SHP2 is involved in the occurrence, development and prognosis of cancer

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Abstract. Src homology-2 domain-containing protein tyrosine phosphatase (SHP2), encoded by protein tyrosine phosphatase non-receptor type 11 (PTPN11), is widely expressed in several human tissue types, and plays an important role in a variety of diseases. The present study assessed the impact of SHP2 on the occurrence, development and prognosis of solid tumors. The transcriptome sequencing data of 33 cancer types were downloaded from The Cancer Genome Atlas database. Clinical information of the corresponding patients, tumor mutational burden and information pertinent to microsatellite instability were also downloaded. The log-rank test and univariate Cox's regression test were used to evaluate patient survival. The 'ESTIMATE' method was used to assess the tumor microenvironment, and the 'CIBERSORT' algorithm was used to evaluate tumor immune cell infiltration. Spearman's correlation analysis was used to evaluate the correlation between SHP2 expression and the targets identified. ELISA was used to assess the SHP2 expression levels in peripheral blood samples of patients with breast, ovarian, endometrial and cervical cancer. The data indicated that the expression levels of SHP2 were increased in a variety of tumor tissues, and were associated with tumor progression and prognosis. In peripheral blood, the positive rates of SHP2 expression in breast cancer (71.43%) and ovarian cancer (58.82%) were significantly higher than those in the corresponding control groups. However, the positive rates of SHP2 expression in patients with endometrial cancer (31.03%) and cervical cancer (41.30%) were significantly lower than those in the corresponding control groups. Increased SHP2 expression improved overall survival (OS) and disease free survival (DFS) time in patients with kidney renal clear cell carcinoma. However, increased SHP2 expression reduced OS and DFS in patients with urothelial carcinoma, and cervical and endocervical cancer types. Moreover, the elevated expression of SHP2 could also reduce the OS of patients with breast invasive carcinoma, mesothelioma and liver hepatocellular carcinoma. PTPN11 expression was associated with the tumor microenvironment of various tumor types. The tumor mutational burden of various tumor types was associated with microsatellite instability. PTPN11 inhibited T-cell activation and promoted M2 macrophage activation in several tumors. Therefore, SHP2 may be used in the evaluation of tumor progression and prognosis, and it may be an optimal potential biological target for cancer therapy.

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Abbreviations: ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancer; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DFS, disease-free survival; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal 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 leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; NSCLC, non-small cell lung cancer; OS, overall survival; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TAMs, tumor-associated macrophages; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma

Key words: protein tyrosine phosphatase non-receptor type 11, src homology-2 domain-containing protein tyrosine phosphatase, prognosis, tumor microenvironment

Introduction

Src homology-domain-containing protein tyrosine phosphatase (SHP) is a protein tyrosine phosphatase (PTP) family member. SHP comprises SHP1, encoded by protein tyrosine

phosphatase non-receptor type (PTPN) 6, and SHP2, encoded by PTPN11 (1,2). SHP2/PTPN11 is considered a signaling molecule that is involved in the regulation of a number of cellular processes, such as cell growth, differentiation, the mitotic cycle and oncogenic transformation. SHP2 is widely expressed in a variety of human tissues, such as breast and endometrial cancer (3,4).

SHP2 regulates cell proliferation, differentiation, apoptosis and survival, affecting multiple signaling pathways, such as the mitogen-activated protein kinase pathway. The abnormal expression and mutation of SHP2 are related to human developmental disorders, leukemia and solid tumors (such as lung, breast, ovarian and endometrial cancer) (3,4). In different cancer types, activation of SHP2 has been proposed as a disease cause. For example, Kim *et al* (5) indicated that SHP2 was not expressed in normal gastric mucosal cells, while it was found to be expressed at high levels in 87% of gastric cancer tissues, and was markedly related to the progression of gastric cancer. Patients with gastric cancer and high SHP2 expression demonstrate higher pathological grades, tumor stage (T stage) and lymph node stage. Elevated expression of SHP2 indicates a poor prognosis in patients with gastric cancer. SHP2 is widely upregulated in invasive ductal carcinoma of the breast, and is associated with HER2 expression, androgen receptor nuclear localization, T stage and lymph node metastasis (6,7). Inhibition of SHP2 expression can hinder the development of receptor tyrosine kinase (RTK)-driven malignant growth cells, such as those derived from KRAS-mutated breast carcinoma. In non-small cell lung cancer (NSCLC) with a KRAS mutation, the blockage of SHP2 expression is enough to induce tumor senescence, which triggers the clearance of cancer cells by the immune system (8). A limited number of studies have demonstrated that SHP2 expression is decreased in certain cancer types. For example, Jiang *et al* (9) observed that SHP2 expression was reduced in 70.6% of patients with liver cancer, and decreased SHP2 expression was associated with a poor prognosis in hepatocellular carcinoma. Therefore, given the close relationship between SHP2/PTPN11 and human diseases, and its wide expression in tumor tissues, as well as the dual function of SHP2/PTPN11 as a tumor suppressor and tumor-promoting gene in tumors, the comprehensive discussion of SHP2/PTPN11 has important clinical significance. The present study assessed the expression levels of SHP2 in different tumors, and its relationship with prognosis, immunity and the tumor microenvironment.

Materials and methods

Data acquisition and pre-processing. SHP2 expression levels of the 33 types of tumor with associated data in The Cancer Genome Atlas (TCGA) database and the corresponding tumor RNA-sequencing data were downloaded from the Genomic Data Commons data portal website (https://portal.gdc.cancer.gov/). Clinical information (SHP2 expression level, follow-up time and tumor type), tumor mutational burden and microsatellite instability of the corresponding patients were also downloaded. The 33 types of tumors included the following: Adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical and endocervical cancer (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML), brain lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), mesothelioma (MESO), ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectal adenocarcinoma (READ), sarcoma (SARC), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), testicular germ cell tumors (TGCT), thyroid carcinoma (THCA), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), uterine carcinosarcoma (UCS) and uveal melanoma (UVM) (10).

Expression levels, prognostic evaluation and the tumor microenvironment. The R 4.2.2 software (R Core Team; www.r-project.org) was used for statistical analysis. Univariate Cox regression analysis was used to evaluate the association between SHP2 gene expression, overall survival (OS) and disease free survival (DFS); the use of forest plots through the 'forest plot' R package (version 3.1.1) aimed to display the P-value, hazard ratio (HR) and 95% confidence interval (CI) of every variable (11). According to the median value of the SHP2/PTPN11 gene expression, patients were divided into SHP2/PTPN11 high and SHP2/PTPN11 low expression groups. The Kaplan-Meier method was used to draw the survival curve of every tumor type, and the log-rank test was used to analyze survival status.

