Prognostic Implications and Immune Infiltration Analysis of SSBP1 in Lung Adenocarcinoma

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Research Article

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Abstract

Background

Single-Stranded DNA Binding Protein 1(SSBP1) has been found closely related to the malignant biological process of the tumor. However, the functions of SSBP1 in lung adenocarcinoma (LUAD) are still unclear. The present study explored the prognosis of SSBP1 in LUAD.

Methods

Oncomine, TIMER, and UALCAN were used to analyze the difference of SSBP1 expression in normal and tumor tissues, and HPA was used to analyze the immunohistochemical staining in tumor tissues. We also used Kaplan-Meier analysis to evaluate the impact of SSBP1 on LUAD patients' survival. In addition, we used the TIMER database to explore the correlation between the expression of SSBP1 and tumor-infiltrating immune cells (TILs). Furthermore, we used STRING and GeneMANIA to build a network of protein-protein interaction (PPI) and perform GO analysis. Finally, we used CARE to evaluate the relationship between the expression of SSBP1 and the response of the drug.

Results

The expression of SSBP1 was up-regulated in the patients with LUAD and was related to the worse overall survival (OS) probability. And, SSBP1 was negatively associated with ImmuneScore, ESTIMATEScore, and StromalScore. In addition, SSBP1 expression has a negative correlation with the infiltration level of B cells, dendritic cells, CD4 + T cells, and macrophages. And, the expression of SSBP1 in B cells, CD8 + T cells, and dendritic cells (DC) was related to OS. Furthermore, a positive correlation was found between the expression SSBP1 and CARE.

Conclusion

SSBP1 is a potentially poor prognostic biomarker of LUAD and correlated with immune infiltrates in LUAD.

Background

Lung cancer is a highly heterogeneous malignant tumor worldwide, and its mortality and morbidity rate rank first among tumors, which poses a considerable threat to human health and life [1, 2]. Non-small cell lung cancer (NSCLC) is the main histological subtype of lung cancer, accounting for approximately 85%, and the most common pathological subtype of NSCLC is lung adenocarcinoma (LUAD)[3], and LUAD's five-year survival rate is only 22.1%[4]. With the rapid development of targeted therapy and immunotherapy, the therapeutic strategies of advanced-stage lung cancer have entered an era of the emerging concept of precision and individualization in recent years. Unsurprisingly, although the diagnosis and treatment technology has made significant progress, the prognosis of patients with lung cancer is still relatively poor due to the high recurrence and metastasis rate of lung cancer[5]. Therefore, to help clinical work and improve the prognosis of patients with lung tumors, it is imperative to find new prognostic markers and potential targets. Mitochondrial single-strand DNA binding protein 1 (mitochondrial single-strand binding protein 1, SSBP1) is a key molecule in mitochondrial DNA synthesis, a housekeeping gene involved in mitochondrial biogenesis, and a single-stranded DNA (ssDNA) binding complex that maintains genome stability[6]. Subunits, mitochondrial DNA replication, repair, and transcription play an important role and affect the biological processes of cells[7–9]. Abnormal expression of SSBP1 will directly lead to abnormal mitochondrial DNA copy number or gene mutation, and ultimately cause the occurrence and development of malignant tumors. Recent studies have found that abnormally expressed SSBP1 affects the proliferation, invasion, metastasis, and other malignant biological behaviors of breast cancer, colon cancer, gastric cancer, and other malignant tumors[10–13]. However, the biological function and prognostic significance of LUAD are still unclear.

To explore whether the expression level of SSBP1 is related to the prognosis of LUAD, we used databases such as TIMER, Oncomine, HPA, etc. The association between immune infiltration and SSBP1 expression was also assessed by TIMER. In addition, GSEA was applied to the TCGA-LUAD dataset, revealing the underlying molecular mechanism of SSBP1. Our results demonstrated the role of SSBP1 expression in the prognosis of LUAD, as well as the possible correlation between SSBP1 expression and immune infiltration in the tumor microenvironment, and the interaction mechanism between them.

Materials And Methods

Analysis of SSBP1 gene expression in LUAD patients

Compare SSBP1 expression in tumor and normal tissues by the Oncomine database[14] (https://www.oncomine.org/) and the TIMER database[15] (https://cistrome.shinyapps.io/timer/). Use UALCAN to analyze the expression of SSBP1 mRNA and protein in the TCGA database of LUAD patients, and obtain the expression of SSBP1 in LUAD patients with different clinical characteristics[16]. Immunohistochemical images of SSBP1 protein in lung adenocarcinoma and healthy lung tissue are from the HPA database [17] (https://www.proteinatlas.org/).

Association of SSBP1 expression with the prognosis of LUAD

Kaplan-Meier plotter database (http://www.kmplot.com) was used to analyze the prognostic effect of SSBP1 in LUAD patients[18]. At the same time, we also used UALCAN and GEPIA to analyze the prognosis. GEPIA is a developed interactive website that can perform differential expression analysis, profiling plotting, correlation analysis, patient survival analysis, similar gene detection, and dimensionality reduction analysis[19] (http://gepia2.cancer-pku.cn/#index).

