, it is necessary to identify novel molecular markers to improve the early diagnosis and treatment of LADC.

The enzyme carbamoyl phosphate synthetase 1 (CPS1) forms carbamoyl phosphate from bicarbonate, ammonia, and adenosine triphosphate (ATP) and is activated allosterically by N-acetylglutamate. The neonatal presentation of bi-allelic mutations of *CPS1* results in hyperammonemia with reduced citrulline (7). Moreover, CPS1 is the rate-limiting enzyme in the first step of the urea cycle and an indispensable enzyme in human liver metabolism (8). Therefore, *CPS1* is closely related to liver disease, including evolution of chronic HCV infection and hepatic fibrosis (9). In addition, emerging studies have shown that the metabolic gene *CPS1* exhibits significantly reduced expression in poorly differentiated hepatocellular carcinoma cell lines (10). Another report also indicates that the overexpression of *CPS1* is correlated with both of the adverse therapeutic responses in colorectal cancer patients receiving neoadjuvant concurrent chemoradiotherapy (CCRT) (11). At the same time, Pham-Danis *et al.* recently found that downregulation of *CPS1* with EGFR inhibitors could further reduce the proliferation of *EGFR*-mutant non-small cell lung cancer (NSCLC) cells and prevent cell cycle progression (12). However, the specific role of *CPS1* in LADC and its detailed mechanisms necessitate further study.

The purpose of our study was to investigate the roles of *CPS1* in human LADC and its relationship with clinical treatment and prognosis, so as to provide a new option for further effective treatment of LADC patients.

Methods

Data collection and reanalysis using different bioinformatics methods

We summarize several bioinformatics databases used to evaluate the *CPS1* expression levels in LADC tissues and cell lines in *Table S1*.

The differential biomarker screening and clinicopathologic based analysis could be carried out using the Oncomine database containing 65 gene expression datasets from most major types of cancer, along with their normal tissues and various cancer subtypes (13). We performed an exploration of *CPS1* expression between LADC and normal tissues. In addition, two databases, Gene Expression Profiling Interactive Analysis (GEPIA) (14) and UALCAN (15), were used to validate the results. Through these public bioinformatics webtools, we could clearly understand the expression profiles of *CPS1* in human LADC tissues and cell lines.

Wanderer is an intuitive webtool allowing real-time access and visualization of gene expression and DNA methylation profiles from The Cancer Genome Atlas (TCGA) (16). This allows us to screen for possible methylation sites in the full sequence of *CPS1* DNA and to analyze the associations between clinical features of LADC patients, *CPS1* expression and its methylation values.

Kaplan-Meier survival analysis was used to assess the impact of genes on patient survival (17). We used Kaplan-Meier Plotter to describe the relationships between *CPS1* expression levels and first-progression (FS) and postprogression survival time (PPS) (18). Otherwise, the determination of associations between *CPS1* expression and disease-free survival (DFS) and overall survival (OS) was achieved through the GEPIA and UALCAN databases, respectively.

The Gene Expression Omnibus (GEO) database is an international public repository that archives and freely distributes high-throughput gene expression and other functional genomics datasets (19). We obtained

two therapeutically relevant transcriptome microarrays, GSE2189 (20) and GSE54712 (21), from the GEO database. Subsequently, the effect of *CPS1* expression on LADC chemotherapy was analyzed.

cBioPortal, a web-based resource for analyzing multidimensional cancer genomics data (22), was used to screen for genes coexpressed with *CPS1* in LADC tissues. Furthermore, we used a STRING database (23) and Cytoscape tool (24) to construct a protein-protein interaction network (PPI) of these coexpressed genes. We then performed Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the *CPS1* coexpressed genes in LADC samples using the web-based GEne SeT AnaLysis toolkit (WebGestalt) (25) and Pathview algorithm (26), respectively.

MethHC is a database comprising a systematic integration of DNA methylation data and mRNA/microRNA expression profiles in human cancers (27). We use it to evaluate the correlation analysis between *CPS1* expression and its methylation values. For the correlation between disease prognosis and *CPS1* methylation values, the MethSurv tool was used to examine the effect of *CPS1* methylation on patient survival time (28).

Statistical analyses

We used Student's t-test and statistical package SPSS (SPSS) 12.0 (IBM Analytics) to analyze genes differentially expressed between cancerous and noncancerous tissues. The relationship between *CPS1* expression and clinicopathological features was also analyzed using ANOVA and K independent sample tests, and correlations between genes were assessed using Pearson correlation coefficients. Meanwhile, significance of survival analysis was performed using Kaplan-Meier analysis with the Log-rank test. $P<0.05$ was considered to be statistically significant.