The matrix and immune scores of every patient were calculated using the 'ESTIMATE' software package (version 1.0.13) to evaluate the infiltration of immune cells in tumor tissue and tumor purity. For reliable immune score evaluation, the 'CIBERSORT' algorithm was used to evaluate tumor immune cell infiltration (12). Spearman's correlation analysis was used to evaluate the correlation between SHP2/PTPN11 gene expression and the target, including matrix and immune scores, immune cell infiltration, tumor mutation load and microsatellite instability (13). Student's unpaired t-test was used to evaluate the SHP2/PTPN11 gene expression levels between tumor and corresponding non-tumor normal tissues. $P<0.05$ was considered to indicate a statistically significant difference.

Patients and ELISA. A total of 42 breast cancer and 42 normal human serum specimens were collected. A total of 34 ovarian cancer and 36 normal human serum specimens were collected. A total of 29 endometrial cancer and 33 normal human serum specimens were collected. A total of 46 cervical cancer and 40 normal human serum specimens were collected. All samples were collected from the Chongqing Health Center for Women and Children (Chongqing, China) from February 2021 to December 2022. The patients' personal information was not collected. The patients provided written informed consent for the collection of blood samples. All specimens were stored at -80˚C and tested by ELISA in January 2023. The present study was approved by the Ethics Association of

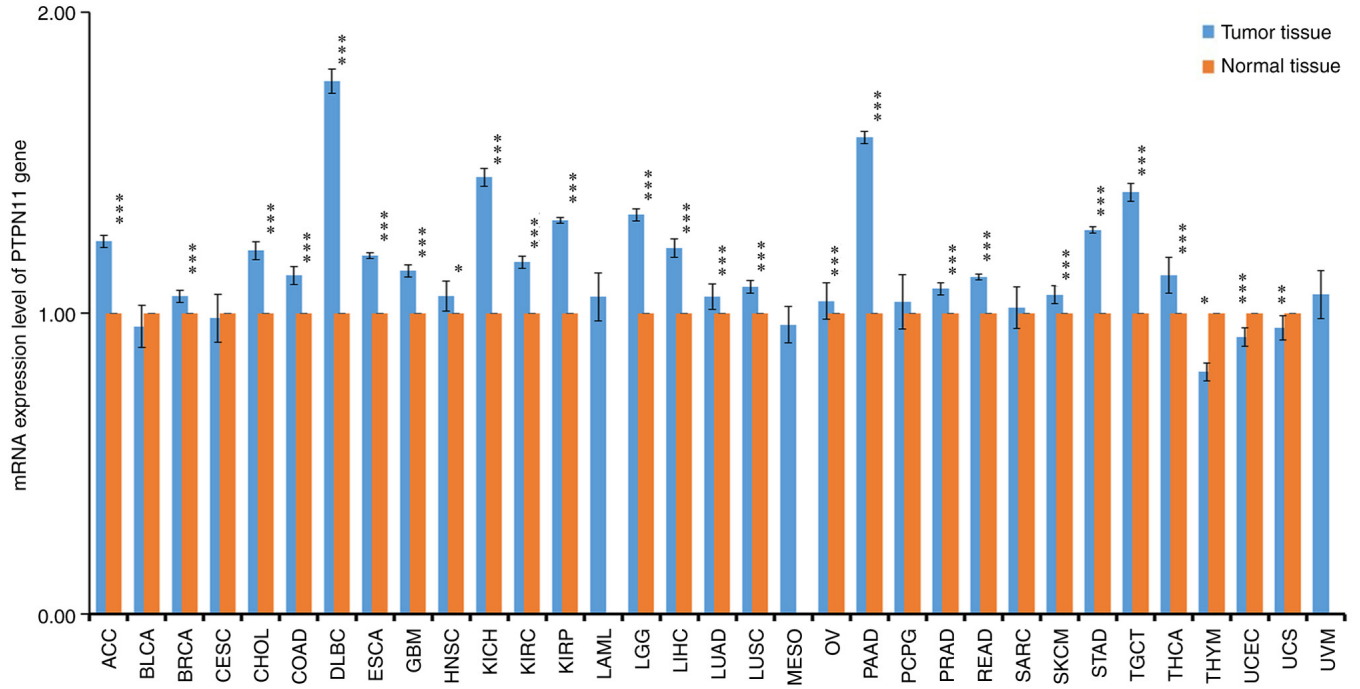

Figure 1. Expression level of PTPN11 gene in 33 types of tumors and their corresponding normal tissues. SPSS (version 25.0; IBM Corp.) was used for data normalization processing (Z-score normalization). Unpaired Student's t-test was used to analyze the data. *P<0.05, **P<0.01 and ***P<0.001 vs. normal tissues. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancers; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal 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 leukemia; 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, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma; PTPN11, protein tyrosine phosphatase non-receptor type 11.

Chongqing Health Center for Women and Children (approval no. cstc2020-jyk2.0).

The expression levels of SHP2/PTPN11 in serum were detected by double antibody one-step sandwich ELISA. The PTPN11 ELISA kit (cat. no. P20220809; Shanghai Enzyme-linked Biotechnology Co., Ltd.) was used at room temperature. A total of 50 μl standard at different concentrations was used, and 50 μl sample was added into the sample well. A total of 100 μl horseradish peroxidase-labeled antibody was added to the standard and sample wells, and incubated in a water bath at 37˚C for 60 min. Following washing, 50 μl substrates A and B was added to the plate wells and incubated at 37˚C for 15 min. A total of 50 μl stop solution was added to each well, and the optical density (OD) value was measured at a wavelength of 450 nm within 15 min. The test was repeated three times.

Statistical analysis. SPSS version 25.0 (IBM Corp.) was used to analyze the experimental data. The Shapiro-Wilk test was used to evaluate whether experimental data conformed to a normal distribution. Unpaired Student's t-test was used to evaluate the expression levels of the SHP2/PTPN11 gene between tumor and normal tissues. Spearman's correlation analysis was used to evaluate the correlation between the SHP2/PTPN11 gene expression and the target (matrix score, immune score, immune cell infiltration, tumor mutation load and microsatellite instability) (13). The χ^2 test was used to analyze the difference in SHP2 expression level in the peripheral blood of patients with breast, ovarian, endometrial and cervical cancer. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression levels of the PTPN11 gene in tumors. Among the 33 types of tumors that exhibit PTPN11 in TCGA, 30 types of tumors could be compared with their corresponding normal tissues as controls (LAML, MESO and UVM had no controls). For a total of 26 out of the 30 types of tumors, statistically significant differences were noted in the expression levels of the PTPN11 gene compared with those of the normal tissues (P<0.05). Among them, PTPN11 was expressed at higher levels in ACC, BRCA, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, OV, PAAD, PRAD, READ, SKCM, STAD, TGCT and THCA, while the expression levels of PTPN11 were decreased in THYM, UCEC and UCS (Fig. 1).