Assessment of tumor microenvironment and infiltrated immune cells

TIMER is an interactive web application that can analyze and visualize the abundance of tumor-infiltrating immune cells comprehensively and flexibly (http://timer.compgenomics.org/). TISIDB is a database that allows users to query the function of specific genes in tumor-immune interactions. [20] (http://cis.hku.hk/TISIDB/index.php). Using Sangerbox, we evaluate the immune score and stromal score of each TCGA tumor sample(http://sangerbox.com/Index).

Gene set enrichment analysis (gsea) of SSBP1 high and low expression group

Based on the TCGA database, use the GSEA function in Sangerbox to analyze the KEGG and HALLMARK pathways of the SSBP1 high expression group and low expression group.

Assess the drug response of LUAD

CARE is a computational method that focuses on targeted therapy, which can infer genome-wide transcriptome characteristics of drug efficacy from cell line compound screening. The correlation between the gene expression profile of cancer samples and the CARE scoring vector is analyzed by Pearson correlation test[21].

Statistical analysis

Spearman correlation analysis was used to describe the correlation between SSBP1 and tumor mutation burden (TMB). The P-value less than 0.05 was considered statistically significant.

Results

SSBP1 high expression in LUAD

Compared with the adjacent normal tissues detected by the Oncomine database, the expression of SSBP1 in various tumors increased or decreased (Fig. 1A). And, TIMER online database analysis showed that compared with adjacent tissues or normal lung tissues, the expression of SSBP1 in LUAD was significantly up-regulated (Fig. 1B; * p <0.05, ** p <0.01, *** p <0.001). In addition, to explore the gene and protein expression of LUAD patients, we used UALCAN to analyze SSBP1 expression in LUAD patients from the TCGA database (Fig. 1C, D; P <0.01). Furthermore, we also used the HPA database to analyze the expression of SSBP1 in tumor tissues (Fig. 1E).

Clinical characteristics and poor prognosis of LUAD with high expression of SSBP1

UALCAN was used to detect the relationship between SSBP1 and the clinical characteristics of patients with LUAD. As shown in Fig 2A,2B, and 2C, SSBP1 expression in LUAD tissues was significantly higher than that in normal tissues. In addition, SSBP1 expression is also closely related to the clinical characteristics of patients, such as tumor stage, lymph node metastasis, and TP53 mutation. Moreover, compared with normal tissues, regardless of race, gender, or age, the expression of SSBP1 increased(Fig S1). GEPIA, UALCAN, and KM were used to analyze the prognostic value of SSBP1 expression on LUAD. The results showed that OS and SSBP1 expression in LUAD patients were significantly negatively correlated. (HR= 2, log-rank p = 1.2e-05) (Fig. 2D). However, the correlation between DFS and SSBP1 expression was not significant (HR = 1.3, log-rank p = 0.065) (Fig. 2E). The UALCAN database showed that high SSBP1 expression was also associated with poorer OS in LUAD patients (p = 0.037), and the Kaplan-Meier plotter database showed the prognosis of LUAD patients with worsened OS (HR = 1.38, log-rank p = 1e-07) (Fig. 2F).

Relationship between SSBP1 expression and tumor-infiltrating immune cells

A positive correlation was detected by Spearman's correlation analysis between the expression of SSBP1 and TMB (P = 5.9E-6) (Fig. 3A).TISIDB database was used to comprehensively analyze the relationship between the expression of SSBP1 and chemokines to further elucidate the underlying mechanism of SSBP1 in immune cells migration. Meanwhile, based on the TISIDB database, we analyzed the relationship of SSBP1 expression and 28 TILs in multiple cancer types (Fig. S2), and most tumor infiltrating cells in LUAD are negatively correlated with the expression of SSBP1 (Fig. 3B). Furthermore, in order to explore the relationship between SSBP1 expression and immune score, we used the ESTIMATE algorithm in the SangerBox tool showed that the expression of SSBP1 had a significantly negatively correlation with StromalScore(r = -0.191, P =1.29e-05), ESTIMATEScore (r = -0.205, P = 2.81 e-06) and ImmuneScore (r = -0.179, P =4.69e-05) (Fig. 3C). TIMER database was used to analysis of the correlation between the expression of SSBP1 and the level of immune cells infiltrating showed that SSBP1 had a negatively correlation with B cells (r = -0.32, P =1.24e-13), CD4+T (r = -0.321, P = 1.02E-13), Neutrophil (r = -0.101, P = 0.024), Macrophages (r = -0.145, P = 0.00104) and Dendritic (r = -0.166, P = 0.00016). However, the expression of SSBP1 has no statistically significant correlation with CD8+ T cells (Fig. 3D).Moreover, the high expression of SSBP1 in B cells (P = 0.001), CD8+ T cells (P = 0.045) and dendritic cells (P = 0.007) was better correlated with the OS of LUAD by survival analysis (Fig. 3E).

SSBP1 interaction network

We used STRING to analyze the relationship between SSBP1 and upstream and downstream proteins. A corresponding relational network diagram can be obtained (Fig. 4A). PPI enrichment P-value is < 1.05e-5. There are 11 nodes containing INTS3, SSBP2, INIP, SSBP1, GABPA, GABPB1, TP53, TFAM, POLRMT, POLG, C10orf2.

