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Comprehensive evaluation of *SPATS2* expression and its prognostic potential in liver cancer

Jin Xing, PhD[a], Yijun Tian, PhD[b], Wu Ji, PhD[a,*], Xinying Wang, PhD[a,*]

Abstract

Spermatogenesis associated serine rich 2 (SPATS2) has been reported to be dysregulated in few types of cancer; however, no reports have investigated SPATS2 in liver cancer. The aim of the present study was to investigate SPATS2 expression in liver cancer and to analyze its association with the prognosis of liver cancer patients.

We examined the differential expression of *SPATS2* in liver cancer by exploring The Cancer Genome Atlas (TCGA) database. The diagnostic efficiency of *SPATS2* was obtained by Receiver Operating Characteristic (ROC) curve. The Chi-Squared test was used to assess clinical relevance. Survival analysis and Cox regression model were used to detect the effect of *SPATS2* on the survival of liver cancer patients. Gene Set Enrichment Analysis (GSEA) was used to identify signaling pathways related to *SPATS2* expression.

SPATS2 is highly expressed in liver cancer ($P < 2.2e\text{-}16$) and has the high diagnostic ability (AUC = 0.964). Survival analysis showed that patients with high *SPATS2* expression have an apparently shorter overall survival (OS, $P < .0001$) and relapse-free survival (RFS, $P < .0001$). Cox regression analysis showed that high *SPATS2* expression might be an independent risk factor for liver cancer (OS, HR = 2.41, $P = .000$; RFS, HR = 1.90, $P < .001$). GSEA analysis identified 3 signaling pathways (Mitotic spindle, G2M checkpoint, E2F targets) that were enriched in the presence of high *SPATS2* expression.

SPATS2 expression could be a novel diagnostic and prognostic biomarker in liver cancer.

Abbreviations: AUC = area under curve, FDR = false discovery rate, GSEA = Gene Set Enrichment Analysis, NES = normalized enrichment score, OS = overall survival, RFS = relapse-free survival, ROC = Receiver Operating Characteristic, SPATS2 = Spermatogenesis associated serine rich 2, TCGA = The Cancer Genome Atlas.

Keywords: diagnosis, liver cancer, prognosis, *SPATS2*

1. Introduction

Liver cancer is the sixth most common malignant tumor in the world.[1] Epidemiological data show that there are more than 780,000 new cases of liver cancer worldwide every year, which makes liver cancer become the second most common-seen cancerous death.[2,3] Although great progress has been made in the treatment of liver cancer in recent years, the prognosis remain poor.[4,5] Therefore, a reliable prognostic marker is needed to improve the liver cancer patients' prognosis.

Spermatogenesis associated serine rich 2 (*SPATS2*) most expressed in adult testis and slightly expressed in liver and other tissues. Previous studies have found that *SPATS2* is involved in sperm development, and subsequently found that it promotes the progression of prostate cancer.[6] However, the role of *SPATS2* in liver cancer is unknown, it is necessary to explore the role of *SPATS2* in liver cancer.

In this study, we tested the *SPATS2* mRNA expression difference in liver cancer by exploring TCGA database. ROC curve was drawn to evaluate the diagnostic value. The Chi-Squared test was used to evaluate the clinical correlation. Survival analysis and Cox regression model were executed to identify the effect of *SPATS2* on liver cancer patients' survival rate. GSEA was used to identify signaling pathways related to *SPATS2* expression

2. Materials and methods

2.1. Data source

We obtained the currently available clinical as well as the RNAseq data of 50 normal and 373 liver cancer tissues form TCGA databases (https://cancergenome.nih.gov/). The gene-level was estimated as log2(x+1) transformed RSEM normalized count. The HCCDB dataset (http://lifeome.net/database/hccdb/download.html) was used for validation. No ethical conflict is needed because all the data in this study are from public database and available for research.

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The authors declare that they have no conflict of interest.

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2.2. Statistical analysis

We used R (version 3.5.3)[7] for statistical analysis. Ggplot2 package was used to plot the boxplot.[8] The ROC curve drawn by pROC package evaluated the capability of diagnosis as well as setting the optimal cutoff value to separate high *SPATS2* expression group from the low *SPATS2* expression group accordingly.[9] Besides, the Chi-Squared test was used to evaluate the possible correlation between clinical features and the *SPATS2* expression. Then we used a survival package to plot survival curves and performed a logarithmic rank test.[10] The univariate Cox model and multivariate Cox model were used to clarify the prognostic role of *SPATS2* independently from other clinical features.[11]

2.3. Gene set enrichment analysis (GSEA)

GSEA determines whether an a priori defined set of genes has statistically significant differences in expression under 2 different biological conditions.[12,13] This analysis, performed using GSEA software 3.0 from the Broad Institute, was used for analysis of RNAseq data from TCGA-LIHC. The gene set of "h.all.v6.2.symbols.gmt", which summarizes and represents specific, well-defined biological states or processes, was downloaded from the Molecular Signatures Database (http://software.broadinstitute.org/gsea/msigdb/index.jsp). The normalized enrichment score (NES) was determined by analysis of 1000 permutations. A gene set was considered significantly enriched when the *P* value was less than .05 and the false discovery rate (FDR) was less than .25.

