

# Promoter methylation attenuates SHP1 expression and function in patients with primary central nervous system lymphoma

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**Abstract.** The Src homology region 2 domain-containing phosphatase-1 (SHP1) is a critical negative regulator involved in the JAK/STAT signaling pathway. The SHP1 gene has been proposed as a candidate tumor suppressor in solid and hematological malignancies and promoter methylation is an important biological process in controlling tumorigenesis. However, the detailed roles of SHP1 promoter methylation in the pathogenesis of primary central nervous system lymphoma (PCNSL) is largely unknown. In the present study, we evaluated the correlation between SHP1 expression and promoter methylation in patients with PCNSL. Thirty-three patients with PCNSL were included. We evaluated SHP1 protein expression levels by immunohistochemistry and the SHP1 promoter methylation profile by pyrosequencing analysis. For cases (n=8) with a good yield of total protein, SHP1 phosphorylation (pSHP1) and STAT3 protein expression levels were further analyzed by western blot analysis to uncover the molecular impact of SHP1 promoter methylation on downstream signaling pathways. In this study, a lower expression of SHP1 protein level was observed in 16/33 cases (48.5%) of PCNSL. SHP1 promoter

methylation was predominant in 29/33 cases (87.9%) with a mean methylation level of 31.7±36.5%. The mean methylation level of the SHP1 promoter was significantly elevated in patients with a lower SHP1 protein expression, compared with those showing a higher SHP1 protein expression (50.3±38.9 vs. 14.2±24.0%, p=0.004). Further analysis showed that SHP1 protein expression was significantly decreased in patients with a higher SHP1 promoter methylation status (p=0.001), and such attenuation was correlated with a downregulation of pSHP1 (p=0.005) and an upregulation of STAT3 protein expression (p=0.020). Our data demonstrated that epigenetic alterations in the promoter region downregulated SHP1 expression in PCNSL patients. SHP1 promoter methylation was correlated with tyrosine phosphorylation and activation of transcription factor STAT3, which may contribute to the pathogenesis of PCNSL. Therapeutic regimens with epigenetic modifiers may be a potential option for patients with PCNSL.

## Introduction

Primary central nervous system lymphoma (PCNSL) is a distinct type of non-Hodgkin's lymphoma located in the brain, leptomeninges, spinal cord, cerebrospinal fluid (CSF) and intraocular structures, accounting for 2-3% of all brain tumors and is associated with a dismal prognosis (1,2). Approximately 95% of PCNSL cases are histologically characterized as diffuse large B-cell lymphoma (DLBCL) (3). Although PCNSL shares some common characteristics with systemic DLBCL, it has unique features based on the transcription profiles and therapeutic protocols (4-8). In clinical practice, patients with PCNSL have greatly benefitted from well-established routine chemotherapy and radiotherapy strategies. However, treating refractory and relapsed PCNSL is still a huge challenge. Novel therapeutical strategies including immunotherapy and targeted therapy by clinically available epigenetic modifiers such as 5-aza-2'-deoxycytidine have been adopted as potential options for these B-cell malignancies, which may shed light on the potential clinical management of PCNSL.

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The development of functional B cells is dependent on the maturation of progenitor cells in the bone marrow, and transcription factor networks play a fundamental role in B cell differentiation. Recent evidence has revealed that most PCNSL cells demonstrate an activated B-cell-like phenotype with constitutive activation of the JAK/STAT and NF- $\kappa$ B signaling pathways (4,9,10). Cytokines including IL-4 and IL-10 were found to stimulate B cell proliferation and survival through upregulation of JAK1 and constitutive activation of STAT3 and STAT6 in PCNSL (11-13). In addition, oncostatin M (OSM) was found to trigger activation of the JAK family, which in turn activated the STAT family in PCNSL, while downregulation of SOCS1 and SOCS3 led to activation of the JAK/STAT signaling pathway (9,14). Molecules involved in the JAK/STAT signaling pathway may be regulating factors contributing to the pathogenesis of PCNSL.