Results

CPS1 is upregulated in LADC tissues and cell lines

To examine changes in *CPS1* expression between LADC and adjacent nontumor tissues, we analyzed their expression profiles in a number of independent bioinformatics databases. Using the Oncomine database, we found that the *CPS1* transcription levels in tumor tissues from two individual microarray datasets increased significantly (*Figure 1A*). To further confirm these results, we analyzed the expression of *CPS1* in LADC using GEPIA and UALCAN: we obtained the same change trends as above (*Figure 1B,C*). Expectedly, we obtained a GSE54712 dataset (21) from the GEO database and found that *CPS1* could induce tumor development and metastasis under the synergy of cancer stem cells (CSCs) (*Figure 1D*). All of the above data indicated that the upregulation of *CPS1* expression level promotes the development and progression of LADC.

CPS1 expression is associated with the clinical characteristics of LADC patients

To date, almost no literature has reported a relationship between the expression of *CPS1* and the clinical prognosis of human LADC. The effect of *CPS1* expression on survival index was assessed using a Kaplan-Meier plotter tool, confirming that upregulation of *CPS1* expression was significantly associated with shorter FP and PPS (P=0.028 and 0.038, respectively) (*Figure 2A,B*). Furthermore, the UALCAN database showed that patients with high CPS1 levels had shortened OS (P=0.0057) (*Figure 2C*). At the same time, from the GEPIA database, we also found that patients with high levels of *CPS1* expression had shorter DFS (P=0.042) (*Figure 2D*). Using the Wanderer database, we obtained a series of clinical data, summarized as *Table 1*. Correlations between *CPS1* expression with gender (P=0.000) and primary therapy outcome (P=0.048) are shown in *Table 1*. No correlation was observed between *CPS1* expression and other clinical features, such as *KRAS* mutation status, *EGFR* mutation status, etc. Multivariate analysis using the COX regression model showed that the primary therapy outcome was independently associated with *CPS1* transcription levels in LADC samples (*Table 2*). In conclusion, *CPS1* could serve as a potential biomarker for diagnosis and prognosis of LADC patients.

The role of CPS1 in LADC therapies

Next, we screened the chemotherapy-related microarray datasets from the GEO database to further determine the roles of *CPS1* in the treatment of LADC patients. From the GSE2189 dataset (20), we found that the expression level of *CPS1* was significantly decreased at 4 and 12 hours after