2.4. Ethical approval

Ethics committee approval was not necessary because all clinical data used in this study were obtained from a public database and are available for research.

3. Results

3.1. Patient characteristic

The *SPATS2* expression and clinical features including age, stage, new type, histologic grade, longest dimension, subdivision, sample type, lymphatic invasion, hepatitis virus, vital status, and *SPATS2* expression were shown in Table 1.

3.2. Differential expression of SPATS2 in liver cancer

Boxplots showed that *SPATS2* expression was high in liver cancer compared with which in normal liver tissues ($P < 2.2e\text{-}16$, Fig. 1), which was also validated by HCCDB dataset (Fig. 1). In addition, *SPATS2* was also expressed differently in different groups of stage ($P = .00051$), vital status ($P = .013$), age ($P = .049$), gender ($P = .021$), T classification ($P = .00029$), and histologic grade ($P = 4.5e{-}10$).

3.3. The diagnostic capability of SPATS2

According to the performance of the ROC curve, we found the area under curve (AUC) was 0.964, which represents the high diagnostic ability. In addition, we reached the same results by analyzing the subgroups of different stages (AUC: stage I was 0.957, stage II was 0.976, stage III was 0.979, stage IV was 0.872; Fig. 2).

Table 1

Clinical characteristics of the liver cancer patients.

characteristics	Number of patients (%)
Age	
<55	117 (31.45)
>=55	255 (68.55)
Gender	
FEMALE	121 (32.44)
MALE	252 (67.56)
Histological type	
Fibrolamellar Carcinoma	3 (0.8)
Hepatocellular Carcinoma	363 (97.32)
Hepatocholangiocarcinoma (Mixed)	7 (1.88)
Histologic grade	
NA	5 (1.34)
G1	55 (14.75)
G2	178 (47.72)
G3	123 (32.98)
G4	12 (3.22)
Stage	
NA	24 (6.43)
I	172 (46.11)
II	87 (23.32)
III	85 (22.79)
IV	5 (1.34)
T classification	
NA	2 (0.54)
T1	182 (48.79)
T2	95 (25.47)
T3	80 (21.45)
T4	13 (3.49)
TX	1 (0.27)
N classification	
NA	1 (0.27)
N0	253 (67.83)
N1	4 (1.07)
NX	115 (30.83)
M classification	
M0	267 (71.58)
M1	4 (1.07)
MX	102 (27.35)
Hepatitis virus	
FALSE	219 (58.71)
TRUE	154 (41.29)
Radiation therapy	
NA	25 (6.7)
NO	340 (91.15)
YES	8 (2.14)
Residual tumor	
NA	7 (1.88)
R0	326 (87.4)
R1	17 (4.56)
R2	1 (0.27)
RX	22 (5.9)
Vital status	
DECEASED	130 (34.85)
LIVING	243 (65.15)
relapse	
NO	179 (55.94)
YES	141 (44.06)
SPATS2	
High	139 (37.27)
Low	234 (62.73)

