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Multiomic analysis of the function of *SPOCK1* across cancers: an integrated bioinformatics approach

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Abstract

Objective: To investigate SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene expression across The Cancer Genome Atlas (TCGA) cancers, both in cancer versus normal tissues and in different stages across the cancer types.

Methods: This integrated bioinformatics study used data from several bioinformatics databases (Cancer Cell Line Encyclopedia, Genotype-Tissue Expression, TCGA, Tumor Immune Estimation Resource [TIMER]) to define the expression pattern of the *SPOCK1* gene. A survival analysis was undertaken across the cancers. The search tool for retrieval of interacting genes (STRING) database was used to identify proteins that interacted with *SPOCK1*. Gene Set Enrichment Analysis was conducted to determine pathway enrichment. The TIMER database was used to explore the correlation between *SPOCK1* and immune cell infiltration.

Results: This multiomic analysis showed that the *SPOCK1* gene was expressed differently between normal tissues and tumours in several cancers and that it was involved in cancer progression. The overexpression of the *SPOCK1* gene was associated with poor clinical outcomes. Analysis of gene expression and tumour-infiltrating immune cells showed that *SPOCK1* correlated with several immune cells across cancers.

Conclusions: This research showed that *SPOCK1* might serve as a new target for several cancer therapies in the future.

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Introduction

Cancer is among the most lethal diseases worldwide and it is an increasing threat to human health.[1] The number of cancer patients continues to expand due to the growth and aging of the global population.[2] Over the last few decades, many efforts have been invested in cancer prevention, early diagnosis and treatments to reduce the disease burden.[3] The process of carcinogenesis is extremely complex, with normal cells becoming pathological with disrupted apoptosis and dysregulation of metabolism.[4] The accumulation of gene alterations is key to oncogenesis and closely related to the prognosis of cancer patients.[5] Hence identification of genes that are involved can be utilized as new markers.[4] An understanding of the mechanism of the altered expression of these genes will enable them to be exploited as novel therapeutic targets and shed new light on cancer treatments.

The SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene encodes a protein that is a member of the secreted protein acidic and rich in cysteine (*SPARC*) family.[6] Other members of the gene family include *SPOCK2*, *SPOCK3*, SPARC like 1 (*SPARCL1*), SPARC related modular calcium binding 1 (*SMOC1*) and *SMOC2*.[7] The proteins encoded by the members of the *SPARC* gene family are similar in structure, consisting of an N-terminus, follistatin-like domain and a C-terminus.[8] They have similar functions, for example they play important roles in the process of cancer cell adhesion and cell–matrix interactions, cell proliferation, migration and apoptosis.[9] Several studies have explored the function of *SPOCK1* in light of its similar structure to *SPARC* and found that it participates in cancer cell invasion in oesophageal squamous cell carcinoma,[10] colorectal cancer[11,12] and gallbladder cancer.[13] Research has also shown that *SPOCK1* plays an important role in prostate cancer recurrence.[14] The over-expression of the *SPOCK1* gene promoted glioma cell proliferation, migration and invasion via the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/protein kinase B (AKT) and Wnt/β-catenin signalling pathways; and *SPOCK1* silencing reversed this process.[15] Further research showed that *SPOCK1* was upregulated in osimertinib-resistant lung cancer cells and knockdown of *SPOCK1* inhibited osimertinib-resistant cell growth and overcame resistance.[16] Recent research demonstrated that *SPOCK1* could induce mesenchymal epithelial transition factor receptor-dependent epithelial–mesenchymal transition (EMT) signalling in lapatinib-resistant gastric cancer, which is responsible for gastric cancer drug resistance.[17] The over-expression of *SPOCK1* also promoted proliferation and invasion and blocked apoptosis of HCC cells.[18] *SPOCK1* can function on its own, as well as by working together with other elements like fibronectin.[19] Since the expression of *SPOCK1* is low among normal tissue specimens,[10–13] it could be used as a potential biomarker for the early detection and precise prognosis in a variety of cancers. The underlying oncogenic mechanisms of the *SPOCK1* gene during cancer initiation and progression needs further investigation and evaluation.

All of the previous studies suggested that *SPOCK1* plays a critical role in human

tumorigenesis,[10–19] but details about *SPOCK1*-mediated cancer progression are lacking. This current bioinformatics analysis investigated *SPOCK1* gene expression across The Cancer Genome Atlas (TCGA) cancers, both in cancer versus normal tissues and in different stages across cancers. It also explored important genes and proteins that have close interactions with *SPOCK1*.

Materials and methods

Expression of the SPOCK1 *gene across diverse cancer types*

Clinicopathological data, RNA-Seq data (HTSeq–FPKM) and immune subtypes data acquisition was achieved by downloading from TCGA[20] and The Genotype-Tissue Expression (GTEx).[21] The GTEx database was used as a supplementation to the noncancerous control group for cancer types that had less than 30 adjacent normal tissues in TCGA. An online tool, the Broad Institute Cancer Cell Line Encyclopedia (CCLE),[22] was used for visualization of the pan-cancer expression of *SPOCK1* across diverse cancer types.[23]

The cancer types that were investigated were bladder urothelial carcinoma (BLCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), lung adenocarcinoma (LUAD), pancreatic adenocarcinoma (PAAD), prostate adenocarcinoma (PRAD), sarcoma (SARC), stomach adenocarcinoma (STAD) and uveal melanoma (UVM).