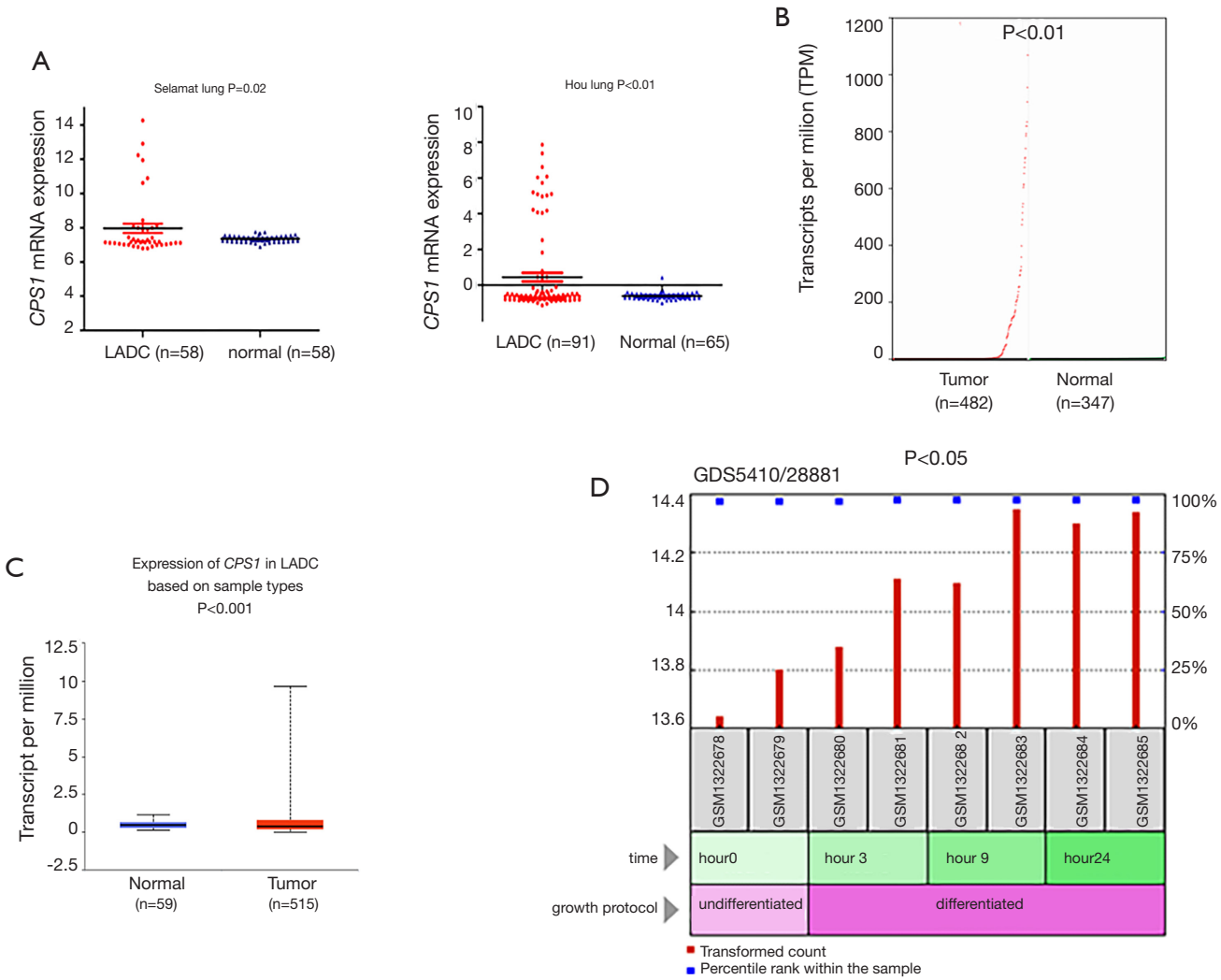

Figure 1 Analysis of carbamoyl phosphate synthetase 1 (*CPS1*) expression level in lung adenocarcinoma (LADC) samples. (A) The expression of *CPS1* messenger RNA (mRNA) in the two datasets, Selament Lung and Hou Lung; (B,C) the mRNA expression of *CPS1* was detected from the GEPIA and UALCAN public databases; (D) expression level of *CPS1* induced by the synergy of cancer stem cells (CSCs).

treatment with the chemotherapy drug motexafin in the A549 human lung cancer cell line (P<0.05) (*Figure 3*). These findings indicated that changes in *CPS1* expression levels might be related to cancer treatment response.

Functional enrichment analysis of CPS1 coexpressed genes

We downloaded the information of genes coexpressed with *CPS1* using the cBioPortal database and obtained 174 coexpressing differential genes (co-DEGs) (http://fp.amegroups.cn/cms/d7e742548b77ff1fe1b3d13978dec587/atm.2020.02.146-1.doc). The PPI networks of these 174 co-DEGs were then constructed by STRING and Cytoscape (*Figure 4A*). To further understand the biological functions of these co-DEGs, we performed GO annotation and KEGG pathway analysis. The GO annotation identifies the major biological processes (response to stimulus and biological regulation), cellular components (nucleus) and molecular functions (protein binding) of *CPS1* biology (*Figure 4B*). The Pathview database was used to analyze the KEGG pathway: as shown in *Table S2*, the corresponding pathways (osteoclast differentiation, regulation of actin cytoskeleton, apoptosis) were obtained.

