Original Article

Overexpression of abnormal spindle-like microcephaly-associated (ASPM) increases tumor aggressiveness and predicts poor outcome in patients with lung adenocarcinoma

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Background: Cumulative evidence points to abnormal spindle-like microcephaly-associated (ASPM) protein being overexpressed in various cancers, and the aberrant expression of ASPM has been shown to promote cancer tumorigenicity and progression. However, its role and clinical significance in lung adenocarcinoma (LUAD) remains unclear. This study aimed to determine the expression patterns of ASPM and its clinical significance in LUAD.

Methods: In total, 4 original worldwide LUAD microarray mRNA expression datasets (N=1,116) with clinical and follow-up annotations were downloaded from The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) databases. The expression of ASPM protein in LUAD patients was detected by immunohistochemistry. Survival analysis and Cox regression analysis were used to examine the prognostic value of ASPM expression. Gene set enrichment analysis (GSEA) was performed to investigate the relationship between ASPM and LUAD.

Results: Dataset analyses and immunohistochemistry revealed that ASPM expression was significantly higher in the LUAD tissues compared with normal lung tissues, especially in the advanced tumor stage. Additionally, overexpression of ASPM was significantly correlated with shorter overall survival (OS) and relapse-free survival (RFS) in LUAD. Univariate and multivariate Cox regression analyses revealed that the overexpression of ASPM was a potential independent predictor of poor OS and RFS. However, ASPM overexpression was not significantly associated with predicting OS in lung squamous cell carcinoma. GSEA analysis demonstrated that ASPM was significantly enriched in the cell cycle, DNA replication, homologous recombination, RNA degradation, mismatch repair, and p53 signaling pathways.

Conclusions: These findings demonstrate the important role of ASPM in the tumorigenesis and progression of LUAD.

Keywords: Abnormal spindle-like microcephaly-associated (ASPM); lung adenocarcinoma; The Cancer Genome Atlas (TCGA); Gene Expression Omnibus (GEO); prognosis

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Introduction

Lung cancer is one of the most common cancers worldwide, with a high mortality rate. Lung adenocarcinoma (LUAD) is the most predominant subtype of lung cancer (1). Increasing our understanding of the biology and mechanisms of lung cancer has resulted in advancements in early detection and multimodal care. Furthermore, over the last 2 decades, there have been tremendous improvements in care for patients with advanced-stage lung cancer (1). However, the genesis and development of lung cancer involve the accumulation of multiple molecular events. Genetic alterations in lung cancer are associated with abnormal cell proliferation, inhibition of cell differentiation, and aggressiveness (2,3). Identifying the aberrant expression of certain genes in tumors is essential for the identification of prognostic biomarkers and the development of novel therapies (4).

Abnormal spindle-like microcephaly-associated (ASPM) protein is involved in normal mitotic spindle function. However, defects in ASPM are associated with autosomal recessive microcephaly, which is a neurodevelopmental disease (5-7). An evolutionary mechanism has been suggested whereby ASPM regulates cortical expansion by controlling the affinity of ventricular radial glial cells for the ventricular surface (8). Increasing evidence indicates that ASPM can serve as a regulator of cancer stem cells. A recent study by Tsai's group revealed that ASPM modulates cancer stem cells to give rise to stemness properties and tumorigenic potential through its co-regulation of the classic Wnt-β-catenin signaling pathway in pancreatic ductal adenocarcinoma (9). Similarly, ASPM has been found to enhance tumor stemness and aggressiveness in glioblastoma (10), LUAD (11), gastric cancer (12-14), and prostate cancer (15).

Previous studies have also found that mRNA and protein expression levels of ASPM were significantly upregulated in glioblastoma tissues and glioblastoma cell lines (10,16). Chen and colleagues (17) reported that the highest fold change in the differentially expressed genes was associated with ASPM, which were highly expressed in glioblastoma tissue. Furthermore, ASPM expression patterns from The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) databases have shown that glioblastoma patients with high expression of ASPM have a poor prognosis. Mechanistically, ASPM promotes glioblastoma growth by regulating G1 restriction point progression and Wnt-β-catenin signaling. ASPM knockdown is reported to enhance radiosensitivity in glioblastoma cells by influencing DNA double-strand break repair. Therefore, ASPM could be a potential target for combination therapy with radiation in glioblastoma (18). Several studies have revealed that increased expression of ASPM is significantly associated with poor outcomes in hepatocellular carcinoma (19), epithelial ovarian cancer (20), pancreatic tumor (21), prostate cancer (22), and bladder cancer (23). Therefore, ASPM could be a novel prognostic biomarker and therapeutic target. However, the role and clinical significance of ASPM in LUAD have not been established.

The present study explored the expression pattern of ASPM and its association with tumor progression and prognosis in LUAD patients using bioinformatics analysis. We present the following article in accordance with the MDAR checklist (available at http://dx.doi.org/10.21037/tcr-20-2570).

Methods

Worldwide microarray gene expression datasets

Original worldwide microarray mRNA expression datasets and corresponding clinical information of lung cancer patients were downloaded from the GEO (https:// www.ncbi.nlm.nih.gov/geo/) and TCGA (https://portal.gdc.cancer.gov/) databases, respectively. TCGA-LUAD (515 patients) and 3 LUAD-related GEO datasets including GSE31210 (204 LUAD patients) (24), GSE30219 (85 LUAD patients) (25), and GSE72094 (442 LUAD patients) (26) were subjected to gene expression analyses and survival analysis. Preprocessing and analysis of raw data were performed using R software version 3.6.3. Additionally, the expression of ASPM mRNA in the TCGA-LUAD and GSE31210 datasets were log2-transformed for further analysis. Patients with a survival time shorter than 3 months were excluded, and a total of 1,116 LUAD patients were enrolled for survival analysis. A total of 502 lung squamous cell carcinoma (LUSC) samples and 49 normal samples were downloaded from the TCGA-LUSC dataset for gene expression analysis. Out of the 504 LUSC patients with a follow-up survival time longer than 3 months, 431 LUSC patients were included in the survival analysis. Patient information derived from the TCGA and GEO datasets is summarized in *Table 1*.