Role of SPOCK1 *gene over-expression in cancer prognosis*

The expression levels of *SPOCK1* across several types of cancers and their subtypes and its differential gene expression analysis at different pathological stages was analysed. The current study also explored the correlation between the expression of *SPOCK1* and cancer prognosis in cancers. Overall survival (OS) and the disease-free survival (DFS) analysis was undertaken on the basis of

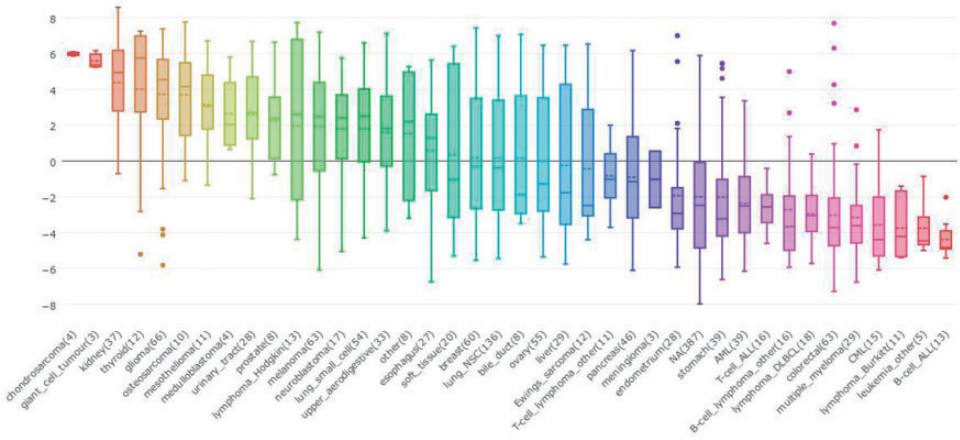

Figure 1. The Broad Institute Cancer Cell Line Encyclopedia was used to visualize the pan-cancer expression of the SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene across diverse cancer types. Central bold horizontal lines represent the median values. Extremities of the box represent the 25th and 75th percentiles. Error bars represent minimum and maximum outliers. The colour version of this figure is available at: http://imr.sagepub.com

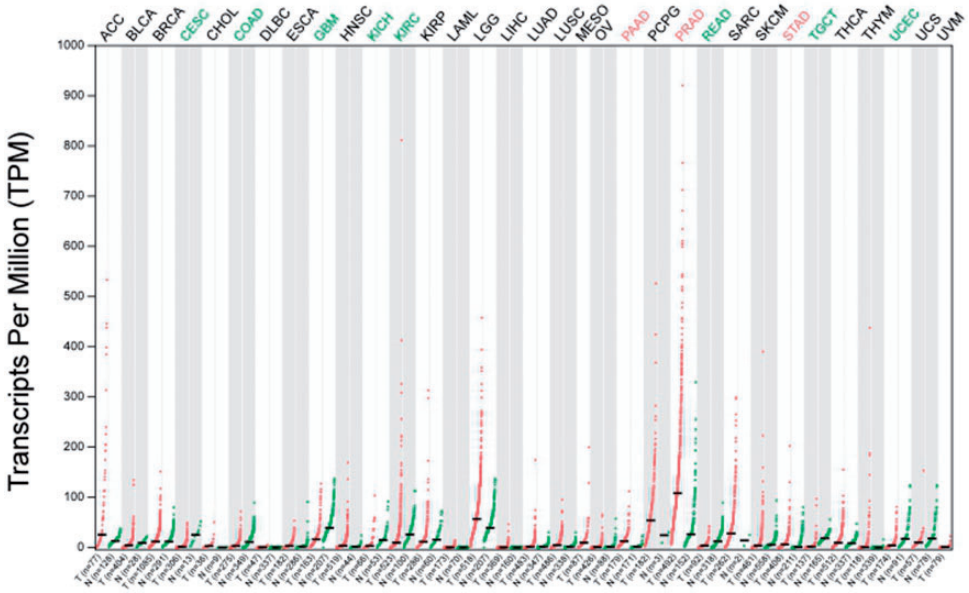

Figure 2. SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene expression in Genotype-Tissue Expression normal tissues, The Cancer Genome Atlas (TCGA) normal tissues and TCGA cancer tissues. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, oesophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukaemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumours; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma. The colour version of this figure is available at: http://imr.sagepub.com

SPOCK1 expression using Gene Expression Profiling Interactive Analysis.[24] Specifically, paired Student's *t*-test was applied to compare the mRNA expression from the *SPOCK1* gene in normal and tumour samples. Correlation between *SPOCK1* expression and different individual cancer stages was analysed using analysis of variance. The OS and DFS of two groups (cut-off set as the median expression levels of *SPOCK1*) were compared using the Kaplan–Meier method. Survival maps were plotted with a significance level of $P < 0.05$ and false discovery rate (FDR) adjustment.

Protein–protein interaction network analysis

The search tool for retrieval of interacting genes (STRING; https://string-db.org/) database was utilized to generate protein–protein interaction (PPI) networks.[25] Functional interactions between *SPOCK1* and other genes were analysed using Pearson's correlation coefficient. In the PPI network, nodes represent proteins and the edges represent interactions between the proteins. The median confidence score was set at 0.4. R software was used to

Table 1. SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene expression in Genotype-Tissue Expression normal tissues and The Cancer Genome Atlas normal tissues across various cancers.

	Transcripts per million				
Cancer type	Maximum	Upper quartile	Median	Lower quartile	Minimum
BLCA	23.98	11.44	8.66	6.86	4.42
BRCA	32.92	13.96	8.99	5.29	0.71
CESC	38.1	24.33	15.43	9.15	5.26
CHOL	0.01	0.05	0.02	0.01	0
COAD	11.53	3.44	1.60	0.87	0.31
ESCA	10.02	2.05	0.84	0.45	0.22
HNSC	7.99	2.29	1.35	0.49	0.13
KIRC	115.16	51.56	34.93	21.22	6.33
LIHC	0.1	0.03	0.02	0.01	0
LUAD	1.19	0.61	0.45	0.27	0.03
LUSC	2.45	1.06	0.55	0.35	0.09
PAAD	25.65	24.55	19.88	12.76	5.89
PRAD	438.28	118.18	90.46	54.56	11.16
STAD	71.76	8.86	2.33	0.44	0.11
READ	18.79	5.34	4.61	1.22	0.64

BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, oesophageal carcinoma; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; STAD, stomach adenocarcinoma; READ, rectum adenocarcinoma.

