# Critical role of SHP2 (PTPN11) signaling in germinal center-derived lymphoma

Xin Jiang,[1] Honggang Guo,[1] Jianguo Wu,[1] Qiang He,[2] Yiqiao Li,[2] Miao Wang,[3] Hongyang Pan,[4] Wande Li,[5] Jinjie Wang,[1] Qingqing Wang,[6] Jing Shen,[1] Yuehai Ke,[1] and Ren Zhou[1]*

[1]Department of Pathology and Pathophysiology, Zhejiang University School of Medicine, Hangzhou, China; [2]Department of Nephrology, Zhejiang Province People's Hospital, Hangzhou, China; [3]Department of Pathology, Basic Medical College, Capital Medical University, Beijing, China; [4]Central Laboratory, Epitomics (Hangzhou) Biotech Co. Ltd, Hangzhou, Zhejiang, China; [5]Department of Biochemistry, Boston University School of Medicine, MA, USA; and [6]Department of Immunology, Zhejiang University School of Medicine, Hangzhou, China

## ABSTRACT

Germinal center lymphoma is a heterogeneous human lymphoma entity. Here we report that constitutive activity of SHP2 (PTPN11) and its downstream kinase ERK is essential for the viability of germinal center lymphoma cells and disease progression. Mechanistically, SHP2/ERK inhibition impedes c-Myc transcriptional activity, which results in the repression of proliferative phenotype signatures of germinal center lymphoma. Furthermore, SHP2/ERK signaling is required to maintain the CD19/c-Myc loop, which preferentially promotes survival of a distinct subtype of germinal center lymphoma cells carrying the *MYC/IGH* translocation. These findings demonstrate a critical function for SHP2/ERK signaling upstream of c-Myc in germinal center lymphoma cells and provide a rationale for targeting SHP2 in the therapy of germinal center lymphoma.

## Introduction

The germinal center (GC) within secondary lymphoid organs is crucial for the generation of thymus-dependent humoral immune responses.[1] B cells that enter the GC proliferate and undergo somatic hypermutation and class switch recombination to generate high-affinity antibodies or go towards the apoptotic program.[2] However, both somatic hypermutation and class switch recombination are major threats to GC B-cell genomic integrity and stability.[3] Thus, the beneficial features of GC B cells are, to some extent, counterbalanced by their potential adverse roles in lymphomagenesis because the majority of malignant lymphomas derive from GC B cells (GC lymphoma).[4,5] Prototypic examples include the translocation which places oncogenic *MYC* under the control of the *IGH* promoter in Burkitt lymphoma[6] and some diffuse large B-cell lymphomas[7] as well as the *BCL2/IGH* translocation in follicular lymphoma and GC B-cell-like diffuse large B-cell lymphoma.[8]

The entry of B cells into the GC requires stimulation by foreign pathogens through both BCR/CD19/CD21[9] and antigen-specific T helper cell/CD40L/CD40 signals.[10] GC B cells use multiple stimulatory signals, but their corresponding contributions remain poorly understood to date. The B-cell membrane antigen CD19 modulates low-affinity B-cell receptor signals and enhances GC formation in adaptive immunity through PI3K signaling.[11,12] Although CD19 overexpression in B cells inducing autoantibody production in CD19 transgenic mice and the preferential cell expansion of malignant B cells expressing high intensity CD19 are attributed to their competitive advantage in eliciting a response to external and internal antigens,[13-15] the regulation of CD19 signals controlling B-cell transformation is still difficult to define clearly. Co-aggregate B-cell receptor and CD19/CD21 co-receptors are required for the survival of both resting and cycling B cells by regulating the cellular accumulation of the anti-apoptotic protein Bcl-2.[16,17] Additionally the B-cell receptor, by stabilizing the amount and activity of the oncoprotein c-Myc,[18,19] might facilitate B-cell lymphomagenesis through the CD19/RAS/ERK pathway, which can promote the expression of anti-apoptotic proteins.[19,20] Indeed, previous experimental data acquired through the use of Eμ-Myc transgenic mice demonstrated that the c-Myc/ERK/CD19 feedback signaling loop is important for the malignant transformation of B cells and in determining the severity of lymphomas.[19,20] Chromosomal overexpression of translocation-elicited c-Myc induces the transcription of cyclin and cyclin-dependent kinase (CDK) genes which also relies significantly on CD19/ERK cascade-mediated phosphorylation of c-Myc on Ser62.[19] Specifically, CD19-mediated *MYC/IGH* translocation-induced (*MYC/IGH*+) B-cell lymphomagenesis can be inhibited by deleting the scaffold protein kinase suppressor of RAS-1 (KSR1) or treatment with MEK/ERK inhibitors.[20] Nevertheless, it remains unclear whether CD19 expression itself contributes alone to the regulation of c-Myc protein stability and activity in GC lymphoma, or whether CD19 expression is directly regulated by c-Myc transcriptional activity. The characterization of these molecular mechanisms might provide novel insights into the biology of GC lymphoma and reveal prognostic factors and therapeutic targets.

The first identified ubiquitous protein tyrosine phosphatase SHP2 (encoded by *PTPN11*) has a critical but poorly defined function in the regulation of immune responses.[21,22] SHP2 binds to receptor tyrosine kinases, either directly or through scaffolding adapter molecules such as IRS2 and GAB family proteins.[23,24]

 doi:10.3324/haematol.2014.106401
The online version of this article has a Supplementary Appendix.
Manuscript received on February 24, 2014. Manuscript accepted on September 2, 2014.
Correspondence: zhouren@zju.edu.cn

It then undergoes phosphorylation at regulatory tyrosines in its C-terminus, particularly the signal-initiating Tyr542 or Tyr580 and recruits the GRB2/SOS complex to the plasma membrane,[25] which in turn leads to ERK activation. SHP2 mediates most cytokine- and growth factor-stimulated activation of the MAPK/ERK pathway.[26] Other kinase pathways such as JAK-STAT and PI3K regulated by SHP2 are cell-specific and/or receptor-specific.[26-29] In malignancies, SHP2 is aberrantly activated downstream of oncoproteins or by mutation.[30-33] Germline and somatic gain-of-function mutations of SHP2 are found in nearly 50% of the instances of Noonan syndrome and 35% of cases of juvenile myelomonocytic leukemia but are rarely reported in solid tumors.[34,35] Overexpression of SHP2 is also detected in samples from patients with leukemia and diffuse large B-cell lymphoma.[36-38] However, the relative expression and functional impact of SHP2 in B-cell lymphomagenesis remains unknown.

This study provides evidence that SHP2/ERK signaling is essential for viability and c-Myc activation in GC lymphoma lines. Furthermore, the CD19/c-Myc signaling loop, which is preferentially associated with GC lymphoma lines that carry the *MYC/IGH* translocation, are dependent on SHP2 catalytic activity. This strongly suggests that specific biological features of GC lymphoma are likely to be determined by the simultaneous activation of the SHP2/ERK pathway.

## Methods

This study was approved by the Ethics Committee of Zhejiang University School of Medicine. The methods used for quantitative real-time polymerase chain reaction analysis of GC lymphoma proliferative signatures are described in detail in the *Online Supplementary Methods* and in *Online Supplementary Table S1*. The *Online Supplementary Methods* section also includes information on the cell cultures, antibodies, chemical reagents, short-hairpin RNA, procedures for immunohistochemistry, chromatin immunoprecipitation, luciferase activity assay and MTT assay.

### Cell viability, proliferation and cell cycle assays

Cell viability was quantified by counting cells up to 5 days after trypan blue staining.[20] Cell cycle analysis was determined by flow cytometry after staining with 50 μg/mL propidium iodide (Invitrogen). Cell proliferation rate was also determined, at the indicated times, by flow cytometry after staining with carboxyfluorescein succinimidyl ester (CFSE; Invitrogen). Cells were acquired on a FC 500 MCL (Beckman Coulter) and analyzed using FlowJo software (Tree Star, Inc.).

### Confocal immunofluorescence staining

Primary antibodies anti-CD19, anti-SHP2, and anti-GAB2 (all from Cell Signaling Technology) were diluted 1:50 before use. Nuclei were stained for 10 min at room temperature with 300 ng/mL DAPI (Sigma). All sections were independently evaluated with a LSM 700 confocal microscope (Carl Zeiss) by three investigators.

### Immunoblotting, immunoprecipitation and electrophoretic mobility shift assay

Cells were lysed on ice for 30 min with 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, and protease inhibitors (Roche). Immunoblotting and immunoprecipitation analysis were performed as described previously.[38] The electrophoretic mobility shift assay (EMSA) was conducted with $^{32}$P-dATP-labeled oligonucleotide probes (*Online Supplementary Table S2*) and the products were separated on a native polyacrylamide gel.

### Statistical analysis

Statistical analyses were performed using the Student *t* test with 95% confidence intervals.

## Results

### SHP2 is necessary for the progression of germinal center lymphoma

To monitor whether SHP2/ERK signaling was activated in B-lymphoma cells, we first assessed the phosphorylation status of SHP2 and ERK in six lymphoma lines whose cell of origin was known (Figure 1A, *Online Supplementary Table S3*). The four GC lymphoma lines expressed high levels of total and activated forms of SHP2 protein, whereas SHP2 was activated at very low levels in two non-GC lymphoma lines; however, ERK1/2 and its phosphorylation were similar in all six lymphoma lines. We also detected significant amounts of either total or activated AKT in $^{MYC/IGH-}$GC lymphoma and non-GC lymphoma lines, whereas $^{MYC/IGH+}$GC lymphoma lines had very little phosphorylation of AKT. Activation of IκBα and STAT3 was detected in $^{MYC/IGH-}$GC lymphoma (Pfeiffer) and non-GC lymphoma lines, but not in $^{MYC/IGH-}$GC lymphoma (SU-DHL5) and $^{MYC/IGH+}$GC lymphoma lines.