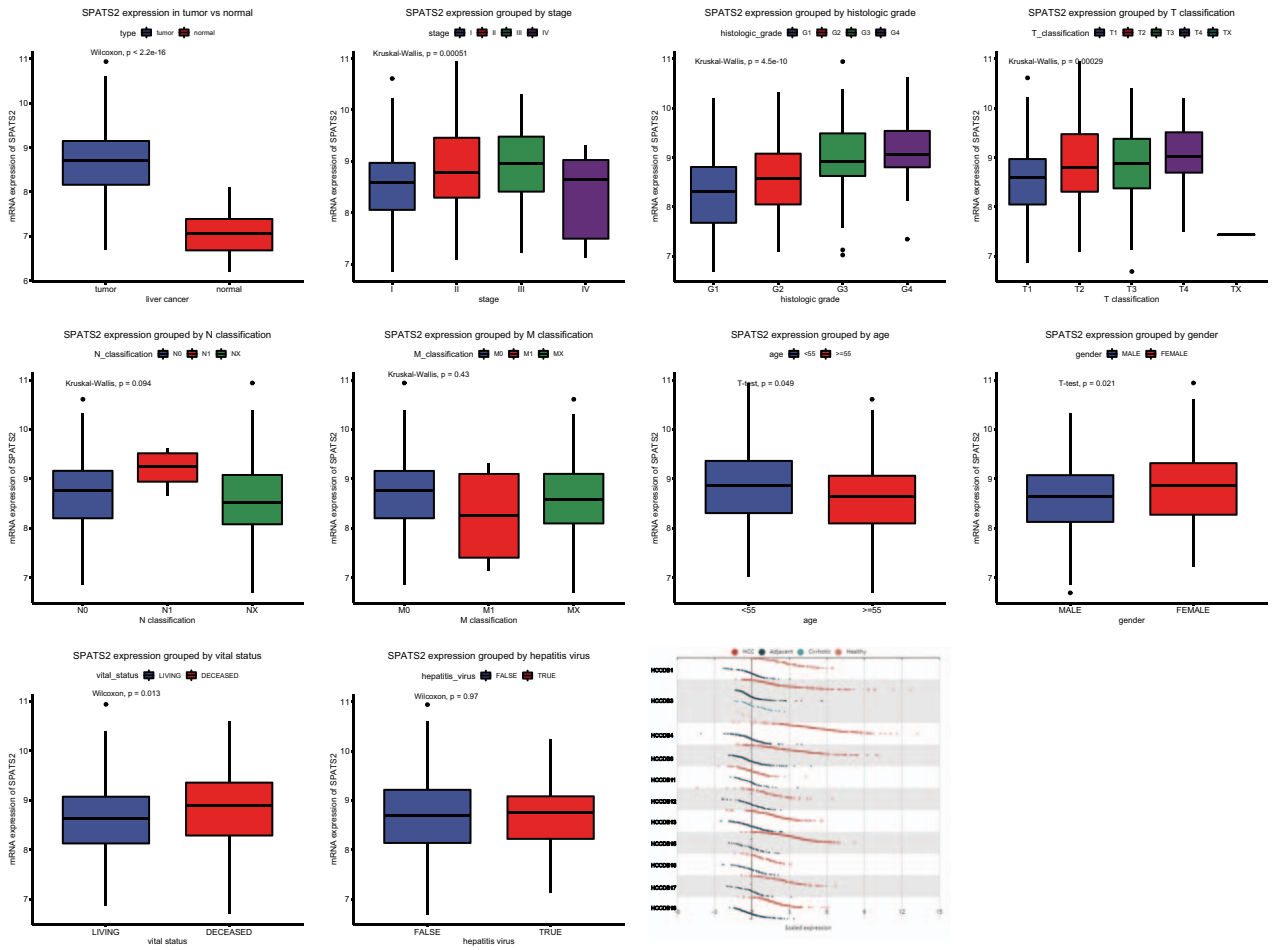

Figure 1. SPATS2 expression in liver cancer. SPATS2 expression between normal tissues and liver cancer, stages, histologic grade, TNM classification, age, gender, vital status. SPATS2 expression in liver cancer validated by HCCDB datasets.

3.4. Relationship between SPATS2 expression and clinical features of liver cancer

As show in Table 2, the expression of *SPATS2* was strongly associated with age ($P=.024$), vital status ($P=.001$), stage ($P=.002$), T classification ($P=.002$) and histologic grade ($P=.000$) of liver cancer patients.

3.5. High SPATS2 expression is related to liver cancer patients' poor overall survival

As shown in Figure 3, patients with higher *SPATS2* expression had particularly shorter OS ($P<.0001$), which was validated by HCCDB datasets (Fig. 3) and consistent with results of subgroup analysis, especially in stage I/II ($P=.03$), stage III/IV ($P<.001$), stage G1/G2 ($P<.0001$), stage G3/G4 ($P=.0054$), male ($P<.0001$), female ($P=.0047$), younger ($P<.0001$), older ($P=1e{-}04$), hepatitis virus positive ($P=.013$), and hepatitis virus negative ($P<.0001$). Cox model and Multivariate Cox model suggested that high *SPATS2* expression was an independent risk factor for the OS of liver cancer (HR = 2.47, $P<.001$, Table 3).

3.6. High SPATS2 expression is related to liver cancer patients poor relapse-free survival

As shown in Figure 4, patients with higher *SPATS2* expression had particularly shorter RFS ($P<.0001$), which was consistent with results of subgroup analysis, especially in stage I/II ($P=.016$), stage III/IV ($P=.037$), stage G1/G2 ($P=.00014$), stage G3/G4 ($P=.016$), male ($P=.00014$), female ($P=.048$), younger ($P<.0036$), older ($P=.0028$), and hepatitis virus negative ($P<.0001$). Univariate Cox model and Multivariate Cox model suggested that high *SPATS2* expression was an independent risk factor for the RFS of liver cancer (HR = 1.92, $P<.001$, Table 4).

