New understanding of SPAG4 as a potential tumor prognostic and immunotherapeutic marker

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Abstract

Human sperm-associated antigen 4 (SPAG4), as a member of the cancer-testis antigen(CTA) family, is considered to be a potential cancer marker. However, It is not known whether SPAG4 functions in the tumor immune microenvironment through some common mechanism. Here, we explored the oncogenic role of SPAG4 in 33 human tumors based on TCGA and GEO databases using web-based tools such as TIMER2.0, GEPIA2, TISIDE, ROC plotter, TIDE, and Western Blot experiments. The results showed that SPAG4 was highly expressed in 21 tumors and predicted a poor clinical prognosis in most tumors. Moreover, SPAG4 has an important role in the tumor microenvironment and is likely to be involved in the regulation of the immune microenvironment. Most importantly, SPAG4 expression was significantly associated with drug resistance and immune checkpoint blockade therapy in tumor patients, and could potentially regulate the occurrence of immune escape. In conclusion, our study suggests that SPAG4 is an immune oncogenic molecule that can be used as a biomarker for cancer detection, prognosis, treatment design and follow-up.

Introduction

Cancer testicular antigens (CTA) are a family of multifunctional proteins expressed mainly in tissues such as the testis and placenta, with little or low expression in other normal tissues, but the high expression in various tumor tissues. Gibbs, Z.A et al[1] suggested that the CTA family plays an important role in the regulation of cell proliferation, stem cell differentiation and carcinogenesis. However, in response to the current successful clinical practice of tumor immune-targeted therapy, the CTA family with specific expression patterns and tumor antigenicity has received the most attention in the study of tumor immunotherapy. The CTA family members TTK, NUF2, ACRBP, CEP55 and MPHOSPH1 have been reported to elicit cellular or humoral immune responses [2–5]. In addition, there is precedent for the use of MAGE and NY-ESO-1 antigenic peptides or proteins in the treatment of various tumors in past clinical work, with good efficacy. For example, in a clinical study, the MAGE-A3 antigen vaccine showed good efficacy against melanoma and exhibited low biotoxicity [6]. To improve the anti-tumor efficacy of CTA vaccines, some researchers have supplemented the vaccines with various adjuvants to activate CD4 + T cell immunity and support the activation, and maintenance of CD8 + CTL cells with tumor cytotoxicity [7]. All of the above studies suggest that members of the CTA family have great potential for tumor diagnosis, improved clinical prognosis and immunotherapy.

Sperm-associated antigens (SPAG) are a group of proteins that are hardly expressed in normal tissues except for sperm, but exhibit varying degrees of tumor-specific expression in various tumors. For example, SPAG1, SPAG5, SPAG8 and SPAG17 are highly expressed in tumor tissues such as pancreatic ductal carcinoma, cervical cancer, gastric cancer, lymphocytic leukemia and thyroid cancer, and are closely associated with tumourigenesis, metastasis, prognosis and immunotherapy [8–12]. Among them, Sperm-associated antigens 4 (SPAG4) has been increasingly reported in tumors since Kennedy, C et al [13] observed its high expression in tumor tissues by MTE microarrays and identified it as a tumor marker. It has been suggested that SPAG4 is an oxygen-regulated gene that regulates the hypoxic environment

through HIF [14] and is closely associated with glycolytic events in the tumor microenvironment [15]; high SPAG4 expression promotes migration and invasion of kidney cancer cells and may independently affect the clinical prognosis of kidney cancer patients [14, 16, 17]. In addition, SPAG4 is a tumor marker of glioblastoma and lung squamous cell carcinoma, and is also an important predictor of prognosis for glioblastoma, lung squamous cell carcinoma, pancreatic cancer and colorectal cancer [15, 18–20].

SPAG4, a member of the CTA family, has been hypothesized to be similarly linked to the tumor immune microenvironment[21–24]. However, there are no systematic analyses and reports on the association of SPAG4 with tumor immunity. Therefore, in this study, we first analyzed the expression of SPAG4 in 33 human tumors using the Oncomine, TIMER and GEPIA database. And verified the high SPAG4 expression in colon, liver and breast cancers by Western Blot. The correlation between SPAG4 expression and tumor prognosis was then analyzed by Kaplan-Meier Plotter and HPA databases. SPAG4 expression was also found to correlate significantly with the degree of immune infiltration, immune cell markers, 8 common immune checkpoints, tumor mutational burden(TMB) and microsatellite instability (MSI) in a variety of tumors by TIMER and GEPIA2. In addition, SPAG4 was found to be differentially expressed in immune and molecular subtypes from the TISIDB database in various human cancer types. Finally, using the ROC mapper, GDSC and TIDE database, we found that SPAG4 expression was associated with sensitivity to chemotherapeutic agents and immune checkpoint-targeted therapies in a variety of tumors. Most importantly, SPAG4 may regulate the occurrence of immune escape. In conclusion, this study aimed to explore the potential of SPAG4 in anti-tumor immunotherapy and to provide new targets for tumor immunotherapy.