To extend our study to primary lymphomas, we also assessed the phosphorylation status of SHP2 and ERK1/2 using immunohistochemical methods on 56 paraffin-embedded biopsies from B-lymphomas. As expected, we observed that virtually all GC lymphomas stained positive for SHP2 Tyr542 phosphorylation (*Online Supplementary Figure S1A,B*). Conversely, non-GC lymphoma cases had very few phospho-Tyr542 SHP2-positive stained malignant cells (*Online Supplementary Figure S1A,B*). There was no significant difference ERK1/2 phosphorylation status between the two groups of B-lymphomas (*Online Supplementary Figure S1A,B*).

To determine the role of SHP2 in the progression of GC lymphomas, we constructed lentiviral vectors expressing two independent short hairpin RNA (shRNA) targeting SHP2 (SHP2 shR1 and shR2) and obtained stable B-lymphoma lines expressing SHP2 shRNA (SHP2Δkd). Two SHP2 shRNA were highly toxic for four GC lymphoma lines with or without *MYC/IGH* translocation but not for two non-GC lymphoma lines (Figure 1B,C and *Online Supplementary Figure S2A*). Consistent with the depletion of SHP2, Erk1/2 phosphorylation was also reduced in most $^{SHP2\Delta kd}$B-cell lymphoma lines except $^{SHP2\Delta kd}$OCI-LY3 cells (*Online Supplementary Figure S2A*). In subsequent survival assays, two MEK inhibitors (U0126 and PD0325901) were toxic for four GC lymphoma lines but not for two non-GC lymphoma lines, whereas LY294002 (PI3K inhibitor) and BAY 11-7082 (IκBα inhibitor) were selectively toxic to two non-GC lymphoma cell lines (*Online Supplementary Figure S2B*). Our results also showed that depletion of SHP2 resulted in a consistent downregulation of the GC lymphoma phenotype protein BCL6 in GC lymphoma cells, whereas the non-GC lymphoma cells had very little BCL6 protein (*Online Supplementary Figure S2A*). After inhibition of either SHP2 or ERK, the gene expression of GC lymphoma proliferative phenotype signatures was significantly downregulated (*Online Supplementary Figure S3A,B* and *Online Supplementary*

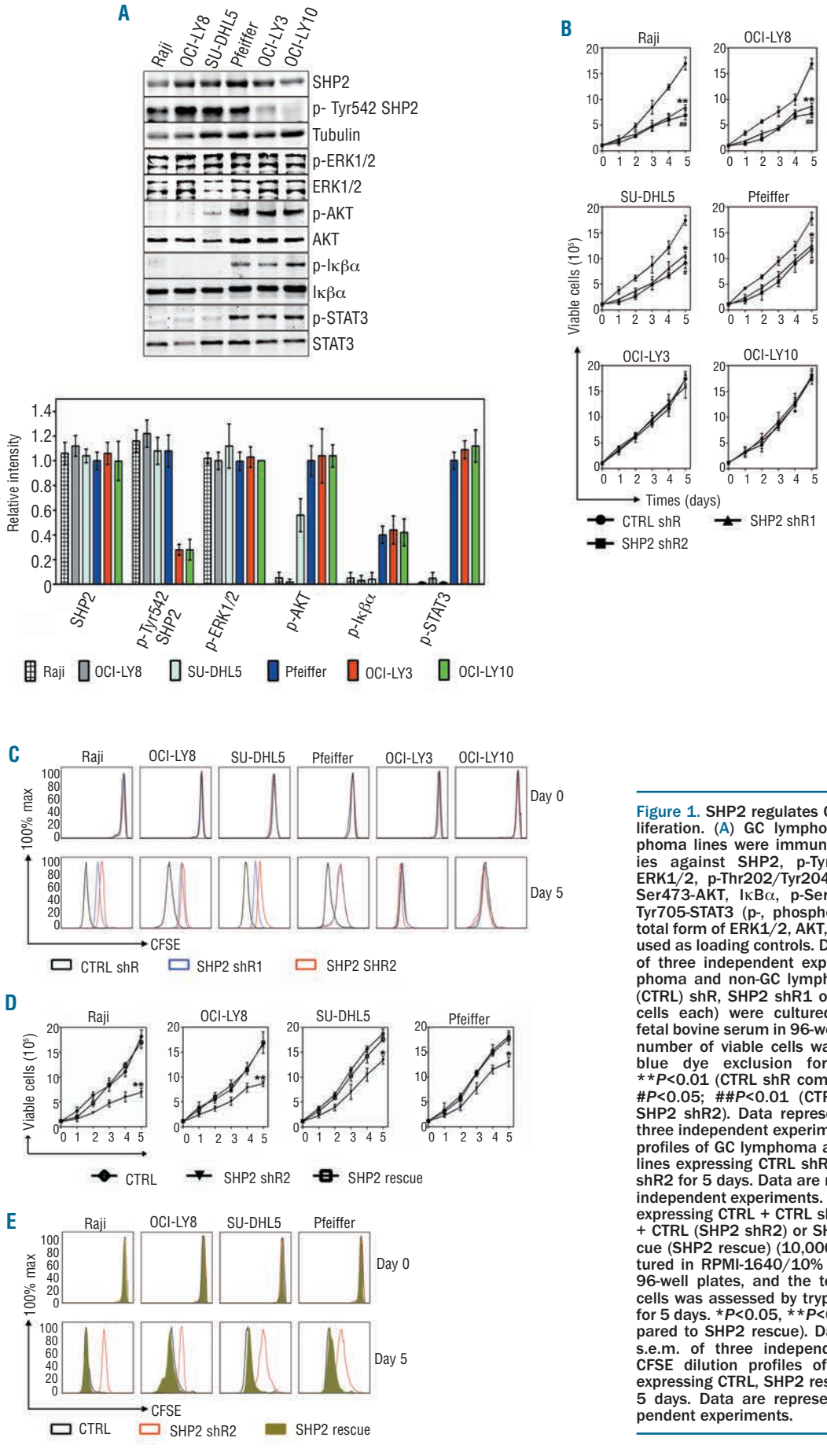


**Figure 1.** SHP2 regulates GC lymphoma cell proliferation. (A) GC lymphoma and non-GC lymphoma lines were immunoblotted with antibodies against SHP2, p-Tyr542 SHP2, tubulin, ERK1/2, p-Thr202/Tyr204-ERK1/2, AKT1/2, p-Ser473-AKT, IκBα, p-Ser32- IκBα, STAT3, p-Tyr705-STAT3 (p-, phosphorylated). Tubulin and total form of ERK1/2, AKT, IκBα, and STAT3 were used as loading controls. Data are representative of three independent experiments. (B) GC lymphoma and non-GC lymphoma lines expressing (CTRL) shR, SHP2 shR1 or SHP2 shR2 (10,000 cells each) were cultured in RPMI-1640/10% fetal bovine serum in 96-well plates, and the total number of viable cells was assessed by trypan blue dye exclusion for 5 days. **P*<0.05, ***P*<0.01 (CTRL shR compared to SHP2 shR1). #*P*<0.05; ##*P*<0.01 (CTRL shR compared to SHP2 shR2). Data represent mean ± s.e.m. of three independent experiments. (C) CFSE dilution profiles of GC lymphoma and non-GC lymphoma lines expressing CTRL shR, SHP2 shR1 or SHP2 shR2 for 5 days. Data are representative of three independent experiments. (D) GC lymphoma cells expressing CTRL + CTRL shR (CTRL), SHP2 shR2 + CTRL (SHP2 shR2) or SHP2 shR2 + SHP2 rescue (SHP2 rescue) (10,000 cells each) were cultured in RPMI-1640/10% fetal bovine serum in 96-well plates, and the total number of viable cells was assessed by trypan blue dye exclusion for 5 days. **P*<0.05, ***P*<0.01 (SHP2 shR2 compared to SHP2 rescue). Data represent mean ± s.e.m. of three independent experiments. (E) CFSE dilution profiles of GC lymphoma cells expressing CTRL, SHP2 rescue or SHP2 shR2 for 5 days. Data are representative of three independent experiments.

