Reduction of Hematopoietic Cell-Specific Tyrosine Phosphatase SHP-1 Gene Expression in Natural Killer Cell Lymphoma and Various Types of Lymphomas/Leukemias

Combination Analysis with cDNA Expression Array and Tissue Microarray

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To investigate the lymphomagenesis of NK/T lymphoma, we comprehensively and systematically analyzed the expression pattern of the human NK/T cell line (NK-YS) genome by cDNA expression array and tissue microarray. We detected significant changes in the gene expression of NK-YS cell line: an increase in 18 and a decrease in 20 genes compared to normal NK cells or peripheral blood mononuclear cells. Among these genes, we found a strong decrease in hematopoietic cell specific protein-tyrosine-phosphatase SH-PTP1 (SHP1) mRNA by cDNA expression array and reverse transcriptase-polymerase chain reaction. Further analysis with standard immunohistochemistry and tissue microarray, which used 207 paraffin-embedded specimens of various kinds of malignant lymphomas, showed that 100% of NK/T lymphoma specimens and more than 95% of various types of malignant lymphoma were negative for SHP1 protein expression. On the other hand, SHP1 protein was strongly expressed in the mantle zone and interfollicular zone lymphocytes in reactive lymphoid hyperplasia specimens. In addition, various kinds of hematopoietic cell lines, particularly the highly aggressive lymphoma/leukemia lines, lacked SHP1 expression *in vitro*, suggesting that loss of SHP1 expression may

be related to not only malignant transformation, but also tumor cell aggressiveness. SHP1 expression could not be induced in either of two NK/T cell lines by phorbol ester, suggesting that genetic impairment or modification with methylation of SHP1 DNA could be one of the critical events in the pathogenesis of NK/T lymphoma. This evidence strongly suggests that loss of SHP1 gene expression plays an important role in multistep tumorigenesis, possibly as an anti-oncogene in the wide range of lymphomas/leukemias as well as NK/T lymphomas. (Am J Pathol 2001, 159:1495–1505)

Nasal/nasopharyngeal NK/T cell lymphoma, currently referred to as an angiocentric lymphoma in the REAL classification,¹ is now recognized as a distinctive clinicopathological entity. Frequent cases of this lymphoma have been reported in Asian and South American countries; these were characterized as polymorphous pleomorphic morphologies with azurophilic granules, the immunophenotypic profile of CD2⁺, CD3[–], CD56⁺, lack of rearranged T cell receptor genes, and association with the Epstein-Barr virus (EBV).² These tumors are regarded as one of the most aggressive lymphoma types currently known because of their strong resistance against third generation combination chemotherapy and their rapid, unfavorable clinical course. As a consequence of their genetic instability or spontaneous mutation, malignant cells accumulate an increasing number of genetic aberrations during the course of tumor progression, such as chromosomal rearrangements, deletions of anti-onco-

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genes, amplification or activation of oncogenes and various epigenetic changes, that result in altered gene expression. Somatic mutations of the genes for the variable region genes of B- and T-lymphocyte antigens have been shown to be a hallmark of lymphomas and leukemia, including germinal center B cells and their descendants. For several types of B-cell lymphomas, chromosomal translocations into immunoglobulin heavy chain switch regions have been described, such as translocation of *c-myc* in Burkitt's lymphomas,³ *Bcl-6* in diffuse large cell lymphomas,⁴ *Bcl-2* in follicular lymphomas,⁵ *Bcl-1* in mantle-zone lymphomas,⁶ and fibroblast growth factor receptor-3 in multiple myeloma.⁷ Such kinds of conserved genetic changes for these own lineage have been revealed, however universally conserved genetic changes and gene expression alterations on malignant lymphoma/leukemia have not been identified yet.

Completion of the genome sequences of model organisms and humans will provide us with complete blueprints of these genomes. The study of gene expression on a genomic scale is the most obvious opportunity made possible by complete genome sequences, and the most experimentally straightforward. cDNA microarrays make it easy to measure the transcripts for every gene at once.⁸⁻¹¹ Due to the tight connection between the function of a gene product and its expression pattern, each gene is expressed in specific cells and under specific conditions. Promoters of genes function as transducers, responding to input information about the identities, environment, and internal state of a cell by changing the level of transcription of specific genes. The group of genes expressed in a cell determines what the cell is made of, what biochemical and regulatory systems are operative, how the cell is built, and what it can and cannot do. Many genes and signal transduction pathways that control cellular proliferation, differentiation and programmed cell death, as well as genomic integrity, are also involved in cancer development. cDNA microarrays have enabled measurement of the expression of thousands of genes in a single experiment for the systematic and comprehensive exploration of genome-wide alterations in cancer cells.⁸⁻¹⁰ Recently, distinct types of diffuse large B cell lymphoma (DLL), which show markedly different clinical courses and treatment responses apparently reflecting the variation in tumor proliferation rate, host response, and differentiation state of tumor, have been identified and diagnosed by gene expression profiling with cDNA microarrays.¹⁰ However, analysis of hundreds of specimens from patients from different stages is essential to establish the diagnostic, prognostic, and therapeutic importance of each of the many oncogene or anti-oncogene candidates. To overcome this difficulty, an array-based high-throughput tissue microarray technique has been established to facilitate analysis of gene expression and DNA copy number of large numbers of tumors.¹²⁻¹⁴ We developed this technique independently, modified it, and here apply the modified method to lymphoma analysis in combination with a cDNA expression array.