Immunohistochemistry

All patients treated with chemotherapy or radiotherapy before surgery were excluded from this study. Paired

Table 1 Patients information from the TCGA and GEO datasets

Clinical characteristics	TCGA-LUAD	GSE31210	GSE30219	GSE72094	TCGA-LUSC
Tumor tissue samples	535	226	85	442	502
Normal tissue samples	59	20	14	0	49
Total patients	456	204	82	374	431
Age at diagnosis (y)	66 (33–88)	60 (30–76)	60 (44–86)	66 (38–89)	68 (39–85)
Gender					
Male	208	95	65	164	320
Female	248	109	17	210	111
Smoking					
Never	–	105	–	90	–
Ever	–	99	–	284	–
TNM stage					
I	243	162	–	242	208
II	108	42	–	59	139
III	74	–	–	54	74
IV	24	–	–	14	6
Unknow	7	–	–	5	4
T stage					
T1	151	–	68	–	99
T2	245	–	12	–	247
T3	40	–	2	–	66
T4	17	–	–	–	19
TX	3	–	–	–	–
N stage					
N0	293	–	79	–	275
N1	86	–	3	–	113
N2	64	–	–	–	35
N3	2	–	–	–	4
Unknown	11	–	–	–	4
M stage					
M0	300	–	–	–	356
M1	23	–	–	–	6
Unknown	133	–	–	–	69
Relapse					
Yes					
No	-	54	27	–	–
Survival status					
Dead	151	30	42	101	170
Alive	305	174	40	273	261

TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

normal lung tissues were obtained from 32 LUAD patients who underwent surgical resection between April 2018 and February 2019. All patients were randomly selected to participate in this study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013), and was approved by the research ethics committees of our hospital (Ek2020012). Written informed consent was obtained from all the participants. Cellular localization and expression levels of the ASPM protein in clinical LUAD tissues and paired normal lung tissues were analyzed using immunohistochemistry (IHC) as previously described (27,28). The composite expression score (CES) was calculated from immunostaining patterns, yielding a range of 0–12. The primary rabbit anti-ASPM antibody ab238106 (1:100; Abcam Corp., Cambridge, UK) and goat anti-rabbit IgG H&L ab205718 (1:20,000; Abcam Corp., Cambridge, UK) were used for immunohistochemical analysis. The 2016 World Health Organization classification guidelines were used to perform histopathological diagnosis. The detailed clinical parameters of hospital LUAD patients are presented in Table S1.

Oncomine database analysis

The Oncomine database (https://www.oncome.org/) is a unique oncogene chip database and integrated data-mining platform offering access to published transcriptome data for various types of cancers (29). In this study, the Oncomine database was used to examine the differences in ASPM mRNA expression between tumor tissues and normal tissues in LUAD. The set parameters when filtering the data were as follows: (I) Gene: ASPM; (II) analysis type: cancer *vs.* normal analysis; (III) data type: mRNA; (IV) cancer type: lung adenocarcinoma; (V) threshold by P value <1e-4, fold-change >2, gene rank=top 10%.

Tumor immune estimation resource (TIMER) database analysis

The Tumor Immune Estimation Resource (TIMER; https://cistrome.shinyapps.io/timer/) web application (version 2.0) is an interactive resource for comprehensive analysis of immune infiltrates across different kinds of cancer types from TCGA dataset. Analysis of the differential expression of the ASPM gene between tumor and normal tissues in 32 different kinds of cancer types was performed in the Diff Exp module (30). The survival module was used to explore the associations between clinical outcome and ASPM gene expression.

Gene set enrichment analysis (GSEA)

The median ASPM mRNA expression level was used as the cutoff point for each of the datasets. The 535 LUAD samples from TCGA database were divided into a high-risk ASPM group and a low-risk ASPM group. Gene set enrichment analysis (GSEA) version 3.0 (http://software.broadinstitute.org/gsea/) was used to assess the ASPM gene-associated biological pathways (31). Annotated gene datasets c2.cp.kegg.v7.0.symbols.gmt were selected as the reference gene sets. The number of permutations was set to 1,000. Resulting pathways were selected using gene size ≥20 and a false discovery rate (FDR) q value <0.05, and ranked using normalized enrichment score (NES).

Statistical analysis

SPSS version 23.0 software (IMB, Armonk, NY, USA) and GraphPad Prism 8.02 were used to perform statistical analysis. The Shapiro-Wilk test was used to determine the normality of ASPM mRNA expression data. The Mann-Whitney U test was used for the determination of the expression levels of ASPM between tumor tissues and normal lung tissues, and the Kruskal-Wallis test was used to compare the mean expression levels of ASPM between multiple groups. Continuous variables were grouped based on their median values. The Kaplan-Meier curve method and Cox proportional hazards regression were used to analyze overall survival (OS) and relapse-free survival (RFS). Univariate and multivariate survival analyses were used to perform further analysis. A P value <0.05 was considered to be statistically significant.

Results

ASPM expression was upregulated in LUAD tissues

By exploring the expression data from the TCGA-LUAD, GSE31210, and GSE30219 datasets, we found that ASPM mRNA expression was significantly higher in LUAD tissues compared to normal lung tissues (*Figure 1A*,*B*,*C*,*D*). ASPM transcription levels, which were validated using the Oncomine database (*Figure 1E*,*F*,*G*), were found to be significantly upregulated in LUAD tissues. Furthermore, results showed that ASPM mRNA expression was significantly upregulated in LUSC tissues compared with normal lung tissues based on the TCGA-LUSC dataset (Figure S1A,B). The expression of ASPM at the protein level was examined in the 32 LUAD patient samples. ASPM