The SSBP family (SSBP1, SSBP2, SSBP3, and SSBP4) gene interaction network was further constructed by GeneMANIA (Fig. 4B). According to GO analysis, SSBP1 is a key molecule in mitochondrial DNA synthesis, mainly involved in the replication, transcription, and post-injury repair of mitochondrial DNA. The main cellular components are Mitochondrial nucleoid, Mitochondrial matrix, SOSS complex, and Intracellular organelle lumen. (Fig. 4C)

High SSBP1 expression significantly increases cancer malignancy

The Sangerbox tool was used to perform GSEA on KEGG and HALLMARK to explore the biological pathways of the two groups. We found that the top 3 significantly enriched KEGG pathways in the high expression group of SSBP1 were pyrimidine metabolism, purine metabolism, and Huntington's disease (Fig. 5A). While, in the low expression group of SSBP1, the top 4 significantly enriched KEGGs pathways are related to hematopoietic cell lineage, aldosterone-regulated sodium reabsorption, linoleic acid metabolism, and α-linoleic acid metabolism (Fig. 5B). In addition, the GSEA of the HALLMARK pathway showed that the top 3 pathways related to the high expression of SSBP1 were glycolysis, DNA repair, and mTORC. And, the top 4 pathways related to the low expression of SSBP1 were the IL-6 JAK STAT3 signaling pathway, KRAS signaling pathway, myogenesis, and hedgehog signaling pathway (Fig. 5C, D). Moreover, CancerSEA database[22] was used to explore the single-cell RNA (scRNA) analysis of LUAD showed that the expression of SSBP1 had significantly positive correlation with tumor malignant features including DNA damage(r = 0.48, P =0.000), DNArepair (r =0.47, P =0.000), invasion (r = 0.45, P =0.000), cell-cycle (r =0.40, P =0.000), and metastasis (r = 0.36, P =0.000) (Fig. 6 A, B, C, D and E). These findings implied that high SSBP1 expression is significantly related to the malignancy of LUAD. The t-SNE plot shows siRNA analysis of SSBP1 expression (Fig. 6 F).

High expression of SSBP1 is associated with anti-angiogenesis response

From the Cancer Cell Line Encyclopedia (CCLE), Genomics of Drug Sensitivity in Cancer (GDSC, previously named CGP), and The Cancer Therapeutics Response Portal (CTRP) cohorts' analysis, Analysis from the Cancer Cell Line Encyclopedia (CCLE), The Cancer Therapeutics Response Portal (CTRP), and Genomics of Drug Sensitivity in Cancer (GDSC) cohorts showed that among the top5 chemical components, the expression of SSBP1 had a positive correlation with the CARE score, which was mainly including Tacrolimus, Dabrafenib, Elocalcitol, CHEMBL3186197, and GSK525762A. At the same time, it was found that these drugs act on anti-angiogenesis and other malignant biological processes, indicating the treatment strategy for LUAD patients based on the high expression of SSBP1 could add anti-angiogenesis drugs.(Fig. 7, and Table 1)

Discussion

Mitochondria are important places for energy conversion and metabolism in eukaryotes and mitochondrial dysfunction will lead to a series of diseases including tumors[23]. Moreover, the key features of solid tumors are hypoxia and mitochondrial metabolism disorders, and are related to metabolic reprogramming is related to disease progression[24, 25]. SSBP1 is a key molecule in the synthesis of mitochondrial DNA, a housekeeping gene involved in mitochondrial biogenesis, and a single-stranded DNA (ssDNA) binding complex that maintains the stability of the genome[6, 9]. Abnormal expression of SSBP1 will lead to instability of mitochondrial DNA and ultimately cause the occurrence of malignant tumors. This study found that the expression of SSBP1 was high in patients with LUAD and was significantly related to worsening prognosis. In addition, the potential mechanism of the high expression of SSBP1 was explored. This study showed that mutated TP53 caused a significant up-regulation of SSBP1 expression. The incidence of TP53 mutation in LUAD patients was 46% [26]. Studies have proven that hSSB1, a human ssDNA binding protein in the SSB protein family, can bind to and protect p53 from proteasome degradation, and also regulate DNA damage checkpoints, thereby regulating the transcriptional activity of p53[27, 28].

Cancer metabolism and behavior are regulated by cellular internal factors and metabolites in the tumor microenvironment (TME)[29]. At the same time, TME can be used as a predictor factor of immune checkpoint inhibitor response, and SSBP1 has a significantly positive correlation with TMB. And, in LUAD, high immune scores and estimates were related to better progression-free survival (PFS)[30]. We found that SSBP1 had a significantly negative correlation with ImmuneScore and ESTIMATEScore. This finding indicates that SSBP1 plays a significant effect on TIME's immune regulation. In addition, infiltrating immune cells such as T cells, B cells, and macrophages account for a large proportion of TME in NSCLC tumor[31]. The infiltration of T and B cells in NSCLC predicts a good prognosis in some studies[32, 33]. And the infiltration of DCs in LUAD is associated with protective immunity demonstrated in previous studies[34]. Our analysis showed that SSBP1 in LUAD had a negative correlation with the infiltration level of B cells, dendritic cells, macrophages, and CD4 + T cells. Finally, survival analysis found that tumor-associated B cells, CD4 + T cells, and dendritic cells in LUAD had a good effect.