The Src homology region 2 domain-containing phosphatase-1 (SHP1) is an important negative regulator involved in the JAK/STAT signaling pathway through cytokine/growth factors (15). SHP1 is a non-transmembrane protein tyrosine phosphatase predominantly expressed in hematopoietic cells (16-19), participating in various pathways which negatively regulate molecular signals involved in cell activation, proliferation, differentiation, and migration through three categories of receptors (20). These receptors include growth factor receptors with an intrinsic tyrosine kinase activity such as c-kit, EGF, and CSF-1; cytokine receptors such as IL-2R, IL-3R, and Epo-R; and receptor complexes involved in immune response such as TCR and CD5 (21-23). A previous study reported that SHP1 expression in PCNSL reflects the origin of PCNSL from a late germinal center to an early postgerminal center stage (24). It has previously been shown that DLBCL expresses a very low level of SHP1 protein, suggesting that loss of SHP1 may result in the development of DLBCL (25). In hematologic malignancies, the SHP1 gene silenced by aberrant CpG promoter methylation was found to commonly induce a marked decrease in the SHP1 protein level, indicating that SHP1 expression is predominantly regulated by DNA methylation. Nevertheless, the impact of SHP1 methylation on the development of PCNSL has not been comprehensively characterized.

In this study, we applied immunohistochemistry and pyrosequencing analysis to 33 PCNSL samples to investigate the SHP1 protein expression profile and promoter methylation status. By evaluating molecular alterations in the JAK/STAT signaling pathway through western blot analysis, we aimed to clarify the influence of SHP1 and SHP1 promoter methylation on the pathogenesis of PCNSL.

## Patients and methods

**Patients.** Clinical data and tumor specimens from 33 immunocompetent patients with PCNSL were analyzed retrospectively. Diagnosis of DLBCL was carried out by histologic review for all specimens according to the Revised European-American Lymphoma and WHO classification (26). For this study, patients were selected on the basis of the availability of paraffin-embedded tumor tissues and fresh-frozen tumor tissues. All patients received chemotherapy regimens based on high-dose methotrexate (HD-MTX). Patients who

did not achieve complete remission to HDMTX-based chemotherapy were given rescue whole brain radiotherapy (WBRT). Informed consent from all the patients was obtained. This study was approved by the Beijing Tiantan Hospital Ethics Committee, Capital Medical University.

**Tumor specimens.** Tumor specimens were obtained during stereotactic biopsy or surgery. Formalin-fixed and paraffin-embedded tumor specimens were used for immunohistochemistry, whereas fresh-frozen tumor specimens were stored at -80°C until extraction of DNA for pyrosequencing analysis. Of the 33 fresh-frozen samples, only 8 samples for protein extraction were used for further western blot analysis due to the quantity of tissue available after pyrosequencing analysis. For normal control samples, we used 11 paraffin-embedded reactive hyperplasia of lymph nodes for immunohistochemistry, peripheral blood from the 33 PCNSL patients for pyrosequencing analysis and 1 fresh-frozen normal lymph node for western blot analysis.

**Immunohistochemical analysis.** A panel of immunohistochemical staining was performed on 4- $\mu$ m thick sections (EnVision method). Sections were deparaffinized in xylene and dehydrated with ethanol. Antigen retrieval was carried out in a microwave oven for 15 min. And then sections were incubated with a working dilution of each monoclonal antibody: mouse anti-SHP1 monoclonal antibody (Sc-7289, 1:500 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), monoclonal antibodies against CD10 (ZM-0283, 1:100 dilution), BCL-6 (ZM-0011, 1:50 dilution), MUM1 (ZM-0399; 1:50 dilution) and BCL-2 (ZA-0536, 1:100 dilution) from Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd. (Beijing, China). Reactive hyperplasia of lymph nodes was used as positive controls.

Two pathologists assessed the immunohistochemical staining independently. Fifteen to 20 fields at x400 magnification were analyzed per specimen. SHP1 immunohistochemical staining was evaluated semiquantitatively by estimating the fraction of positive cells according to the cut-off point as published by Sugita *et al* (24). Tumor tissues with more than 60% positively stained cells were considered (3+), 30-60% (2+), 0-30% (1+), and no staining was considered (-). Only cytoplasmic staining of cells was considered for grading. Staining was considered positive for CD10, BCL-6, and MUM1 when more than 30% of tumor cells were positively stained (27). For BCL-2, staining was considered positive when more than 50% of tumor cells expressed the BCL-2 protein according to a previously published method for systemic DLBCL (28).