Expression levels of the PTPN11 gene are associated with patient prognosis. According to the median value of the PTPN11 gene expression, patients with 33 types of tumors were divided into PTPN11 high and low expression groups. Survival analysis demonstrated that the OS of patients in the PTPN11 high and low expression groups was significantly

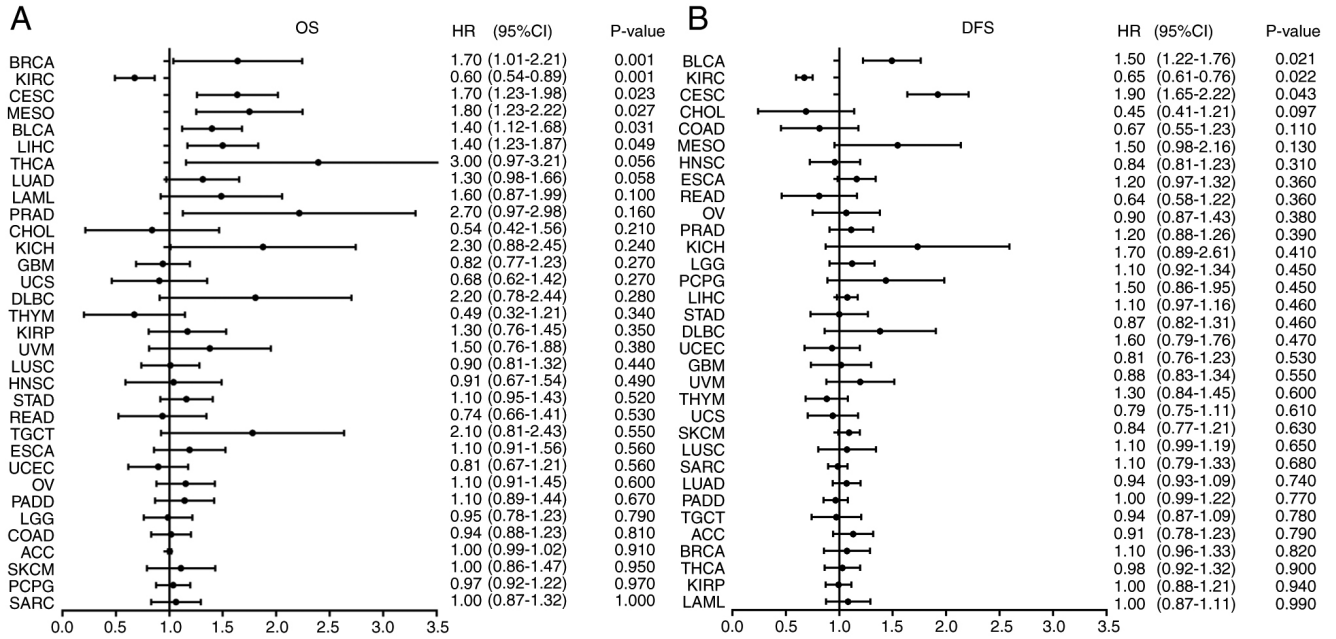

Figure 2. Relationship between the expression level of protein tyrosine phosphatase non-receptor type 11 gene and prognosis in 33 types of tumors. (A) OS and (B) DFS. Univariate Cox regression analysis was used to analyze the data; 'forest' plots were used to display the P-value, HR and 95% CI of each variable. HR, hazard ratio; CI, confidence interval; OS, overall survival; DFS, disease-free survival; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancers; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal 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 leukemia; 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, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

different among the BRCA (HR, 1.70; 95% CI, 1.01-2.21; P=0.001), CESC (HR, 1.70; 95% CI, 1.23-1.98; P=0.023), MESO (HR, 1.80; 95% CI, 1.23-2.22; P=0.027), BLCA (HR, 1.40; 95% CI, 1.12-1.68; P=0.031) and LIHC (HR, 1.40; 95% CI, 1.23-1.87; P=0.049) tumor types. Patients in the PTPN11 high expression group exhibited shorter OS times than those in the control group (P<0.05). However, high expression levels of PTPN11 improved the OS time of patients with the KIRC tumor type (HR, 0.60; 95% CI, 0.54-0.89; P=0.001) (Fig. 2A). Considering that OS may be affected by non-tumor-associated deaths, the relationship between PTPN11 gene expression and DFS was evaluated further. High expression of the PTPN11 gene reduced the DFS time of patients with the BLCA (HR, 1.50; 95% CI; 1.22-1.76; P=0.021) and CESC (HR, 1.90; 95% CI, 1.65-2.22; P=0.043) tumor types. This phenomenon was not observed in patients with other tumor types. However, high expression levels of PTPN11 improved the DFS time of patients with KIRC (HR, 0.65; 95% CI, 0.61-0.76; P=0.022) (Fig. 2B).

PTPN11 gene expression correlates with immune checkpoints and immune scores. By using Spearman's correlation analysis, it was found that the expression levels of the PTPN11 gene were significantly correlated with cancer types in the presence of immune cells. In BLCA, BRCA, CESC, CHOL, COAD, HNSC, KIRC, KIRP, LGG, PRAD, READ, SKCM, STAD, THCA, THYM and UCEC, the expression levels of the PTPN11 gene were significantly negatively correlated with regulatory, follicular helper and cluster of differentiation (CD)8^+ T cells (P<0.05). In BRCA, CESC, COAD, ESCA, HNSC, KICH, KIRC, LAML, LGG, LUAD, LUSC, OV, PCPG, PRAD, READ, SARC, TGCT, THCA, THYM and UCEC, the expression levels of the PTPN11 gene were significantly and positively correlated with the number of $CD4^+$ T cells (P<0.05). In BLCA, BRCA, HNSC, LIHC, LUAD, PRAD and THCA, the expression levels of the PTPN11 gene were significantly and positively correlated with the number of neutrophils (P<0.05). In BRCA, KIRC, SKCM, STAD, TGCT, THCA, THYM and UCEC, the expression levels of the PTPN11 gene were significantly and positively correlated with the number of macrophages M2 (P<0.05; Fig. 3).

Sialic acid binding Ig like lectin 15, indoleamine 2,3-dioxygenase 1, CD274, hepatitis A virus cellular receptor 2, programmed cell death (PDCD) 1, cytotoxic T-lymphocyte associated protein 4, lymphocyte activating 3 (LAG3) and PDCD 1 ligand 2 are transcripts related to immune checkpoints. The expression levels of these eight immune checkpoint-related genes were obtained and assessed. Spearman's correlation analysis was used to analyze the immune checkpoints genes related to PTPN11. Results were found in the DLBC, PCPG, PAAD, PRAD, LIHC, UVM and READ tumor types, where the expression levels of the PTPN11 gene were significantly and positively correlated with CD274, which had a large number of associations (P<0.05). However, in UCS, THCA and MESO (correlation coefficient >±0.3), the expression levels of the PTPN11 gene were significantly and negatively correlated with LAG3 (P<0.05; Fig. 4; Table I).