Table 2. SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene expression in The Cancer Genome Atlas cancer tissues.

	Transcripts per million				
Cancer type	Maximum	Upper quartile	Median	Lower quartile	Minimum
BLCA	131.61	5.68	5.26	1.56	0.01
BRCA	149.75	23.92	12.58	5.42	0.06
CESC	36.48	4.39	1.84	0.73	0.02
CHOL	16.03	5.63	2.31	1.2	0.14
COAD	58.22	8.45	3.40	0.75	0
ESCA	68.84	12.13	3.66	1.08	0.11
HNSC	90.14	10.08	3.52	1.44	0.04
KIRC	396.28	29.76	10.23	2.96	0
LIHC	2.87	0.53	0.14	0.04	0
LUAD	39.28	4.27	1.43	0.54	0.01
LUSC	71.55	10.79	4.86	2.50	0.05
PAAD	101.61	22.93	13.86	6.38	0.25
PRAD	874.52	219.25	127.71	73.75	15.77
STAD	235.22	16.29	7.19	2.02	0.05
READ	42.26	5.73	1.98	0.82	0.05

BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; ESCA, oesophageal carcinoma; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; STAD, stomach adenocarcinoma; READ, rectum adenocarcinoma.

perform Gene Set Enrichment Analysis (GSEA; https://www.gsea-msigdb.org/gsea/index.jsp) enrichment analysis where an FDR <0.05 and an enrichment score >0.65 were set as the cut-off criteria.

Correlation between SPOCK1 *and tumour-infiltrating immune cells*

In order to explore the correlation between *SPOCK1* and tumour-infiltrating immune cells, the Tumor Immune Estimation Resource (TIMER) was utilized.[26] Spearman's rank correlation coefficient was used and a P-value < 0.05 was considered statistically significant.

Results

The CCLE database was used to visualize the expression of the *SPOCK1* gene across diverse cancer types (Figure 1). Figure 2 illustrates the expression of the *SPOCK1* gene (transcripts per million) in GTEx normal tissues, TCGA normal tissues and TCGA cancer tissues (Tables 1 and 2). In the dot plot in Figure 2, the dots represent the expression levels of the samples.

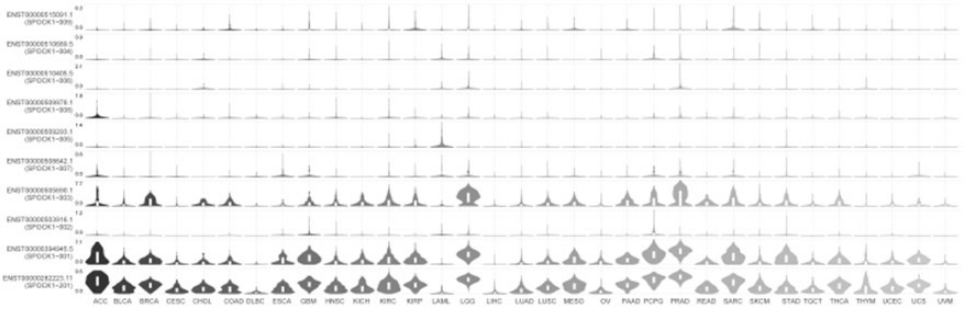

Figure 3. Different isoforms of the SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene.

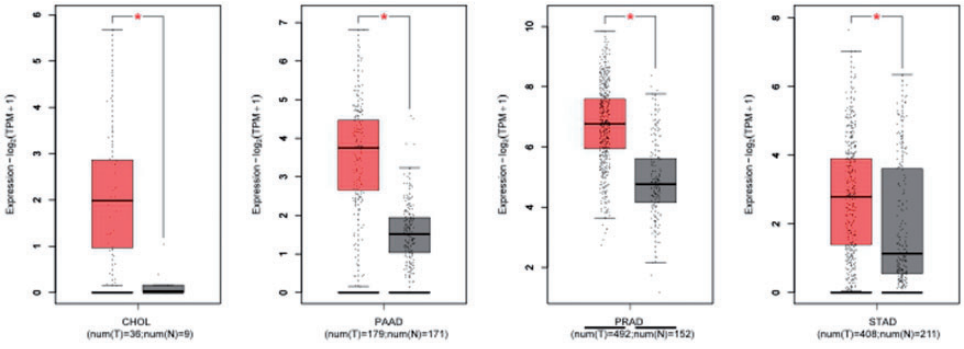

Figure 4. Comparison of SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene expression between tumour tissues (red boxes) and normal tissues (grey boxes). CHOL, cholangiocarcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; STAD, stomach adenocarcinoma. *$P < 0.05$, paired Student's t-test. Central black horizontal lines represent the median values. Extremities of the box represent the 25th and 75th percentiles. Error bars represent minimum and maximum outliers. The colour version of this figure is available at: http://imr.sagepub.com

The SPOCK1 gene has ten splice variants: SPOCKl-201, SPOCK1-001, SPOCK1-002, SPOCK1-003, SPOCK1-004, SPOCK1-005, SPOCK1-006, SPOCK1-007, SPOCK1-008, SPOCK1-009. The predominant SPOCK1 isoforms expressed in all contexts were SPOCK1-201, SPOCK1-001 and SPOCK1-003 (Figure 3).