**Pyrosequencing detection.** Genomic DNA was extracted from 33 PCNSL frozen tumor tissues and paired peripheral blood by using the QIAamp DNA Mini kit (Qiagen). Pyrosequencing analysis was carried out by Gene-Tech Co., Ltd. (Shanghai, China). For SHP1, 4 CpG sites were studied. PCR primer sequences and sequencing primer are listed as follows: GGTT GTGGTGAGAAATTAATTAGA (forward primer) and CTCC AAACCCAAATAATACTTCA (reverse primer); and GGAG GAGGGAGAGATG (sequencing primer). The annealing temperature was 54°C. A bisulfite-treated sample (2  $\mu$ l) was amplified in 40  $\mu$ l of reaction

mixture, containing primers and 2.5 U of Takara Hot Start Taq (Takara Biotechnology Co., Ltd.). The PCR product was purified to obtain single-stranded DNA mixed with 40  $\mu$ l sequencing buffer, containing 0.5  $\mu$ M sequencing primers. Sequencing analysis used the PyroMark ID technique. Methylation data are presented as the percentage of average methylation in all observed CpG sites. Positive rate using the mean methylation level plus two times the standard deviation (SD) of the control samples as a cut-off point (29).

**Western blot analysis.** The 8 fresh-frozen tumor tissues and 1 normal lymph node were ground and lysed in lysis buffer and proteinase inhibitor (50:1). After incubation for 30 min on ice, the lysates were subjected to centrifugation at 15,000 rpm for 15 min at 4°C. The supernatants were collected after centrifugation. Proteins were quantified using the Bicinchoninic Acid Protein Assay kit (Beyotime, Nantong, China). A total of 40  $\mu$ g protein was loaded onto each lane of 12% polyacrylamide gel followed by electrotransfer onto polyvinylidene fluoride membranes (Thermo Fisher Scientific, Waltham, MA, USA). The membranes were then blocked with skimmed milk powder in phosphate-buffered saline with 0.05% Tween-20 (PBST) for 2 h and incubated with the following primary antibodies at 4°C overnight: mouse monoclonal antibody against SHP1 (Sc-7289) was purchased from Santa Cruz Biotechnology, Inc. and used at a dilution of 1:500; rabbit monoclonal antibody against SHP1 phosphorylation (pSHP1) was purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA) and used at a dilution of 1:1,000; mouse monoclonal antibody against STAT3 was purchased from Cell Signaling Technology, Inc. and used at a dilution of 1:1,000; antibody against GADPH was purchased from TransGen Biotech Co., Ltd. (Beijing, China) and used at a dilution of 1:2,000. Membranes were rinsed in PBST three times and incubated with the secondary antibody at a dilution of 1:2,000 (horseradish peroxidase-conjugated monoclonal goat anti-mouse IgG or horseradish peroxidase-conjugated monoclonal goat anti-rabbit IgG; TransGen Biotech Co., Ltd.) at room temperature for 1 h. Membranes were rinsed in PBST three times again and were developed using an enhanced chemiluminescence detection system (GE Healthcare, Piscataway, NJ, USA).

**Statistical analysis.** Distribution of patient characteristics was applied by the Chi-squared ( $\chi^2$ ) test. The relationship between SHP1 protein expression and the clinicopathologic variables was evaluated by the Fisher's exact test and  $\chi^2$  test. Two group comparison was carried out using the Student's t-test or Wilcoxon signed-rank test. All statistical analyses were performed using the SPSS 17.0 software package. A value of  $p < 0.05$  was considered statistically significant.

## Results

**Patient characteristics.** The general information of the PCNSL patients involved in this study is described in Table I. All PCNSLs were proven to be DLBCL by pathological analysis and 29 (87.9%) specimens were obtained from stereotactic biopsies. The median age was 57 years (range, 28-83 years) with a gender ratio of male-to-female of 1.75. Ten patients (30.3%)

Table I. Clinical characteristics of the PCNSL patients.