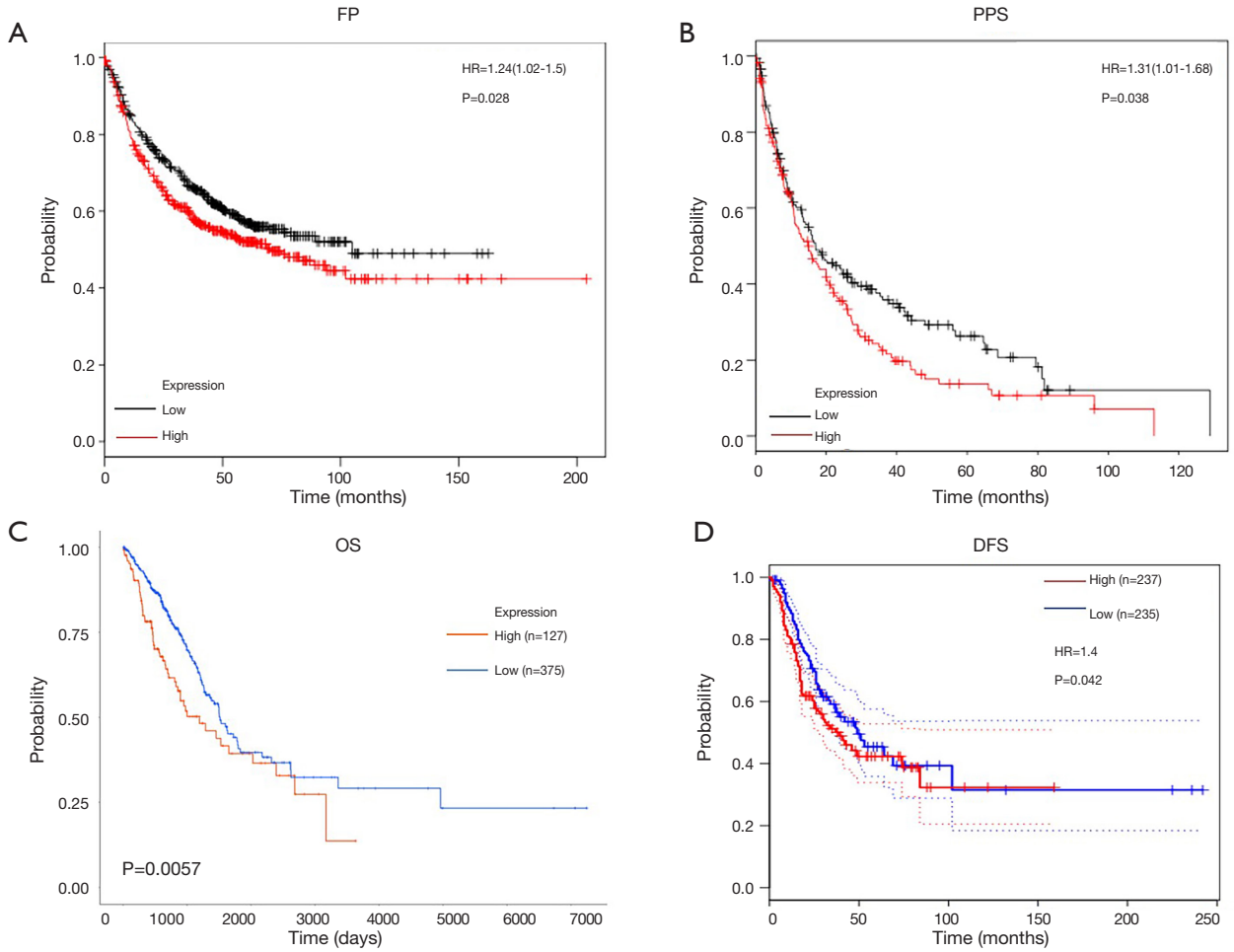

Figure 2 The effects of carbamoyl phosphate synthetase 1 (*CPS1*) expression on prognosis in lung adenocarcinoma (LADC) patients. (A,B) Kaplan-Meier analysis of first-progression (FP) and postprogression survival time (PPS) in LADC patients based on *CPS1* expression; (C) the association between *CPS1* expression and OS determined with the UALCAN database; (D) the association between *CPS1* expression and DFS is evaluated with the GEPIA database.

Relationship between CPS1 methylation and clinical features of patients with LADC

It is well known that there is a negative correlation between DNA methylation and gene expression (29-31). From the MethHC database, we observed that the global *CPS1* methylation level in the LADC samples was lower than that in normal samples (P<0.05) (*Figure 5A*) and was negatively correlated with expression (P<0.05, r=–0.080) (*Figure 5B*), supporting the high expression of *CPS1* in LADC samples. Next, we used the UALCAN and DiseaseMeth databases to further validate the results of *CPS1* methylation level. The results showed that *CPS1* presented low methylation levels in human LADC patients (*Figure 5C,D*). Subsequently, we screened the methylation site cg06888547 from the Wanderer database as the most statistically significant candidate site (*Table 3*). Using the MethSurv network tool analysis, it was found that higher methylation values of cg06888547 in LADC patients were associated with longer OS (P=0.0085) (*Figure 5E*). These data demonstrated that DNA hypomethylation, a major epigenetic modification, might lead to *CPS1* overexpression at the transcriptional level, thus plays important roles in carcinogenesis and progression of LADC patients.