The levels of SPOCK1 gene expression between TCGA tumour tissues and normal tissues were significantly different in cancers such as CHOL, PAAD, PRAD and STAD ($P < 0.05$) (Figure 4 and Table 3). The expression of the SPOCK1 gene was significantly different among pathological stages in cancers such as BLCA, KIRC, LUAD and STAD ($P < 0.05$) (Figure 5 and Table 4).

The OS time between cancers with higher and lower SPOCK1 gene expression were compared in TCGA cancer types (Figure 6). Data demonstrated a shorter OS with a worse prognosis in patients with cancers with higher SPOCK1 gene expression levels compared with cancers with lower SPOCK1 gene expression levels for the following cancers: COAD, HNSC, KIRC, LUAD and UVM ($P < 0.05$). For DFS time in TCGA tumour types (Figure 7), data demonstrated that higher SPOCK1 gene expression levels resulted in worse DFS prognosis compared with lower levels of SPOCK1 gene expression in the following tumours: BLCA, COAD, KIRC, SARC and UVM ($P < 0.05$).

The gene interaction network showed that the SPOCK1 gene was co-expressed with the TNFAIP6, MLLT10, MEGF11, SGCB, DCTN1, SLC24A2, CNTN1 and LSAMP genes (Figure 8). The SPOCK1 gene had physical interactions with the GTF2E2 and TNF genes. The SPOCK1 gene had shared protein domains with the TACSTD2, SPOCK3 and SPOCK2 genes; and co-localized with the SLC25A33, SFXN3, MEGF11, SGCB, GRIA4, SLC24A2 and LSAMP genes. Data from the STRING database analysis revealed

Table 3. The levels of SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene expression in cancer tissues compared with normal tissues.

Cancer type	Maximum (log_2TPM+1)	Upper quartile (log_2TPM+1)	Median (log_2TPM+1)	Lower quartile (log_2TPM+1)	Minimum (log_2TPM+1)	Statistical significance[a]
CHOL normal	0.212	0.071	0.033	0.019	0	$P < 0.0001$
CHOL tumour	4.09	2.73	1.73	1.14	0.19	
COAD normal	3.647	2.151	1.382	0.902	0.387	$P < 0.0001$
COAD tumour	5.888	3.241	2.139	0.807	0	
HNSC normal	3.168	1.72	1.232	0.577	0.175	$P < 0.0001$
HNSC tumour	6.51	3.47	2.177	1.284	0.056	
KIRC normal	6.86	5.716	5.167	4.474	2.874	$P < 0.0001$
KIRC tumour	8.634	4.943	3.489	1.985	0.014	
LIHC normal	0.133	0.037	0.023	0.013	0	$P < 0.0001$
LIHC tumour	1.953	0.614	0.187	0.059	0	
LUSC normal	1.787	1.046	0.633	0.434	0.125	$P < 0.0001$
LUSC tumour	6.181	3.559	2.551	1.809	0.066	
PRAD normal	8.779	6.897	6.515	5.796	3.604	$P < 0.0001$
PRAD tumour	9.774	7.783	7.008	6.224	4.068	

[a]Student's *t*-test.

TPM, transcripts per million; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUSC, lung squamous cell carcinoma; PRAD, prostate adenocarcinoma.