Characteristics	Patients, n (%)
Age (years)	
>60	12 (36.4)
≤60	21 (63.6)
Gender	
Male	22 (66.7)
Female	11 (33.3)
LDH	
Elevated	13 (39.4)
Normal	20 (60.6)
Immunophenotype	
GCB	4 (12.1)
Non-GCB	29 (87.9)
No. of lesions	
1	12 (36.4)
At least 2	21 (63.6)
ECOG performance status	
0 to 1	10 (30.3)
At least 2	23 (69.7)
Deep structure involvement	
Presence	24 (72.7)
Absence	9 (27.3)
Stereotactic biopsy	29 (87.9)
Surgery	4 (12.1)
Treatment	
HD-MTX based chemotherapy	33 (100)
Pathology	
DLBCL	33 (100)

PCNSL, primary central nervous system lymphoma; LDH, lactate dehydrogenase; GCB, germinal center B-cell-like; ECOG, Eastern Cooperative Oncology Group; HD-MTX, high-dose methotrexate; DLBCL, diffuse large B-cell lymphoma.

were scored 0 or 1 by the Eastern Cooperative Oncology Group (ECOG) performance status and the other 23 patients (69.7%) scored 2-4 by the same scoring system. Multiple brain lesions were observed in 63.6% of the patients (21/33) and deep brain structures were compromised in 72.7% of the patients (24/33). Among routine biochemical markers, an elevated serum lactate dehydrogenase (LDH) level was observed in 13 of the 33 patients (39.4%). For all samples from these patients, CD10, BCL-6, MUM1 and BCL-2 positive expression was noted in 12.12% (4/33), 45.46% (15/33), 87.88% (29/33) and 78.57% (22/28) of the cases, respectively. According to the Hans classification criteria (30), 4 cases (12.12%) were classified as the germinal center B-cell-like (GCB) subgroup, and the other 29 cases (87.88%) were non-GCB.

**SHP1 protein expression was confirmed by immunohistochemical staining.** Immunohistochemical staining was conducted for SHP1 protein expression. Immunoreactivity for

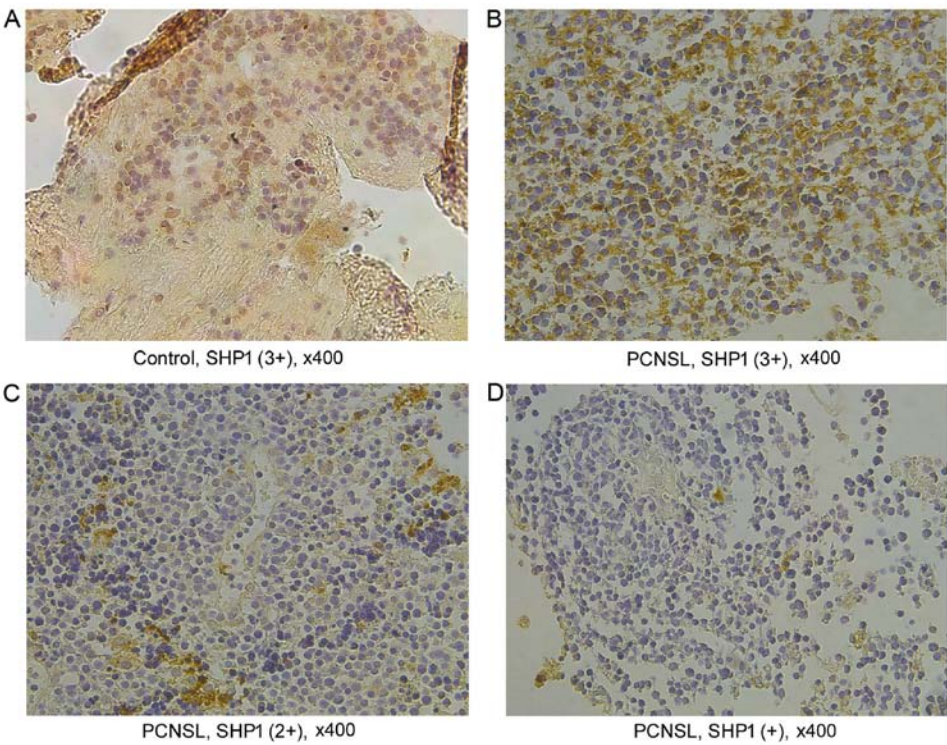


Figure 1. SHP1 expression profiles in primary central nervous system lymphoma (PCNSL) and hyperplasia of lymph nodes. (A) Representative high cytoplasmic SHP1 expression (3+) in hyperplasia of lymph nodes. (B) SHP1-positive lymphoma showing cytoplasmic staining (3+). (C) SHP1-positive lymphoma showing cytoplasmic staining (2+). (D) SHP1-positive lymphoma showing cytoplasmic staining (+). SHP1, Src homology region 2 domain-containing phosphatase-1.