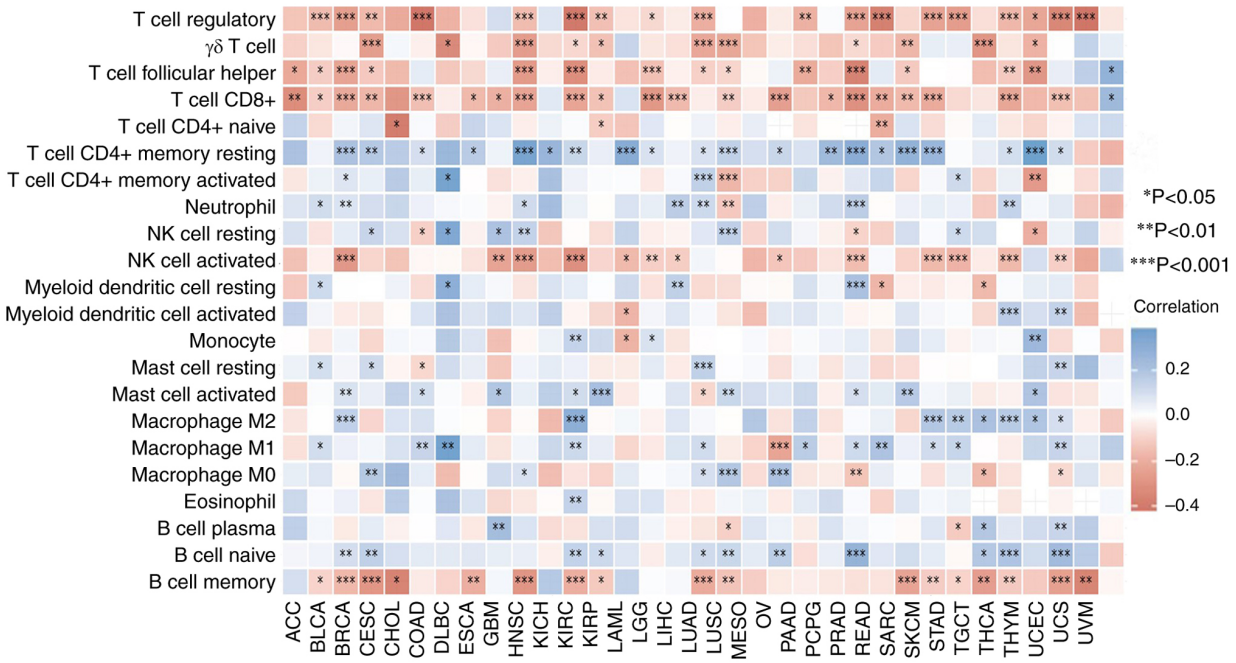

Figure 3. Correlation between the expression level of protein tyrosine phosphatase non-receptor type 11 gene and immune cells in different cancer types. Spearman's correlation analysis was used to assess the data. NK, natural killer; CD, cluster of differentiation; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancers; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal 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 leukemia; 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, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

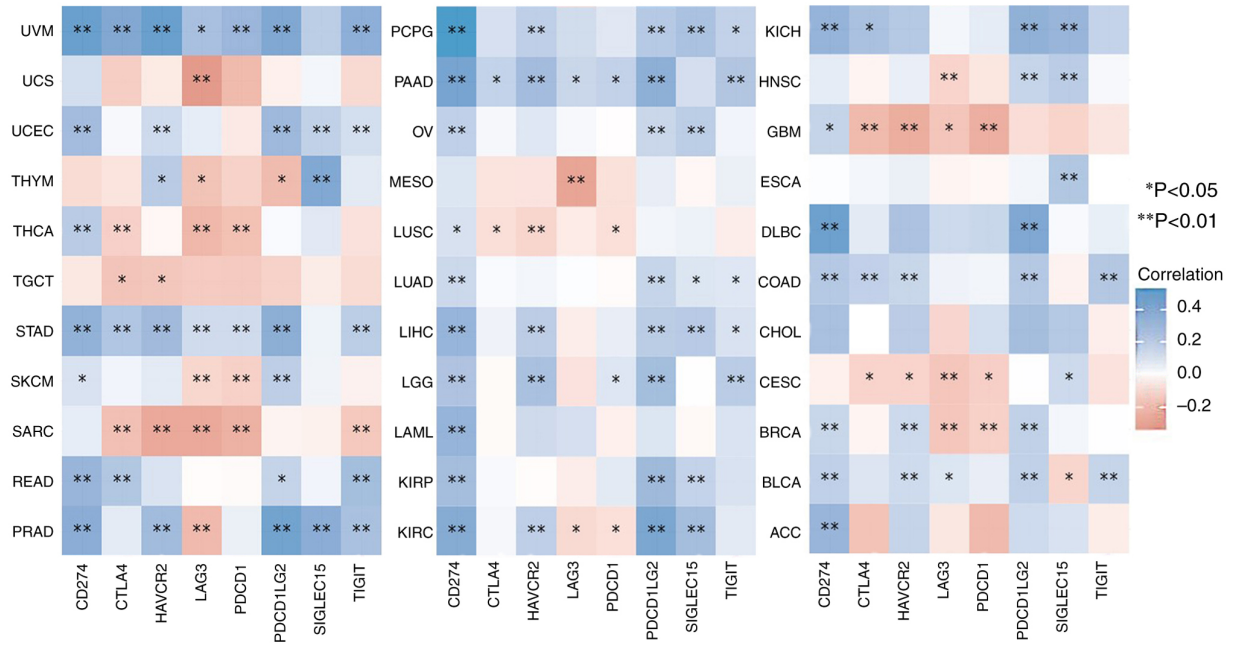

Figure 4. Correlation between the expression level of protein tyrosine phosphatase non-receptor type 11 gene and immune checkpoints in different cancer types. Spearman's correlation analysis was used to assess the data. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancers; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal 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 leukemia; 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, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

Expression levels of the PTPN11 gene are related to microsatellite instability and tumor mutational burden. The expression levels of the PTPN11 gene in LAML, LUAD, SKCM and THYM were significantly and positively correlated with microsatellite instability. However, in UVM and LGG, the expression levels of the PTPN11 gene were significantly and negatively correlated with microsatellite instability. Among them, the correlation coefficient between the expression levels of the PTPN11 gene and microsatellite instability was the highest in UVM (ρ=-0.313), THYM (ρ=0.212) and LAML (ρ=0.203) (Fig. 5A).

The expression levels of the PTPN11 gene were significantly and positively correlated with the tumor mutation burden in ACC, COAD, LUAD, LUSC, MESO, OV, READ, TGCT and UCEC. However, the expression levels of the PTPN11 gene in DLBC and SARC were significantly and negatively correlated with the tumor mutational burden. Among them, the correlation coefficient between the expression levels of the PTPN11 gene and the tumor mutational burden was the highest in DLBC (ρ=-0.561), SARC (ρ=-0.400), ACC (ρ=0.449), MESO (ρ=0.315), LUSC (ρ=0.289) and READ (ρ=0.281) (Fig. 5B).