3.7. GSEA identifies SPATS2-related signaling pathway

We compared the data sets for low and high *SPATS2* expression using GSEA to identify signaling pathways activated during liver cancer. The results indicated significant differences (FDR < 0.25, NOM P value < .05) in the enrichment of the MSigDB collection (h.all.v6.2.symbols.gmt; Table 5). We selected the most significantly enriched signaling pathways based on normalized enrichment score (NES) (Fig. 5, Table 5).

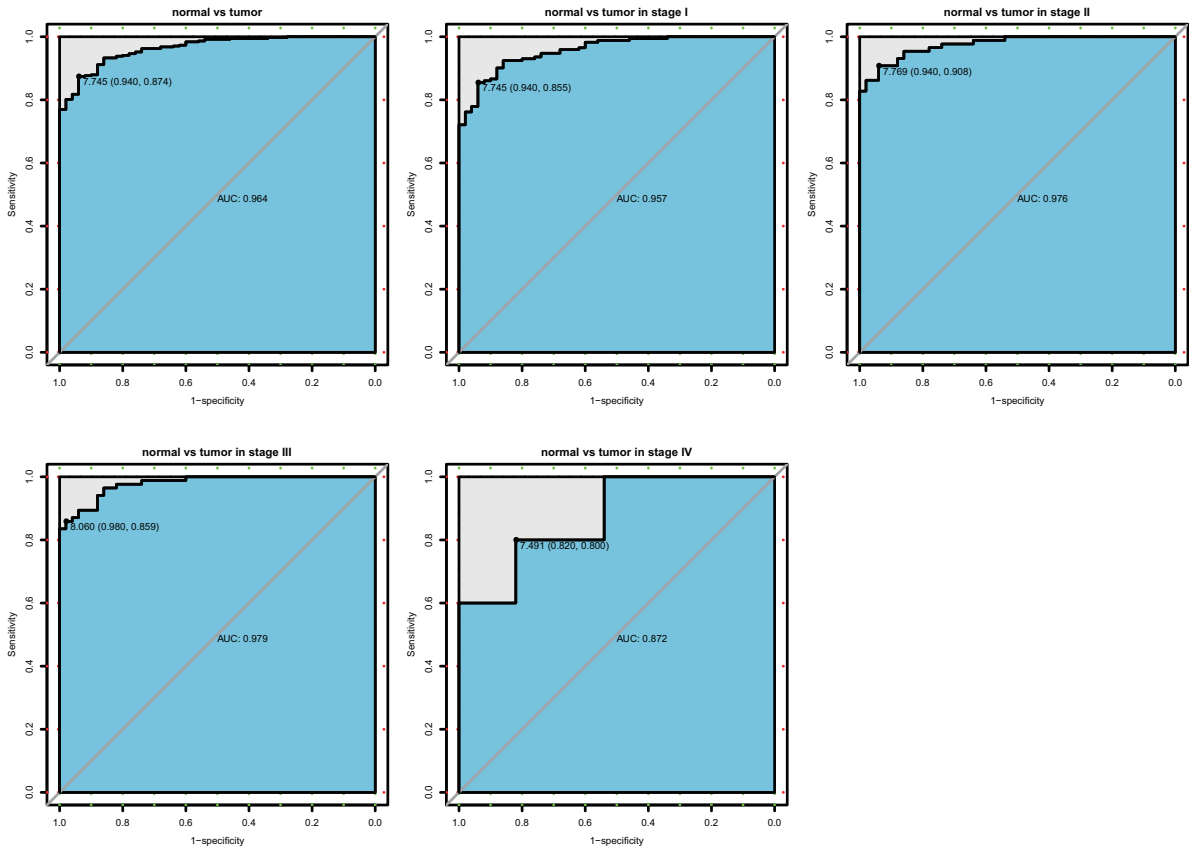

Figure 2. Diagnosis value of SPATS2. The ROC curve of normal tissues and liver cancer, and subgroup analysis of stage I, II, III, IV.

Table 2

Relationship between the clinical features and SPATS2 expression in liver cancer patients.