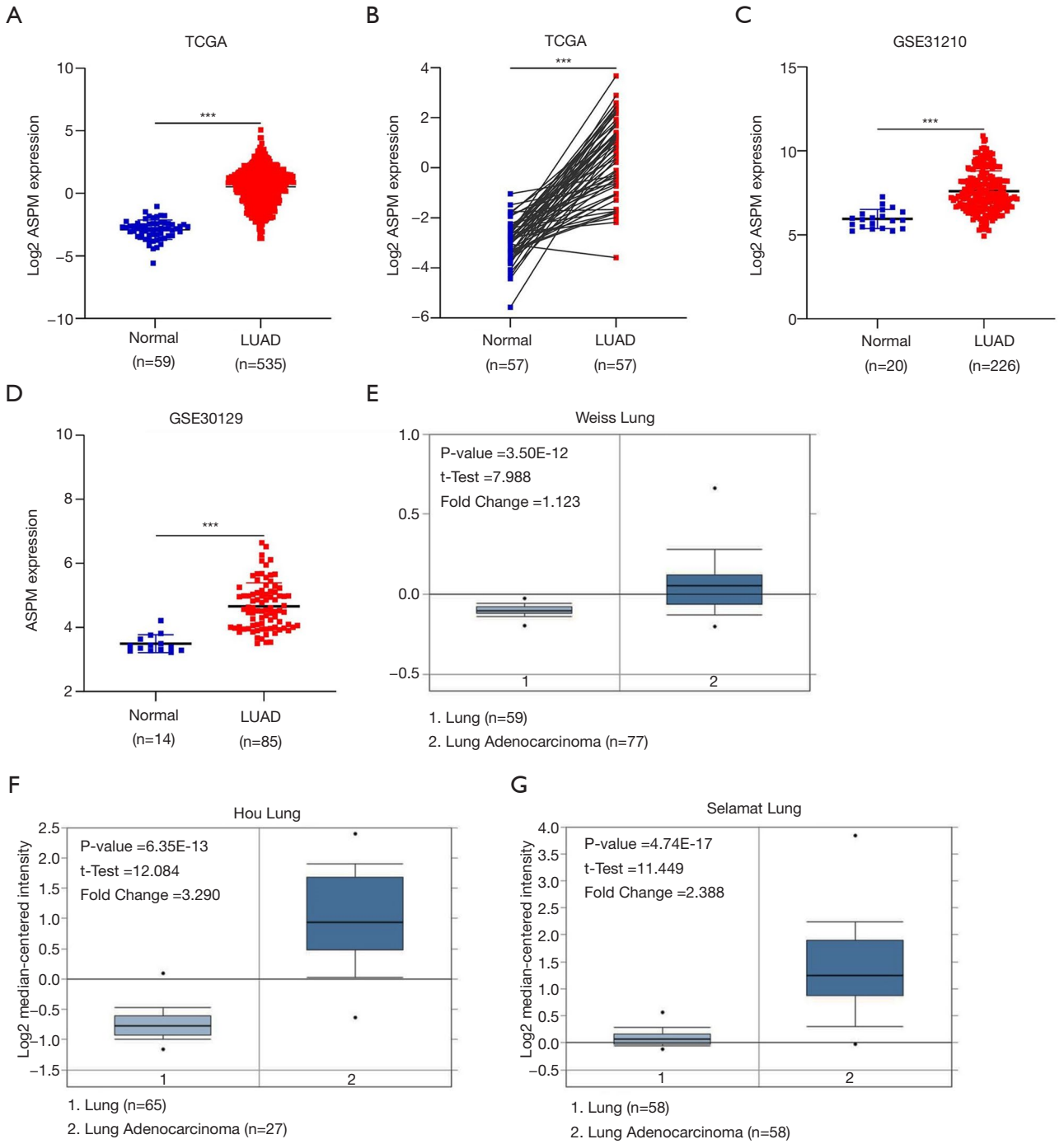

Figure 1 ASPM expression levels in LUAD based on the TCGA-LUAD and GEO datasets. (A) Expression of ASPM mRNA in LUAD and normal lung tissues in the TCGA-LUAD dataset. (B) ASPM mRNA expression in 57 LUAD tissues and paired normal lung tissues in the TCGA-LUAD dataset. (C,D) The expression difference of ASPM mRNA between LUAD and normal lung tissues in the GSE31210 and GSE30219 datasets. (E,F,G) Box plot showing ASPM mRNA levels in the Weiss Lung, Hou Lung, and Selamat Lung datasets (Oncomine). LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus. ***P<0.001.

was predominantly found in the nucleus of LUAD cells and normal lung tissue cells (*Figure 2A*). Compared with the normal lung tissues, the expression level of ASPM protein was significantly higher in LUAD tissues (*Figure 2B*; CES: normal =3.250±2.423 *vs.* LUAD =6.156±2.996, t=7.506, P<0.0001).

ASPM expression levels in different types of tumors

The Oncomine and TIMER databases were queried for ASPM expression in different types of human cancers. In the Oncomine database, ASPM expression was upregulated in most human cancers including bladder cancer, brain and central nervous system (CNS) cancer, breast cancer, cervical cancer, colorectal cancer, gastric cancer, and lung cancer, amongst others (*Figure 3A*). However, ASPM expression was found to be downregulated in breast cancer and leukemia. Furthermore, in the TIMER database, expression of ASPM was found to be significantly upregulated in almost all TCGA cancer types (*Figure 3B*). These results indicated that ASPM is overexpressed in most human cancers.

Upregulation of ASPM was an aggressive factor in LUAD

The expression of ASPM was associated with advanced TNM stage (P=0.036) (*Figure 4A*) after exploring the expression data across all patient characteristics from the TCGA-LUAD dataset. The results also showed that ASPM overexpression was significantly associated with advanced TNM stage in LUAD patients (*Figure 4B*; P<0.001) and early recurrence (*Figure 4C*; P<0.001) in the GSE31210 dataset. Similarly, further analyses of the GSE30219 dataset revealed that increased ASPM expression was significantly associated with advanced T stage (*Figure 4D*; P<0.001), N stage (*Figure 4E*; P=0.044), and early recurrence (*Figure 4F*; P<0.0001) in LUAD patients. These results strongly suggest that upregulated ASPM expression was significantly correlated with advanced pathological stage and early recurrence of LUAD. However, these associations were not found in LUSC patients from the TCGA-LUSC dataset (Figure S1C).

Upregulated expression of ASPM predicted poor prognosis in LUAD

Patients were separated into low- and high-expression groups based on the median mRNA expression values of ASPM. LUAD patients with high ASPM expression had significantly poorer prognosis in the TCGA-LUAD dataset (*Figure 5A*; n=456, P<0.001). Statistical analysis among stage I–II patients showed a significant difference in OS between high- and low-expression groups in the TCGA-LUAD dataset (*Figure 5B*; n=351, P=0.002). The results were validated in 3 GEO datasets. The results indicated that the OS (*Figure 5C,D*; n=204, P<0.01) and RFS (*Figure 5E,F*; n=203, P<0.01) were significantly lower in the ASPM mRNA high expression group in the GEO31210 dataset. A similar analysis in GSE30219 with 82 primary LUAD cases showed that high expression levels of ASPM were significantly correlated with a poor OS (*Figure 5G*; P=0.007) and RFS (*Figure 5H*, P=0.002). Furthermore, high expression of ASPM mRNA was associated with a significantly shorter OS for stage I–IV patients in GSE72094 (*Figure 5I*; n=374, P=0.001). Stratification analysis indicated a significant difference in OS between high and low ASPM expression groups in early-stage patients from the GSE72094 dataset (*Figure 5J,K*; all P<0.01). However, no significant association was found between ASPM expression and OS in LUSC patients from the TCGA-LUSC dataset (Figure S2).

The relationship between ASPM expression and prognosis in different cancers based on TIMER is shown in Figure S3. In this study, increased expression of ASPM was associated with poor OS in various cancers, including adrenocortical carcinoma, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, lower-grade glioma, liver hepatocellular carcinoma, mesothelioma, pancreatic adenocarcinoma, pheochromocytoma and paraganglioma, and uterine corpus endometrial carcinoma (Figure S3A,B,C,D,E,F,G,H,I). In contrast, high expression of ASPM was associated with better OS in head and neck squamous cell carcinoma (HPV positive) and thymoma (Figure S3J,K).