Discussion

Our research first has found that *CPS1* can be used as a

Table 1 Single factor clinical data analysis related to *CPS1*

Characters	Population	Mean ± SD	P value
Gender			0
Male	208	7.57±4.42	
Female	249	5.91±3.19	
Kras mutation status			0.351
Yes	14	6.83±4.28	
No	34	3.37±0.58	
Egfr mutation status			0.844
Yes	65	6.86±4.12	
No	175	6.74±4.06	
Vital status			0.144
Alive	342	6.44±3.67	
Dead	115	7.34±4.40	
Person neoplasm cancer status			0.054
Tumor free	249	6.18±3.49	
With tumor	98	7.41±4.41	
Pathologic T			0.243
T1/T1a/T1b	140	6.13±3.36	
T2/T2a/T2b	256	6.86±4.06	
T3	41	6.77±3.91	
T4	18	7.56±4.42	
TX	2	9.68±9.65	
Pathologic N			0.731
N0	290	6.61±3.73	
N1	85	6.43±3.87	
N2	70	7.19±4.52	
N3	2	5.33±1.11	
NX	9	7.17±4.56	
Pathologic M			0.103
M0	313	6.84±3.91	
M1/M1a/M1b	22	7.96±5.07	
MX	118	6.00±3.52	
Primary therapy outcome			0.048
Complete remission/ response	73	6.84±3.91	
Partial remission/ response	1	7.96±5.07	
Stable disease	4	6.00±3.52	

Table 2 Clinical multivariate data related to *CPS1*

Source	Type III sum of squares	df	Meansquare	F	P value
Gender	50.903	1	50.903	3.577	0.063
Primary therapy outcome	127.668	2	63.834	4.486	0.015

proto-oncogene of LADC and can be used as a potential biomarker through comprehensive mining of public databases. We also analyzed the differential coexpression of *CPS1* and found a possible signaling pathway for *CPS1* to determine its biological significance in cancer development. The Oncomine, UALCAN and GEPIA datasets indicate that *CPS1* is highly expressed in LADC. Kaplan-Meier survival analysis also showed that patients with elevated *CPS1* levels had shortened OS and PPS. In addition, the methylation level of *CPS1* is opposite to that of protein expression, showing a low level.

CPS1 is the first rate-limiting mitochondrial enzyme in the urea cycle. Lack of CPS1 due to mutation can lead to life-threatening hyperammonemia (32,33). A recent study by Çeliktas *et al.* has shown that knockdown of *CPS1* induced accumulation of ammonia and reduction of nucleic acid synthesis pathway, leading to the decreased cancer cell growth. At the same time, they have proven the increased of *CPS1* expression was statistically related with the worse OS both at mRNA and protein levels, which is consistent with what we found (32). Therefore, *CPS1* may become a potential molecular biomarker of poor prognosis and facilitate the individualized treatment for LADC. In addition, in hepatocellular carcinoma (HCC), *CPS1* is downregulated after treatment with aflatoxin B1 (AFB1), an effective hepatocarcinogen, and may serve a differentiation function (34). The researchers discovered the hypermethylation and decreased RNA expressions of *CPS1* in most HCC patients. They speculated that *CPS1* might serve an inhibitory action in the tumorigenesis and development of HCC cancer cells (35). However, our study indicated that *CPS1* might be an oncogenic factor for LADC biology, which may be due to the activated Janus kinases (JAKs)/Signal transducers and activators of transcription (STAT) signaling pathway (32). Generally, JAK-STAT signaling activation has