Materials And Methods

2.1 Description of data analysis tools

Oncomine(https://www.oncomine.org/resource/login.html)[25] is a tool for analyzing gene expression differences and finding possible molecular markers based on known gene-drug analysis. TIMER2.0(http://timer.cistrome.org/)[26] is a web used to analyze the relationship between genes and the immune of TCGA tumors. GEPIA2 (http://gepia2.cancer-pku.cn)[27], a web-matched TCGA and GTEx, is an online server for gene expression analysis based on tumor and normal samples from the TCGA and the GTEx databases. HPA (http://www.proteinatlas.org/) was examined in detail for the expression of each protein in 64 cell lines, 48 human normal tissues, and 20 tumor tissues using immunoassay techniques. Kaplan-Meier Plotter(http://kmplot.com/analysis/index)[28] integrates gene expression information and clinical prognostic data for Meta-analysis and discovery of survival-related molecular markers. TISIDB (http://cis.hku.hk/TISIDB/index.php)[29] is an integrated repository portal for tumor-immune system interactions. GSCA(http://bioinfo.life.hust.edu.cn/GSCA/#/)[30] is an online algorithm for integrating genomic and immunogenomic data of TCGA tumors, drug responses from Genomics of Drug Sensitivity in Cancer (GDSC). The ROC plotter (http:// www. rocplot. org/)[31] is capable to link gene expression and response to therapy using transcriptome-level data. TIDE(http://tide.dfci. Harva rd. edu/)[32] is used to infer the function of genes that regulate tumor immunity, to synthesize the immune

dysfunction and rejection of tumor immune escape mechanism, and thus to effectively predict the effect of immune checkpoint suppression therapy. And Sangerbox (http://www.sangerbox.com/) is a free and helpful online platform for data analysis.

2.2 The expression analysis

We first used Oncomine and TIMER2.0 to compare the expression levels of SPAG4 in tumor tissues and paired normal tissues. There are not include normal tissues for some cancers in TIMER2.0 are used GEPIA2 to reveal the expression. Then three cases of colon cancer, four cases of liver cancer and four cases of breast cancer tumor tissue collected from *The Third Xiangya Hospital of Central South University,* were used to verify the protein expression of SPAG4 by standard Western Blot steps. Primary antibody: The rabbit anti-SPAG4 antibody (Prpteintech, Wuhan,China), The mouse anti-GAPDH antibody (Prpteintech,Wuhan,China). Besides, GEPIA2 was also used to explore the expression level of SPAG4 in different stages (stage I, II, III, IV) of different cancers.

2.3 The immune and molecular subtypes analysis

Using TISIBD, we analyzed the association of SPAG4 expression with the immune and molecular subtypes of each tumor, Differences with a p-value < 0.05 were considered to be statistically significant.

2.4 The survival prognosis analysis

We used Kaplan-Meier plotter to obtain the prognostic value of SPAG4 expression in patients with each tumor. The relationship between SPAG4 expression levels and overall survival (OS) (n=7462) and relapse-free survival(RFS) (n=4420) of tumors, while 95% confidence intervals and log-rank p-values of Hazard ratios (HRs) were obtained. Moreover, we also obtained the prognostic value of SPAG4 expression levels in patients with endometriosis, colorectal, glioma, renal, and urothelial cancer from the HPA website.

2.5 The immune correlation analysis

TIMER and GEPIA2 were used to analyze the relationship of SPAG4 expression with immune cells, immune cell markers and 8 common immune checkpoints(CD274、CTLA4、HAVCR2、LAG3、PDCD1、PDCD1LG2、SIGLEC15、TIGIT) in tumors. In addition, we obtained radar plots of tumor mutational burden (TMB) and microsatellite instability (MSI) of SPAG4 in each tumor from the Sangerbox website.

2.6 The drug sensitivity and therapeutic responsiveness analysis

We analyzed the effect of SPAG4 expression on the sensitivity of each tumor cell line to GDSC drugs via the GSCA web tool, and the top30 correlation results are shown. To analyze the effect of SPAG4 on treatment response, The ROC plotter was used to analyze the correlation between SPAG4 at the transcriptome level and the response to the corresponding drug treatment in patients with breasts, glioblastoma, colorectal and ovarian cancers. And we also obtained the correlation of SPAG4 with immune checkpoint inhibitor therapy, the tumour immune checkpoints include PD-1 (CD279), PD-L1

(CD274) and CTLA4 (CD152). Finally, we analyzed the correlation between SPAG4 expression levels and cytotoxic T cell levels (CTL) using the TIDE website, and also assessed the relationship between SPAG4 expression and responsiveness to immune checkpoint blockade therapy in patients with BLCA, GBM, KIRC, and Melanoma using Kaplan-Meier curves.

2.7 The statistic analysis

Western Blot phenotypic results were quantified using *imagej*, and then statistically analysed using *Graphpad Prism 8* for paired t-tests. And all resulting images were embellished using *Adobe Photoshop 2020* and *Adobe Illustrator 2020*. In this study, $p<0.05$ is considered to have significant correlation, where * represents $p < 0.05$, ** represents $p <0.01$, *** represents $p <0.001$.