Through GSEA analysis, we explored the biological function of SSBP1 in LUAD. Functional analysis showed that the first three pathways related to the high expression of SSBP1 were glycolysis, DNA repair, and mTORC1 signaling pathways[35]. Subsequently, we verified the above-mentioned biological functions from the perspective of single cells. Subsequently, we verified the above-mentioned biological functions from a single cell perspective, showing that SSBP1 had a significantly positive correlation with malignant biological behaviors such as DNA damage, DNA repair, tumor invasion, cell cycle, and metastasis, which indicated that SSBP1 might play a significant effect on LUAD tumor promotion. Furthermore, from the CCLE, GDSC, and CTRP cohort analysis, it was found that among the top5 chemical components, the expression of SSBP1 was positively correlated with CARE score, mainly including Tacrolimus, Dabrafenib, Elocalcitol, CHEMBL3186197, and GSK525762A. At the same time, it was found that these drugs act on anti-angiogenesis and other malignant biological processes, indicating the treatment strategy for LUAD patients based on the high expression of SSBP1 could add anti-angiogenesis drugs.

Conclusions

In general, our study found that the expression of SSBP1 is high in LUAD and is significantly related to the worsened prognosis of LUA. And, SSBP1 may affect TME by reducing the levels of immune infiltrating cells such as B cells, CD4 + T cells, macrophages, and dendritic cells, leading to worsening prognosis.

Abbreviations

SSBP1
Single-Stranded DNA Binding Protein 1
LUAD
Lung adenocarcinoma
NSCLC
Non-small cell lung cancer
TCGA
The cancer genomeatlas
TIMER
The tumor immune estimation resource
MHC
Major histocompatibility complex
GTE
Genotype-tissue expression
CCLE
Cancer cell line encyclopedia
IHC
Immunohistochemistry
THPA
The human protein atlas
GEPIA
Gene expression profiling interactive analysis
CARE
Computational Analysis of REsistance
GEO
Gene expression omnibus
ECOG
Eastern Cooperative Oncology Group
GSEA
Gene set enrichment analysis
scRNA
single-cell RNA
TMB
Tumo mutation burden
CCLE
Cancer Cell Line Encyclopedia
GDSC
Genomics of Drug Sensitivity in Cancer
CTRP
Cancer Therapeutics Response Portal
TILs
Tumor-infiltrating immune cells
TME
Tumor microenvironment OS:Overall survival
FPS
First-progression survival
PPS
Post-progression

Declarations

Acknowledgments

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Authors' contributions

NZY and LZ Data Collection and Curation, Writing-Original Manuscripts

HML, JXW, and YDX Preliminary Review and Editing

JGM Final Reviewing and Editing

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Availability of data and materials

All data in this study were retrieved from public and open-source databases. All databases and its web address were listed below: GEPIA(http://gepia2.cancer-pku.cn/#index),Oncomine(https://www.oncomine.org/),KMplotter(http://www.kmplot.com),UALCAN(http://ualcan.path.uab.edu/index.html),OncoLnc(http://w (http://sangerbox.com/Index), miRMap (https://mirmap.ezlab.org/), TargetScan(http://www.targetscan.org/vert_72/),miRWalk(http://mirwalk.umm.uniheidelberg.de/),CancerSEA(biocc.hrbmu.edu.cn/CancerSEA/goSearch).

Declarations

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Table

Table 1

	Drug	Target	t-value	p-value
CGP	PHA-793887	CDK9	2.86159	0.00431338
	CPD	AURKB	3.29237	0.0010322
	GSK1070916	AURKB	3.27617	0.00109386
	Lapatinib	ERBB2	3.71834	0.000230647
	Dabrafenib	BRAF_V600E.Mutation	4.40355	1.20E-05
	SB590885	BRAF_V600E.Mutation	3.74894	0.000190195
	PLX4720	BRAF_V600E.Mutation	2.91603	0.00363021
	INC424	JAK1_missense.Mutation	-5.27343	1.68E-07
	1256580-46-7	ALK	3.4289	0.000633689
	GSK525762A	BRD4	2.4646	0.013904
	GSK525762A	BRD2	5.36013	1.06E-07
	PHA-793887	CDK1	3.21562	0.00134824
	870483-87-7	CSF1R	-6.79671	1.95E-11
	CAL-101	PIK3CD	-2.50612	0.0123822
	BMS345541	IKBKB	2.38929	0.0170864
CTRP	Sorafenib	FLT3	2.60946	0.00924791
	Neratinib	ERBB2	-2.65739	0.00803677
	CHEMBL243659	FNTA	2.40672	0.0163339
	BIBR1532	TERT	2.90522	0.0037748
	JQ-1	BRD2	2.76324	0.00585796
	SMR001317659	PDE4B	2.75529	0.00600107
	BDBM50127227	FNTA	2.55165	0.0109156
	PAC-1	CASP3	2.81574	0.00499814
	SCHEMBL14981874	KDM4C	2.49103	0.0130457
	Erlotinib	EGFR	-2.90381	0.00379279
	UNII-40E3AZG1MX	INSR	3.32337	0.000931792
	Elocalcitol	VDR	4.85045	1.50E-06
	BAS02002358	GPER1	2.72223	0.00663225
	180002-83-9	CNR2	3.41095	0.000681668
	I-BET151	BRD2	2.97505	0.00302046
	KU-55933	ATM	2.44228	0.0148216
	ABT-737	BCL2	-2.77445	0.00567643
	NVP-BSK805	JAK2	-2.34099	0.0194909
	Rapamycin	MTOR	2.64841	0.00825001
	ABT-199	BCL2	-3.68452	0.000255917
	GSK525762A	BRD2	2.68044	0.00750908
	Nelarabine	POLA1	-2.67655	0.00760591
	CHEMBL3186197	IGF1R	2.9645	0.00312615
	BCP9000801	MDM2	2.84919	0.00450817
	PHA-793887	CDK9	2.3054	0.0214066
	CHEMBL3186197	INSR	5.21495	2.37E-07
	NSC373989	MDM2	3.25732	0.00117552
	INC424	JAK2	-3.71356	0.000219251
	Entinostat	HDAC1	2.69117	0.00727629
	BMS-754807	IGF1R	3.73142	0.000204478
	UNII-UZ77T1VFBM	BIRC5	4.01313	6.64E-05
	Tacrolimus	PPP3CC	4.28849	2.04E-05
	Tacrolimus	PPP3CB	2.43487	0.0151355
	Dasatinib	ABL1	-3.42715	0.000642461
	Cerulenin	HMGCS1	3.74138	0.000196827
CCLE	Sorafenib	FLT3	-7.61177	1.43E-13
	NVP-AEW541	IGF1R	3.15612	0.00169764