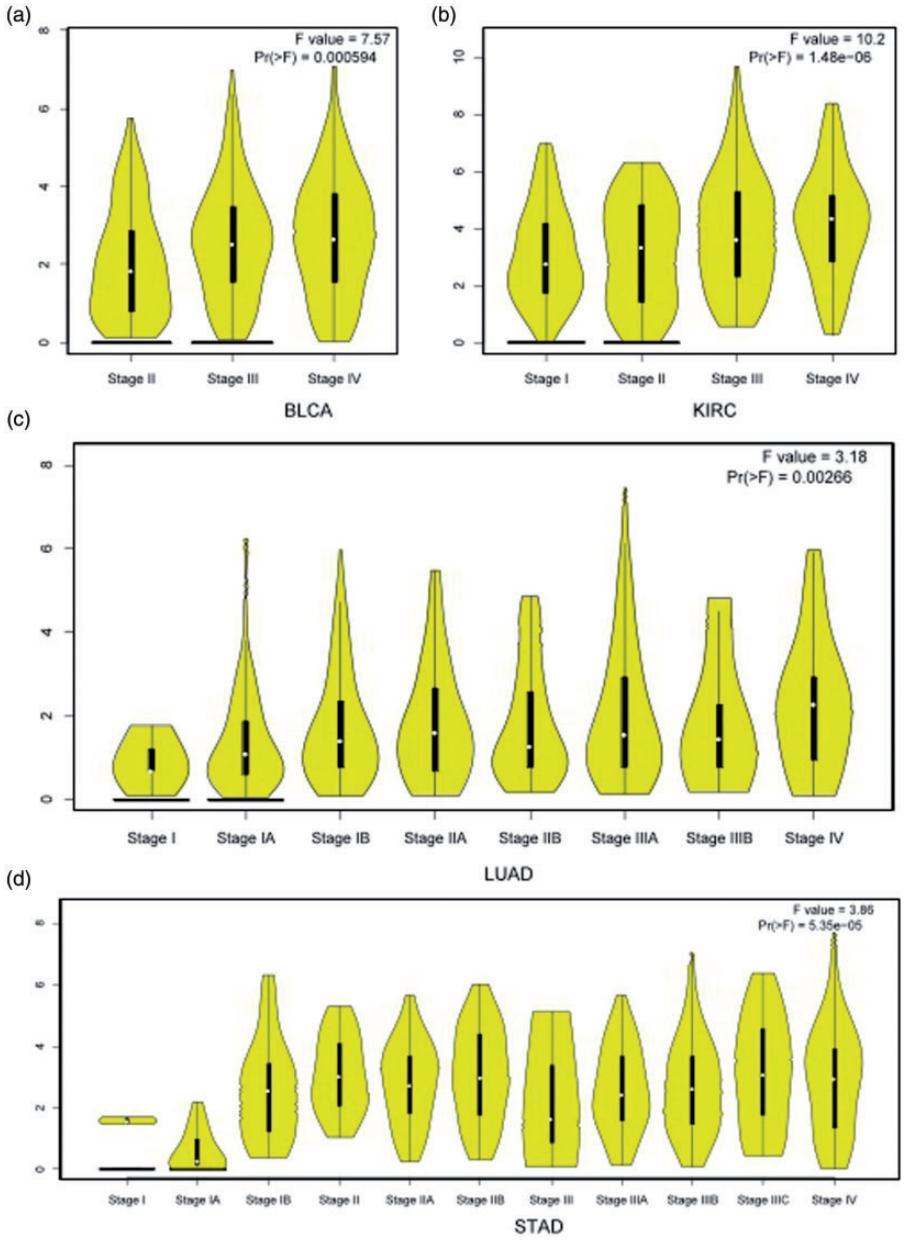

Figure 5. Violin plots showing the comparison of SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene expression between tumour tissues at different pathological stages. (a) BLCA, bladder urothelial carcinoma; (b) KIRC, kidney renal clear cell carcinoma; (c) LUAD, lung adenocarcinoma; (d) STAD, stomach adenocarcinoma. Cancer stages were compared using analysis of variance. The colour version of this figure is available at: http://imr.sagepub.com

that all of the proteins from these genes (CHD1L, CGREF1, SERTM1, MMP14, MMP16, SPARC, SMOC1, TG, STS, and SPARCL1) were in the same protein network with SPOCK1 (Figure 9).

The GSEA enrichment analysis revealed that the enrichment of the SPOCK1 gene was found to mediate upstream glycolysis, G2M checkpoint, mammalian target of rapamycin complex 1 signalling, mitotic spindle activities, EMT and myc targets; and to mediate downstream E2 factor targets, tumour necrosis factor-α signalling via nuclear factor kappa-light-chain-enhancer of activated B cells, oestrogen response, hypoxia and the interleukin-2-signal transducer and activator of transcription 5 signalling process (Figure 10).

Figure 11 presents the correlation between the expression of the SPOCK1 gene and tumour-infiltrating immune cells in BLCA, KIRC, LUAD and STAD based on data from the TIMER database. The results showed that in BLCA, the expression of the SPOCK1 gene had significant inverse correlations with tumour purity (partial correlation = –0.352, $P < 0.0001$) and the infiltration of B cells

Table 4. The levels of SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene expression in tumour tissues at different pathological stages.

Cancer type	Individual cancer stage comparison	Statistical significance[a]
BLCA	Stage 2 versus stage 3	$P < 0.0001$
	Stage 2 versus stage 4	$P < 0.0001$
CESC	Stage 1 versus stage 3	$P < 0.0001$
COAD	Stage 1 versus stage 3	$P < 0.0001$
ESCA	Stage 1 versus stage 2	$P < 0.0001$
	Stage 1 versus stage 3	$P < 0.0001$
HNSC	Stage 2 versus stage 4	$P < 0.0001$
	Stage 3 versus stage 4	$P < 0.0001$
KIRC	Stage 1 versus stage 3	$P < 0.0001$
	Stage 1 versus stage 4	$P < 0.0001$
	Stage 2 versus stage 3	$P < 0.0001$
	Stage 2 versus stage 4	$P < 0.0001$
LUAD	Stage 1 versus stage 3	$P < 0.0001$
READ	Stage 1 versus stage 2	$P < 0.0001$
	Stage 1 versus stage 3	$P < 0.0001$
STAD	Stage 1 versus stage 2	$P < 0.0001$
	Stage 1 versus stage 3	$P < 0.0001$
	Stage 1 versus stage 4	$P < 0.0001$
UCS	Stage 1 versus stage 4	$P < 0.0001$
UCEC	Stage 1 versus stage 4	$P < 0.0001$
	Stage 2 versus stage 3	$P < 0.0001$
	Stage 2 versus stage 4	$P < 0.0001$
	Stage 3 versus stage 4	$P < 0.0001$
UVM	Stage 3 versus stage 4	$P < 0.0001$

[a]Analysis of variance.

BLCA, bladder urothelial carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; COAD, colon adenocarcinoma; ESCA, oesophageal carcinoma; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; LUAD, lung adenocarcinoma; READ, rectum adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

(partial correlation = –0.152, $P < 0.0001$; and significant positive correlations with the infiltration of CD4+ T cells (partial correlation = 0.17, $P = 0.001096$) and macrophages (partial correlation = 0.352, $P < 0.0001$).

In KIRC, the expression of the SPOCK1 gene had a significant inverse correlation with tumour purity (partial correlation = –0.171, $P = 0.000224$); and significant positive correlations with the infiltration of B cells (partial correlation = 0.175, $P = 0.000164$), neutrophils (partial correlation = 0.134, $P = 0.003934$) and dendritic cells (partial correlation = 0.123, $P = 0.00889$).

In LUAD, the expression of the SPOCK1 gene had significant inverse correlations with tumour purity (partial correlation = –0.169, $P = 0.000166$) and the infiltration of B cells (partial correlation = –0.151, $P = 0.000881$); and significant positive correlations with the infiltration of neutrophils (partial correlation = 0.179, $P < 0.0001$) and dendritic cells (partial correlation = 0.114, $P = 0.011486$).