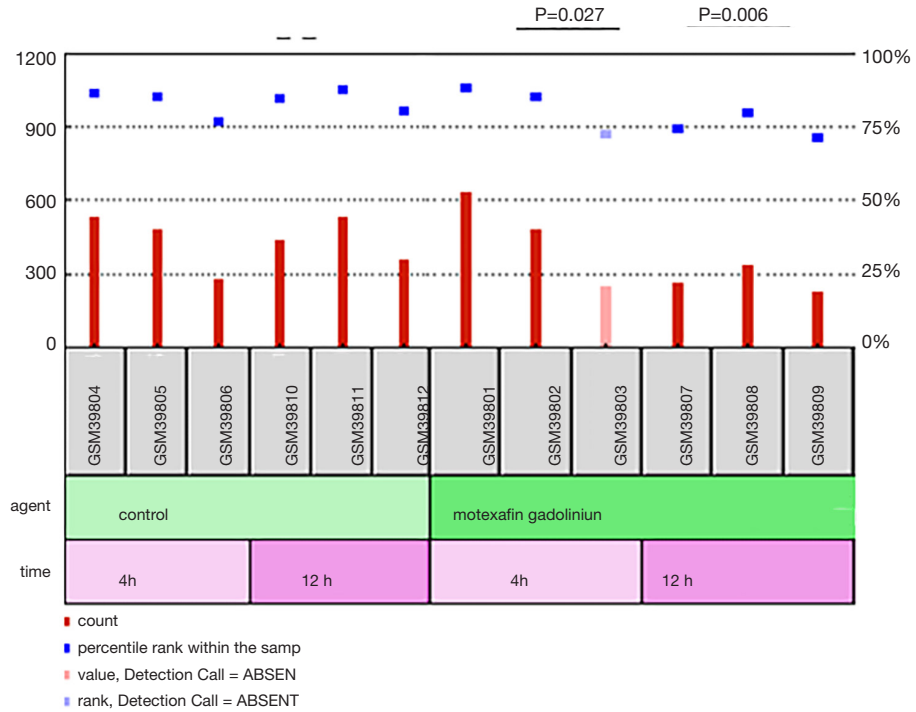

Figure 3 The role of carbamoyl phosphate synthetase 1 (*CPS1*) in lung adenocarcinoma (LADC) therapies. GSE2189 downloaded from the GEO database assesses the effect of CPS1 on LADC treatment response.

been proved to promote cancer progress through modulating the inflammation, invasion and immunosuppression (36).

Although a large body of literature indicates that *CPS1* plays a crucial role in human malignancies, little is known about the detailed roles of *CPS1* in human LADC. Our research has fully demonstrated that *CPS1* promotes tumors in LADC samples. Moreover, we have demonstrated that patients with high expression of *CPS1* have worse OS and PPS. This provides an idea for further exploration of *CPS1* as a potential biomarker for LADC. Human LADC tumorigenesis is impacted by epigenetic changes, a situation which contributes to the abnormal changes of specific genes. Moreover, the other promising cause for LADC diagnostics could be the methylation values of DNA sequences in certain biomarkers (37). To date, the most meaningful and common epigenetic modifications in the mammalian genome are DNA methylation events. Oshima *et al.* found that the methylation of metastasis-suppressive miRNAs could upregulate these miRNA expression levels, leading to the metastasis profiles of cancer cells in lung carcinogenesis (38). DNA hypermethylation-mediated downregulation of LINC00261 plays an important proliferation-inducing role in LADC progression (39). By reanalyzing the datasets from several user-friendly webtools, we found the significant low-methylated values of *CPS1* and identified the negative association between its methylation and expression levels, thus confirming that DNA hypomethylation was indeed responsible for the increased *CPS1* expression in LADC tissues.

However, our study has several limitations. First of all, our research is mainly based on the analysis of biological databases, lacking effective external experimental validation. Secondly, it is necessary to include more prognostic variables to improve the accuracy of survival analysis. Finally, further validation of CPS1 in multicenter clinical trials and prospective studies is much needed.

Conclusions

Taken together, our results indicated that *CPS1* is a candidate tumor proto-oncogene for human LADC. The chemotherapy results of LADC also indicate that *CPS1* is a potential indicator of cancer treatment efficacy. In addition, reanalysis based on public databases provides new insights into the screening of potential biomarkers associated with human malignant diseases, especially human lung cancers.