Figures

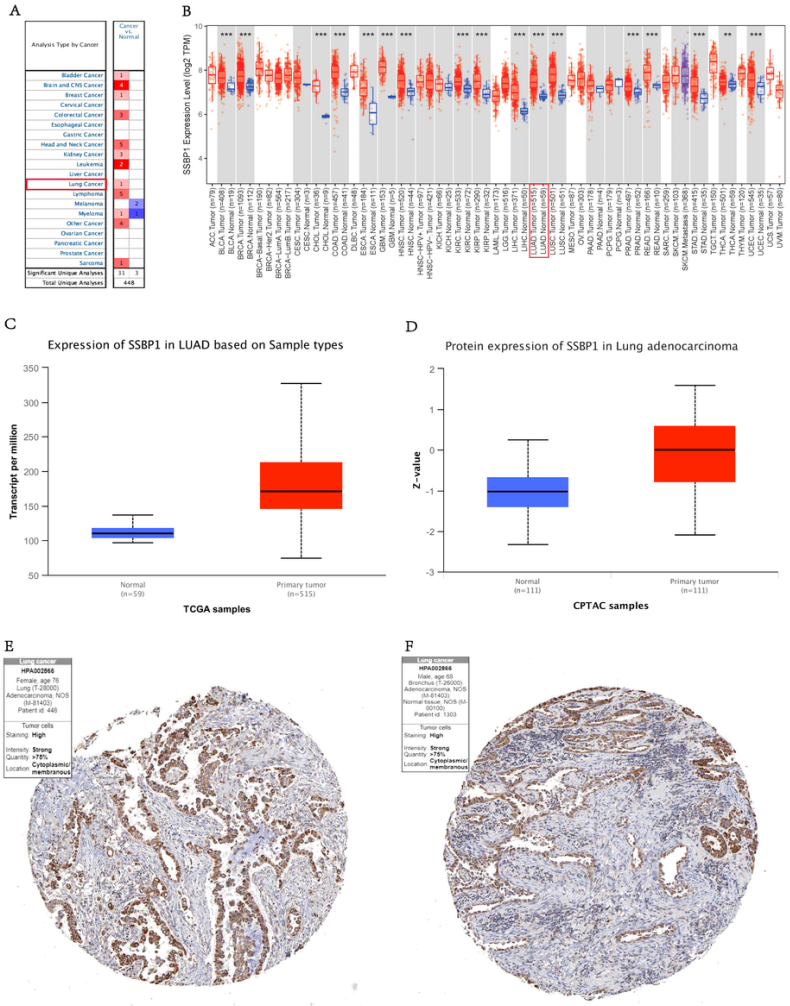

Figure 1

SSBP1 expression in LUAD. (A)Compared with adjacent tissues using the Oncomine database, SSBP1 expression in various tumors increased or decreased, (B)SSBP1 expression in pan-cancer analysis using TCGA dataset analyzed by TIMER (* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$), (C)Use UALCAN to analyze the expression of SSBP1 in the TCGA database($P < 0.01$), (D)Analyze the expression of SSBP1 protein in the TCGA database using UALCAN($P < 0.01$), and (E) Immunohistochemical staining of SSBP1 of LUAD in HPA database