Result

3.1 SPAG4 is highly expressed in most human tumors

Oncomine database was used to analyze the expression levels of SPAG4 in human tumors. We found that the SPAG4 expression is higher in kidney, lung, bladder, brain and CNS, breast, cervical, colorectal, esophageal, liver, ovarian cancers, and leukemia compared to normal tissues (Fig.1A). And its lower expression was observed in pancreatic cancer, myeloma, and other cancers. We also used the TIMER2.0 database to obtain the expression level of SPAG4 in all TCGA tumors, and the result of GEPIA2 is displayed as the supplementary results of the tumors without adjacent normal tissues. The difference in SPAG4 expression in tumors and adjacent normal tissues was shown in the Fig.1B and Fig.1C, SPAG4 is significantly higher in 21 tumor tissues than adjacent tissues. While in PRAD ,LAML, LGG , and TGCT tumor tissue, is lower than adjacent tissues. And SPAG4 highly expressed in SKCM metastasis tissues than in SKCM tumor tissues, in HNSC-HPV+ tumors also higher than HNSC-HPV- tumors. To further analysis the expression of SPAG4 in human tumors, we performed western blot using clinical tumour samples to confirm the SPAG4 protein level in tumour samples and adjacent tissues (Figure.1D). The results showed that SPAG4 protein was upregulated in COAD, LIHC, BRCA samples. And then, the GEPIA2 database was used to study the SPAG4 expression level in different stage of TCGA tumors. There is an obvious difference in different tumor stages of BRCA, HNSC, KICH, KIRC, LIHC, PAAD, and THCA (Fig.1E, all $p<0.05$).

3.2 SPAG4 is differentially expressed in different molecular and immune subtypes

As previously described, SPAG4 is differentially expressed in SKCM metastatic and non-metastatic, HPV- and HPV+ HNSC, and in different tumor stages, but it is not known whether SPAG4 is differentially expressed in different molecular and immune subtypes of tumors. Next, we explored the SPAG4 expression in different immune and molecular subtypes among human tumors with the TISIDB website. We can observe that the expression of SPAG4 is correlated with the immune subtypes, and there is a different SPAG4 expression level in the different immune subtypes of tumors (Fig.2A). Take the KIRC as an example, we can find that SPAG4 was expressed in all six subtypes C1 (wound healing), C2 (IFN-

gamma dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet), C6 (TGF-b dominant), and its expression was gradually decreased in C1-C5 subtypes but increased in C6 subtypes. For different molecular subtypes of cancers, significant differences for SPAG4 expression can be observed in ACC, BRCA, COAD, LGG, ESCA, LUSC, STAD, and UCEC(Fig.2B), all the $p<0.05$.

3.3 SPAG4 is a potential prognosis biomarker for human tumors

In order to better understand the relevance of SPAG4 expression in tumors, the prognosis value of SPAG4 was analyzed in Kaplan-Meier plotter and HPA database. In Fig.3A, higher SPAG4 expression is associated with adverse overall survival(OS) in 8 tumors: CESC, KIRC, LIHC, LUSC, PAAD, READ, SARC and THYM. But in BLCA, PCPG, OV and UCEC, is associated with better OS. To further examine the prognostic potential of SPAG4, We also investigated the correlation between SPAG4 and relapse-free survival(RFS) in different tumors. As shown in Fig.3B, high SPAG4 expression implies poor RFS in CESC, KIRC, LIHC, LUSC and STAD, while low SPAG4 expression has a worse RFS in UCEC and KIRP. Moreover, in HPA database(Fig.3B), SPAG4 highly expression is an unfavorable prognostic maker association with patient survival in colorectal, glioma and renal cancer ($p <0.001$), but in endometrial cancer and urothelial cancer is favorable ($p<0.001$). These results confirmed the prognostic value of SPAG4 in some specific types of cancers, and that increased and decreased SPAG4 expression have different prognostic value depending on the type of tumor.

3.4 SPAG4 correlates with immune cell infiltration in the TME

Tumor-infiltrating immune cells, which are the important components of the tumor microenvironment(TME), were closely linked to the occurrence and development of tumors. Therefore, after demonstrating the differential expression of SPAG4 in different immune subtypes, we explored the potential relevance of SPAG4 expression to immune cell infiltration in human tumors. Over here, the TIMER2.0 database was used to evaluate the correlation in every type of TCGA tumor by different algorithms. In general, the results showed that SPAG4 was positively correlated with the degree of infiltration of B cells, macrophages, CD8+ T cells and CD4+T cells in a variety of tumors(Fig.4). Such as we find that SPAG4 positively correlate with the B cell infiltration level in most TCGA tumors in at least five or more algorithms. But the interesting thing is that in GBM, PAAD,THYM, BLCA and KIRP, SPAG4 exhibited a negative correlation with B cells, macrophages, CD8+T cells and CD4+T cells, regardless of which algorithm was used. The trend of this correlation was slightly different, which may be due to the other immune infiltration rates of certain tumors. Similarly, we also found that SPAG4 expression showed a positive correlation with immunosuppressive regulatory cells(macrophages-M2, CAF, Tregs, MDSC) in most tumours, this implies that SPAG4 has the potential to play a role in regulating immune escape in tumours.