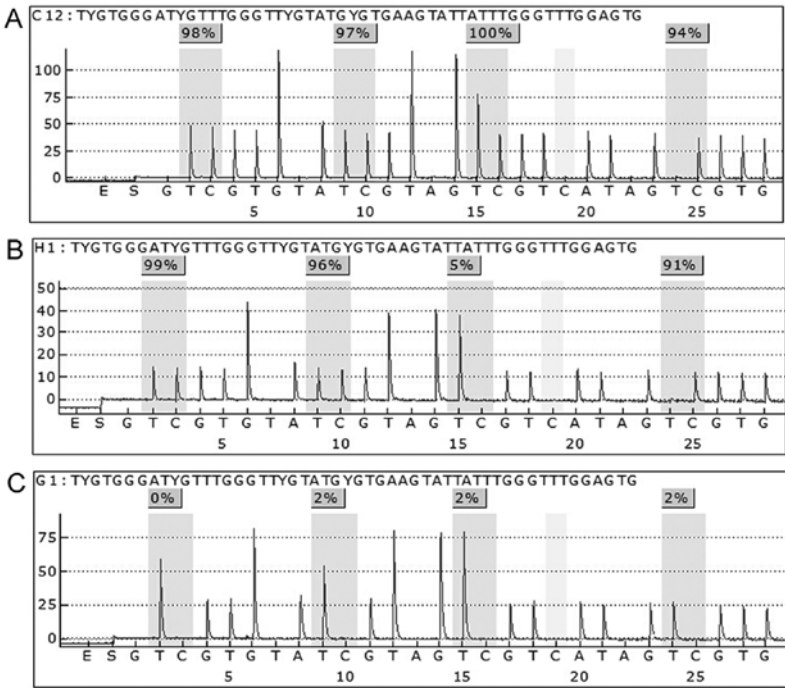


Figure 2. SHP1 promoter methylation status in primary central nervous system lymphoma (PCNSL) by pyrosequencing analysis. (A) Methylation levels ranged from 94 to 100%, with a mean of 97.3%. (B) Methylation levels ranged from 5 to 99%, with a mean of 72.8%. (C) Methylation levels ranged from 0 to 2%, with a mean of 1.5%. The gray highlighted bar marks the tested CpG sites and the percentage readings of cytosine and thymine. SHP1, Src homology region 2 domain-containing phosphatase-1.

SHP1 was strong in reactive hyperplasia of lymph node as a positive control and scored as (3+) (Fig. 1A). SHP1-positive immunostaining was detected in 30 PCNSL tissues (90.9%),

of which, 17 positive cases showing a high SHP1 expression (over 60% positive cells) were scored as (3+) (Fig. 1B); while the remaining 13 cases showed a low SHP1 expression with

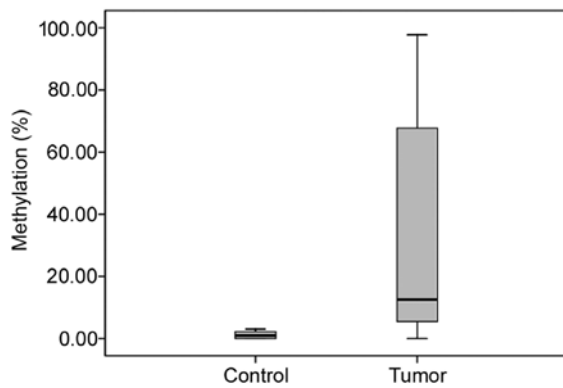


Figure 3. Comparison of promoter methylation levels of SHP1 in primary central nervous system lymphoma (PCNSL) tumor tissues and paired peripheral blood. PCNSL tumor tissues had higher methylation levels of SHP1 ( $31.7 \pm 36.5$  vs.  $1.2 \pm 1.1\%$ ,  $p < 0.001$ ). SHP1, Src homology region 2 domain-containing phosphatase-1.