In STAD, the expression of the SPOCK1 gene had significant inverse correlations with tumour purity (partial correlation = –0.129, $P = 0.011966$) and the infiltration of B cells (partial correlation = –0.111, $P = 0.033804$); and significant positive correlations with the infiltration of CD8+ T cells (partial correlation = 0.116, $P = 0.026166$), CD4+ T cells (partial correlation = 0.265, $P < 0.0001$), macrophages (partial correlation = 0.583, $P < 0.0001$), neutrophils (partial correlation = 0.212, $P < 0.0001$) and

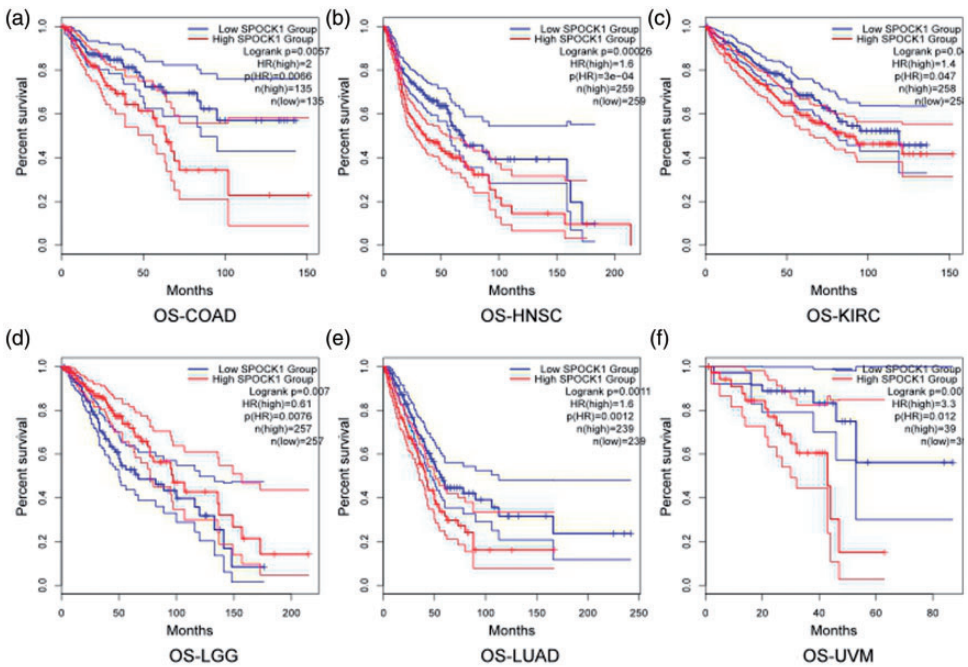

Figure 6. Kaplan–Meier analysis of the overall survival (OS) time between higher SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene expression and lower *SPOCK1* gene expression in The Cancer Genome Atlas tumour types. The other lines represent the 25% and 75% cut-offs. (a) COAD, colon adenocarcinoma; (b) HNSC, head and neck squamous cell carcinoma; (c) KIRC, kidney renal clear cell carcinoma; (d) LGG, brain lower grade glioma; (e) LUAD, lung adenocarcinoma; (f) UVM, uveal melanoma. The colour version of this figure is available at: http://imr.sagepub.com

dendritic cells (partial correlation = 0.368, $P < 0.0001$).

Discussions

The *SPOCK1* gene is overexpressed in prostate cancer, lung cancer, ovarian cancer, gastric cancer, colorectal cancer and breast cancer and plays a key role in cancer progression,[9,17,27–32] which suggests that *SPOCK1* could be a novel gene of interest in the search of new therapeutic targets. The *SPOCK1* gene participates in regulating cancer cell proliferation, cell cycle regulation, apoptosis, adhesion, cell–matrix interactions, metastasis and drug resistance in these cancers.[9,17,21–32] However, the underlying mechanism of action of the *SPOCK1* gene is still not completely understood. To address this lack of data, this current study implemented a comprehensive bioinformatics approach to discover the function of the *SPOCK1* gene in a pan-cancer setting. Analysis of *SPOCK1* gene expression was performed across all TCGA cancer types compared with TCGA normal tissues in addition to GTEx normal tissues. The results of this current study demonstrated that the *SPOCK1* gene was upregulated in several types of cancer, showing that the *SPOCK1* gene is functionally active in these tumours. The current analysis showed that in CHOL, PAAD, PRAD and STAD, the expression of the *SPOCK1* gene was notably higher in cancer tissues than in normal tissues. In BLCA, KIRC, LUAD and STAD, the differences between the pathological stages were statistically significant. The stages of the disease and the degree of deterioration are related, indicating that the *SPOCK1*