3.5 Correlation analysis between SPAG4 and immune marker genes, ICP, TMB and MSI

To further elucidate the relationship between SPAG4 expression and diverse immune infiltrating cells. We focused on the correlation between SPAG4 and various immune marker genes of CD4+T cells, CD8+T

cells, B cells, macrophages(M1 and M2), neutrophil, dendritic cells(DC) in each tumor of TIMER2.0(Fig.5A) and overall human tumors of GEPIA2(Table.1). The result shows that the expression of SPAG4 is positively related to the least two more makers of immune cells in most tumors except the BRCA-Her2, DLBC, KICH, KIRC, KIRP, MESO, OV, PAAD, and UVM. Among these tumors, such as in BRCA-Her2, SPAG4 is only positively related to the VSIG4 maker of macrophage M2 but is only negatively related to the NOS2 maker of macrophage M1 in KICH(Fig.5A). As shown in the Table.1, we were surprised to find that SPAG4 showed positive correlation with almost all DC cell marker genes(HLA-DPB1, HLADQB1, HLA-DRA, HLA-DPA1, NRP1) in overall human tumors of GEPIA2. These results seem to indicate a strong relationship between SPAG4 and DC infiltration, but this needs to be confirmed by further studies.

We know that immune checkpoints(ICP) are essential for immune cell infiltration and immunotherapy. Subsequently, we also analyzed the correlation between SPAG4 expression and the 8 common ICP genes in every tumor and overall human tumors via TIMER2.0 and GEPIA2. The result of the correlation between them in every tumor can be observed in the Fig.5B, take the PRAD, SKCM, and THCA as example, there is a positive correlation between SPAG4 and the 7 common checkpoints, only except the CD274 checkpoint, but in the KIRC and PAAD is a negative correlation between them. In Fig.5C, we can find that SPAG4 is positively related to the HAVCR2, LAG3 and PDCD, but negatively related to the CD274 in overall human tumors.

There are many genes can exert anti-tumor immune effects by regulating tumor mutational burden (TMB) and microsatellite instability (MSI) in the tumor microenvironment. Therefore, to explore the anti-tumor immune mechanism of SPAG4 and its role in immune response, we analyzed the correlation between SPAG4 expression and TMB and MSI in each tumor(Fig.5D). For TMB, there is a statistically positive correlation in THYM, UCEC, ESCA, KIRC, KIRP, LGG, LIHC, LUAD and PAAD, and negative correlation in COAD, PCPG and THCA. For MSI, which is statistically positive correlate with SPAG4 expression in DLBC, KICH, THCA, but statistically negative correlation in COAD, LUSC and STAD. The above results tentatively validate our speculation that SPAG4 may affect antitumor immunity by regulating TMB and MSI in TME.

3.6 SPAG4 is associated with therapeutic responses

Finally, we analyzed the association between SPAG4 expression and the sensitivity of different clinical chemotherapeutic agents to various human tumor cell lines. We found that high SPAG4 expression was associated with reduced sensitivity of cancer cell lines to more than 20 antitumor drugs, such as BX-912 (PDK1 inhibitor), I-BET762 (BRD inhibitor), TPCA-1 (IKK-2 inhibitor) and Vorinostat (HDAC6 inhibitor). However, it resulted in increased sensitivity of various cancer cell lines to CHIR-99021 (GSK-3α/β inhibitor), FTI 277 (FTase inhibitor), and TGX-221 (PI3K inhibitor) (Fig.6A). Furthermore, we evaluated the effect of SPAG4 expression on chemotherapeutic responses in clinical cancer cohorts. We found that glioblastoma(Topoisomerase inhibitor), ovarian(Platin & Taxane), and breast(Fluorouracil, Epi-Amycin, Cyclophosphamide, FEC) cancer patients with higher SPAG4 expressions benefited more from

chemotherapies, but colorectal cancer(Oxaliplatin) patients were resistant to chemotherapies than cohorts with lower expression(Fig.6B).

In addition, based on the above results, we analyzed the predictive role of SPAG4 in cancer patients treated with immune checkpoint inhibitors (ICIs). We found that high SPAG4 expression would lead to reduced sensitivity of tumor patients to immunosuppression of PD-1 and CTLA-4, while showing the opposite result for PD-L1(Fig.6C). Next, to observe the effect of SPAG4 on the efficacy of immune checkpoint inhibitor therapy in specific tumour patients, we explored the relationship between gene expression and treatment outcome in clinical studies of immune checkpoint blockade using the TIDE database. We can find that in GBM, KIRC and Melanoma, SPAG4 expression was positively correlated with the level of cytotoxic T lymphocytes(CTLs) infiltration, but that patients with low SPAG4 expression had a better prognosis after immune checkpoint blockade(PD-1 or PD-L1) treatment. Moreover, the results were different in BLCA (Fig.6D, upper image). In the BLCA clinical cohort, SPAG4 expression negatively correlated with the level of CTL infiltration, but its high expression predicted a better prognosis after immune checkpoint blockade(ICB) treatment (Fig.6D, lower image). Although they were not statistically significant, this could still suggest that SPAG4 may have an important regulatory role in both immune escape, which needs to be studied in depth for different types of tumors.

Discussion

SPAG4 is also an important member of the SUN protein family, also known as SUN4. It is a nuclear membrane protein consisting of a conserved SUN structural domain at the C-terminus, a transmembrane region at the N-terminal and a helical structural domain in the middle. In recent years, the role of SPAG4 in tumors has received much attention from researchers, but there are fewer relevant systematic analyses. Here, we used the Oncommine, TIMER2.0 and GEPIA2 databases for the first time to determine the expression levels of SPAG4 in tumors and normal tissues, and found that SPAG4 was significantly highly expressed in most tumors, which is consistent with previous findings in renal and lung cancers [14, 16, 33]. However, in tumor tissues such as pancreatic cancer, myeloma, low-grade brain tumor, acute myeloid leukemia, testicular cancer and prostate cancer, the expression levels were lower than adjacent tissues. This suggests that probably in the same way that downregulation of SUN1, a member of the same family with SUN4, affects the formation of LINC complexes and increases the motility of breast cancer cells thus promoting distant metastasis [34]. And SUN2, another member, downregulation promotes colon cancer development through the SUN2-SIRT1-MeCP2-BDNF signaling axis [35], downregulation of SPAG4 expression may also be an important mechanism for tumorigenic and progressive.