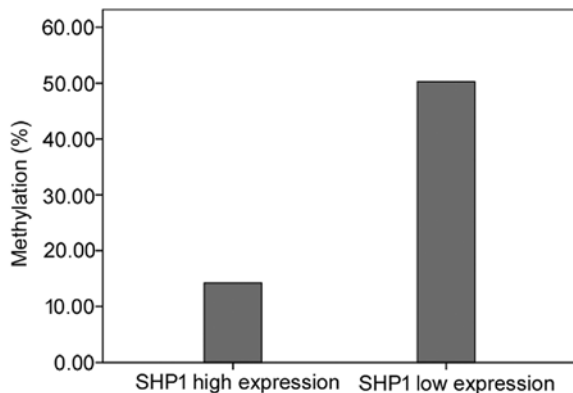


Figure 4. Comparison of promoter methylation levels of SHP1 in the SHP1 protein low expression group and high expression group. The methylation level was higher in the low expression SHP1 protein group than this level in the high expression SHP1 protein group ( $p = 0.004$ ). SHP1, Src homology region 2 domain-containing phosphatase-1.

less than 60% of positive cells in the tissues, including 9 cases of (2+) and 4 cases of (+), respectively (Fig. 1C and D).

**SHP1 protein expression is correlated with promoter methylation status in PCNSL patients.** The SHP1 mean methylation level in the control samples was  $1.2 \pm 1.1\%$ . SHP1 promoter methylation was detected in 29/33 of the tumor tissues (87.9%) with a mean methylation level of  $31.7 \pm 36.5\%$ . In this study, PCNSL tissues with a mean methylation level of  $>3.4\%$  (mean  $\pm$  2SD of the control samples) were considered to have a bona fide methylation state, otherwise they were considered unmethylated (Fig. 2). Compared with the methylation level of the PCNSL samples, no significant methylation was observed in the control samples ( $p < 0.001$ ) (Fig. 3).

In 15 patients, the tumor tissues showed a low level of SHP1 expression and promoter methylation, and 3 unmethylated patients showed a high SHP1 expression. However, one unmethylated patient had a decreased SHP1 protein expression. The methylation levels in patients with a low SHP1 protein expression were higher than those with a high SHP1 protein expression ( $50.3 \pm 38.9$  vs.  $14.2 \pm 24.0\%$ ,  $p = 0.004$ ) (Fig. 4).

Table II. Clinical characteristics of the patients with PCNSL in relation to SHP1 protein expression (Fisher's exact test).

Variables	SHP1 protein expression (n=33), n (%)		P-value
	Low (n=16)	High (n=17)	
Age (years)			
>60	7 (58.33)	5 (41.67)	0.48
≤60	9 (42.86)	12 (57.14)	
Gender			
Male	11 (50.00)	11 (50.00)	1.00
Female	5 (45.45)	6 (54.55)	
LDH			
Elevated	10 (76.92)	3 (23.08)	0.03
Normal	7 (35.00)	13 (65.00)	
Immunophenotype			
GCB	3 (75.00)	1 (25.00)	0.34
non-GCB	13 (44.83)	16 (55.17)	
No. of lesions			
1	5 (41.67)	7 (58.33)	0.72
At least 2	11 (52.38)	10 (47.62)	
ECOG performance status			
0 to 1	5 (50.00)	5 (50.00)	1.00
At least 2	11 (47.83)	12 (52.17)	
Deep structure involvement			
Presence	11 (45.83)	13 (54.17)	0.71
Absence	5 (55.56)	4 (44.44)	

PCNSL, primary central nervous system lymphoma; SHP1, Src homology region 2 domain-containing phosphatase-1; LDH, lactate dehydrogenase; GCB, germinal center B-cell-like; ECOG, Eastern Cooperative Oncology Group.

Low SHP1 expression was present more frequently in PCNSL patients with elevated LDH than those with a normal LDH level (76.92 vs. 35.00%,  $p = 0.03$ ) (Table II), while SHP1 protein expression was not related to the indicated clinicopathologic variables routinely detected in PCNSL.