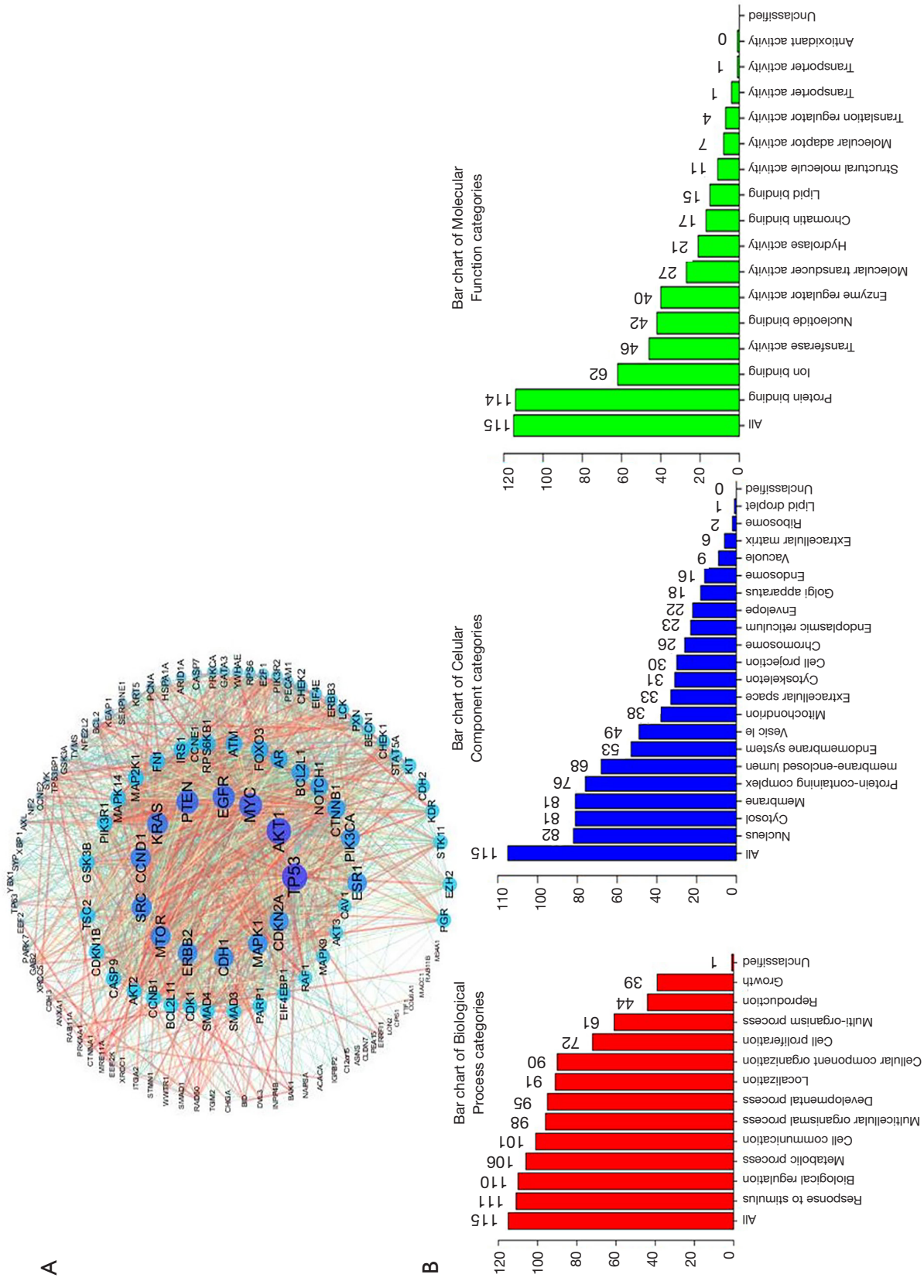

Figure 4 Coexpression network analysis for carbamoyl phosphate synthetase 1 (*CPS1*). (A) The PPI network of *CPS1* interaction partners generated by two frequently used algorithms, STRING and Cytoscape; (B) primary molecular functions, biological processes and cell components related to the biology of *CPS1* were identified by WebGestalt.