SHP2 is associated with the risk of breast, ovarian, endometrial and cervical cancer. The peripheral blood samples from patients with 4 different tumor types were collected. ELISA was used to assess the expression levels of SHP2. The standard OD value was 0.143. If the OD value of the sample was higher than that of the standard OD value, it was considered positive for SHP2, otherwise it was considered negative. By using the χ^2 test, it was found that SHP2 was expressed at higher levels in breast cancer (71.43%; P<0.001) and ovarian cancer (58.82%; P<0.001) than in the control group; however, it was expressed at lower percentages in endometrial cancer (31.03%; P<0.001) and cervical cancer (41.30%; P=0.001) than in the control group (Table II; Fig. 6). This finding is consistent with the results of the pan-cancer analysis in the present study.

Table I. Correlation between SHP2/PTPN11 and immune checkpoint-related genes.

Cancer	Immune checkpoint-related genes	ρ	P-value
THYM	SIGLEC15	0.394	<0.01
PRAD	SIGLEC15	0.325	<0.01
DLBC	PDCD1LG2	0.442	<0.01
PRAD	PDCD1LG2	0.439	<0.01
THCA	PDCD1	-0.338	<0.01
UCS	LAG3	-0.319	<0.05
THCA	LAG3	-0.351	<0.01
MESO	LAG3	-0.361	<0.01
UVM	HAVCR2	0.323	<0.01
SARC	HAVCR2	-0.315	<0.01
UVM	CTLA4	0.342	<0.01
DLBC	CD274	0.473	<0.01
PCPG	CD274	0.402	<0.01
PAAD	CD274	0.367	<0.01
PRAD	CD274	0.342	<0.01
LIHC	CD274	0.331	<0.01
UVM	CD274	0.326	<0.01
READ	CD274	0.302	<0.01

SHP2, src homology-2 domain-containing protein tyrosine phosphatase; SIGLEC15, Sialic acid binding Ig like lectin 15; PDCD1, programmed cell death 1; PDCD1LG2, PDCD 1 ligand 2; LAG3, lymphocyte activating 3; HAVCR2, hepatitis A virus cellular receptor 2; CTLA4, cytotoxic T-lymphocyte associated protein 4; THYM, thymoma; PRAD, prostate adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; THCA, thyroid carcinoma; UCS, uterine carcinosarcoma; MESO, mesothelioma; UVM, uveal melanoma; SARC, sarcoma; PCPG, pheochromocytoma and paraganglioma; PAAD, pancreatic adenocarcinoma; LIHC, liver hepatocellular carcinoma; READ, rectal adenocarcinoma.

Discussion

SHP2 consists of the N-SH2 and C-SH2 domains, a protein tyrosine phosphatase (PTP) domain and a C-terminal tail with two tyrosine phosphorylation sites (14). The deviations in its biological function can cause various disorders in the regulation of normal body functions (such as normal development of the body, cardiovascular production and immune response), and lead to the formation of cancer, diabetes and autoimmune diseases, among others (15). SHP2 serves a number of roles in the formation and progression of tumors (4). SHP2 can regulate the proliferation and metastasis of tumor cells by participating in numerous signaling pathways such as the PI3K/AKT and RAS/ERK pathways (16-18). Mutations or changes in the expression levels of PTPN11 can lead to the formation of leukemia and various other tumors, such as liver, cervical, ovarian and endometrial cancer (4,17). Therefore, SHP2/PTPN11 may be an ideal target for cancer intervention (4).

In the present study, the expression levels of SHP2/PTPN11 were analyzed in tumors, and the data indicated that SHP2/PTPN11 was expressed at high levels in KIRC. Concomitantly, the prognostic analysis indicated that high expression of SHP2/PTPN11 resulted in increased OS (HR, 0.60; P=0.001) and DFS (HR, 0.65; P=0.022) times of patients with KIRC. Therefore, SHP2/PTPN11 may be a tumor suppressor gene in KIRC, and its elevated expression can improve patient prognosis. Increased SHP2/PTPN11 expression decreased the OS (BLCA: HR, 1.40; P=0.031; CESC: HR, 1.70; P=0.023) and DFS (BLCA: HR, 1.50; P=0.021; CESC: HR, 1.90; P=0.043) times of patients with BLCA and CESC; moreover, the high expression levels of SHP2/PTPN11 decreased the OS times of patients with BRCA, MESO and LIHC, thereby worsening patient prognosis, suggesting that SHP2/PTPN11 may act as an oncogene in BLCA, CESC, BRCA, MESO and LIHC.

In a number of solid tumors, excessive SHP2 activation has been stated to serve an essential pathogenic role. For example, Feng *et al* (19) indicated that SHP2 was expressed at high levels in 94.1% of patients with NSCLC; its expression was higher in the intratumoral area than in the stromal area. Moreover, high expression of SHP2 is associated with an improved prognosis in patients with NSCLC, and can increase OS and PFS time. Lei *et al* (20) indicated that high