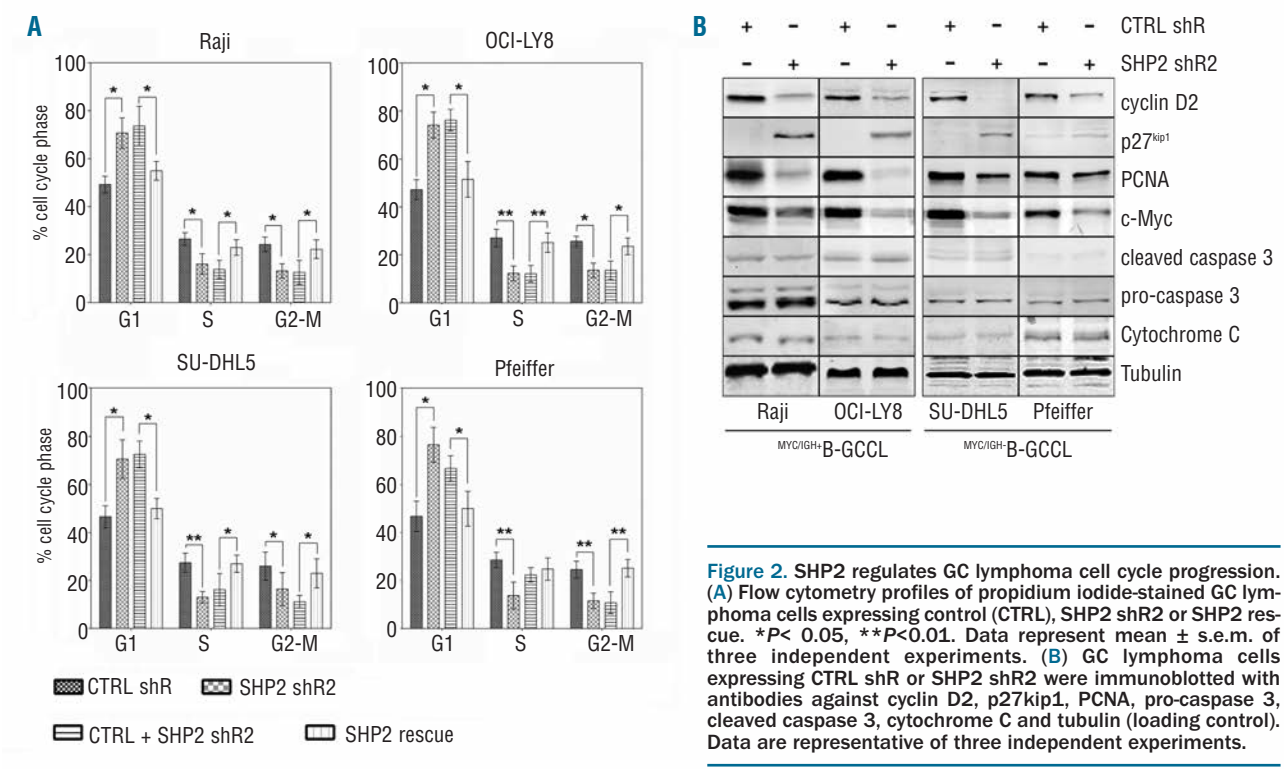


**Figure 2. SHP2 regulates GC lymphoma cell cycle progression.** (**A**) Flow cytometry profiles of propidium iodide-stained GC lymphoma cells expressing control (CTRL), SHP2 shR2 or SHP2 rescue. **P*< 0.05, ***P*<0.01. Data represent mean ± s.e.m. of three independent experiments. (**B**) GC lymphoma cells expressing CTRL shR or SHP2 shR2 were immunoblotted with antibodies against cyclin D2, p27kip1, PCNA, pro-caspase 3, cleaved caspase 3, cytochrome C and tubulin (loading control). Data are representative of three independent experiments.

*Tables S4* and *S5*). To exclude off-target effects, we rescued the proliferation of GC lymphoma cells by expressing a non-targetable SHP2 complementary DNA (rescue) in cells expressing SHP2 shR2 (Figure 1D,E and *Online Supplementary Figure S4A*), thereby confirming that SHP2/ERK signaling inhibition blocks GC lymphoma cell proliferation.

To identify whether the observed low growth rate in $^{SHP2\Delta kd}$GC lymphoma cells was due to cell cycle arrest, propidium iodide incorporation was assessed. Cell cycle analysis revealed that SHP2 knockdown led to a significant increase in the G1 phase and a corresponding decrease in the S and G2-M phases in GC lymphoma cells (Figure 2A). In addition, we stained for the proto-oncogene c-Myc, proliferation marker PCNA, cell cycle regulator cyclin D2, CDK inhibitor p27$^{kip1}$ and apoptotic markers caspase 3 and cytochrome C in GC lymphoma cells expressing control or SHP2 shR2 (Figure 2B). SHP2 knockdown resulted in down-regulation of c-Myc, cyclin D2 and PCNA and up-regulation of p27$^{kip1}$. However, the levels of cytochrome C, caspase 3 and its cleaved forms were not changed in $^{SHP2\Delta kd}$GC lymphoma cells. These GC lymphoma proliferative phenotype markers, including BCL6, cyclin D2 and PCNA, and SHP2/ERK signaling had been rescued by exogenous SHP2 (Figure 2A and *Online Supplementary Figure S4B,C*). These data indicate that a lack of SHP2 prevents the hyperproliferative phenotypes of GC lymphoma cells grown in culture without affecting apoptosis of the cells.

### Active CD19 signaling controls viability and proliferation of distinct $^{MYC/IGH+}$GC lymphoma cells

Active CD19 signaling promoted constitutive ERK/c-Myc signaling in various $^{MYC/IGH+}$B-lymphoma samples,[19] but whether CD19 signaling contributes to other types of B-lymphoma remained unclear. Here, a similar expression pattern of CD19 and its activated form was detected in all six B-lymphoma lines and 56 paraffin-embedded B-lymphoma biopsies, which did not correlate with cell of origin status (Figure 3A and *Online Supplementary Figure S1A,B*). A CD19 shRNA was selectively toxic for $^{MYC/IGH+}$GC lymphoma lines but not for $^{MYC/IGH-}$GC lymphomas or for non-GC lymphoma lines (Figure 3B-D, *Online Supplementary Figure S3A* and *Online Supplementary Table S4*). The ERK/c-Myc pathway was activated by CD19 signaling only in $^{MYC/IGH+}$GC lymphoma lines since knockdown of CD19 impaired ERK1/2 phosphorylation and depressed the expression of ERK target c-Myc (Figure 3B, *Online Supplementary Figures S2C* and *S5A*) and proliferation in $^{MYC/IGH+}$GC lymphoma lines but not in $^{MYC/IGH-}$GC lymphoma or the non-GC lymphoma lines (Figure 3C,D). In addition, CD19 signaling had no effect on the AKT, IκBα, STAT3 kinase pathways in these cells (Figure 3B). CD19 knockdown also decreased SHP2 Tyr542 phosphorylation, but not total amount of SHP2 in $^{MYC/IGH+}$GC lymphoma lines (Figure 3E). These data suggest a critical role for CD19/ERK/c-Myc signaling in maintaining the viability and proliferation of distinct $^{MYC/IGH+}$GC lymphomas.

### SHP2 depletion impairs CD19-induced ERK and SRC activity in $^{MYC/IGH+}$GC lymphoma cells

CD19 cytoplasmic tyrosines mediate the recruitment of signaling regulatory molecules, such as protein tyrosine kinases, PI3K, and GRB2, to the B-cell surface in normal and malignant hematopoiesis.[39,40] SHP2 has a positive effect on MAPK signaling mainly through its scaffolding adapter proteins GAB2 and GRB2 in the hematopoietic system.[24,25,38] CD19 deficiency resulted in a loss of SHP2 catalytic activity and was associated with a reduction in $^{MYC/IGH+}$GC lymphoma cell proliferation but not in either $^{MYC/IGH-}$GC lymphoma or non-GC lymphoma lines. We then explored whether SHP2

was phosphorylated in a complex with CD19 in the MYC/IGH+GC lymphoma lines. CD19 ligation resulted in the recruitment of SHP2 and GAB2 to CD19 in two MYC/IGH+GC lymphoma lines but not in either MYC/IGH−GC lymphoma or non-GC lymphoma lines (Figure 4A,B and *Online Supplementary Figure S6A,B*). CD19 engagement with anti-CD19 led to a strong interaction of SHP2 with GRB2 in MYC/IGH+GC lymphoma lines (Figure 4A), which had been described to interact with CD19 in the Daudi cell line.[39] CD19 ligation also regulated SHP2 Tyr542 and GAB2