Clinical characteristics	Variable	No. of patients	SPATS2 expression				$\chi 2$	*P* value
			High	%	Low	%		
Age	<55	117	54	−38.85	63	−27.04	5.0981	**.024**
	>=55	255	85	−61.15	170	−72.96		
Gender	FEMALE	121	54	−38.85	67	−28.63	3.6998	.054
	MALE	252	85	−61.15	167	−71.37		
Histological type	Fibrolamellar Carcinoma	3	0	0	3	−1.28	5.2958	.096
	Hepatocellular Carcinoma	363	134	−96.4	229	−97.86		
	Hepatocholangiocarcinoma (Mixed)	7	5	−3.6	2	−0.85		
Histologic grade	G1	55	12	−8.7	43	−18.7	21.6385	**0**
	G2	178	56	−40.58	122	−53.04		
	G3	123	62	−44.93	61	−26.52		
	G4	12	8	−5.8	4	−1.74		
Stage	I	172	48	−37.21	124	−56.36	14.4648	**.002**
	II	87	35	−27.13	52	−23.64		
	III	85	44	−34.11	41	−18.64		
	IV	5	2	−1.55	3	−1.36		
T classification	T1	182	51	−36.69	131	−56.47	16.0251	**.002**
	T2	95	42	−30.22	53	−22.84		
	T3	80	38	−27.34	42	−18.1		
	T4	13	8	−5.76	5	−2.16		
	TX	1	0	0	1	−0.43		
N classification	N0	253	97	−70.29	156	−66.67	3.4399	.166
	N1	4	3	−2.17	1	−0.43		
	NX	115	38	−27.54	77	−32.91		
M classification	M0	267	102	−73.38	165	−70.51	0.7578	.629
	M1	4	2	−1.44	2	−0.85		
	MX	102	35	−25.18	67	−28.63		
Hepatitis virus	FALSE	219	82	(58.99)	137	(58.55)	0	1
	TRUE	154	57	(41.01)	97	(41.45)		
Radiation therapy	NO	340	131	−99.24	209	−96.76	1.2796	.258
	YES	8	1	−0.76	7	−3.24		
Residual tumor	R0	326	116	−85.29	210	−91.3	4.305	.19
	R1	17	9	−6.62	8	−3.48		
	R2	1	0	0	1	−0.43		
	RX	22	11	−8.09	11	−4.78		
Vital status	DECEASED	130	64	−46.04	66	−28.21	11.4474	**.001**
	LIVING	243	75	−53.96	168	−71.79		