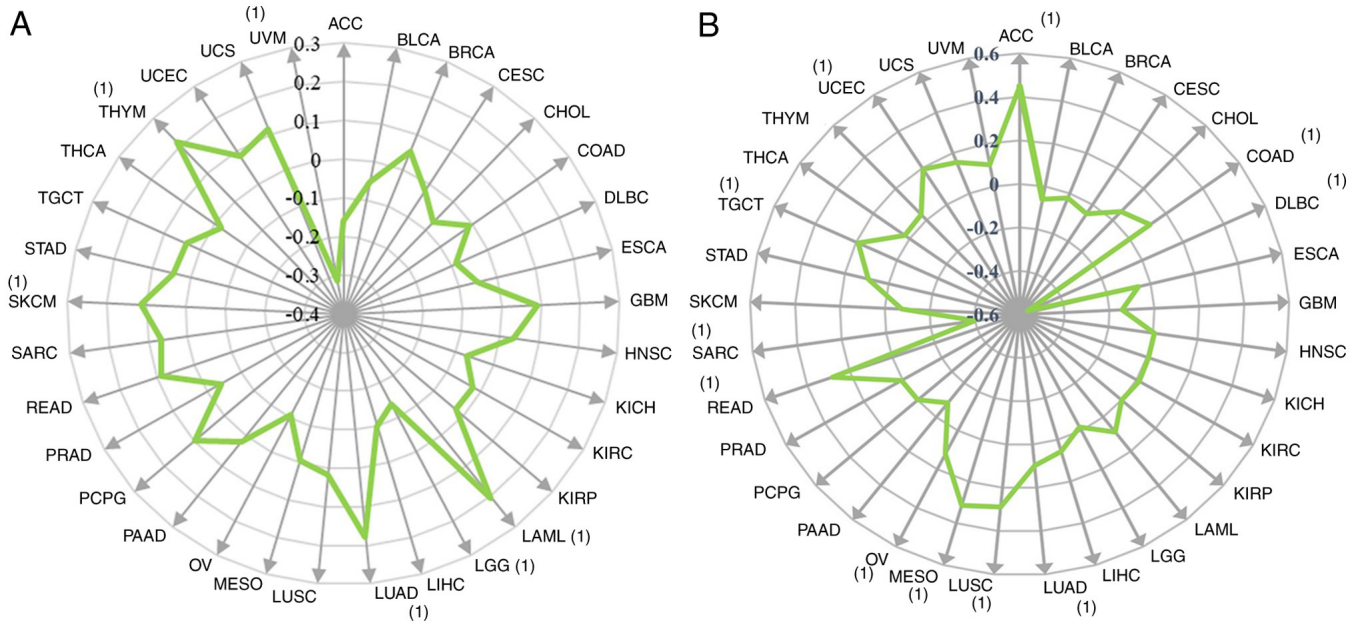

Figure 5. Correlation between protein tyrosine phosphatase non-receptor type 11 gene expression level and microsatellite instability and tumor mutation burden. (A) Microsatellite instability. (B) Tumor mutation burden. (1) indicates P<0.05. Spearman's correlation analysis was used to assess the data. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancers; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal 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 leukemia; 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, rectal adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

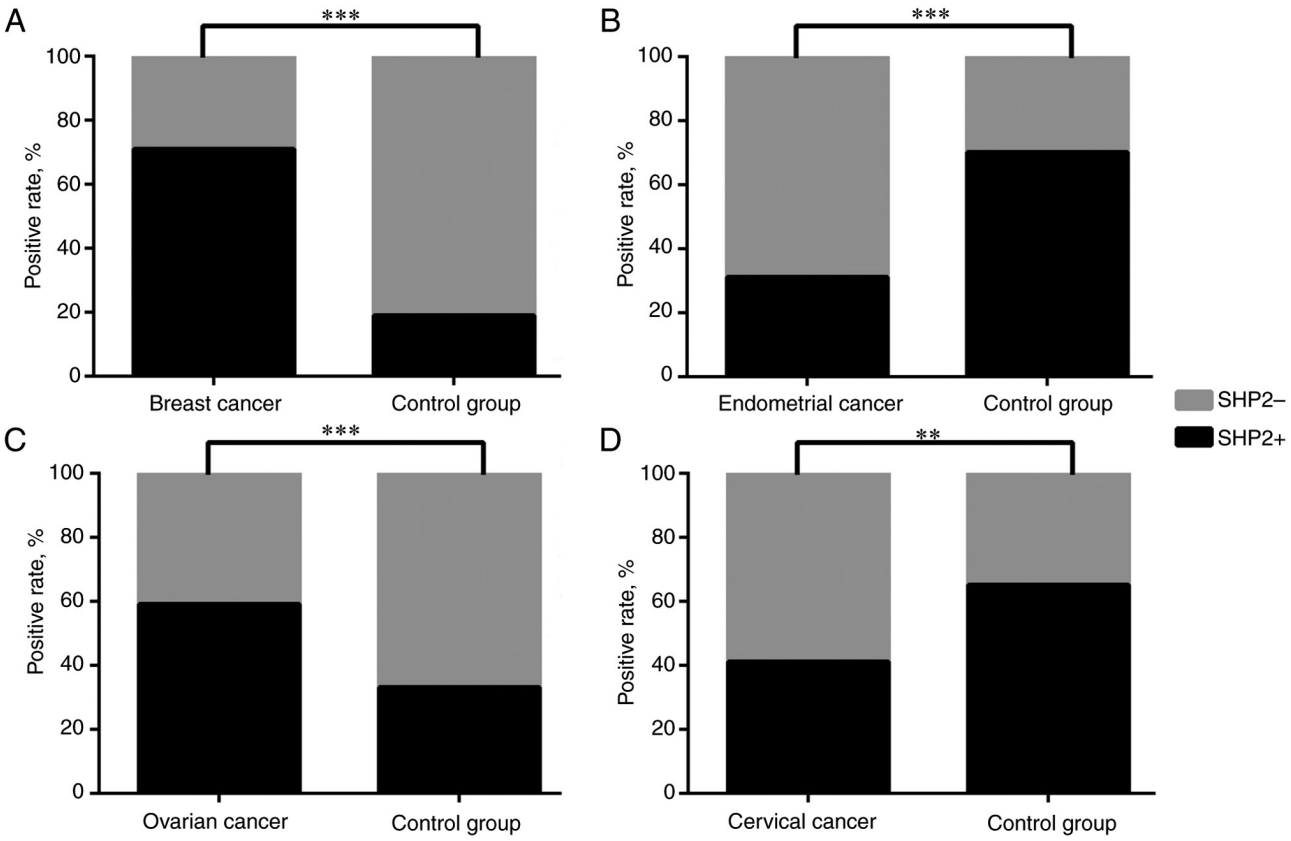

Figure 6. Association between the expression of SHP2 and the risk of tumorigenesis in peripheral blood. (A) Breast cancer. (B) Endometrial cancer. (C) Ovarian cancer. (D) Cervical cancer. The χ^2 test was used to analyze the data. SHP2, src homology-2 domain-containing protein tyrosine phosphatase. **P<0.01 and ***P<0.001.

Table II. Expression of SHP2 in peripheral blood.

	SHP2						Optical density value[a]	
Cancer type	+	-	Total, n	Odds ratio	95% confidence interval	P-value	+	-
Breast								
Cancer group	30	12	42	10.44	5.391-20.210	<0.001	0.378	0.010
Control group	8	34	42				0.303	0.017
Ovarian								
Cancer group	20	14	34	2.92	1.641-5.202	<0.001	0.458	0.022
Control group	12	24	36				0.404	0.020
Endometrial								
Cancer group	9	20	29				0.450	0.017
Control group	23	10	33	0.19	0.105-0.352	<0.001	0.488	0.011
Cervical								
Cancer group	19	27	46				0.523	0.029
Control group	26	14	40	0.37	0.211-0.663	0.001	0.479	0.026

[a]Optical density value is the average value of every tumor and its corresponding control group. The χ^2 test was used to analyze the data. SHP2, src homology-2 domain-containing protein tyrosine phosphatase.

expression of SHP2 could promote the occurrence of breast cancer and reduce lymph node metastasis. Hu *et al* (21) observed that the positive rate of SHP2 in ovarian cancer tissues reached 81.67%, while the positive rate of SHP2 in normal ovarian tissues was 0.00%. Moreover, SHP2 expression was associated with lymph node metastasis, clinical stage and histological grade. The current study indicated that SHP2 was expressed in various tumor types. The expression levels of SHP2 were increased in the majority of tumors, with the exception of THYM, UCEC and UCS. By using peripheral blood samples of patients with cancer, it was found that SHP2 was not expressed at high levels in all tumors; by contrast, it indicated tumor-promoting or -suppressing functions in different tumors. The expression levels of SHP2 were increased in breast and ovarian cancer, whereas they were expressed at low levels in endometrial and cervical cancer. Therefore, the role of SHP2 in the remaining tumors requires additional investigation.