ASPM expression was an independent prognostic predictor for poor OS and RFS in LUAD

Univariate and multivariate Cox regression analyses were used to evaluate the clinical prognostic value of ASPM. Univariate analysis revealed that the expression levels of ASPM mRNA (P<0.01 in all datasets), TNM stage (P<0.01 in the GSE72094 and GSE31210 datasets), T stage (P<0.01 in the TCGA-LUAD dataset), and N stage (P<0.01 in TCGA-LUAD dataset) were significantly associated with OS in LUAD (*Tables 2,3*). Multivariate analysis showed a high level of ASPM mRNA expression (P<0.05 in all

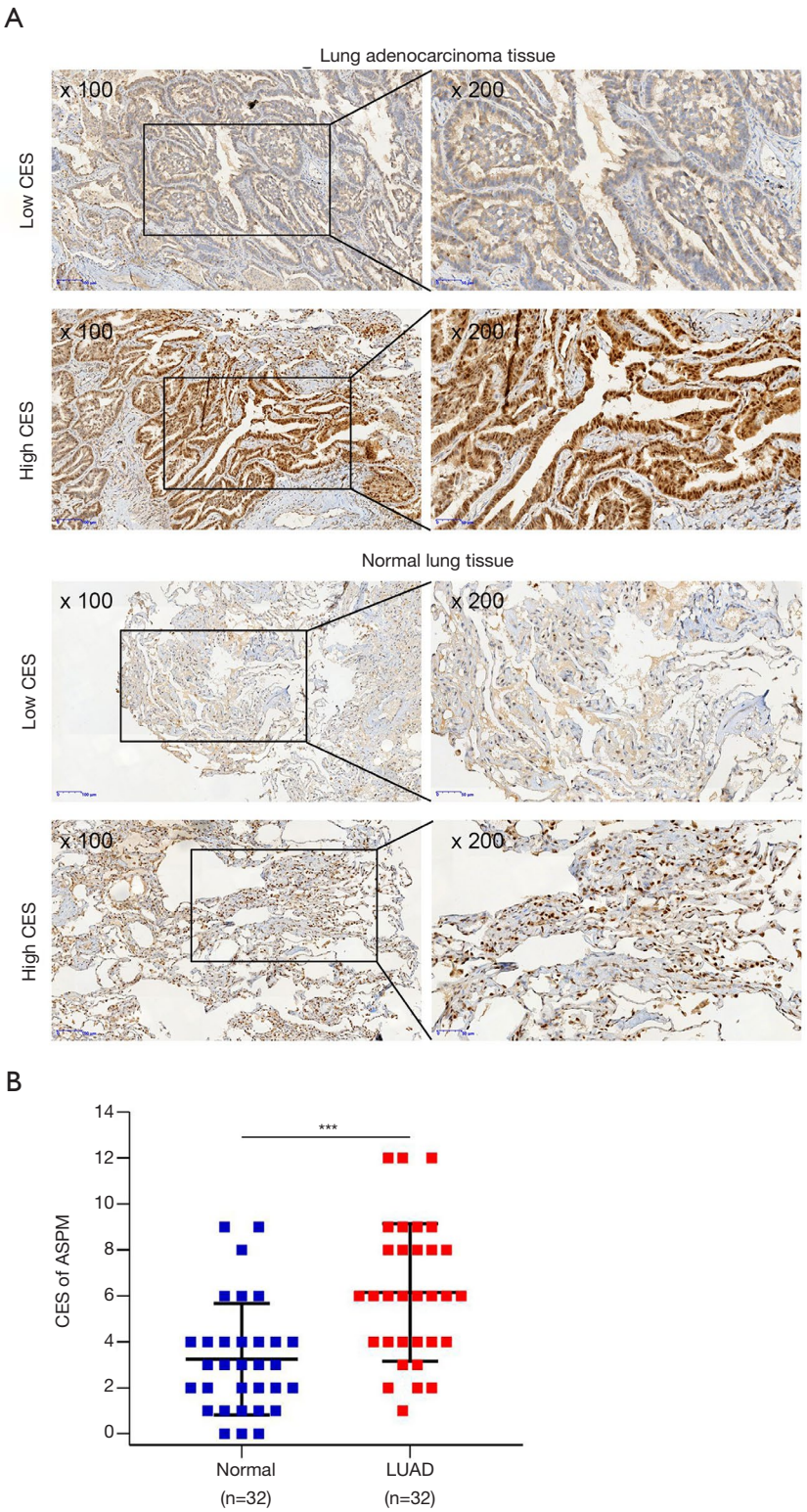

Figure 2 Immunohistochemistry analysis of LUAD tissues from 32 hospital patients. (A) Representative images of IHC staining for ASPM protein in LUAD and paired normal lung tissues (magnification, ×200, ×100). (B) CES of ASPM in LUAD and paired normal lung tissues. LUAD, lung adenocarcinoma; IHC, immunohistochemistry; CES, composite expression score. ***P<0.001.