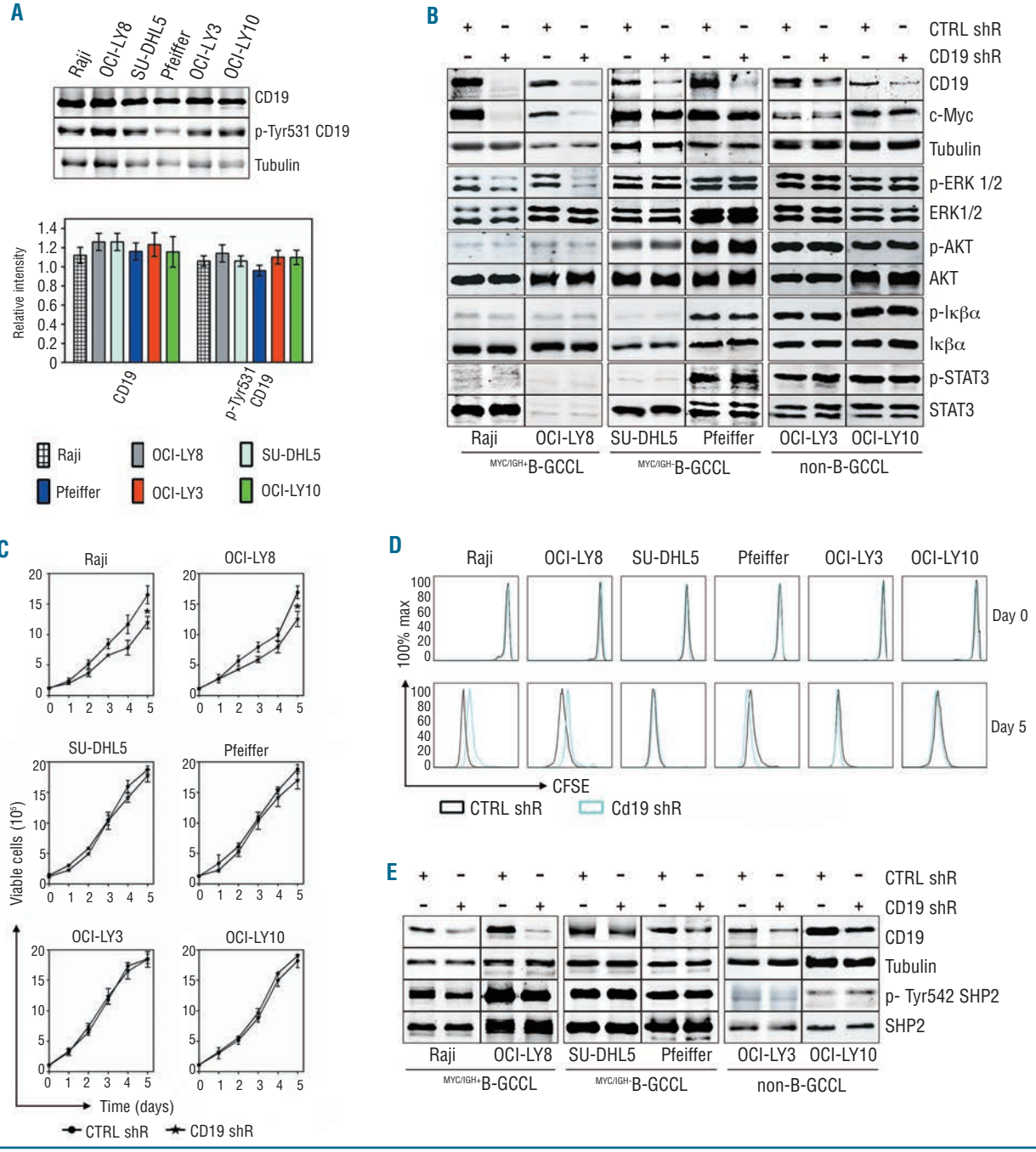


**Figure 3.** CD19 shR is toxic to MYC/IGH+GC lymphoma cells. (A) GC lymphoma and non-GC lymphoma lines were immunoblotted with antibodies against CD19, p-Tyr531 CD19 and tubulin (loading control). p-, phosphorylated. Data are representative of three independent experiments. (B) GC lymphoma and non-GC lymphoma lines expressing control (CTRL) shR or CD19 shR were immunoblotted with antibodies against CD19, c-Myc, tubulin, ERK1/2, p-Thr202/Tyr204-ERK1/2, AKT1/2, p-Ser473-AKT, IκBα, p-Ser32-IκBα, STAT3, p-Tyr705-STAT3 (p-, phosphorylated). Tubulin and total form of ERK1/2, AKT, IκBα, and STAT3 were used as loading controls. Data are representative of three independent experiments. (C) GC lymphoma and non-GC lymphoma lines expressing CTRL shR or CD19 shR (10,000 cells each) were cultured in RPMI-1640/10% fetal bovine serum in 96-well plates, and the total number of viable cells was assessed by trypan blue dye exclusion for 5 days. **P*<0.05 (CTRL shR compared to CD19 shR). Data represent mean ± s.e.m. of three independent experiments. (D) CFSE dilution profiles of GC lymphoma and non-GC lymphoma lines expressing CTRL shR or CD19 shR for 5 days. Data are representative of three independent experiments. (E) GC lymphoma and non-GC lymphoma lines were immunoblotted with antibodies against CD19, SHP2, p-Tyr542 SHP2 and tubulin (loading control). Data are representative of three independent experiments.

Tyr452 phosphorylation in $^{MYC/IGH+}$GC lymphoma lines (Figure 4C). Thus, it was shown that CD19 formed a complex with SHP2, GAB2 and GRB2, and that SHP2 phosphorylation was partially correlated with CD19 ligation in $^{MYC/IGH+}$GC lymphoma cells.

Furthermore, we examined CD19-induced MAPK signaling pathways in these $^{SHP2\Delta kdMYC/IGH+}$GC lymphoma cells. SHP2 knockdown impaired activation of ERK and SRC in response to treatment with anti-CD19 in $^{MYC/IGH+}$GC lymphoma cells. In contrast, $^{SHP2\Delta kdMYC/IGH+}$GC lymphoma cells showed no substantial defects in anti-CD19-induced activation of c-Jun N-terminal kinases or p38 kinases (Figure 4D). Thus, SHP2 is involved in CD19-induced activation of ERK and SRC and makes little or no contribution to CD19-

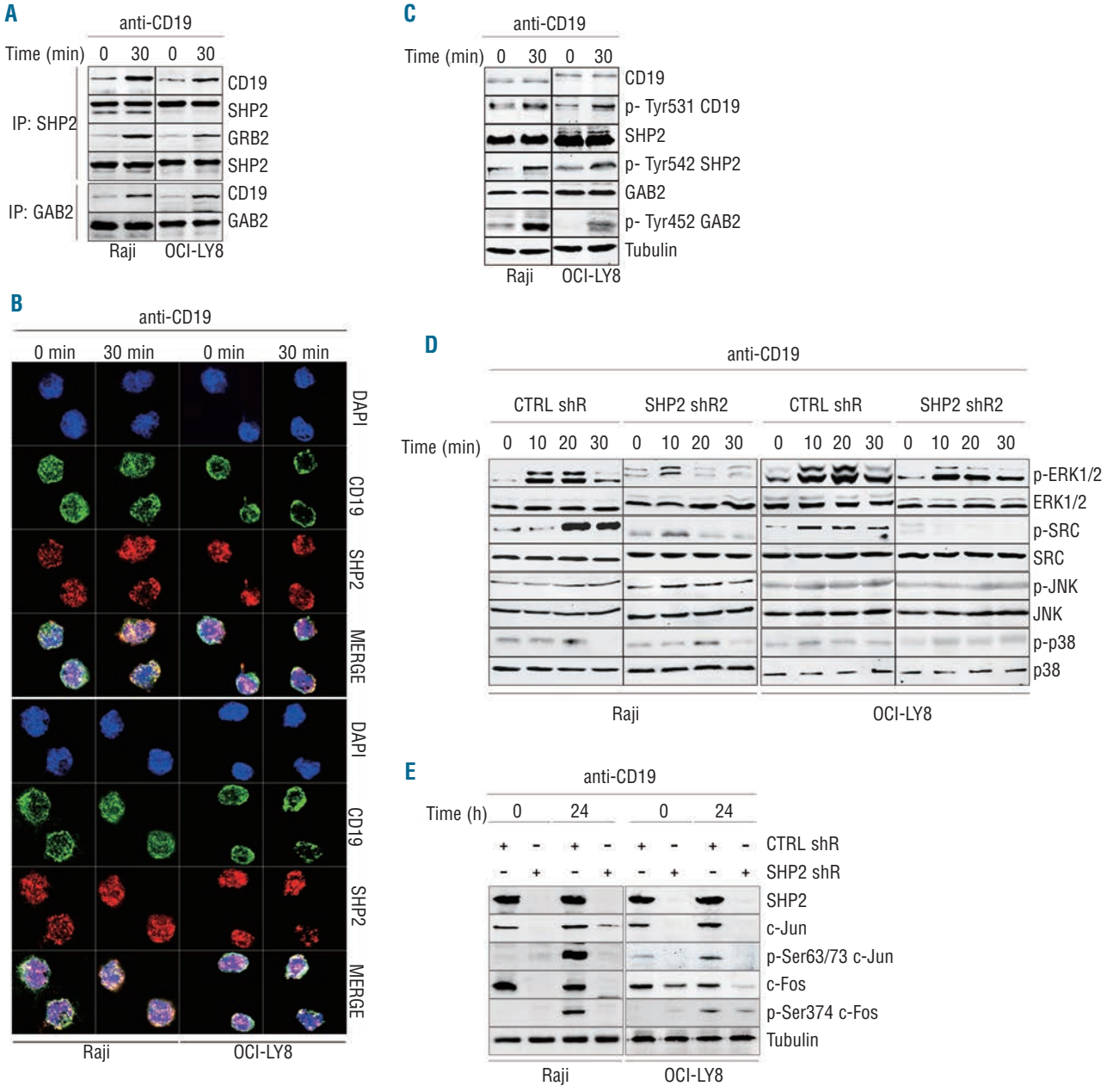


**Figure 4.** SHP2 is required for CD19-mediated SRC, ERK and AP-1 activation in $^{MYC/IGH+}$GC lymphoma cells. (A) SHP2 and GAB2 co-precipitate with CD19 and SHP2 co-precipitates with GRB2 in wild-type $^{MYC/IGH+}$GC lymphoma cells before and after CD19 crosslinking (10 μg/mL). (IP, immunoprecipitate). Data are representative of three independent experiments. (B) Confocal immunofluorescence images showing the cellular localization of CD19 (green), DAPI (blue), and SHP2 (red) or GAB2 (red) in wild-type $^{MYC/IGH+}$GC lymphoma cells before and after CD19 crosslinking (10 μg/mL). (C) Immunoblot analysis of $^{MYC/IGH+}$GC lymphoma cells incubated with anti-CD19 (10 μg/mL) for 0 and 30 min, assessed with anti-CD19, anti-p-Tyr531-CD19, anti-SHP2, anti-p-Tyr542 SHP2, anti-GAB2, anti-p-Tyr452-GAB2, and anti-tubulin (loading control). (p-, phosphorylated). Data are representative of three independent experiments. (D) $^{MYC/IGH+}$GC lymphoma cells expressing CTRL shR or SHP2 shR2 were stimulated with anti-CD19 (10 μg/mL) and lysates were analyzed by immunoblot to measure expression of MAPK and SRC. Total form of ERK1/2, p38, JNK and SRC were used as loading controls. Data are representative of three independent experiments. (E) $^{MYC/IGH+}$GC lymphoma cells expressing CTRL shR or SHP2 shR2 were stimulated with anti-CD19 (10 μg/mL) for 0 or 24 hours (h) and lysates were analyzed by immunoblot to measure expression of p-Ser63/73 c-Jun, c-Jun, p-Ser374 c-Fos, c-Fos and tubulin (loading control). Data are representative of three independent experiments.

induced activation of p38 or c-Jun N-terminal kinases.

Next we explored the basis of signaling for the defective cell cycle progression of $^{SHP2\Delta kd}$*MYC/IGH*$^{+}$GC lymphoma cells. ERK activation led to phosphorylation and activation of the AP-1 component c-Fos and c-Jun transcription factors, which in turn activated the transcription of the cyclin D gene and controlled cellular proliferation and survival in Hodgkin lymphoma and Burkitt lymphoma cells.[41,42] In fact, compared with control cells, $^{SHP2\Delta kd}$*MYC/IGH*$^{+}$GC lymphoma cells showed defective CD19-induced expression of c-Fos, c-Jun and phosphorylation of c-Fos Ser374 and c-Jun Ser63/73 in these cells (Figure 4E). To determine whether ERK and SRC phosphorylation was necessary for the regulation of the CD19/SHP2 complex-induced *MYC/IGH*$^{+}$GC lymphoma cell cycling and proliferation of stimulus-responsive genes encoding mRNA stability, *MYC/IGH*$^{+}$GC lymphoma cells were treated with dasatinib, PD0325901 and U0126. As anticipated, real-time polymerase chain reaction analyses showed that dasatinib, PD0325901, and U0126 enhanced *CDKN1B* gene expression at the mRNA transcription level and inhibited *CCND2*, *FOS* and *JUN* transcriptional levels in *MYC/IGH*$^{+}$GC lymphoma cells (*Online Supplementary Figure S7A*). Similar patterns were observed for cyclin D2, c-Fos, c-Jun and p27$^{kip1}$ protein expression (*Online Supplementary Figure S7B*). Given the known requirement of the ERK/AP-1/cyclin D2 pathway for GC B-cell proliferation,[41,43] these results might explain the defective progression of $^{SHP2\Delta kd}$*MYC/IGH*$^{+}$GC lymphoma cells.