SHP2 is involved in the regulation of various signaling pathways in organisms. SHP2 binding sites are present in RTKs and backbone adaptors, such as growth factor receptor bound (GRB)2-associated binding protein (GAB), insulin receptor substrate, FAR1-related sequence and other proteins. Therefore, this 'molecular switch' ensures that SHP2 is only activated in the appropriate cellular regions (N-SH2 and PTP domain) (22). During the signal transduction process of several growth factors and cytokines, SHP2 acts upstream of RAS, and enables the full activation of the ERK/MAPK pathway (6). The C-terminal tyrosine of SHP2 is phosphorylated in response to the majority of the agonists, and the tyrosine-phosphorylated SHP2 recruits the adaptor protein GRB2 and the guanine nucleotide exchange factor son of sevenless, which contribute to RAS activation (23). Certain studies (14,16,20) have shown that SHP2, which is expressed at high levels in breast cancer, can activate PI3K/AKT signaling to phosphorylate GSK3-β, thereby promoting the proliferation of breast cancer cells. However, SHP2 can also inhibit the PI3K/AKT signaling pathway. In the EGFR signaling pathway, SHP2 can dephosphorylate the PI3K binding site on GAB1 and decrease the GAB1-mediated activation of the PI3K/AKT proteins. In addition, SHP2 can also bind to p85 to form GAB2/SHP2/p85 complexes, thereby inhibiting the PI3K/AKT pathway (24). SHP2 can inhibit the gp130 pathway mediated by IL-6 and promote STAT3 dephosphorylation, thereby negatively regulating the Janus kinase/STAT3 pathway and eventually leading to juvenile bone marrow monocyte leukemia (25,26). In addition, SHP2 is involved in regulating T-cell activity by binding to the phosphotyrosine motif of the immune checkpoint protein PDC1 through its N-SH2 domain (27). Inhibitors that can block the protein-protein interactions between PD-1 and SHP2 are expected to be used as new tumor immunotherapy agents. Therefore, SHP2 has also become a potential drug target in tumor immunotherapy (28).

SHP2/PTPN11 may play an important role in promoting tumor immune escape (29). As important players in shaping the tumor microenvironment, tumor-associated macrophages (TAMs) mediate tumor angiogenesis and immune escape (30). TAMs are a subset of macrophages with M2-like aggregate properties, which play a major role in tumorigenesis, angiogenesis, grid renovation and metastasis (31). For example, the deletion of SHP2 in TAMs can significantly inhibit the growth of melanoma. In response to IFN-γ or cytokine stimulation, the deletion of SHP2 can notably enhance the ability of macrophages to produce chemokine ligand (CXCL) 9, thereby recruiting additional T cells, promoting the production of CXCL9 in the tumor microenvironment, and forming a macrophage/CXCL9-T cell/IFN-γ feedback loop to facilitate

the antitumor immune function of T cells (32,33). The aforementioned studies have demonstrated that SHP2/PTPN11 is an expected target for managing TAM function in immunotherapy. By inhibiting SHP2 expression, a direct inhibition of tumor formation is facilitated by inhibiting downstream pathways, such as that of RAS/ERK, PI3K/AKT and JAK/STAT. By contrast, SHP2 inhibitors can also inhibit tumors by activating T cells and promoting macrophage phagocytosis. Therefore, SHP2 is a target for both immune and targeted therapies.

In conclusion, the findings of the present study showed that SHP2/PTPN11 was widely expressed in the majority of tumors assessed, and that its expression was related to tumorigenesis, tumor development and disease prognosis. SHP2/PTPN11 upregulation could improve OS and DFS time in patients with KIRC. However, SHP2/PTPN11 upregulation could reduce OS time in patients with BLCA, CESC, BRCA, MESO and LIHC. Moreover, the elevated expression of SHP2 could also reduce patient DFS time in BLCA and CESC. SHP2 serves an important role in maintaining the immunosuppressive microenvironment via the inhibition of T-cell activation and the promotion of M2 macrophage activation. Inhibition of SHP2 may be a novel therapeutic approach with the following dual applications: It can directly inhibit the growth of cancer cells in specific tumors and it can change the tumor microenvironment to promote antitumor immunity. Based on the function of SHP2 in tumor cells, novel and effective antitumor drugs targeting it can be developed.

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Authors' contributions

SL conceptualized the study, visualized the data, developed the methodology used, applied the software, performed the statistical analysis, wrote the original draft, and reviewed and edited the manuscript. JQ applied the software and developed the methodology used. XW developed the methodology used, applied the software and performed the statistical analysis. QZ developed the methodology and performed the statistical analysis. CL conceptualized the study, visualized the data, developed the methodology, and wrote, reviewed and edited the manuscript. SL and CL confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study was approved by the Ethics Association of Chongqing Health Center for Women and Children (approval no. cstc2020-jyk2.0). The patients provided written informed consent for the collection of blood samples and their use in the present study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Yuan H, Zhao J, Yang Y, Wei R, Zhu L, Wang J, Ding M, Wang M and Gu Y: SHP-2 Interacts with CD81 and regulates the malignant evolution of colorectal cancer by inhibiting epithelial-mesenchymal transition. Cancer Manag Res 12: 13273-13284, 2020.
2. Zhou L, Feng Y, Ma YC, Zhang Z, Wu JW, Du S, Li WY, Lu XH, Ma Y and Wang RL: Exploring the mechanism of the potent allosteric inhibitor compound2 on $SHP2^{WT}$ and $SHP2^{F285S}$ by molecular dynamics study. J Mol Graph Model 103: 107807, 2021.
3. Mitra R and Ayyannan SR: Small-Molecule Inhibitors of Shp2 phosphatase as potential chemotherapeutic agents for Glioblastoma: A Minireview. ChemMedChem 16: 777-787, 2021.
4. Li S, Wang X, Li Q and Li C: Role of SHP2/PTPN11 in the occurrence and prognosis of cancer: A systematic review and meta-analysis. Oncol Lett 25: 19, 2023.
5. Kim JS, Shin OR, Kim HK, Cho YS, An CH, Lim KW and Kim SS: Overexpression of protein phosphatase non-receptor type 11 (PTPN11) in gastric carcinomas. Dig Dis Sci 55: 1565-1569, 2010.
6. Kerr DL, Haderk F and Bivona TG: Allosteric SHP2 inhibitors in cancer: Targeting the intersection of RAS, resistance, and the immune microenvironment. Curr Opin Chem Biol 62: 1-12, 2021.
7. Sausgruber N, Coissieux MM, Britschgi A, Wyckoff J, Aceto N, Leroy C, Stadler MB, Voshol H, Bonenfant D and Bentires-Alj M: Tyrosine phosphatase SHP2 increases cell motility in triple-negative breast cancer through the activation of SRC-family kinases. Oncogene 34: 2272-2278, 2015.
8. Mainardi S, Mulero-Sánchez A, Prahallad A, Germano G, Bosma A, Krimpenfort P, Lieftink C, Steinberg JD, de Wit N, Gonçalves-Ribeiro S, *et al*: SHP2 is required for growth of KRAS-mutant non-small-cell lung cancer in vivo. Nat Med 24: 961-967, 2018.
9. Jiang C, Hu F, Tai Y, Du J, Mao B, Yuan Z, Wang Y and Wei L: The tumor suppressor role of Src homology phosphotyrosine phosphatase 2 in hepatocellular carcinoma. J Cancer Res Clin Oncol 138: 637-646, 2012.
10. Xinhua S: Role of CAD gene in the prognosis evaluation of pan-cancer. J Basic Clinical Oncol 33: 485-90, 2020.
11. Zhang Z, Lin E, Zhuang H, Xie L, Feng X, Liu J and Yu Y: Construction of a novel gene-based model for prognosis prediction of clear cell renal cell carcinoma. Cancer Cell Int 20: 27, 2020.
12. Sturm G, Finotello F, Petitprez F, Zhang JD, Baumbach J, Fridman WH, List M and Aneichyk T: Comprehensive evaluation of transcriptome-based cell-type quantification methods for immuno-oncology. Bioinformatics 35: i436-i45, 2019.
13. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, Porta-Pardo E, Gao GF, Plaisier CL, Eddy JA, *et al*: The Immune landscape of cancer. Immunity 51: 411-412, 2019.
14. Gu J, Han T, Ma RH, Zhu YL, Jia YN, Du JJ, Chen Y, Jiang XJ, Xie XD and Guo X: SHP2 promotes laryngeal cancer growth through the Ras/Raf/Mek/Erk pathway and serves as a prognostic indicator for laryngeal cancer. Int J Oncol 44: 481-490, 2014.