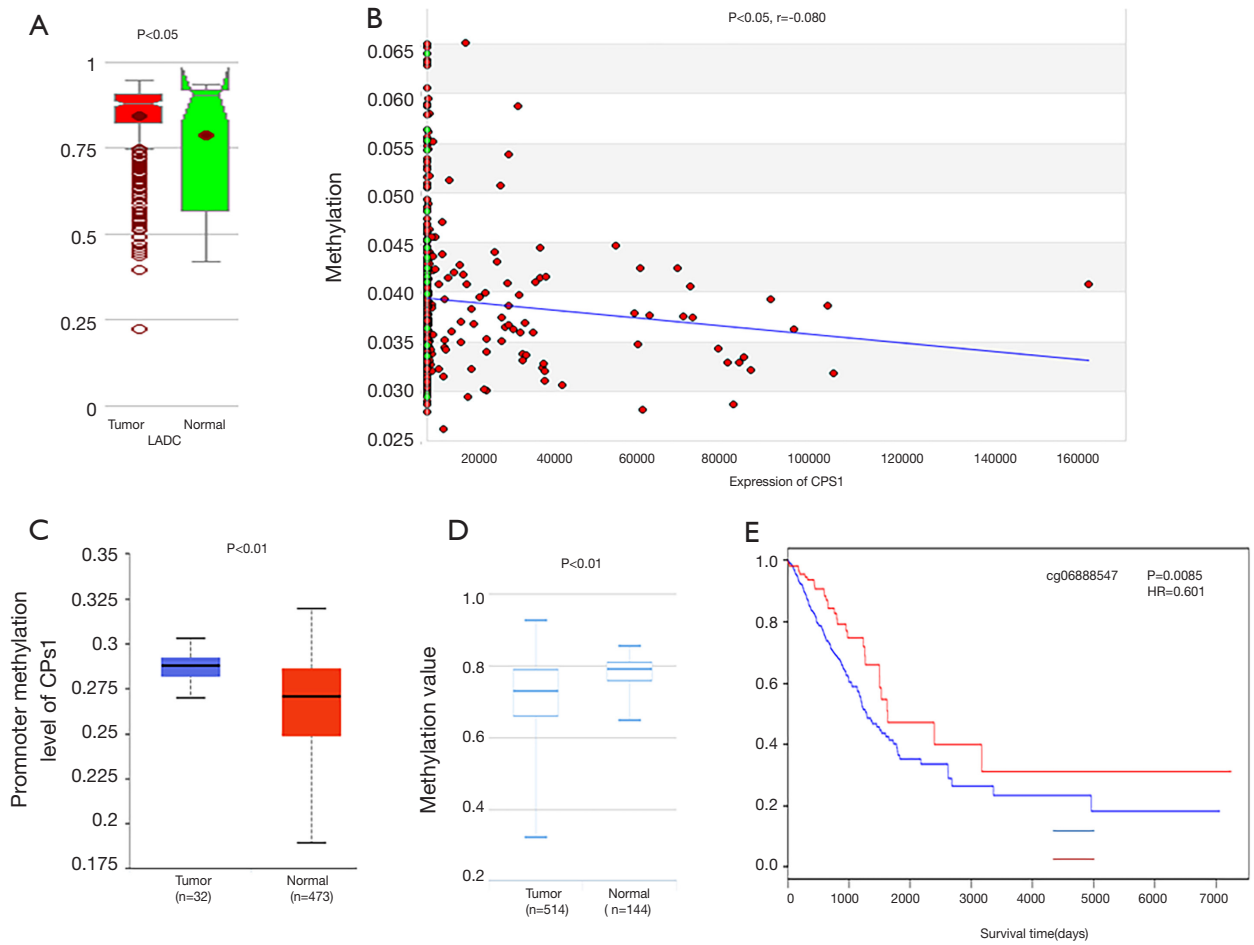

Figure 5 The relationship between carbamoyl phosphate synthetase 1 (*CPS1*) methylation and clinical characteristics of lung adenocarcinoma (LADC) patients. (A) Global *CPS1* methylation in LADC samples compared with the normal samples evaluated by MethHC database; (B) relationship between *CPS1* methylation values and expression level; (C,D) *CPS1* methylation levels downloaded from UALCAN and MethSurv databases; (E) the impact of methylation site cg06888547 of *CPS1* on OS in LADC patients analyzed by the MethSurv webtool.

Table 3 Methylation data downloaded from the Wanderer database

Methylation site	P value
cg06888547	4.75E-11
cg11926456	4.03E-06
cg17971592	8.91E-05
cg06820405	0.000131
cg22996009	0.000967
cg07643321	0.001138
cg21967368	0.001272
cg20604456	0.005654
cg07614486	0.008399
cg21119032	0.008415
cg04350237	0.023947
cg08400511	0.101776
cg21205803	0.127705
cg16205846	0.152554
cg15764953	0.168149
cg07063745	0.342008
ch.2.4215183F	0.380838
cg21846876	0.42634
cg18105667	0.513352
cg14504603	0.773226

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Supplementary

Table S1 The main bioinformatics tools used to analyze the functions of *CPS1* in the biological process of lung adenocarcinoma cells (LADC)

Databases	Samples	URL	Refs
Oncomine	Tissues/Cells	https://www.oncomine.com/resource/login.html	(12)
UALCAN	Tissues	http://ualcan.path.uab.edu/index.html	(14)
GEPIA	Tissues	http://gepia.cancer-pku.cn/	(13)
Kaplan-Meier plotter	Tissues	http://kmplot.com/analysis/	(16)
GEO	Tissues/Cells	https://www.ncbi.nlm.nih.gov/geoprofiles/	(18)
cBioPortal	–	http://www.cbioportal.org/	(21)
STRING	–	https://string-db.org/	(22)
WebGestalt	–	http://www.webgestalt.org/option.php	(24)
Pathview	–	https://pathview.uncc.edu/	(25)
MethHC	–	http://methhc.mbc.nctu.edu.tw/php/index.php	(26)
Methsurv	–	https://biit.cs.ut.ee/methsurv/	(27)
Wanderer	–	http://maplab.imppc.org/	(15)

GEPIA, gene expression profiling interactive analysis; GEO, gene expression omnibus; WebGestalt, the web-based GEne SeT AnaLysis Toolkit.

Table S2 KEGG pathways associated with *CPS1*

Pathway	Stat.mean	Set.size	p.val	q.val
hsa04210 apoptosis	–1.031940522	13	0.31	0.91
hsa04810 regulation of actin cytoskeleton	0.923190599	12	0.91	0.91
hsa04380 osteoclast differentiation	–0.864906765	13	0.91	0.91