### SHP2 regulates the c-Myc and CD19 functional synergistic network in germinal center lymphoma cells

Due to the absence of SHP2, CD19 signaling was impaired in *MYC/IGH*$^{+}$GC lymphoma cells, most notably SHP2 was required for ERK1/2 activation. It was, however, robustly induced upon SHP2 re-expression and could form a SHP2-dependent positive feedback loop that enhanced the transcription factor c-Myc and oncogenic RAS expression in some solid cancers.[30] Because the well-known synergy of c-Myc and CD19 aberrant expression in Eμ-Myc transgenic mice was necessary for malignant B-cell transformation,[19] we examined using immunoblot analyses, whether SHP2 commonly contributed to the reciprocal regulation of CD19 and c-Myc by transcriptional activation and subcellular localization in GC lymphoma cells. As expected, lack of SHP2 resulted in suppression of both CD19 and c-Myc protein and their activation in GC lymphoma cells (Figure 5A), whereas CD19 knockdown had no effect on the expression of SHP2 in GC lymphoma cells but significantly inhibited its Tyr542 phosphorylation in *MYC/IGH*$^{+}$GC lymphoma cells (Figure 3E). In addition, $^{SHP2\Delta kd}$GC lymphoma cells expressing exogenous CD19 protein were unable to reverse the impairment of ERK/c-Myc signaling (Figure 5B and *Online Supplementary Figure S5B*).

We further evaluated the effect of c-Myc on cell viability, proliferation and the activation of CD19 and SHP2 in GC lymphoma cells. A c-Myc shRNA killed all four GC lymphoma lines (Figure 5C-E) and reduced the GC lymphoma proliferative phenotype compared to that in control cells (*Online Supplementary Figure S3A,B* and *Online Supplementary Tables S4* and *S5*). Phosphorylated Tyr531 and total CD19 protein were decreased in $^{c\text{-}Myc\Delta kd}$GC lymphoma cells (Figure 5C). SHP2 Tyr542 phosphorylation was reduced in $^{c\text{-}Myc\Delta kd}$GC lymphoma lines with *MYC/IGH* translocation (Raji, OCI-LY8) but not in two others cell lines (SU-DHL5, Pfeiffer), which correlates with its phosphorylation and activity induced by ligation of CD19 in *MYC/IGH*$^{+}$GC lymphoma cells (Figure 4C), while c-Myc inhibition had no influence on SHP2 protein expression in any of the GC lymphoma lines (Figure 5C). Notably, although the transcription factor PAX5 was required for CD19 expression in normal B cells,[44] the diminished CD19 expression and phosphorylation in $^{c\text{-}Myc\Delta kd}$GC lymphoma cells did not correlate with an obvious change in PAX5 expression relative to cells treated with control shRNA (Figure 5C). However, overexpression of exogenous SHP2 in GC lymphoma cells did not rescue the block to CD19 that resulted from c-Myc deficiency (Figure 5F), indicating that CD19 might be the direct target of c-Myc and that SHP2 regulates CD19 expression which relies, at least partly, on c-Myc transcriptional activity.

### SHP2/ERK signaling maintains c-Myc transcriptional activity in germinal center lymphoma cells

Given our results suggesting that survival and proliferation of GC lymphoma cells depended on the constitutive activation of canonical SHP2/ERK signaling, and GC lymphoma cells are sensitive to inhibition of c-Myc function, we then investigated whether SHP2/ERK signaling contributes to c-Myc pro-survival transcriptional responses in GC lymphoma cells. Consistent with the fact that ERK-mediated phosphorylation of c-Myc on Ser62 stabilizes c-Myc protein and promotes its transcriptional activity,[19,45] we observed that inhibition of SHP2/ERK signaling reduced c-Myc protein levels with a corresponding decrease in phospho-Ser62 in GC lymphoma lines (Figure 6A,B). Because c-Myc Thr58 phosphorylation mediated by GSK3β is directly associated with the degradation of c-Myc,[45] changes in the phosphorylation levels c-Myc Thr58 and GSK3β Ser389 were detected in GC lymphoma lines. c-Myc Thr58 phosphorylation and GSK3β Ser389 dephosphorylation were observed following SHP2 knockdown or ERK inhibition in GC lymphoma lines (Figure 6A,B). These results were consistent with previous reports that ERK could phosphorylate GSK3β at the C-terminal serine to make it inactive, which protects c-Myc from Thr58 phosphorylation by GSK3β and subsequent degradation.[45,46] Next we measured c-Myc DNA binding by EMSA competition. Intriguingly, GC lymphoma cell treatment with either SHP2 shR2 or the MEK inhibitor U0126 specifically decreased the amount of c-Myc DNA binding in four GC lymphoma lines (Figure 6C,D). It was, therefore, presumed that SHP2/ERK inhibition could induce c-Myc destabilization and proteolysis, which suppress the entry of c-Myc into the nucleus to activate transcription in GC lymphoma lines.

Two conserved c-Myc binding sites (E-boxes) recognized by c-Myc were also identified upstream of the CD19 promoter (*Online Supplementary Figure S8A*). In reporter assays, either a 176-bp (containing E-box1) or a 209-bp (containing E-box2) fragment of the CD19 5' flanking region was observed to activate the luciferase gene in a c-Myc-dependent manner in GC lymphoma cells (*Online Supplementary Figure S8B*). We then performed anti-c-Myc chromatin immunoprecipitation and EMSA to verify that c-Myc was recruited to the CD19 5' flanking region in GC lymphoma cells. Using specific primers that flank E-box1 or E-box2, we observed a significant enrichment by polymerase chain reaction of the DNA fragments immunoprecipitated with a c-Myc-specific antibody in GC lymphoma cells compared with that of the controls (*Online Supplementary Figure S8C*). We then used EMSA to determine whether c-Myc bound

specifically to these E-boxes. The two E-box oligonucleotide binding activities of nuclear extracts from $^{c\text{-}Myc\Delta kd}$GC lymphoma cells were both significantly reduced relative to those of control cells (Figure 6E), which suggests that c-Myc directly regulates CD19 transcription by binding to E-box1 and E-box2 in the CD19 5' flanking region. These data indicate that a high level of nuclear c-Myc in GC lymphoma cells is controlled by SHP2/ERK signaling.

Our findings suggest that SHP2 increases CD19 and c-Myc expression levels in GC lymphomas. To test this model directly, we investigated whether restoring the expression of c-Myc or CD19 rescued the effects of SHP2 deletion. Notably, restoration of c-Myc, but not CD19, could abolish the effects of SHP2 knockdown on the growth of GC lymphoma cells (Figure 7A,B). Moreover, exogenous c-Myc re-expression restored the expression of several known c-Myc target genes, including BCL6, cyclin D2, and PCNA, and decreased expression of the cell cycle inhibitor p27 in GC lymphoma cells lacking SHP2 (Figure 7C). Thus, our data demonstrate that c-Myc is essential for maintaining GC