Meanwhile, we found that high expression of SPAG4 in most tumor tissues predicted shorter survival time. But according to the results of Kaplan-Meier mapper, high expression of SPAG4 in BLCA, PCPG, OV and UCEC had longer survival time. In particular, high SPAG4 expression in BLCA and UCEC was significantly associated with good overall survival (OS) and recurrence-free survival (RFS). In addition, we noted that SPAG4 was lowly expressed in pancreatic cancer and leukemia in the Oncomie database, whereas it was highly expressed in them in the TIMER2.0 database. Taking pancreatic cancer as an

example, we speculate that there are three possibilities to explain this phenomenon. Firstly, this could be caused by algorithmic differences between different databases, and also caused by the differences in the non-cancerous tissues (paracancerous or normal tissues) selected as references. Secondly, previous studies have shown that SPAG4 expression is high in normal pancreatic tissue [13], which may lead to its relatively low expression in cancerous tissues. Finally, the number of samples tested was too small, as only four paired normal pancreatic cancer tissues could be found in TIMER2.0, which may have resulted in the inability to accurately assess the expression level of SPAG4. However, similar to the results reported by several current prediction models for SPAG4 in pancreatic ductal carcinoma, colon cancer and lung cancer [18–20]. Our results in the Kaplan-Meier mapper and the HPA database suggest that high SPAG4 expression predicts a poor prognosis in most tumors. So in any case, we consider SPAG4 as a very promising pan-cancer prognostic biomarker.

Our results showed that SPAG4 significantly correlated with the tumor immune microenvironment. It showed a positive statistical correlation with the degree of immune cell (B cell, CD8 + T cell, CD4 + T cell, macrophages) infiltration and four immunosuppressive cells (CAF, Tregs, M2-TAM, MDSC) in multiple tumors. Moreover, we found that SPAG4 expression levels showed different correlations with the level of CTLs in different tumors and different survival outcomes after ICB treatment. In cohorts with high SPAG4 expression, clinical survival was worse in GBM, KIRC and melanoma patients treated with ICB(PD-1 or PD-1L), even though SPAG4 showed a positive correlation with CTL. In contrast, in the BLCA clinical cohort, SPAG4 showed a negative correlation with CTL, but high SPAG4 expression implied a better outcome. We speculate that this may result from the involvement of SPAG4 in the regulation of tumor immune escape. The two main mechanisms of immune escape are currently considered to be immune cell infiltration leading to T cell deficiency or dysfunction [36, 37] and T cell rejection [38]. The interaction between the tumor microenvironment and its important component, tumor immune cells, is a key mechanism of immune escape, physiological resistance and local and systemic metastasis of tumor cells [39]. Among them, regulatory B cells can suppress the body's immune response to tumors through immunosuppressive factors (IL-10, TGF-β, etc.)[40, 41]. In addition, the presence of immunosuppressive cells (CAF, Tregs, M2-TAM, and MDSC) is usually crucial for the immune escape of tumor cells. For example, tumor-associated macrophages have both M1 (inflammatory macrophages) and M2 (non-inflammatory macrophages) phenotypes. The interaction between the tumor immune microenvironment and tumor immune cells allows tumor-associated macrophages to switch from the M1 phenotype to the M2 phenotype, thereby suppressing the immune response or promoting tumor metastasis [42, 43]. CAF are a group of activated fibroblasts that secrete a variety of active factors that play a role in regulating tumor immune surveillance, local tissue metabolism, tumor cell biological behavior(e.g., survival, proliferation, self renewal and stemness, invasion and metastasis) [44]. Therefore, combined with the results of our study, SPAG4 is likely to be involved in the regulation of both immune escape mechanisms mechanisms.

In summary, our first pan-cancer study of SPAG4 showed that SPAG4 is highly expressed in most tumors and usually implies a poor clinical prognosis. Furthermore, the statistical correlation between SPAG4 expression and tumor molecular subtypes, tumor immune subtypes, degree of immune cell infiltration,

tumor mutational burden, microsatellite instability, immune checkpoints, and susceptibility to immunosuppressive therapy, especially the possible involvement in the regulation of immune escape mechanisms, strongly suggests the possibility of a key pathogenic role of SPAG4 in the immuno-oncological context of TME, and affects the prognosis and therapeutic response of tumor patients. In conclusion, our study suggests that SPAG4 is a significant marker for the prognosis and immunotherapy of tumor patients.

Declarations

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Author contributions

Jianlong Wang and Pan Zhang conceived and designed the overall study. Wanjiang Feng, Guowei Wang, Xiaobo Zhang and Xiaoyue Song collected and reviewed the data. Wanjiang Feng, Guowei Wang, Xiaobo Zhang and Sha Cheng analyzed and visualized the data. Wanjiang Feng and Guowei Wang conducted WB experiments and prepared the original manuscript. All authors reviewed and approved the final manuscript.