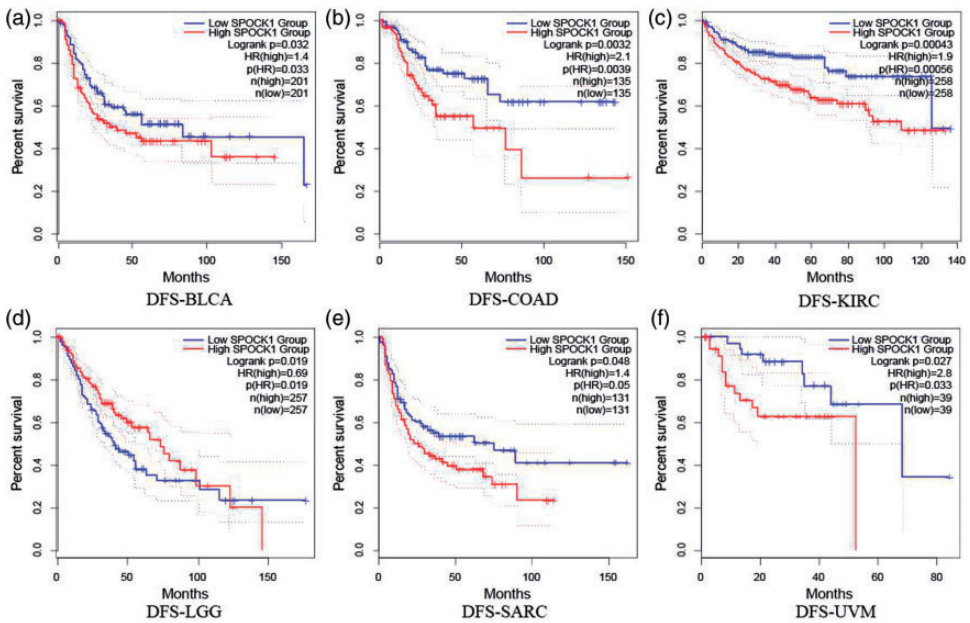

Figure 7. Kaplan–Meier analysis of the disease-free survival (DFS) time between higher SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene expression and lower *SPOCK1* gene expression in The Cancer Genome Atlas tumour types. (a) BLCA, bladder urothelial carcinoma; (b) COAD, colon adenocarcinoma; (c) KIRC, kidney renal clear cell carcinoma; (d) LGG, brain lower grade glioma; (e) SARC, sarcoma; UVM, (f) uveal melanoma. The colour version of this figure is available at: http://imr.sagepub.com

gene might govern the initiation and progression processes of these cancers. In the survival analysis, cancers (COAD, HNSC, KIRC, LUAD and UVM), which have highly elevated the *SPOCK1* gene expression, presented with worse overall survival than those that showed lower *SPOCK1* gene expression. As for the DFS time in the TCGA tumour types, this current research showed that higher expression levels of the *SPOCK1* gene resulted in worse DFS prognosis in BLCA, COAD, KIRC, SARC and UVM, emphasizing the importance of the *SPOCK1* gene for prognosis. Interestingly, in lower grade glioma of the brain, lower expression of the *SPOCK1* gene leads to worse OS and DFS, which might have correlations with the relatively higher expression of the *SPOCK1* gene in the brain;[33] and the underlying reason for this unusual phenomena deserves further investigation.

The *CHD1L*, *CGREF1*, *SERTM1*, *MMP14*, *MMP16*, *SPARC*, *SMOC1*, *TG*, *STS*, and *SPARCL1* genes correlated with the *SPOCK1* gene in the protein–protein network. Among the *SPOCK1* gene protein partners, matrix metalloproteinase 14 (*MMP14*) and *MMP16* are involved in the breakdown of the extracellular

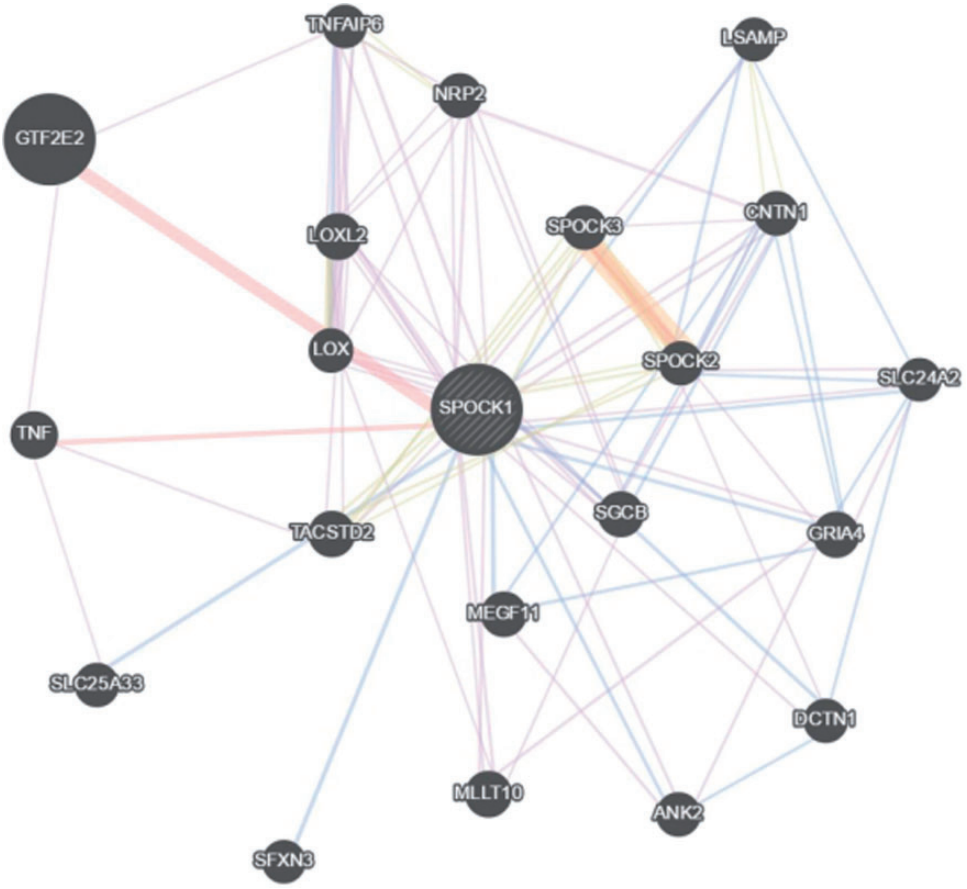

Figure 8. Genes in correlations with the SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene.