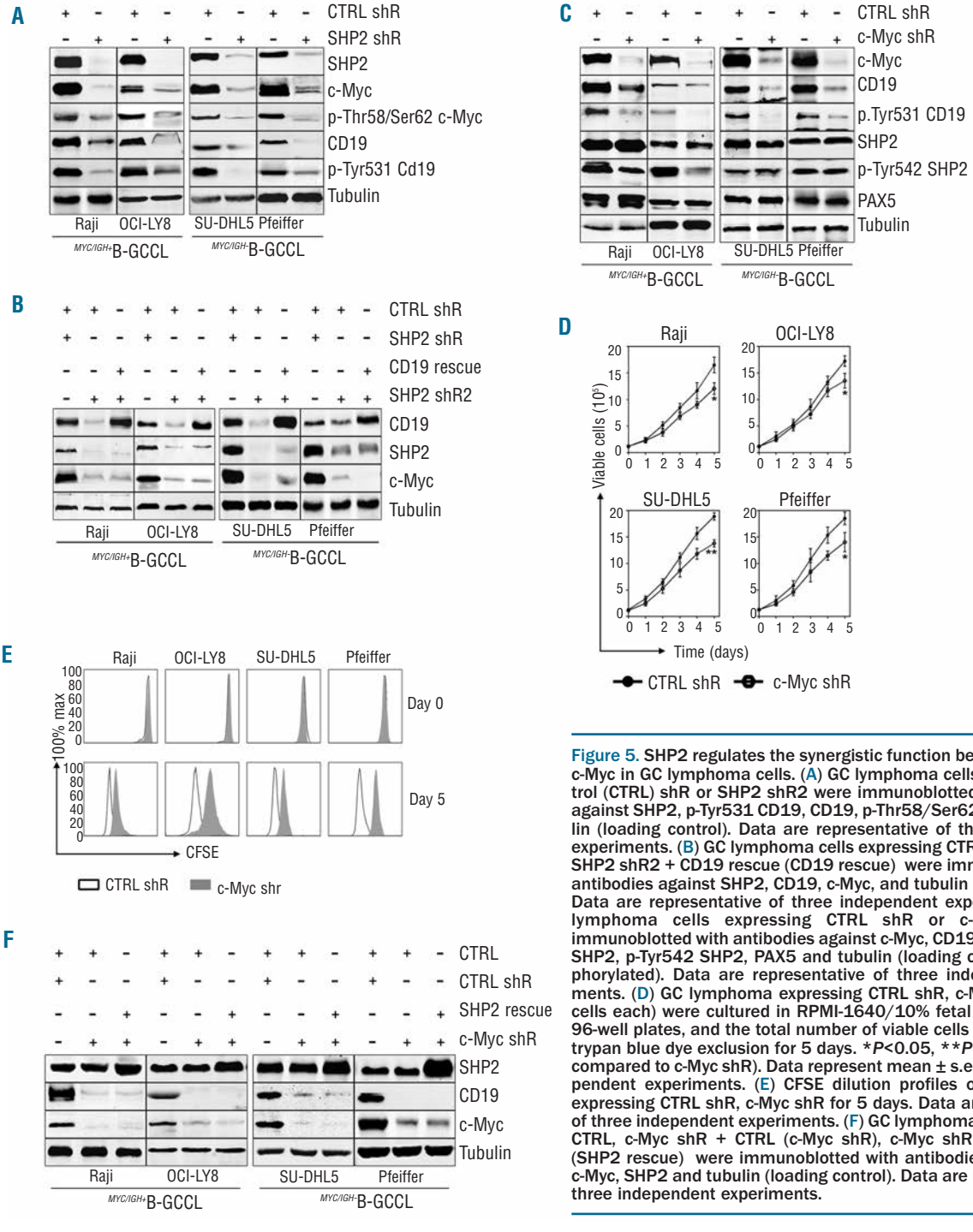


**Figure 5. SHP2 regulates the synergistic function between CD19 and c-Myc in GC lymphoma cells. (A) GC lymphoma cells expressing control (CTRL) shR or SHP2 shR2 were immunoblotted with antibodies against SHP2, p-Tyr531 CD19, CD19, p-Thr58/Ser62 c-Myc and tubulin (loading control). Data are representative of three independent experiments. (B) GC lymphoma cells expressing CTRL, SHP2 shR2 or SHP2 shR2 + CD19 rescue (CD19 rescue) were immunoblotted with antibodies against SHP2, CD19, c-Myc, and tubulin (loading control). Data are representative of three independent experiments. (C) GC lymphoma cells expressing CTRL shR or c-Myc shR were immunoblotted with antibodies against c-Myc, CD19, p-Tyr531 CD19, SHP2, p-Tyr542 SHP2, PAX5 and tubulin (loading control) (p-, phosphorylated). Data are representative of three independent experiments. (D) GC lymphoma expressing CTRL shR, c-Myc shR (10,000 cells each) were cultured in RPMI-1640/10% fetal bovine serum in 96-well plates, and the total number of viable cells was assessed by trypan blue dye exclusion for 5 days. *$P$<0.05, **$P$<0.01 (CTRL shR compared to c-Myc shR). Data represent mean ± s.e.m. of three independent experiments. (E) CFSE dilution profiles of GC lymphoma expressing CTRL shR, c-Myc shR for 5 days. Data are representative of three independent experiments. (F) GC lymphoma cells expressing CTRL, c-Myc shR + CTRL (c-Myc shR), c-Myc shR + SHP2 rescue (SHP2 rescue) were immunoblotted with antibodies against CD19, c-Myc, SHP2 and tubulin (loading control). Data are representative of three independent experiments.**

lymphoma cell viability and proliferation which depend on SHP2-mediated pro-survival signaling (Figure 7D).

## Discussion

The signal transduction networks influencing B-cell lymphomagenesis are not well defined, although their characterization might lead to the development of targeted, potentially curative lymphoma therapies. SHP2 is highly expressed in hematopoietic cells and SHP2 phosphatase positively regulates hematopoietic cell development.[22] Dysregulation of SHP2 signaling is associated with the transformation of blood disorders, including hematologic malignancies.[33,36] Our results here show that SHP2 signaling was required for the survival and progression of GC lymphomas but not of non-GC lymphomas. Our work has also led to the discovery of a SHP2-dependent GC lymphoma proliferative signature comprising a set of genes that are overexpressed in GC lymphomas.[5,6] Depletion of SHP2 led to a similar impairment of GC lymphoma proliferative phenotype signature expression, demonstrating that SHP2 is a high-quality target in GC lymphomas.

Mechanistically, we found that the effects of SHP2 on GC lymphomas require activation of the ERK pathway. In addition, our data indicate that SHP2 activation of ERK1/2 might be responsible for the transcriptional activation of c-Myc, which mediates an increase in the expression of "GC lymphoma phenotype proteins".[5,6] SHP2/ERK signaling mediated c-Myc Ser62 phosphorylation thus stabilizing c-Myc protein and enhancing its transcriptional activity.[19,30] Conversely, ERK activation phosphorylates GSK3β Ser389 in the C-terminus, which causes GSK3β inactivation and inhibits c-Myc Thr58 phosphorylation from mediating c-Myc proteolysis.[45,46] Thus, we believe that SHP2 affected c-Myc activity and stability via a direct signaling mechanism involving activation of the ERK pathway and SHP2/ERK activation-induced c-Myc protein stabilization and entry into the nucleus to initiate gene transcription in GC lymphomas.

Mature conventional B cells in various peripheral lymphoid tissues have a similar surface density of CD19, and activation of B cells with anti-IgM or lipopolysaccharide does not significantly change CD19 expression.[48] As with normal B cells, CD19 is expressed at relatively high levels in nearly all B-cell malignancies.[49] The involvement of ERK signaling and c-Myc in CD19 signaling and biological responses

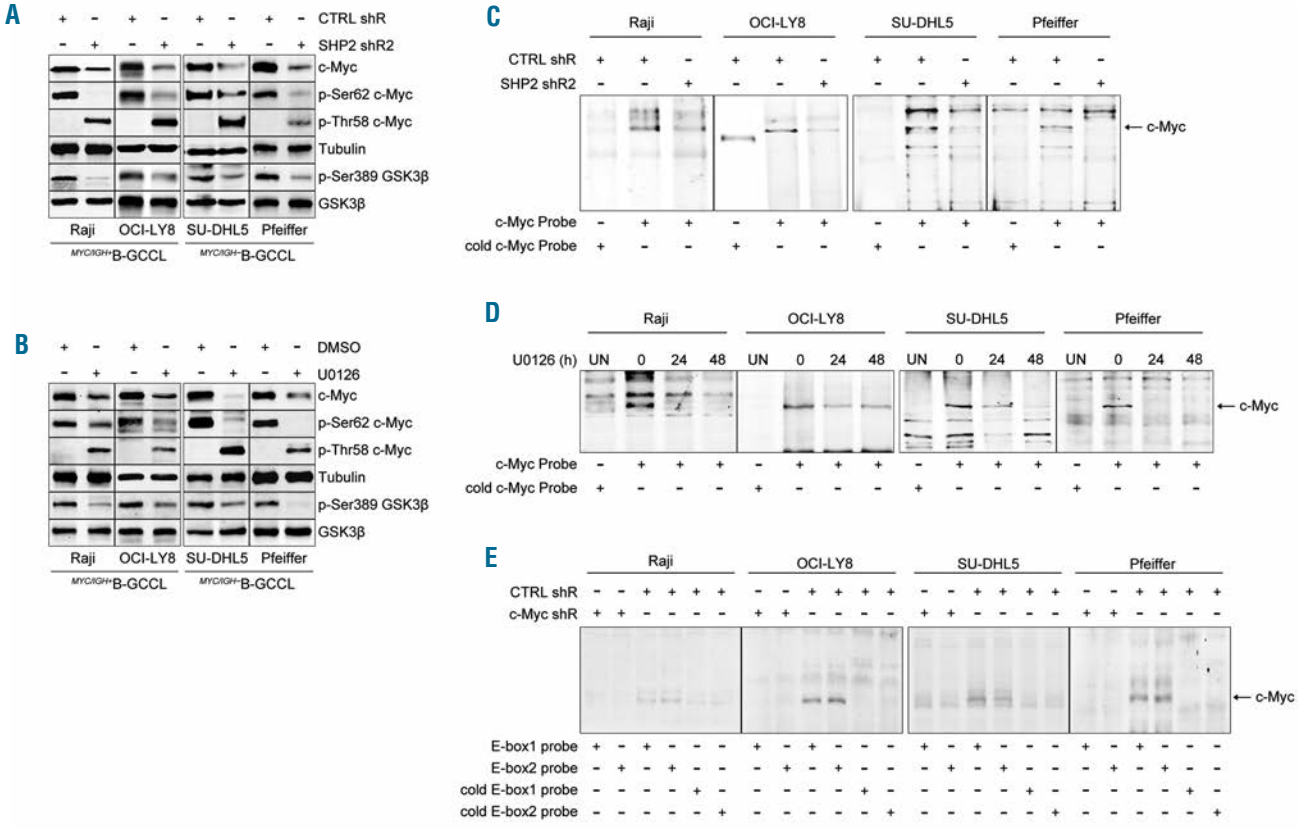


Figure 6. SHP2/ERK signaling is required for c-Myc transcriptional activation in GC lymphoma cells. (A) GC lymphoma cells expressing control (CTRL) shR or SHP2 shR2 were immunoblotted with antibodies against c-Myc, p-Ser62 c-Myc, p-Thr58 c-Myc, p-Ser389 GSK3β, GSK3β and tubulin (loading control) (p-, phosphorylated). Data are representative of three independent experiments. (B) Immunoblot analysis of GC lymphoma cells left unstimulated or stimulated for 6 h with U0126 (10 μM). Cell lysates were analyzed by antibodies to c-Myc, p-Ser62 c-Myc, p-Thr58 c-Myc, p-Ser389 GSK3β, GSK3β and tubulin (loading control). Data are representative of three independent experiments. (C) c-Myc DNA binding was analyzed by EMSA of extracts of GC lymphoma cells transfected with CTRL shR or SHP2 shR2. Migration of c-Myc is indicated. (D) c-Myc DNA binding was analyzed by EMSA of extracts of GC lymphoma cells treated with U0126 (10 μM) for the indicated times. Migration of c-Myc is indicated. (E) EMSA for detection of the activity for c-Myc binding to E-box1 or E-box2 in the CD19 gene, the arrows representing the specific formation of complexes with nuclear extract and E-box1 or E-box2 probes.