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Availability of data and materials

The data sets analysed during this study are available in public, open access repositories listed in this article. Please see the methods and materials section for details.

Ethics approval and consent to participate

The study involving human participants was reviewed and approved by *The Third Xiangya Hospital of Central South University*. Patients/participants gave informed consent to this study.

Consent for publication

All authors agree to publish the manuscript as the final version.

Competing interests

The authors declare no potential conficts of interest.

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Table

Table 1 is available in the Supplementary Files section

Figures

Figure 1

(A).The expression of SPAG4 in different tumor tissues and normal tissues in the Oncomine database. (B)SPAG4 expression in different cancer types from the TCGA database analyzed by the TIMER2.0 database. (*p< 0.05, **p< 0.01, ***p< 0.001). Tumor names marked in yellow: represents no corresponding normal tissue, in cyan: represents different tumour staging, in blue: represents high

expression of SPAG4 in normal tissue. (C)SPAG4 expression in several cancers and paired normal tissue in the GEPIA2 database. Red: represents high expression of SPAG4 in tumours, blue has the same meaning as above. (D)Western blot protein detection of the SPAG4 expression levels in paired COAD, LIHC, BRCA and adjacent normal tissues, the dotted line graph on the right is a statistical analysis of the left graph. (E)SPAG4 expression in different stage(stage I,II,III,IV) of BRCA, KICH, KIRC, LIHC, PAAD and THCA.

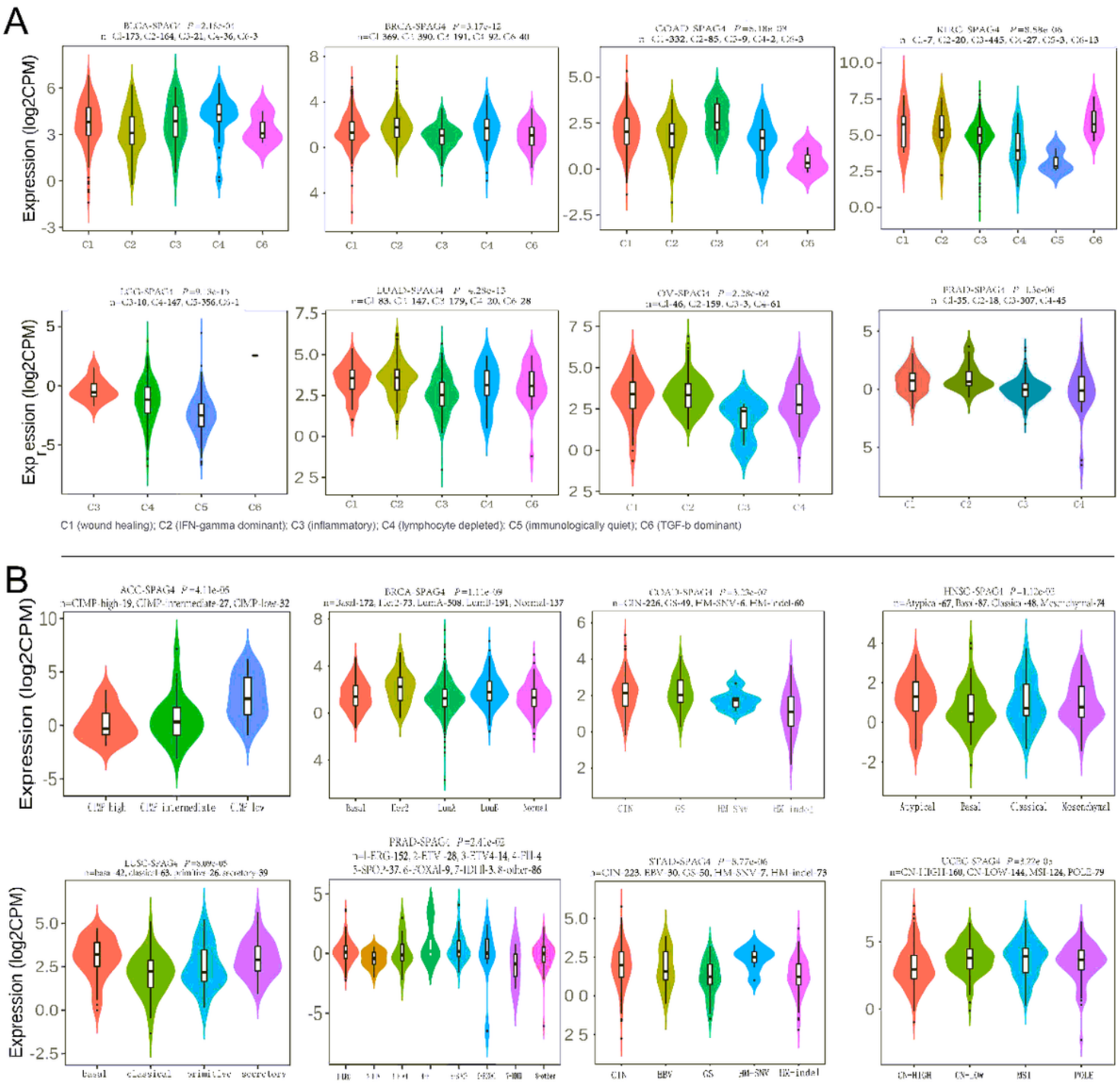

Figure 2

(A).The relationship between SPAG4 expression and pan-cancer immune subtypes in BLCA,BRCA,COAD,KIRC,LGG,LUAD, OV, PRAD. (B).The relationship between SPAG4 expression and pan-cancer immune subtypes in ACC,BRCA,COAD,HNSC,LUSC, PRAD,STAD,UCEC.