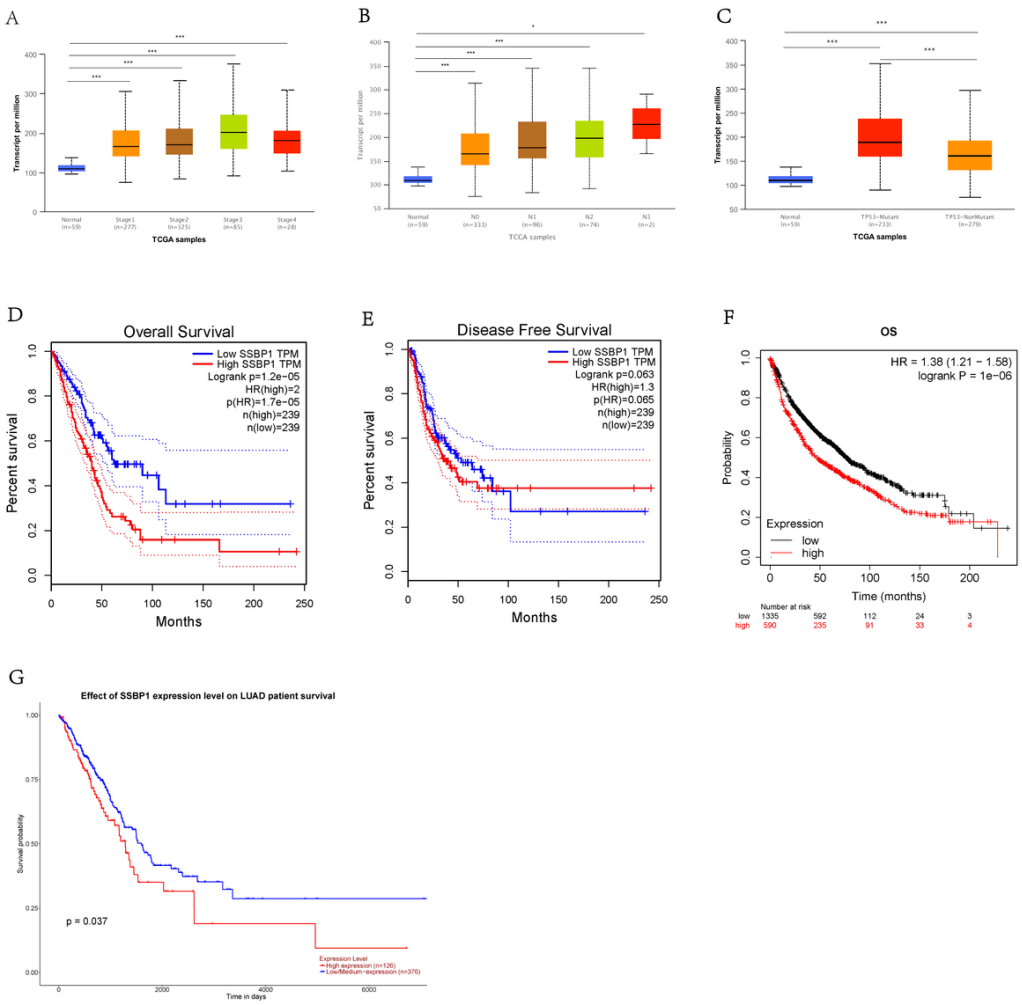

Figure 2

Clinical characteristics and poor prognosis of LUAD patients with high expression of SSBP1.(A) The expression of SSBP in different stages of patients(* P < 0.05，** P < 0.01，*** P < 0.001), (B)The expression of SSBP1 in patients with different lymph node metastases(* *P* < 0.05，** *P* < 0.01，*** *P* < 0.001), (C)The expression of SSBP1 in patients with different TP-53 mutations(* *P* < 0.05，** *P* < 0.01，*** *P* < 0.001); (D) and (E) Use GEPIA to draw KM survival curves of OS and DFS in LUAD patients; (F) and (G) The correlation between the expression of SSBP1 in the KM and UALCAN plotter databases and the overall survival of LUAD.

Figure 3

Correlation between *SSBP1* and tumor-infiltrating immune cells and OS prognosis.

(A) Correlation analysis between *SSBP1* and TMB using Sangerbox tool, (B) Association between *SSBP1* expression level and immune cell infiltration in LUAD from the TISIDB web portal (517 samples), and (C) the immune score from the Sangerbox tool. (D) Correlation between *SSBP1* and TME infiltrating immune cells analyzed using TIMER database, and (E) overall survival