involved in B-lymphomagenesis has been explored.[19] However, isolated enhanced CD19 expression is independent of B-cell lymphomagenesis, and malignant transformation of GC B cells remains a c-Myc-dependent process.[19] Our results here shed new light on the mechanism by which CD19 mediates the activation of ERK and c-Myc in B-lymphomas. Inhibition of CD19 was selectively toxic for $^{MYC/IGH+}$GC lymphoma but not for $^{MYC/IGH-}$GC lymphoma, nor for non-GC lymphoma cells. In $^{MYC/IGH+}$GC lymphoma cells, the Tyr531 of CD19 was progressively phosphorylated and SHP2 was recruited to the CD19 signaling complex, most likely through its association with GAB2. Although our results ruled out the possibility that AKT, IκBα and STAT3 are downstream effectors of CD19, activation of ERK1/2 is severely compromised in the absence of CD19 in $^{MYC/IGH+}$GC lymphoma cells, and SHP2 is a critical regulator of SRC and ERK activation in response to CD19 ligation on $^{MYC/IGH+}$GC lymphoma cells, each of which precedes the induction of c-Myc.[30] Immunoblot analysis also revealed that the reduction of CD19 impaired SHP2 Tyr542 phosphorylation, and that Tyr542 is the major GRB2 binding site.[25] These results are consistent with results of published studies showing that SHP2 acts upstream of the SRC and ERK MAPK pathway,[26,27] possibly by recruiting the GRB2/SOS complex.[39] We also demonstrated that SHP2 could induce the activation of AP-1 after CD19 stimulation through the recruitment and activation of ERK and SRC.[26,27] Given the known requirement of AP-1 activation for progression of Burkitt lymphoma, and ERK1/2 signaling for the activation of AP-1 transcription factors, the involvement of AP-1 in the induction of genes that encode cyclin proteins and in the suppression of $p27^{kip1}$ gene transcription following mitogenic stimulation,[50] the stronger repression of pro-survival genes such as AP-1, c-myc and cyclin D2 might explain the prior sensitivity of $^{MYC/IGH+}$GC lymphoma cells toward CD19/SHP2/ERK inhibition.

Since our data indicate that the SHP2-dependent CD19/ERK/c-Myc loop provides a pathological signal in $^{MYC/IGH+}$GC lymphomas, targeting SHP2 might be effective in GC lymphoma patients with *MYC/IGH* translocation, particularly those who are dependent on active CD19/c-Myc signaling[18,19] which requires SHP2 for full activation of ERK. Interestingly, SHP2/ERK/c-Myc signaling has also been shown to promote the survival and proliferation of GC lymphoma cells without *MYC/IGH* translocation in which SHP2

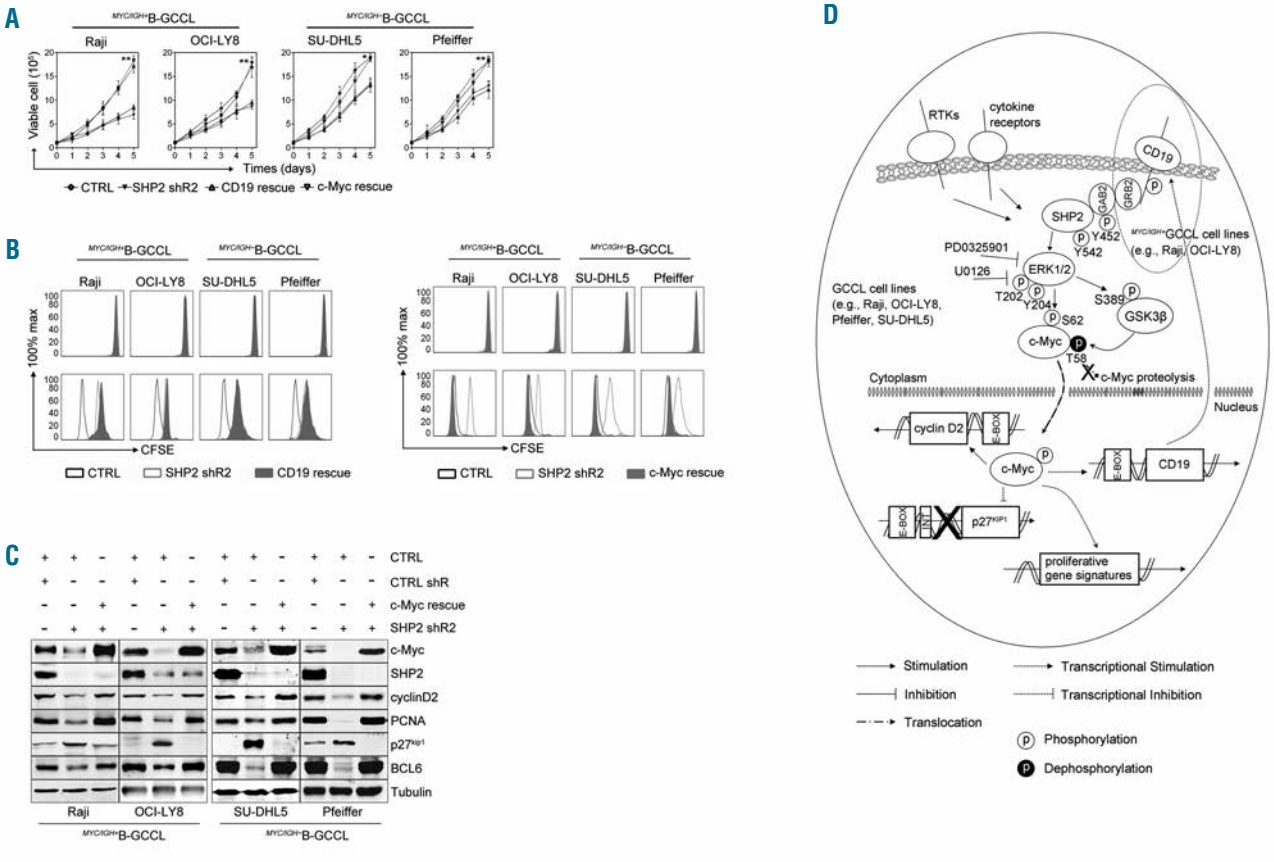


**Figure 7.** c-Myc rescues the effects of SHP2 knockdown on GC lymphoma cell growth. (A) GC lymphoma cells expressing control (CTRL), SHP2 shR2, CD19 rescue or c-Myc rescue (10,000 cells/each) were cultured in RPMI-1640/10% fetal bovine serum in 96-well plates, and the total number of viable cells was assessed by trypan blue dye exclusion for 5 days. **P*< 0.05; ***P*<0.01 (c-Myc rescue compared to SHP2 shR2). Data represent mean ± s.e.m. of three independent experiments. (B) CFSE dilution profiles of GC lymphoma cells expressing CTRL, SHP2 SHP2 shR2, CD19 rescue or c-Myc rescue for 5 days. Data are representative of three independent experiments. (C) GC lymphoma cells expressing CTRL, CTRL shR, SHP2 shR2 or c-Myc rescue were immunoblotted with antibodies against SHP2, c-Myc, cyclin D2, p27kip1, PCNA, BCL6 and tubulin (loading control). Data are representative of three independent experiments. (D) Model of the mechanism of action of SHP2/ERK/c-Myc signaling in GC lymphoma lines.

is hyperactivated, and our findings suggest the need for further experiments to address whether SHP2 influences other B-cell surface markers and signaling elements essential for the maintenance and progression of GC lymphoma. In fact, SHP2 might be important because of its connection with other non-CD19-dependent signals in GC lymphoma lines.

In summary, our data indicate that SHP2 is an important mediator of the CD19/c-Myc signaling loop in distinct $^{MYC/IGH+}$GC lymphomas and is required for the propagation of GC lymphomas.

***Acknowledgments***

*The authors would like to thank Prof. Tong Zhao (Southern Medical University, P.R. China) for her generous gift of the OCI-LY8 and OCI-LY10 cell lines; and Prof. Hans Konrad Muller-Hermelink (University of Würzburg, Germany) for critically reading this manuscript. The authors thank the Chinese Center for Type Culture Collection (CCTCC) for providing the short tandem repeat profiling data. This work was supported by grants from the National Program on Key Basic Research Project (973 program, N. 2014CB542101), the National Science Foundation of China (N. 30670810), the Science Foundation of Zhejiang Province in China (N. Y2090167 and N. 2009C33039) and the Science and Technology Bureau Foundation of Ningbo (N. 2013A610245).*