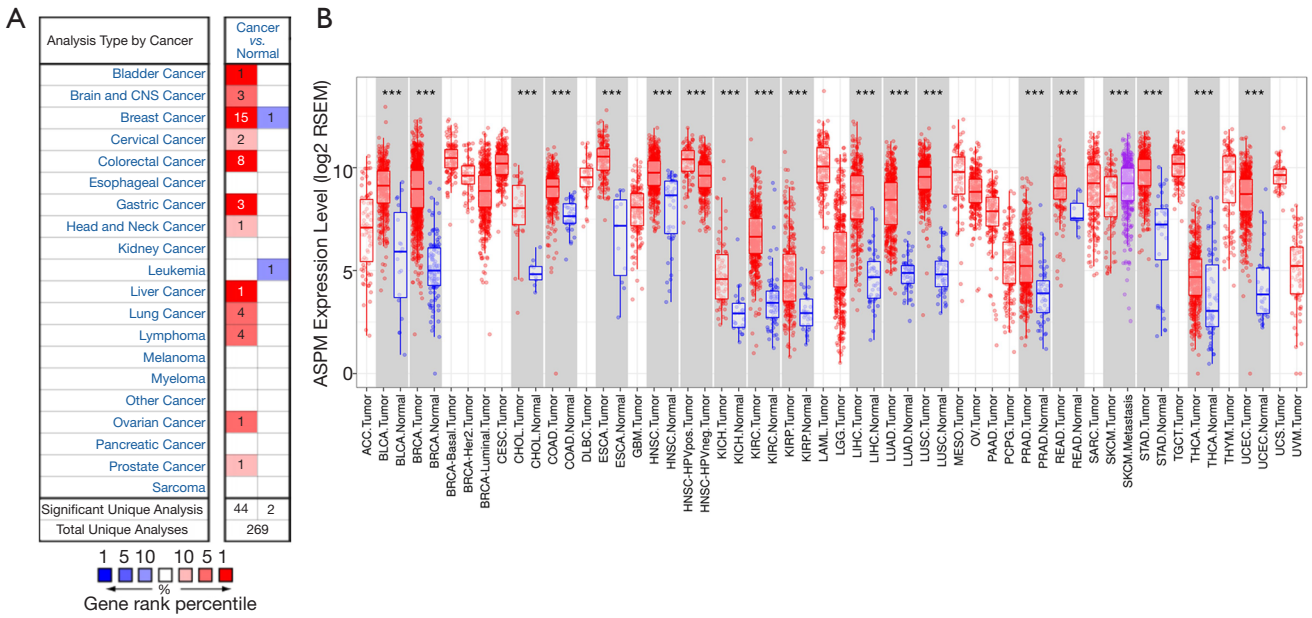

Figure 3 ASPM expression levels in different types of malignancies. (A) Upregulated or downregulated ASPM expression in different tumors compared with normal tissues in the Oncomine database. (B) ASPM expression levels in 32 kinds of cancers from TCGA database evaluated using TIMER. ***P<0.001. TCGA, The Cancer Genome Atlas; TIMER, Tumor Immune Estimation Resource.

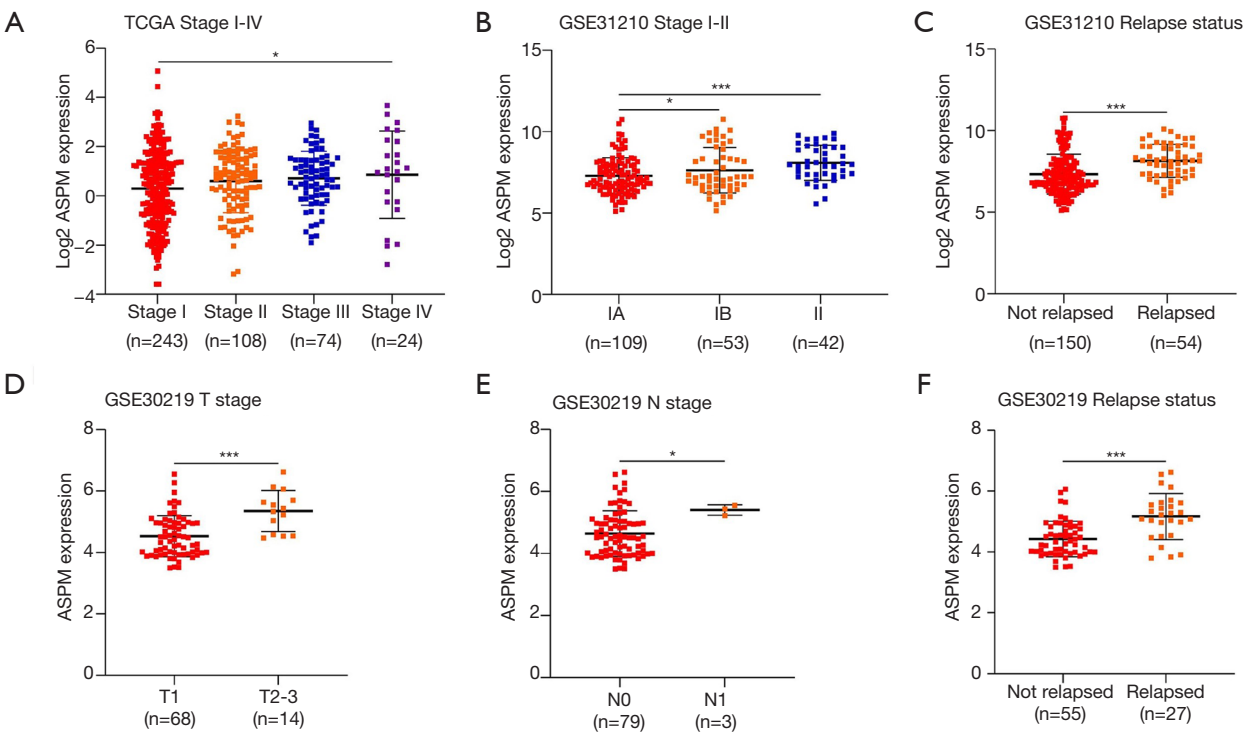

Figure 4 Correlation between ASPM expression and clinicopathological characteristics in LUAD patients. (A) The association between ASPM mRNA level with different TNM stages based on the TCGA-LUAD dataset. (B,C) The association between ASPM mRNA level with different TNM stages and relapse status based on the GSE31210 dataset. (D,E,F) The association between ASPM mRNA level with different T stages, N stages, and relapse status based on the GSE30219 dataset. LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas. *P<0.05; ***P<0.001.