**Promoter methylation downregulates SHP1 expression and phosphorylation levels in PCNSL tissues.** For the samples used for western blot analysis, expression levels of SHP1, pSHP1 and STAT3 were detected and analyzed. Compared with samples with unmethylation of the SHP1 promoter and controls, decreased SHP1 and pSHP1 expression was observed in samples with SHP1 promoter methylation. Contrarily, the STAT3 expression was substantially elevated, showing that SHP1 promoter methylation was associated with a decrease in SHP1 ( $p = 0.001$ ) and pSHP1 ( $p = 0.005$ ), and an increase in STAT3 ( $p = 0.020$ ) in PCNSL. The experiments were performed



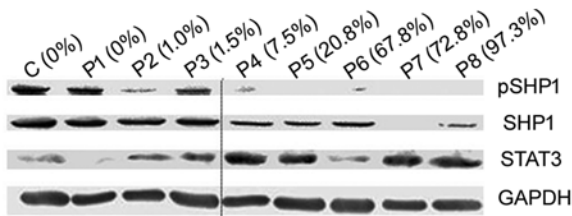


Figure 5. SHP1 expression is associated with pSHP1 and STAT3. Decreased expression of SHP1 induced downregulation of pSHP1, whereas it upregulated STAT3. C, control; P1-8, PCNSL cases; (%), the mean methylation level of the SHP1 promoter. SHP1, Src homology region 2 domain-containing phosphatase-1.

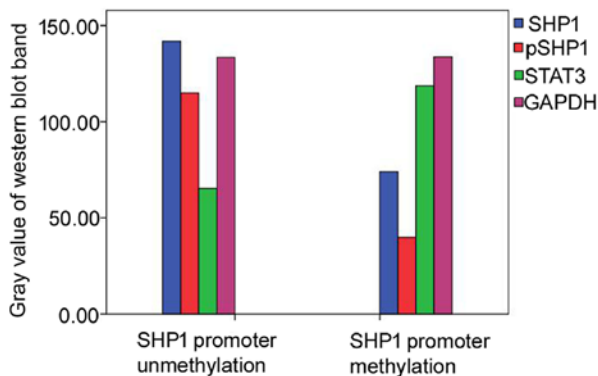


Figure 6. Comparison of gray values of the western blot bands in the methylated and unmethylated SHP1 promoter. The expression of SHP1 and pSHP1 had lower gray values in the western blot band in the methylated SHP1 promoter group compared with the unmethylated SHP1 promoter group ( $p=0.001$  and  $p=0.005$ ), whereas STAT3 had higher gray values in the western blot band in the methylated SHP1 promoter group compared with the unmethylated SHP1 promoter group ( $p=0.020$ ). SHP1, Src homology region 2 domain-containing phosphatase-1.

three times on different samples and a representative result is shown in Figs. 5 and 6.

## Discussion

PCNSL is a rare form of extranodal lymphoma confined to the central nervous system. Largely due to the low incidence and obstacles to obtain tumor tissues, researchers are facing great challenges for a complete recognition of PCNSL via genomic analysis. Although the underlying genetic basis still remains unclear, existing evidence has uncovered the constitutive activation of JAK1, STAT3 and STAT6 in PCNSL. The constitutive activation of the JAK/STAT signaling pathway can induce sustained survival. Defining the key molecular pathways in PCNSL development would provide clues for targeted therapies against these malignancies.

In the present study, we confirmed that SHP1 protein was expressed in PCNSL tissues. The majority of PCNSL patients showed strong SHP1 protein expression, in line with results reported by one previous study on PCNSL (24). Sugita *et al* showed that SHP1 expression in PCNSL reflects the origin of PCNSL from a late germinal center to an early postgerminal center stage (24). Kossev *et al* showed that normal B cells in the mantle and marginal zones as well as plasma cells uniformly

express SHP1. However, specific areas of the germinal centers showed no SHP1 expression (31). These results collectively suggest that SHP1 is downregulated at antigen-dependent stages of B-cell proliferation and differentiation. Moreover, this pattern of SHP1 protein expression was also observed in small B cell lymphomas, including mantle cell lymphoma, marginal zone lymphoma and chronic lymphocytic leukemia/small lymphocytic lymphoma whereas follicle center cell lymphoma did not. In this study, SHP1 expression was positive in the majority of PCNSL cases with lower expression of germinal center marker CD10, confirming their post-germinal center origin. Notably, we found that 17 of 30 SHP1-positive cases were scored as 3+, showing a percentage less than the result reported by Sugita *et al* who found that all SHP1-positive cases strongly expressed and were scored as 3+ (24). By comparing the results vis-à-vis, we believe that the alteration in the SHP1 expression profile in PCNSL per se and experimental variations may contribute to the discrepancy in expression intensity interpretations.