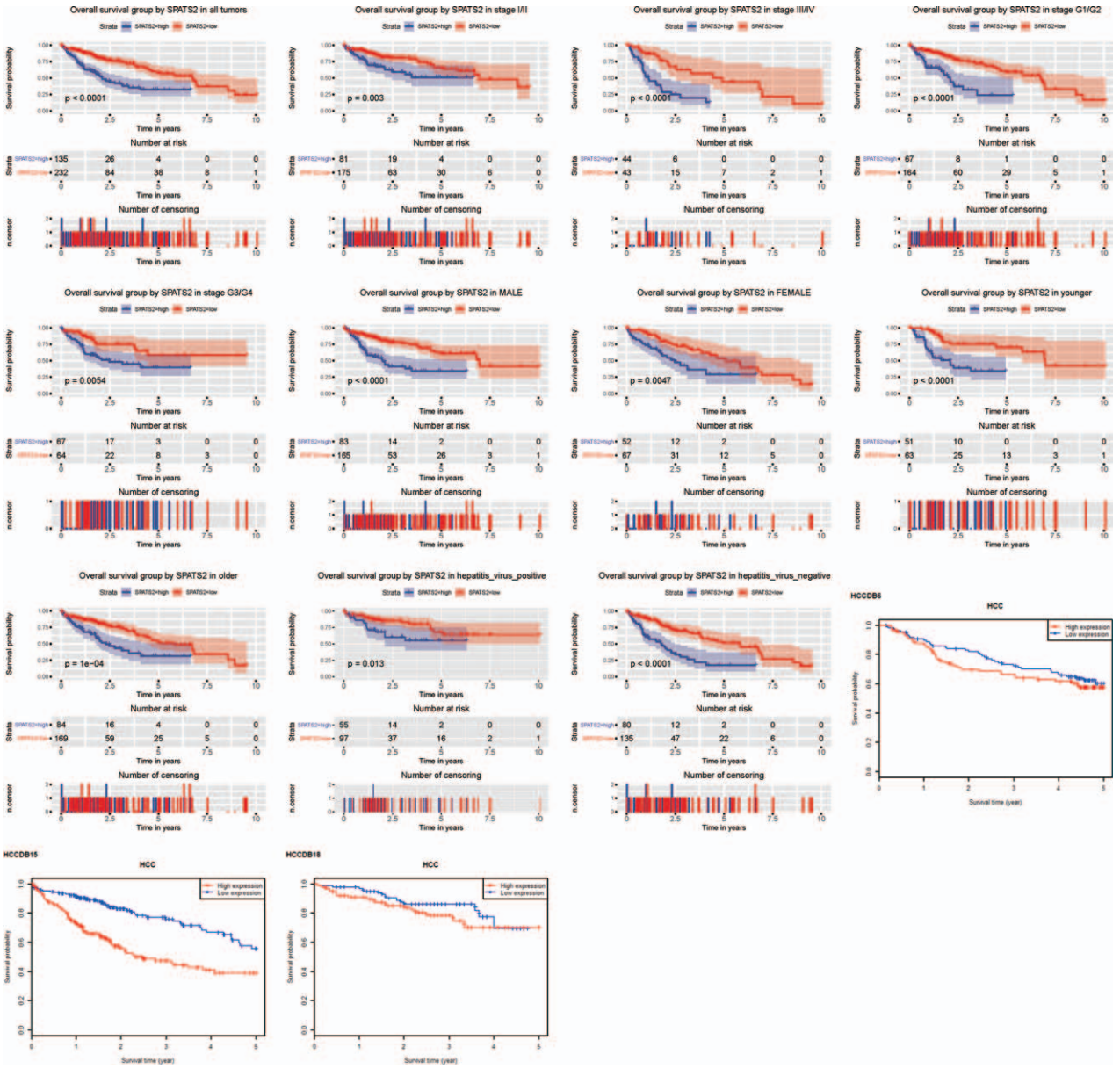

Figure 3. Survival curves of OS in liver cancer. Kaplan–Meier curves of liver cancer patients OS in all tumors, histologic grade G1/G2, G3/G4, clinical stage I/II, III/IV, male, female, younger, and older. Survival curves of OS grouped by SPATS2 in liver cancer validated by HCCDB datasets.

Table 3

Univariate analysis and multivariate analysis of liver cancer patients overall survival.

	Univariate analysis			Multivariate analysis		
Parameters	**Hazard ratio**	**95%CI (lower-upper)**	***P* value**	**Hazard ratio**	**95%CI (lower-upper)**	***P* value**
Age	1	0.69–1.45	.997			
Gender	0.8	0.56–1.14	.22			
Histological type	0.99	0.27–3.66	.986			
Histologic grade	1.04	0.84–1.3	.698			
Stage	1.38	1.15–1.66	.001	0.89	0.71–1.11	.29
T classification	1.66	1.39–1.99	0	1.61	1.26–2.06	0
N classification	0.73	0.51–1.05	.086			
M classification	0.72	0.49–1.04	.077			
Hepatitis virus	0.51	0.35–0.74	.001	0.64	0.43–0.95	.028
Radiation therapy	0.51	0.26–1.03	.06			
Residual tumor	1.42	1.13–1.8	.003	1.35	1.05–1.74	.018
SPATS2	2.72	1.9–3.89	0	2.47	1.71–3.56	0