matrix.[34] The PPI network showed interactions between the *SPOCK1* gene and general transcription factor IIE subunit 2 (*GTF2E2*), tumour necrosis factor (*TNF*), lysyl oxidase like 2 (*LOXL2*) and multiple EGF like domrix in cancer metastasis (*MEGF11*); and with *CHD1L*, which is involved in DNA replication, repair and transcription processes.[35] The GSEA enrichment analysis showed that the *SPOCK1* gene might have positive interactions with the EMT process. Previous research also indicated that the *SPOCK1* gene mediates EMT signalling to regulate cancer cell progression and drug resistance.[36] Checkpoint immunotherapy has been studied comprehensively in recent years.[37] However, the underlying mechanisms of how tumour cells and immune cells interact remains elusive. This current study showed that the correlation between the *SPOCK1* gene and tumour-infiltrating immune cells might be of novel therapeutic value to the immunotherapy of cancers.

In conclusion, the *SPOCK1* gene has been demonstrated to be an oncogene that is involved in major oncogenic processes including cell-cycle control, DNA repair, apoptosis and metastasis. It may affect the migration and invasion of cancer cells in several cancers through the EMT process. It has been confirmed that the overexpression of the *SPOCK1* gene can promote the occurrence and development of tumours, suggesting that it may become a new anti-tumour therapeutic target. This current systematic pan-cancer analysis of *SPOCK1* gene expression in several cancer databases has provided evidence of the relationship between the altered expression of the *SPOCK1* gene and clinical outcomes. This current study uncovered the importance of *SPOCK1* gene expression and possible

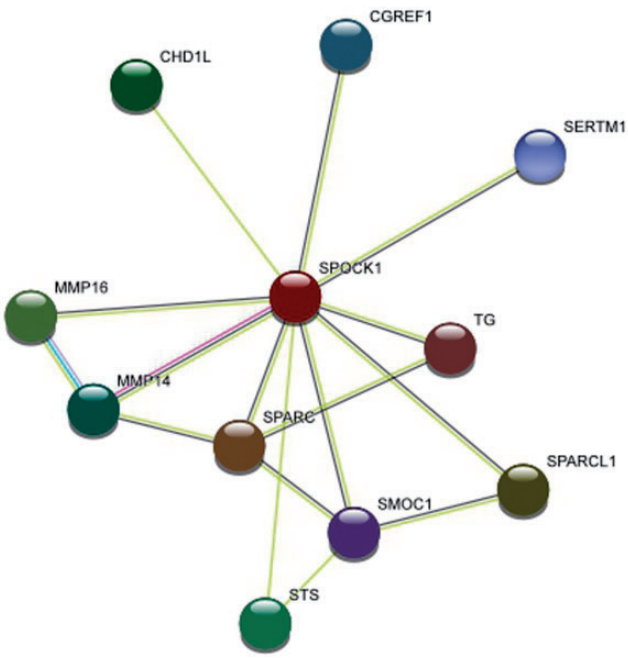

Figure 9. The protein–protein interactions with the SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene.

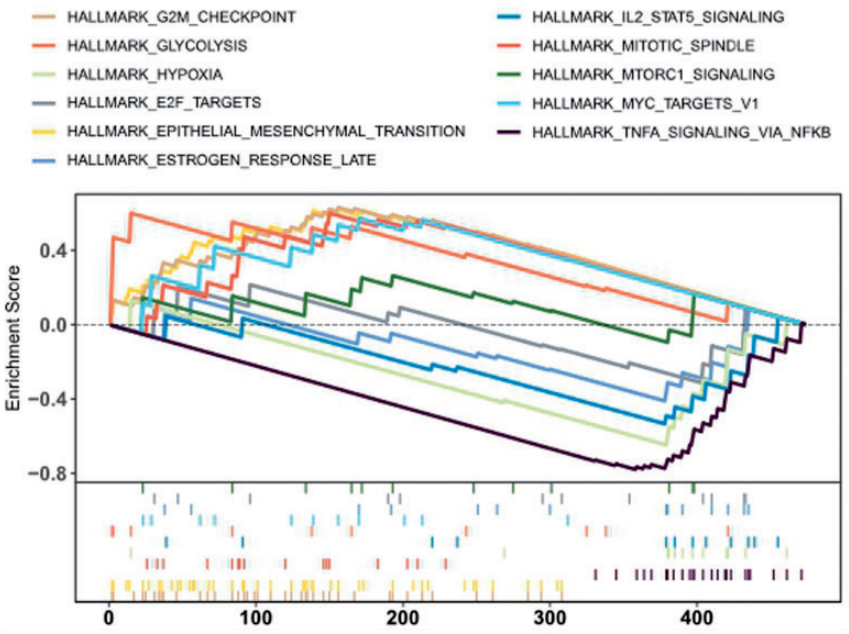

Figure 10. The Gene Set Enrichment Analysis enrichment analysis of the SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene. The colour version of this figure is available at: http://imr.sagepub.com

Figure 11. Correlation analysis between tumour-infiltrating immune cell signatures and the SPARC (osteonectin), cwcv and kazal like domains proteoglycan 1 (*SPOCK1*) gene. Spearman's rank correlation coefficient was used and a *P*-value <0.05 was considered statistically significant. BLCA, bladder urothelial carcinoma; KIRC, kidney renal clear cell carcinoma; LUAD, lung adenocarcinoma; STAD, stomach adenocarcinoma. The colour version of this figure is available at: http://imr.sagepub.com

SPOCK1-related proteins and pathways in cancer progression. Therefore, this current analysis may provide valuable insights into the *SPOCK1* gene as a potential biomarker and therapeutic target for various human cancers.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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