***Authorship and Disclosures***

*Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.*

## References

1. Tarlinton D. Germinal centers: form and function. Curr Opin Immunol. 1998;10(3):245-51.
2. Wolniak KL, Shinall SM, Waldschmidt TJ. The germinal center response. Crit Rev Immunol. 2004;24(1):39-65.
3. Klein U, Dalla-Favera R. Germinal centres: role in B-cell physiology and malignancy. Nat Rev Immunol. 2008;8(1):22-33.
4. Shaffer AL, Rosenwald A, Staudt LM. Lymphoid malignancies: the dark side of B-cell differentiation. Nat Rev Immunol. 2002;2(12):920-32.
5. Lenz G, Wright G, Dave SS, Xiao W, Powell J, Zhao H, et al. Stromal gene signatures in large-B-cell lymphomas. N Engl J Med. 2008;359(22):2313-23.
6. Scheller H, Tobollik S, Kutzera A, Eder M, Unterlehberg J, Pfeil I, Jungnickel B. c-Myc overexpression promotes a germinal center-like program in Burkitt's lymphoma. Oncogene. 2010;29(6):888-97.
7. Niitsu N, Okamoto M, Miura I, Hirano M. Clinical features and prognosis of de novo diffuse large B-cell lymphoma with t(14;18) and 8q24/c-MYC translocations. Leukemia. 2009;23(4):777-83.
8. Huang JZ, Sanger WG, Greiner TC. The t(14;18) defines a unique subset of diffuse large B-cell lymphoma with a germinal center B-cell gene expression profile. Blood. 2002;99(7):2285-90.
9. Haas KM, Tedder TF. Role of the CD19 and CD21/35 receptor complex in innate immunity, host defense and autoimmunity. Adv Exp Med Biol. 2005;560:125-39.
10. Lane P, Brocker T, Hubele S, Padovan E, Lanzavecchia A, McConnell F. Soluble CD40 ligand can replace the normal T cell-derived CD40 ligand signal to B cells in T cell-dependent activation. J Exp Med. 1993;177(4):1209-13.
11. Tuveson DA, Carter RH, Soltoff SP, Fearon DT. CD19 of B cells as a surrogate kinase insert region to bind phosphatidylinositol 3-kinase. Science 1993; 260(5110):986-9.
12. van Zelm MC, Reisli I, van der Burg M, Castaño D, van Noesel CJ, van Tol MJ, et al. An antibody-deficiency syndrome due to mutations in the CD19 gene. N Engl J Med. 2006;354(18):1901-12.
13. Sato S, Steeber DA, Jansen PJ, Tedder TF. CD19 expression levels regulate B lymphocyte development: human CD19 restores normal function in mice lacking endogenous CD19. J Immunol. 1997;158(10):4662-9.
14. Sato S, Ono N, Steeber DA, Pisetsky DS, Tedder TF. CD19 regulates B lymphocyte signaling thresholds critical for the development of B-1 lineage cells and autoimmunity. J Immunol. 1996;157(10):4371-8.
15. Rui L, Schmitz R, Ceribelli M, Staudt LM. Malignant pirates of the immune system. Nat Immunol. 2011;12(10):933-40.
16. Ghassemifar R, Forster L, Finlayson J, Calogero T, Augustson B, Joske D, et al. Differential expression of the Bcl-2 and Bax isoforms in CD19 positive B-lymphocytes isolated from patients diagnosed with chronic lymphocytic leukaemia. Pathology. 2012;44(7):632-7.
17. Roberts T, Snow EC. Cutting edge: recruitment of the CD19/CD21 coreceptor to B cell antigen receptor is required for antigen-mediated expression of Bcl-2 by resting and cycling hen egg lysozyme transgenic B cells. J Immunol. 1999;162(8):4377-80.
18. Chung EY, Psathas JN, Yu D, Li Y, Weiss MJ, Thomas-Tikhonenko A. CD19 is a major B cell receptor-independent activator of MYC-driven B-lymphomagenesis. J Clin Invest. 2012;122(6):2257-66.
19. Poe JC, Minard-Colin V, Kountikov EI, Haas KM, Tedder TF. A c-Myc and surface CD19 signaling amplification loop promotes B cell lymphoma development and progression in mice. J Immunol. 2012;189(5):2318-25.
20. Gramling MW, Eischen CM. Suppression of Ras/Mapk pathway signaling inhibits Myc-induced lymphomagenesis. Cell Death Differ. 2012;19(7):1220-7.
21. Qu CK, Nguyen S, Chen J, Feng GS. Requirement of Shp-2 tyrosine phosphatase in lymphoid and hematopoietic cell development. Blood. 2001;97(4):911-4.
22. Salmond RJ, Alexander DR. SHP2 forecast for the immune system: fog gradually clearing. Trends Immunol. 2006;27(3):154-60.
23. Miyakawa Y, Rojnuckarin P, Habib T, Kaushansky K. Thrombo-poietin induces phosphoinositol 3-kinase activation through SHP2, Gab, and insulin receptor substrate proteins in BAF3 cells and primary murine megakaryocytes. J Biol Chem. 2000;276(4):2494-502.
24. Nishida K, Yoshida Y, Itoh M, Fukada T, Ohtani T, Shirogane T, et al. Gab-family adapter proteins act downstream of cytokine and growth factor receptors and T- and B-cell antigen receptors. Blood. 1999;93(6):1809-16.
25. Araki T, Nawa H, Neel BG. Tyrosyl phosphorylation of Shp2 is required for normal ERK activation in response to some, but not all, growth factors. J Biol Chem. 2003;278(43):41677-84.
26. Shi ZQ, Yu DH, Park M, Marshall M, Feng GS. Molecular mechanism for the Shp-2 tyrosine phosphatase function in promoting growth factor stimulation of Erk activity. Mol Cell Biol. 2000;20(5):1526-36.
27. Müller JP, Schönherr C, Markova B, Bauer R, Stocking C, Böhmer FD. Role of SHP2 for FLT3-dependent proliferation and transformation in 32D cells. Leukemia. 2008;22(10):1945-8.
28. Ostman A, Hellberg C, Bohmer FD. Protein-tyrosine phosphatases and cancer. Nat Rev Cancer. 2006;6(4):307-20.
29. Shuai K, Liu B. Regulation of JAK-STAT signalling in the immune system. Nat Rev Immunol. 2003;3(11):900-11.
30. Aceto N, Sausgruber N, Brinkhaus H, Gaidatzis D, Martiny-Baron G, Mazzarol G, et al. Tyrosine phosphatase SHP2 promotes breast cancer progression and maintains tumor-initiating cells via activation of key transcription factors and a positive feedback signaling loop. Nat Med. 2012;18(4):529-37.
31. Zhang SQ, Yang W, Kontaridis MI, Bivona TG, Wen G, Araki T, et al. Shp2 regulates SRC family kinase activity and Ras/Erk activation by controlling Csk recruitment. Mol Cell. 2004;13(3):341-55.
32. Takahashi A, Tsutsumi R, Kikuchi I, Obuse C, Saito Y, Seidi A, et al. SHP2 tyrosine phosphatase converts parafibromin/Cdc73 from a tumor suppressor to an oncogenic driver. Mol Cell. 2011;43(1):45-56.
33. Loh ML, Vattikuti S, Schubbert S, Reynolds MG, Carlson E, Lieuw KH, et al. Mutations in PTPN11 implicate the SHP-2 phosphatase in leukemogenesis. Blood. 2004;103(6):2325-31.
34. Tartaglia M, Mehler EL, Goldberg R, Zampino G, Brunner HG, Kremer H, et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet. 2001;29(4):465-8.
35. Grossmann KS, Rosario M, Birchmeier C, Birchmeier W. The tyrosine phosphatase Shp2 in development and cancer. Adv Cancer Res. 2010;106:53-89.
36. Xu R. Shp2, a novel oncogenic tyrosine phosphatase and potential therapeutic target

for human leukemia. Cell Res. 2007;17(4): 295-7.
37. Fridberg M, Servin A, Anagnostaki L, Linderoth J, Berglund M, Söderberg O, et al. Protein expression and cellular localization in two prognostic subgroups of diffuse large B-cell lymphoma: higher expression of ZAP70 and PKC-beta II in the non-germinal center group and poor survival in patients deficient in nuclear PTEN. Leuk Lymphoma. 2007;48(11):2221-32.
38. Voena C, Conte C, Ambrogio C, Boeri Erba E, Boccalatte F, Mohammed S, et al. The tyrosine phosphatase Shp2 interacts with NPM-ALK and regulates anaplastic lymphoma cell growth and migration. Cancer Res. 2007;67(9):4278-86.
39. Brooks SR, Li X, Volanakis EJ, Carter RH. Systematic analysis of the role of CD19 cytoplasmic tyrosines in enhancement of activation in Daudi human B cells: clustering of phospholipase C and Vav and of Grb2 and Sos with different CD19 tyrosines. J Immunol. 2000;164(6):3123-31.
40. Otero DC, Omori SA, Rickert RC. Cd19-dependent activation of Akt kinase in B-lymphocytes. J Biol Chem. 2001;276(2):147-8.
41. Mathas S, Hinz M, Anagnostopoulos I, Krappmann D, Lietz A, Jundt F, et al. Aberrantly expressed c-Jun and JunB are a hallmark of Hodgkin lymphoma cells, stimulate proliferation and synergize with NF-κB. EMBO J. 2002;21(15):4104-13.
42. De Leo A, Arena G, Lacanna E, Oliviero G, Colavita F, Mattia E. Resveratrol inhibits Epstein Barr virus lytic cycle in Burkitt's lymphoma cells by affecting multiple molecular targets. Antiviral Res. 2012;96(2): 196-202.
43. Gallagher E, Enzler T, Matsuzawa A, Anzelon-Mills A, Otero D, Holzer R, et al. Kinase MEKK1 is required for CD40-dependent activation of the kinases Jnk and p38, germinal center formation, B cell proliferation and antibody production. Nat Immunol. 2007;8(1):57-63.
44. Nutt SL, Urbánek P, Rolink A, Busslinger M. Essential functions of PAX5 (BSAP) in pro-B cell development: difference between fetal and adult B lymphopoiesis and reduced V-to-DJ recombination at the IgH locus. Genes Dev. 1997;11(4):476-91.
45. Sears R, Nuckolls F, Haura E, Taya Y, Tamai K, Nevins JR. Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. Genes Dev. 2005;14(19):2501-14.
46. Li X, Frye MA, Shelton RC. Review of pharmacological treatment in mood disorders and future directions for drug development. Neuropsychopharmacology. 2012; 37(1):77-101.
47. Kozmik Z, Wang S, Dörfler P, Adams B, Busslinger M. The promoter of the CD19 gene is a target for the B-cell-specific transcription factor BSAP. Mol Cell Biol. 1992; 12(6):2662.
48. Uckun FM, Jaszcz W, Ambrus JL, Fauci AS, Gajl-Peczalska K, Song CW, et al. Detailed studies on expression and function of CD19 surface determinant by using B43 monoclonal antibody and the clinical potential of anti-CD19 immunotoxins. Blood. 1988; 71(1):13-29.
49. Wang K, Wei G, Liu D. CD19: a biomarker for B cell development, lymphoma diagnosis and therapy. Exp Hematol Oncol. 2012;1(1): 36.
50. Khattar E, Kumar V. Mitogenic regulation of p27(Kip1) gene is mediated by AP-1 transcription factors. J Biol Chem. 2010;285(7): 4554-61.