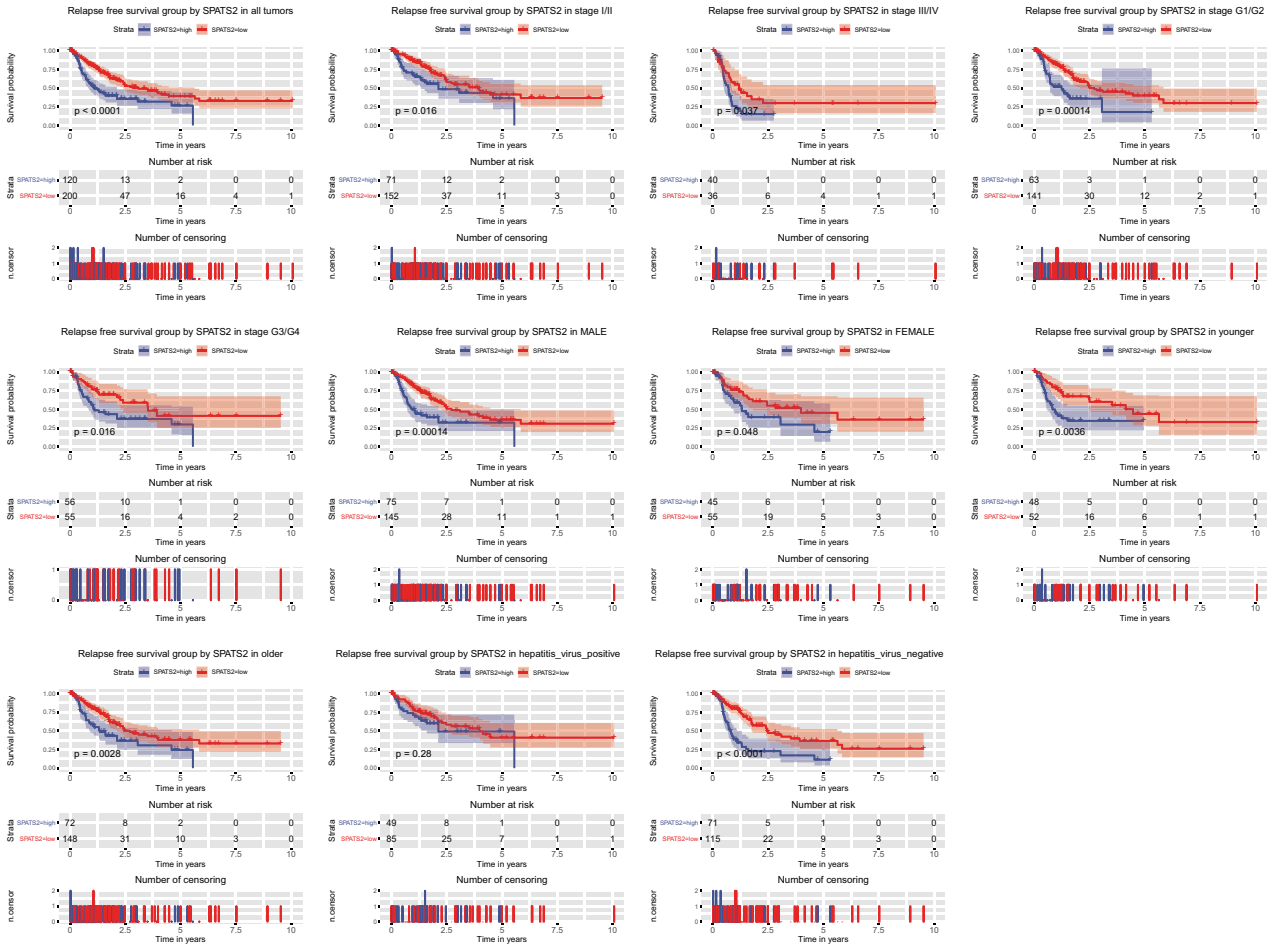

Figure 4. Survival curves of RFS in liver cancer. Kaplan–Meier curves of liver cancer patients RFS in all tumors, histologic grade G1/G2, G3/G4, clinical stage I/II, III/IV, male, female, younger, and older.

The results indicated the data set with high *SPATS2* expression was enriched in Mitotic spindle, G2 M checkpoint, E2F targets.

4. Discussion

At present, liver cancer can be treated in various ways, such as hepatectomy, liver transplantation, ablation, interventional therapy, radiotherapy, drug therapy, and biological immunity. However, the prognosis of patients is still poor. Therefore, it is urgent to find novel markers to predict the prognosis of liver cancer.[14] Many researches have been working to find out the prognostic maker of liver cancer, and aim to guide clinicians to evaluate the prognosis of liver cancer patients recently.[15–22] In this research, we found that *SPATS2*

Table 4

Univariate analysis and multivariate analysis of liver cancer patients' recurrence-free survival.

	Univariate analysis			Multivariate analysis		
Parameters	**Hazard ratio**	**95%CI (lower-upper)**	***P* value**	**Hazard ratio**	**95%CI (lower-upper)**	***P* value**
Age	0.9	0.63–1.28	.55			
Gender	0.99	0.7–1.41	.966			
Histological type	2.02	0.66–6.24	.22			
Histologic grade	0.98	0.8–1.21	.883			
stage	1.66	1.38–1.99	0	1.12	0.87–1.44	.363
T classification	1.78	1.49–2.12	0	1.55	1.18–2.02	.002
N classification	0.97	0.67–1.4	.874			
M classification	1.17	0.79–1.74	.432			
Hepatitis virus	0.63	0.44–0.89	.008	0.83	0.57–1.2	.318
Radiation therapy	0.74	0.26–2.16	.584			
Residual tumor	1.28	1.01–1.61	.042	1.34	1.05–1.71	.018
SPATS2	2.06	1.47–2.89	0	1.92	1.36–2.71	0

Table 5

Gene set enrichment analysis (GSEA) of signaling pathways activated during liver cancer.