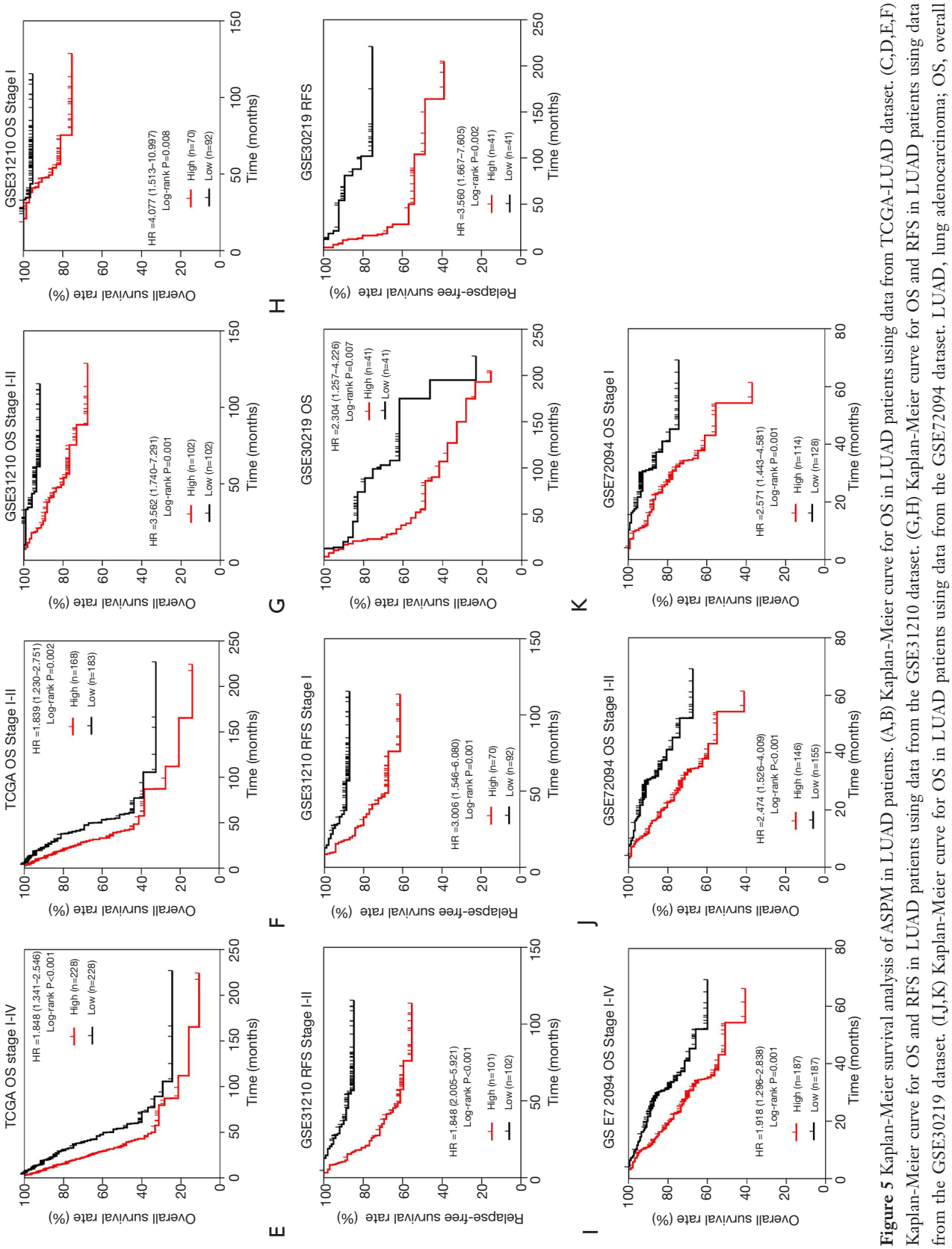

Figure 5 Kaplan-Meier survival analysis of ASPM in LUAD patients. (A,B) Kaplan-Meier curve for OS in LUAD patients using data from TCGA-LUAD dataset. (C,D,E,F) Kaplan-Meier curve for OS and RFS in LUAD patients using data from the GSE31210 dataset. (G,H) Kaplan-Meier curve for OS and RFS in LUAD patients using data from the GSE30219 dataset. (I,J,K) Kaplan-Meier curve for OS in LUAD patients using data from the GSE72094 dataset. LUAD, lung adenocarcinoma; OS, overall survival; TCGA, The Cancer Genome Atlas; RFS, relapse-free survival.

Table 2 Univariate and multivariate analysis of OS in LUAD patients in the TCGA-LUAD dataset

Clinical characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age (>66/≤66)	1.106 (0.760–1.609)	0.599	1.318 (0.890–1.952)	0.168
Gender (famale/male)	1.038 (0.716–1.506)	0.844	1.187 (0.812–1.735)	0.377
TNM stage (IV-III/II-I)	2.918 (1.989–4.283)	<0.001	1.664 (0.965–2.871)	0.067
T stage (4–3/2–1)	2.386 (1.485–3.835)	<0.001	1.497 (0.870–2.575)	0.145
N stage (1–3/0)	2.713 (1.864–3.948)	<0.001	2.128 (1.352–3.350)	0.001
M stage (1/0)	1.651 (0.884–3.085)	0.116	1.011 (0.484–2.112)	0.976
ASPM (high/low)	1.473 (1.196–1.813)	<0.001	1.528 (1.205–1.937)	<0.001

OS, overall survival; LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; CI, confidence interval; HR, hazard ratio.

Table 3 Univariate and multivariate analysis of OS in LUAD patients in the GEO datasets

Clinical characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
GSE72094				
Age (>66/≤66)	1.116 (0.750–1.659)	0.589	1.081 (0.724–1.615)	0.703
Gender (famale/male)	0.632 (0.425–0.938)	0.023	0.513 (0.342–0.770)	0.001
Smoking (ever/never)	0.798 (0.503–1.265)	0.338	0.754 (0.471–1.208)	0.240
TNM stage (IV-III/II-I)	1.663 (1.379–2.005)	<0.001	1.704 (1.406–2.066)	<0.001
ASPM (high/low)	1.274 (1.068–1.520)	0.007	1.270 (1.063–1.519)	0.009
GSE31210				
Age (>60/≤60)	1.271 (0.617–2.619)	0.515	1.580 (0.757–3.300)	0.223
Gender (famale/male)	0.593 (0.288–1.223)	0.157	0.868 (0.309–2.436)	0.788
Smoking (ever/never)	1.908 (0.918–3.966)	0.084	1.143 (0.389–3.360)	0.808
TNM stage (II/I)	4.297 (2.092–8.828)	< 0.001	3.478 (1.655–7.310)	0.001
ASPM (high/low)	1.591 (1.209–2.095)	0.001	1.489 (1.082–2.048)	0.014
GSE30219				
Age (>60/≤60)	1.272 (0.689–2.351)	0.442	1.660 (0.841–3.279)	0.144
Gender (female/male)	0.787 (0.349–1.774)	0.563	1.100 (0.476–2.540)	0.823
T stage (2-3/1)	1.927 (0.977–3.800)	0.058	1.023 (0.427–2.454)	0.959
N stage (1/0)	1.277 (0.306–5.335)	0.738	0.643 (0.139–2.984)	0.573
ASPM (high/low)	1.831 (1.233–2.720)	0.003	2.064 (1.230–3.463)	0.006

OS, overall survival; LUAD, lung adenocarcinoma; GEO, Gene Expression Omnibus; CI, confidence interval; HR, hazard ratio.

datasets), TNM stage (P<0.05 in the GSE72094 and GSE31210 datasets), and N stage (P<0.01 in TCGA-LUAD dataset) were potential independent predictors of OS in LUAD (*Tables 2,3*). The results of the univariate and multivariate Cox regression analyses revealed that the expression level of ASPM was an independent prognostic

Table 4 Univariate and multivariate analysis of RFS in LUAD patients in the GEO datasets