15. Chalfant CE, Kim M, Hartman Z, Geldenhuys WJ and Agazie YM: Novel Small-molecule inhibitor for the oncogenic tyrosine phosphatase SHP2 with anti-breast cancer cell effects. Mol Cancer Res 5: 25113-25124, 2020.
16. Vazhappilly CG, Saleh E, Ramadan W, Menon V, Al-Azawi AM, Tarazi H, Abdu-Allah H, El-Shorbagi AN and El-Awady R: Inhibition of SHP2 by new compounds induces differential effects on RAS/RAF/ERK and PI3K/AKT pathways in different cancer cell types. Invest New Drugs 37: 252-261, 2019.
17. Ahmed TA, Adamopoulos C, Karoulia Z, Wu X, Sachidanandam R, Aaronson SA and Poulikakos PI: SHP2 Drives adaptive resistance to ERK signaling inhibition in molecularly defined subsets of ERK-Dependent tumors. Cell Rep 26: 65-78.e5, 2019.
18. Fedele C, Ran H, Diskin B, Wei W, Jen J, Geer MJ, Araki K, Ozerdem U, Simeone DM, Miller G, *et al*: SHP2 inhibition prevents adaptive resistance to MEK inhibitors in multiple cancer models. Cancer Discov 8: 1237-1249, 2018.
19. Feng HB, Chen Y, Xie Z, Jiang J, Zhong YM, Guo WB, Yan WQ, Lv ZY, Lu DX, Liang HL, *et al*: High SHP2 expression determines the efficacy of PD-1/PD-L1 inhibitors in advanced KRAS mutant non-small cell lung cancer. Thorac Cancer 12: 2564-2573, 2021.
20. Lei W, Qin X, Wang X and Zhu F: Expression and clinical significance of Shp2 protein in breast cancer. Jilin Med J 32: 7692-7694, 2011.
21. Hu Z, Li J, Gao Q, Wei S and Yang B: SHP2 overexpression enhances the invasion and metastasis of ovarian cancer in vitro and in vivo. Onco Targets Ther 10: 3881-3891, 2017.
22. Liotti F, Kumar N, Prevete N, Marotta M, Sorriento D, Ieranò C, Ronchi A, Marino FZ, Moretti S, Colella R, *et al*: PD-1 blockade delays tumor growth by inhibiting an intrinsic SHP2/Ras/MAPK signalling in thyroid cancer cells. J Exp Clin Cancer Res 40: 22, 2021.
23. Zhao Y, Yu H, Ida CM, Halling KC, Kipp BR, Geiersbach K, Rumilla KM, Gupta S, Lin MT and Zheng G: Assessment of RAS dependency for BRAF alterations using cancer genomic databases. JAMA Netw Open 4: e2035479, 2021.
24. Yuan X, Bu H and Zhou J: Recent advances of SHP2 inhibitors in cancer therapy: Current development and clinical application. J Med Chem 63: 11368-11396, 2020.
25. Kim M, Morales LD and Jang IS: Protein tyrosine phosphatases as potential regulators of STAT3 signaling. Int J Mol Sci 19: 2708, 2018.
26. Zhang W, Chan RJ, Chen H, Yang Z, He Y, Zhang X, Luo Y, Yin F, Moh A, Miller LC, *et al*: Negative regulation of Stat3 by activating PTPN11 mutants contributes to the pathogenesis of Noonan syndrome and juvenile myelomonocytic leukemia. J Biol Chem 284: 22353-22363, 2009.
27. Wang J, Pollard K, Allen AN, Tomar T, Pijnenburg D, Yao Z and Rodriguez FJ: Combined inhibition of SHP2 and MEK is effective in models of NF1-Deficient malignant peripheral nerve sheath tumors. Cancer Res 80: 5367-5379, 2020.
28. Zhu G, Xie J, Kong W, Xie J, Li Y, Du L, Zheng Q, Sun L, Guan M, Li H, *et al*: Phase separation of disease-associated SHP2 mutants underlies MAPK hyperactivation. Cell 183: 490-502.e18, 2020.
29. Gong M, Liu P, He T, Zhang M and Li G: Demonstrating the effect of SHP2 inhibitor on cervical squamous cell carcinoma from the perspective of ZAP70. Anticancer Drugs 32: 477-483, 2021.
30. Cassetta L and Pollard JW: Targeting macrophages: Therapeutic approaches in cancer. Nat Rev Drug Discov 17: 887-904, 2018.
31. Xiao P, Zhang H, Zhang Y, Zheng M, Liu R, Zhao Y, Zhang X, Cheng H, Cao Q and Ke Y: Phosphatase Shp2 exacerbates intestinal inflammation by disrupting macrophage responsiveness to interleukin-10. J Exp Med 216: 337-349, 2019.
32. Hui E, Cheung J, Zhu J, Su X, Taylor MJ, Wallweber HA, Sasmal DK, Huang J, Kim JM, Mellman I and Vale RD: T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. Science 355: 1428-1433, 2017.
33. Torres-Ayuso P and Brognard J: Shipping out MEK inhibitor resistance with SHP2 inhibitors. Cancer Discov 8: 1210-1212, 2018.