Gene set	ES	NES	NOM p-val	FDR q-val
HALLMARK_MITOTIC_SPINDLE	−0.601	−1.922	0.000	0.050
HALLMARK_G2M_CHECKPOINT	−0.724	−1.819	0.002	0.064
HALLMARK_E2F_TARGETS	−0.714	−1.748	0.004	0.077

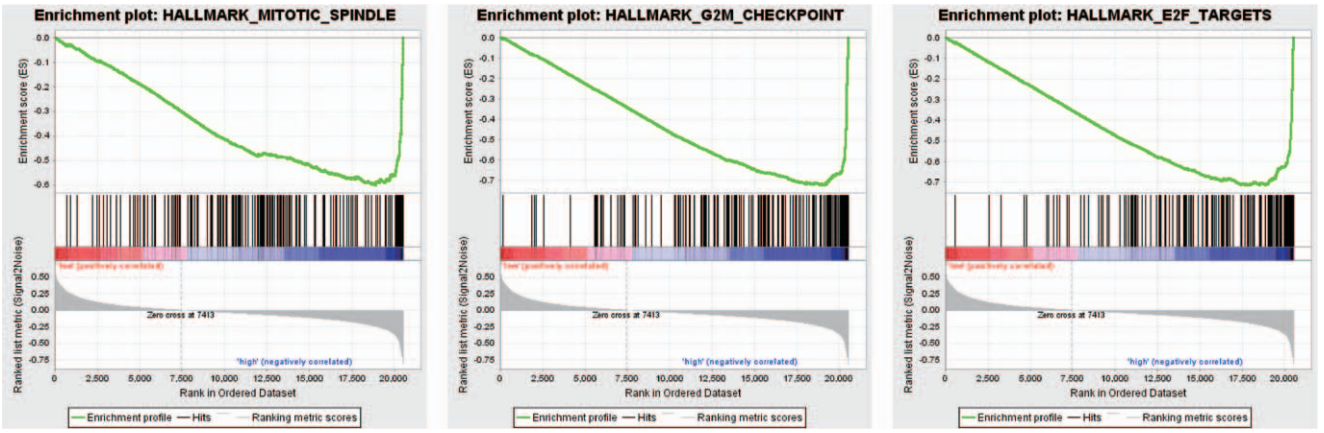

Figure 5. Gene set enrichment analysis (GSEA) of signaling pathways activated during liver cancer. The high *SPATS2* expression phenotype had enrichment of Mitotic spindle, G2M checkpoint, E2F targets.

expression could be a novel diagnostic and prognostic biomarker in liver cancer.

The gene *SPATS2* was firstly found to encode a polypeptide containing 545 amino acid residues in mouse testis, which is involved in sperm growth and development.[23] Subsequently, the researchers found that *SPATS2* was also expressed in 25 human tissues.[24] Recent studies have found that *SPATS2* is highly expressed in squamous cell carcinoma but lowly expressed in non-lepidic AD.[25] In this study, we found that *SPATS2* was highly expressed in liver cancer compared with normal liver tissue. This may be due to the organization specificity. In addition, *SPATS2* can induce the transcription of SNHG5, promoting the survival of colon cancer cells.[26] Meanwhile, we found that the *SPATS2* expression gradually increased in the stage, histologic stage and T classification, indicating that *SPATS2* might promote the progress of liver cancer through helping cancer cell survival. Importantly, *SPATS2* was highly expressed in the deceased than it in the survivors, so further survival analysis is necessary.

The survival of cancer cells is the main cause of cancer recurrence. Previous studies have found that *SPATS2* removes H3K27me3 histone markers and promotes the survival of prostate cancer.[6] Interestingly, patients with high *SPATS2* expression have a significantly shorter OS and RFS in liver cancer. Subgroup analysis also found the prognostic significance in the stage I/II, stage III/IV, G1/G2, G3/G4, male, female, younger, and older. This suggests that *SPATS2* plays an important prognostic role in the whole process of liver cancer development. Consistent with these findings, we found that high *SPATS2* could predict poor prognosis in liver cancer, which may involve in Mitotic spindle, G2 M checkpoint, E2F targets.

To our knowledge, this is the first study to examine the prognostic value of *SPATS2* expression in liver cancer. Together with other studies about the functions of *SPATS2*, we have contributed to a better understanding of the role of *SPATS2*, as well as great possibility of accurately predicting the prognosis of liver cancer. However, the sample size is limited in this study. In the future, more samples need to be involved to validate the prognostic role of *SPATS2* in liver cancer.

5. Conclusion

In conclusion, we mainly focused on the diagnostic and prognostic value of *SPATS2* in patients with liver cancer. *SPATS2* expression could be a novel diagnostic and prognostic biomarker in liver cancer

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Author contributions

Conceptualization: Xinying Wang.
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Investigation: Yijun Tian, Wu Ji.
Methodology: Jin Xing.
Project administration: Yijun Tian.
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Software: Jin Xing.
Validation: Yijun Tian, Wu Ji.
Visualization: Jin Xing.
Writing – original draft: Jin Xing.
Writing – review & editing: Xinying Wang.

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