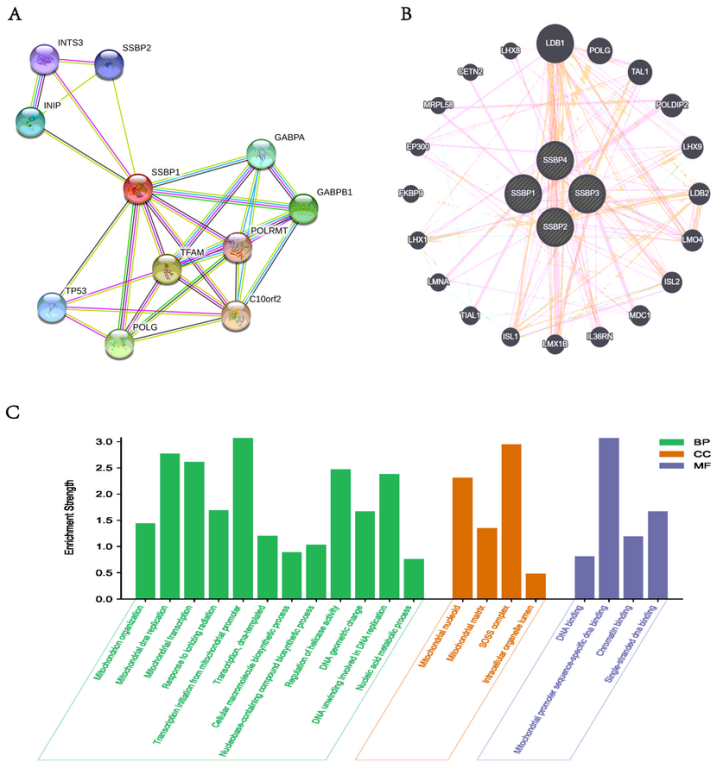

Figure 4

Enrichment analysis. (A) *SSBP1* protein interaction network diagram. (B) *SSBP1* and its family gene interaction network. (C) Analysis about BP, MF, CC.

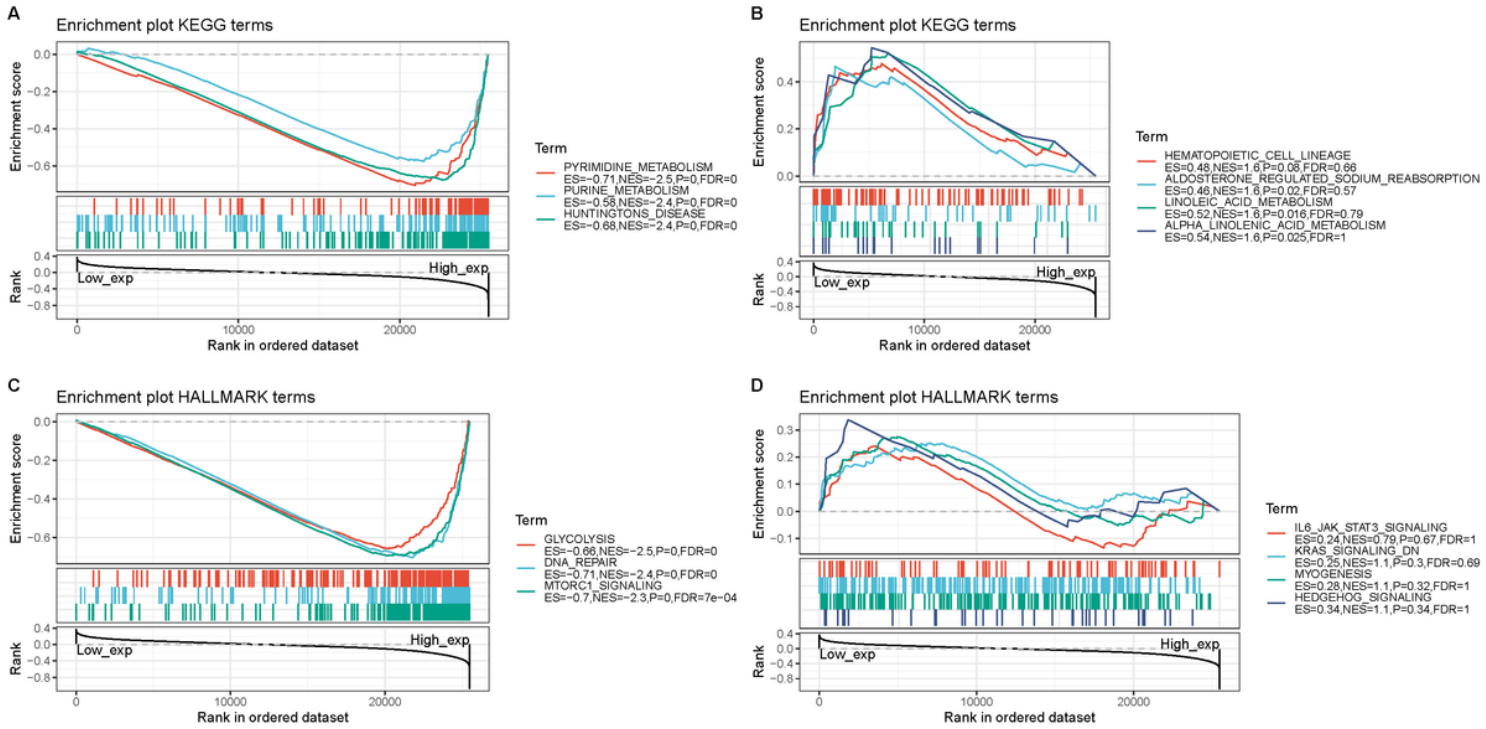

Figure 5

High expression of *SSBP1* significantly enriches proteasome and promotes tumor malignancy in LUAD. (A, B, C, D) High and low *SSBP1* expression groups were presented using the Sangerbox tool and GSEA for KEGG and HALLMARK pathways.