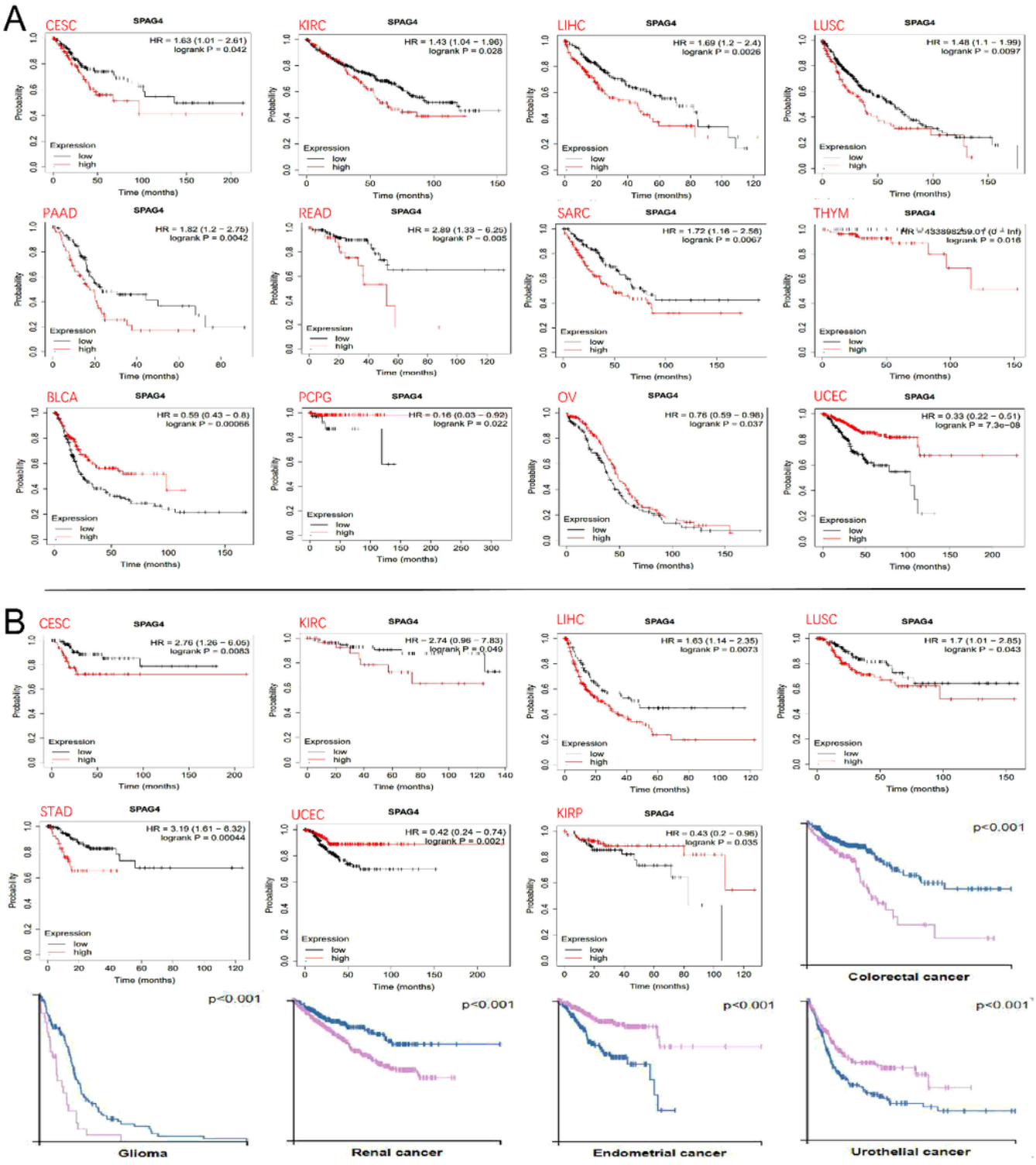

Figure 3

(A).High SPAG4 expression is related to worse OS in CESC, KIRC, LIHC, LUSC, PAAD, READ, SARC and THYM cohorts, but is related to better OS in BLCA, PCPG, OV, and UCEC cohorts. (B).Low SPAG4 expression is related to better RFS in CESC, KIRC, LIHC, LUSC, and STAD cohorts, but worse RFS in UCEC and KIRP cohorts. And high SPAG4 expression(red line) is related to a worse prognosis in colorectal, glioma and renal cancer, low expression(blue line) means worse prognosis in endometrial and urothelial cancer (Fig.3B last 5 survival curve graph).