Clinical characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
GSE31210				
Age (>60/≤60)	1.650 (0.947–2.876)	0.077	2.255 (1.275–3.990)	0.005
Gender (famale/male)	0.687 (0.400–1.178)	0.172	0.745 (0.351–1.581)	0.443
Smoking (Ever/Never)	1.384 (0.807–2.375)	0.238	0.729 (0.332–1.601)	0.431
TNM stage (II/I)	3.297 (1.876–5.793)	<0.001	2.998 (1.671–5.378)	<0.001
ASPM (High/Low)	1.553 (1.271–1.897)	<0.001	1.595 (1.268–2.006)	<0.001
GSE30219				
Age (>60/≤60)	1.242 (0.581–2.656)	0.576	1.781 (0.737–4.306)	0.200
Gender (famale/male)	0.831 (0.314–2.194)	0.707	1.267 (0.465–3.452)	0.644
T stage (2–3/1)	3.293 (1.499–7.221)	0.003	1.020 (0.354–2.937)	0.971
N stage (1/0)	3.795 (1.137–9.672)	0.030	1.420 (0.359–5.612)	0.617
ASPM (high/low)	3.152 (1.905–5.216)	<0.001	3.513 (1.834–6.726)	<0.001

RFS, relapse-free survival; LUAD, lung adenocarcinoma; GEO, Gene Expression Omnibus; CI, confidence interval; HR, hazard ratio.

factor of RFS in LUAD patients after adjustment for these clinical variables (*Table 4*). However, no statistically significant correlation was observed between ASPM and OS in LUSC based on the TCGA-LUSC dataset (Table S2; P=0.161 and 0.210 respectively).

ASPM-associated signaling pathways in LUAD

KEGG pathway analysis by GSEA was performed by using the TCGA-LUAD dataset to better understand the biological role of ASPM in LUAD (*Figure 6*). The results indicated that high ASPM expression was enriched in the cell cycle (q<0.001), DNA replication (q=0.004), homologous recombination (q=0.005), RNA degradation (q=0.006), mismatch repair (q=0.008), and the p53 signaling pathway (q=0.025). Furthermore, there was a significant negative correlation between the expression of ASPM and TP53 (Figure S4A; P<0.05) based on the TCGA-LUAD dataset. Assessment of the relationship between ASPM expression and the TP53 mutation also revealed a significantly higher ASPM expression level in the TP53 mutation group (Figure S4B; P<0.0001).

Discussion

Integrated bioinformatics analysis can be used to identify reliable target genes. Over the years, the application of microarray technology and bioinformatics analysis has provided LUAD microarray data that is currently available in public databases including TCGA and GEO. Using these databases, several studies have recently identified potential biomarkers including ANLN (32), integrin α6 (33), KRT8 (34), and DDX11 (35). These biomarkers have been shown to play potential roles in the diagnosis, staging, and treatment of LUAD.

Recent evidence suggests that the aberrant expression of ASPM promotes cancer tumorigenesis and progression (9,15,17). However, the role and clinical significance of ASPM in LUAD remain unclear. Therefore, this study aimed to determine the expression pattern of ASPM and its clinical significance in LUAD. TCGA-LUAD and 3 original LUAD-related GEO datasets with a large sample size consisting of a total of 1,116 LUAD patients, including 846 LUAD samples and 93 normal samples, were used. The expression levels of ASPM mRNA were found to be upregulated in LUAD tissue. ASPM protein expression was also found to be upregulated in LUAD tissues based on IHC results of the clinical samples. ASPM protein was found to be predominantly located in the nucleus of LUAD cells, and these findings were consistent with those reported by Wang *et al.* (2020) (36). In contrast, immunostaining results showed that ASPM protein was mainly localized in the cytoplasm in gastric (12) and prostate cancer (22) cells. While exploring differential intracellular distributions

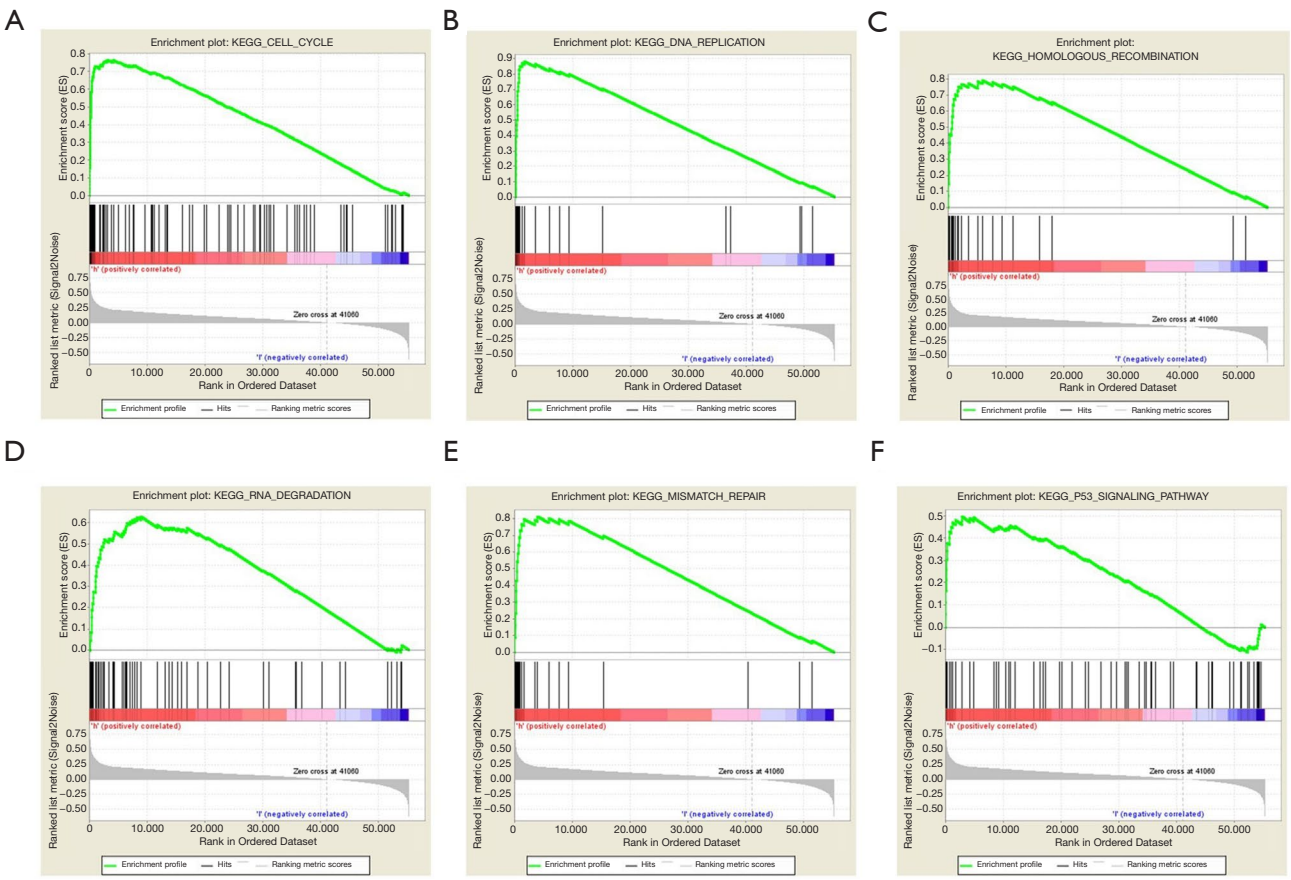

Figure 6 Gene set enrichment analysis plots. (A) Cell cycle, (B) DNA replication, (C) homologous recombination, (D) RNA degradation, (E) mismatch repair, (F) p53 signaling pathway.