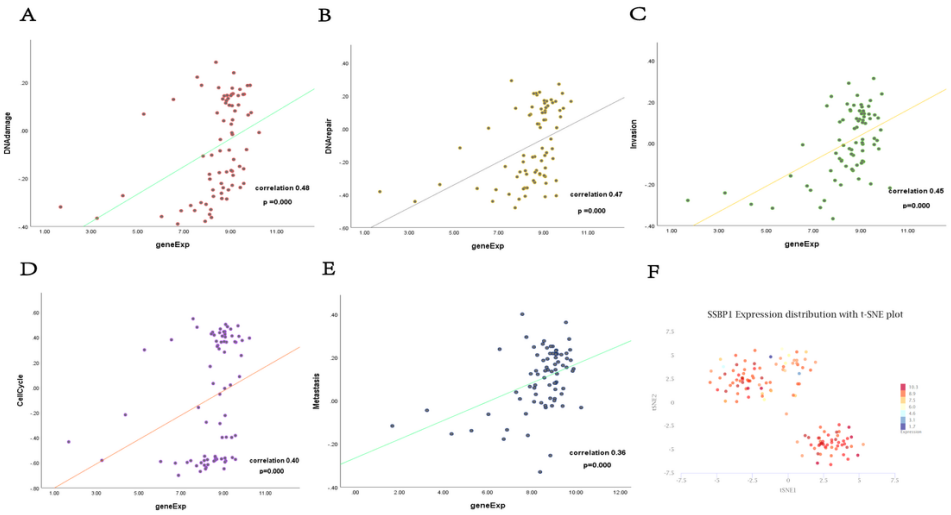

Figure 6

(A), (B), (C), (D) and (E) molecular function of *SSBP1* in LUAD using CancerSEA database shows that *SSBP1* is significantly positively correlated with DNA damage, DNA repair, tumor invasion, cell cycle, and metastasis in LUAD; (F) the t-SNE plot shows siRNA analysis of *SSBP1* expression.

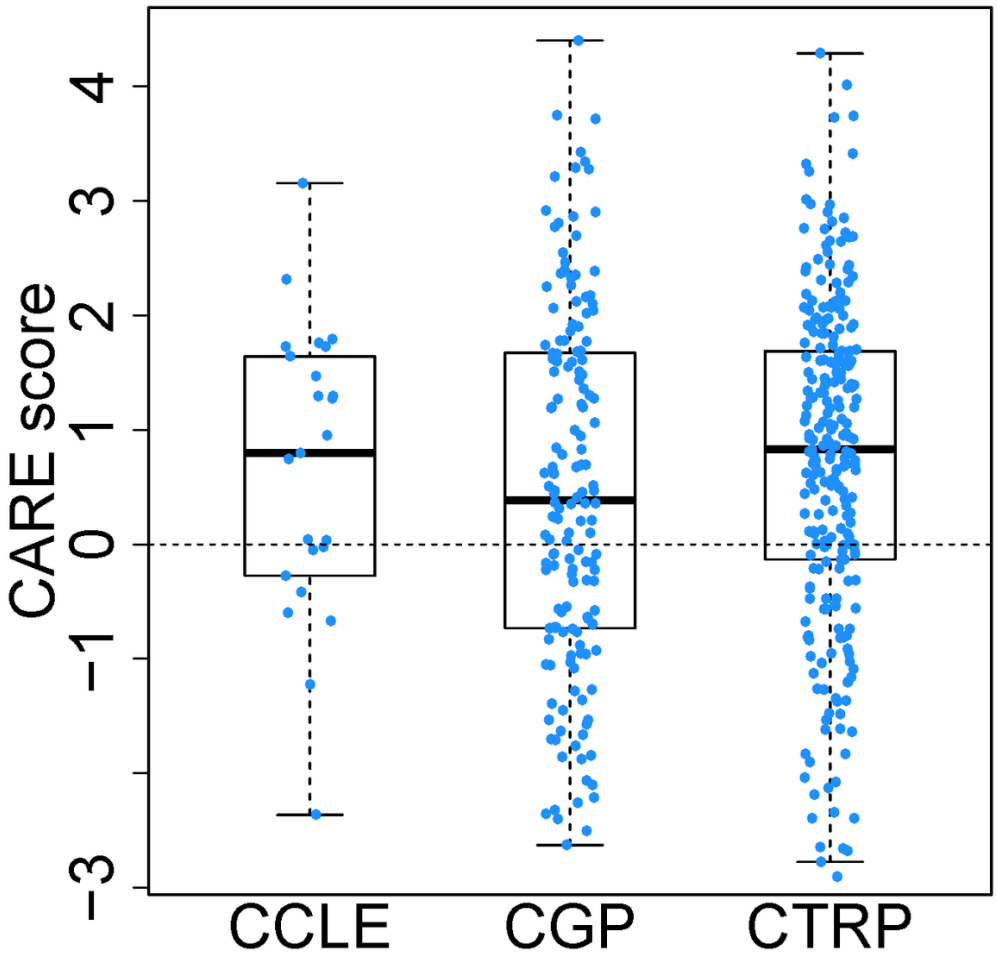

Figure 7

SSBP1 expression is related to drug response**.** SSBP1 expression is significantly positively correlated with CARE score among the many compounds retrieved from the Encyclopedia of Cancer Cell Lines (CCLE), cancer drug sensitivity genomics (GDSC, formerly named CGP), and cancer treatment response portal (CTRP) databases,

Supplementary Files

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

- SupplementalFigures.docx