Our results indicate that SHP1 expression and phosphorylation are suppressed by SHP1 promoter methylation. SHP1 gene methylation associated with loss of SHP1 protein has been demonstrated in many hematologic cancers. To clarify the difference in SHP1 protein expression intensity, we performed pyrosequencing analysis and western blot analysis. We found that SHP1 promoter methylation occurred in 29 of 33 PCNSL cases, and aberrant promoter SHP1 promoter methylation decreased the SHP1 protein expression, in accordance with the results noted in extranodal lymphomas (32,33). SHP1 plays a critical role in the regulation of the JAK/STAT pathway (15,34,35). The biological features of SHP1 were emphasized by the 'moth-eaten' (*mev/mev*) mouse model, which showed markedly decreased SHP1 expression and defects in myeloid cell function with a tendency for developing lymphomas (36,37). SHP1 gene methylation associated with loss of SHP1 protein has been demonstrated in many hematologic cancers. In view of the normal biological significance of SHP1, SHP1 likely plays an important role in the pathogenesis of these cancers. Our data indicate that abnormal SHP1 expression contributes to the constitutive activation of the JAK/STAT signaling pathway in the pathogenesis of PCNSL.

In addition, our study demonstrated that SHP1 promoter methylation did not correlate with a complete absence of SHP1 protein expression. Several studies have also indicated this phenomenon and have suggested that monoallelic promoter methylation may not sufficiently cause complete loss of gene expression or promoter methylation, only in a small portion of tumor cells (38,39), presumably due to the fact that promoter methylation may be one of the multiple causative mechanisms in tumor cells. Notably, this phenomenon was not limited to the SHP1 gene (38-41). In this study, the SHP1 promoter methylation did not cause a complete loss but a decrease in SHP1 protein expression, and the phosphorylation level reflecting the biological activities of SHP1 protein was synchronously decreased. Notably, our further analysis of the downstream signals indicated that the absence of or reduction in the phosphatase activity of SHP1 contributed to STAT3 activation in PCNSL. These results support the notion that promoter methylation contributes to decreased SHP1 protein expression and function, eventually affecting the pathogenesis of PCNSL.

Additionally, in our study, a low-level expression of SHP1 was associated with an elevated LDH level, which was similar to previous reports (42,43). Our preliminary study showed that a high LDH concentration was associated with a poor outcome in PCNSL (44). Currently, the prognostic value of SHP1 protein expression and SHP1 promoter methylation has not been well acknowledged in PCNSL. Nevertheless, in other tumor types including DLBCL, anaplastic large cell lymphoma (ALCL), prostate cancer and high-grade glioma, SHP1 has been proposed as a prognostic factor (42,45,46). In these studies, the SHP1 promoter methylation status and loss of SHP1 protein expression commonly indicate a poor prognosis.

Given that PCNSL increasingly occurs in elderly populations, in which a large proportion of patients are not eligible for routine intensive therapies such as HD-MTX chemotherapy, novel therapies which target key survival molecular points in signaling pathways of PCNSL would enhance the overall patient outcomes. In view of the fact that SHP1 promoter methylation and constitutive activation of the JAK/STAT pathway are co-existent in PCNSL, epigenetic therapeutic strategies by clinically available DNA demethylation agent 5-aza-2'-deoxycytidine may be an effective approach. Most importantly, the demethylation anchor drug 5-aza-2'-deoxycytidine penetrates the blood-brain barrier, providing further merit for the potential clinical application. Currently, how to achieve an effective and safe concentration of such agents in the central nervous system is a critical challenge. Thus, further prospective studies are needed to validate these results and to develop optimal therapies using epigenetic modifiers for PCNSL treatment.

In conclusion, we report in the present study that aberrant methylation of the SHP1 promoter is correlated with decreased SHP1 expression and phosphorylation. Attenuation of the biological functions of SHP1 contributes to the activation of transcription factor STAT3, which may affect the pathogenesis of PCNSL. Epigenetic modifiers such as 5-aza-2'-deoxycytidine may be of significance for the development of methylation-targeted therapeutic regimens.

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