and the functions of 2 major ASPM isoforms, Hsu *et al.* observed that ASPM isoform I was exclusively expressed in the cytoplasm of pancreatic ductal adenocarcinoma cells, while ASPM isoform II was mainly localized in the nucleus. Brüning-Richardson *et al.* (20) also revealed that interphase ASPM was found both in the cytoplasm and nucleus in ovarian cancer cells. Therefore, these differences can be explained by the different ASPM isoforms, cell cycle phases, and types of cancer cells.

The findings in this study demonstrated that overexpression of ASPM was significantly associated with aggressiveness and tumor progression, early recurrence, and poor outcomes in LUAD. We further found that upregulated expression of ASPM predicted poor prognosis in patients with early-stage LUAD (*Figure 5B,C,D,E,F,J,K*). More importantly, overexpression of ASPM was identified as an independent prognostic factor of OS and RFS, especially in patients with early-stage LUAD. These results suggest that ASPM can be used as a biomarker for the early diagnosis of LUAD in combination with the current markers in LUAD patients. These findings also indicate the potential role of ASPM in LUAD cancer progression. Using TIMER, we further confirmed that the expression of ASPM is elevated and associated with poor outcomes in multiple cancers, including adrenocortical carcinoma, kidney renal clear cell carcinoma, and kidney renal papillary cell carcinoma, amongst others. However, ASPM overexpression in LUSC had no significant value in predicting OS based on the TCGA-LUSC dataset. A possible cause of this phenomenon may be that the genetic drivers and tumor control networks at work in LUAD versus LUSC are vastly different (37).

Consistent with these findings, previous studies have demonstrated that ASPM is significantly overexpressed in various types of cancers, including glioblastoma (17), prostate cancer (22), and bladder cancer (23). Therefore, overexpression of ASPM has been considered as a potential prognostic biomarker in many cancers. In a recent study

of 90 Chinese patients with LUAD, ASPM expression was significantly upregulated in LUAD tissues, which was associated with poor OS (36). However, these study findings were limited by the small sample size, and thus the results concerning the association between ASPM and LUAD cannot be considered conclusive. Xu *et al.* (23) demonstrated that ASPM mRNA was overexpressed in bladder cancer compared with paired normal bladder mucosae. Furthermore, ASPM expression was positively associated with grade and TNM stage and short OS and RFS based on 6 bladder cancer-related datasets (n=1,355) from GEO and TCGA. Another study reported that ASPM is a promising biomarker for vascular invasion, early tumor recurrence, and poor OS in hepatocellular carcinoma regardless of p53 mutation status and tumor stage (19). ASPM is also one of the marker genes in several independently established prognostic gene signatures of pancreatic ductal adenocarcinoma (38,39), LUAD (40), and breast cancer (41). Furthermore, integrative network analysis based on large-scale cancer genomics data from TCGA database demonstrated that ASPM is a potential target and anticancer drug repositioning candidate for precision cancer medicine in the treatment of breast and lung cancers (42).

ASPM was found to be enriched in biological pathways associated with LUAD. The 6 pathways associated with oncogenesis included the cell cycle, DNA replication, homologous recombination, RNA degradation, mismatch repair, and p53 signaling pathways. Therefore, ASPM may play a key role in oncogenesis in LUAD. The cell cycle pathway was found to be the most critical pathway based on the low *q* value. Hsu *et al.* (17) reported that ASPM promotes glioblastoma growth by regulating cell cycle progression. They also revealed that the downregulation of ASPM could arrest the cell cycle of GBM cells at the G0/G1 phase and cause a reduction in the expression of cyclin E and β-catenin. Similarly, Hsu *et al.* reported that ASPM isoform II mainly regulates cell cycle progression, especially the G1/S transition by tuning the stability of cyclin E (9). We also observed a negative correlation between the TP53 gene and expression of ASPM in TCGA-LUAD patients, suggesting a potential interaction between ASPM and TP53. Tumor suppressor gene TP53, which is mutated in most cancer cells, plays a central role in the cell cycle of cancer cells (43). Taken together, these findings suggest that ASPM may induce tumorigenesis and progression mainly through the p53 signaling pathway. However, the mechanism of ASPM in promoting LUAD remains unclear. Therefore, further in-depth molecular studies are needed to investigate the mechanism for genetic alterations of ASPM in enhancing LUAD tumorigenesis.

Furthermore, to evaluate the potential effect of ASPM on drug responses, we analyzed the correlation between sensitivity to 84 anticancer drugs and the expression levels of ASPM using the CellMiner database (https://discover.nci.nih.gov/cellminer/home.do) (44). We discovered that sensitivity to paclitaxel, lapatinib, and salinomycin were significantly associated with ASPM, suggesting that ASPM may affect anticancer drug sensitivity in cancer cell lines (Figure S5).

There were two major limitations in this study. First, the roles of different ASPM isoforms in LUAD were not investigated due to a lack of specific antibodies for the different ASPM isoforms. Secondly, chemotherapy and radiotherapy information were not included in the datasets to explore the therapeutic significance of ASPM in LUAD.

In conclusion, integrated bioinformatics analysis of the TCGA-LUAD dataset and 3 GEO datasets confirmed mRNA and protein expression levels of ASPM to be significantly upregulated in LUAD. Overexpression of ASPM was associated with advanced TNM stage and predicted poor outcome in LUAD patients, but not in LUSC patients. Therefore, this study demonstrates that ASPM overexpression exerts significant effects on the tumorigenesis and progression of LUAD. These results, which are in agreement with those of other studies, indicate that ASPM may serve as a promising potential prognostic biomarker and therapeutic target for LUAD, and hence, provides positive prospects for future clinical transformation. However, the molecular mechanisms associated with ASPM in LUAD should be investigated, as well as the role of different ASPM isoforms in the carcinogenesis of LUAD.

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Footnote

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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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013), and was approved by the research ethics committees of the hospital (Ek2020012). Written informed consent was obtained from all the participants.

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