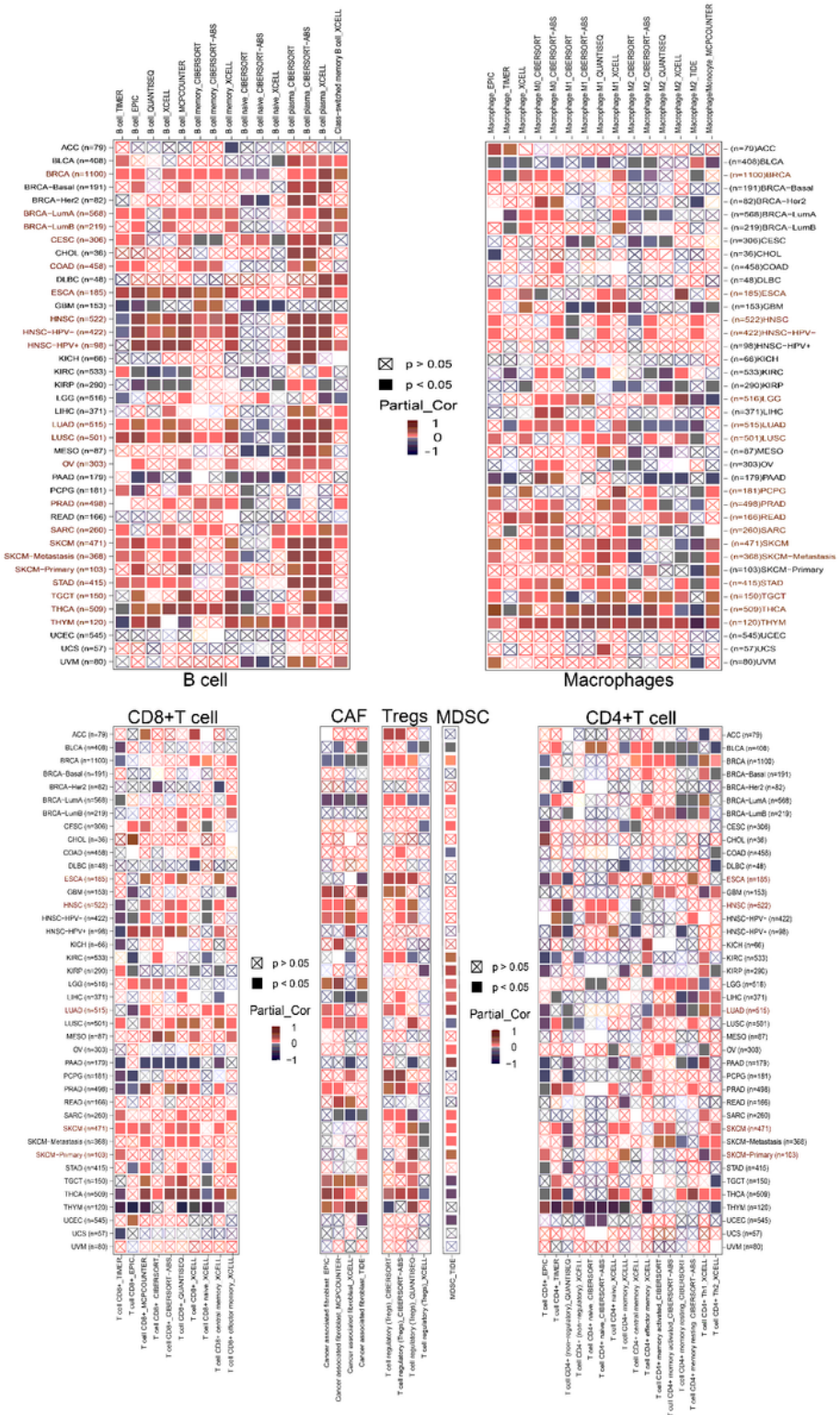

Figure 4

These heatmaps show a significant correlation of SPAG4 expression with immune cells:B cells, macrophages, CD8+T cells, CAF, Treg, MDSC and CD4+ T cells in a variety of human tumours, red represents positive correlation and blue represents negative correlation. Tumor names marked in red represent at least three algorithms indicating that SPAG4 is significantly positively associated with immune cells in this tumor.

Figure 5

(A).The relationship between SPAG4 and immune cell makers genes in every tumor. (B.C).The relationship between SPAG4 and 8 common checkpoint genes in every tumor and in overall human tumors. red represents positive correlation and blue represents negative correlation in the heatmap. (D).The correlation of SPAG4 expression with TMB and MSI in human tumors. Tumor names marked in red represent positive relationship and in blue represent negative relationship. (Table.1).The relationship between SPAG4 and immune cell makers genes in all tumor analyzed by GEPIA2 dataset. In the heatmap, tumor names marked in red or blue represent SPAG4 is positively associated with at least there maker genes or ICP

Figure 6

(A).Bubble plot of the correlation between drug sensitivity and mRNA expression of SPAG4 in the GDSC database. The colours from blue to red indicate mRNA expression versus 50% inhibitory concentration (IC50) values. A positive correlation means that high expression of the gene is resistant to the drug, and vice versa. (B). ROC plots of the association between SPAG4 expression and chemotherapy response in the breast, brain, colorectal and ovarian cancer cohorts. (C). ROC plots of the association between SPAG4 expression and response to anti-immune checkpoint (PD-1,PD-L1,CTLA-4) therapy. (D).Kaplan-Meier curves of survival (top panel) as a measure of immunotherapy response (immune checkpoint blockade) between cancer cohorts with high and low levels of SPAG4 expression. The graph below shows the correlation between SPAG4 expression and cytotoxic T cell levels (CTL) in these cohorts.

Supplementary Files

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

- Table1.xlsx