In the present investigation, we analyzed the expression profiles of human NK cell lines compared to normal

NK cells or peripheral blood mononuclear cells (PBMCs) to elucidate the mechanism of NK/T lymphomagenesis. We found strong suppression of SHP1 gene expression in the various types of lymphomas/leukemias as well as in NK/T cell lymphomas, suggesting that SHP1 is one of the key molecules for the malignant transformation of lymphomas/leukemias.

Materials and Methods

Cell Culture

Human NK cell lines, NK-YS¹⁵ and NK-TY2 (in preparation for publication), were maintained in Iscove's modified Dulbecco's medium (IMDM; GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum [(FCS) Sankou Junyaku, Chiba, Japan], 100 U/ml recombinant human interleukin-2 (Strathman Biotech GMBH, Hannover, Germany), 100 U/ml kanamycin (Meiji, Tokyo, Japan), and 100 μ g/ml streptomycin (LIFE Technologies, Rockville, MD). Other cell lines were maintained in RPMI-1640 supplemented with 10% FCS, 100 U/ml of kanamycin, and 100 μ g/ml streptomycin. PBMCs were isolated by the Ficoll-Hypaque method from healthy volunteer blood donors. Fresh normal NK cell fraction was further purified by the magnetic beads method with anti-CD56 monoclonal antibody (PerSeptive Biosystems, Tokyo, Japan). The purity of this NK cell-enriched fraction was about 60% to 70%. PBMCs were incubated in RPMI 1640 containing 10% FCS with or without 0.1% pokeweed mitogen (PWM) (GIBCO BRL, Rockville, MD) or 5 μ g/ml phytohemagglutinin-P (PHA-P) (Sigma, St. Louis, MO) for 72 hours at 37°C in a CO₂ incubator and used as the source of lymphoblasts. Cells were also treated with 20 ng/ml PMA (TPA; phorbol ester; Sigma, St. Louis, MO) for 72 hours at 37°C in a CO₂ incubator. The KCA cell line was obtained from Dr. E.C. Butcher (Department of Pathology, Stanford University, Stanford, CA). HairM, MOLP2, L428, SKW3, SU-DHL4, RPMI8226, HDLM2, EDS, and HUT102 were from Dr. Y. Matsuo (Fujisaki Cell Center, Hayashibara Biomedical Laboratories, Okayama, Japan).

cDNA Expression Array Analysis

Total RNA of freshly isolated PBMCs from two healthy volunteers were mixed and used as a pooled reference RNA. Each RNA, extracted from NK/T cell lines (NK-YS, NK-TY2), Jurkat cells, purified fresh normal NK cells, or fresh normal PBMCs was ³²P-labeled and used to synthesize probes using the Atlas Pure Total RNA Labeling System (K1038-1; Clontech Laboratories Inc., Palo Alto, CA). Hybridization of the ATLAS Human Cancer 1.2 Array (Clontech Laboratories, Inc., Palo Alto, CA) was performed according to the instruction manual. The results were analyzed on a Fujifilm BAS3000 system using Array Gauge Computer software (Fujifilm Co., Tokyo, Japan). After normalization of the total amounts of gene expression, gene expressions were compared among the NK-YS, NK-TY2, Jurkat cell lines, normal PBMCs, and fresh normal human NK cells.

Analysis of mRNA Expression by RT-PCR

A 2 μ g aliquot of DNase-I treated total cellular RNA was reverse-transcribed with SuperScript II reverse transcriptase (Life Technologies, Rockville, MD) in 20 μ l of 20 mmol/L Tris-HCl (pH 8.3) containing 50 mmol/L KCl, 1.25 mmol/L MgCl₂, 5 mmol/L dithiothreitol, 500 μ mol/L dNTP, and 1 μ mol/L oligo(dT)₁₂₋₁₈ primer. The reaction proceeded at 42°C for 50 minutes and was terminated at 70°C for 15 minutes followed by treatment of RNase H at 37°C for 20 minutes. Portions (0.5 μ l) of single-stranded cDNA were amplified by polymerase chain reaction (PCR) using SHP1 specific primer pairs, the sequences of which were obtained from Clontech Laboratories, Inc. The PCR reaction was performed using Platinum PCR Super Mix (Life Technologies, Rockville, MD). The amplification condition consisted of 2 minutes of pretreatment at 94°C, followed by 30 cycles of denaturation (94°C, 30 seconds), annealing (59°C, 30 seconds), and extension (72°C, 1 minute 30 seconds) and treatment (72°C, 10 minutes).

Tissue Microarray Analysis

We developed independently and modified the method to make tissue microarray without film, which is used in the original method to prevent peel-off of the mounted microtissue specimen.¹² We overcame this difficulty with the fine adjustment of the donor punched out block to recipient pored block. It can be treated as if it is one standard paraffin block. A total of 207 paraffin embedded tissue samples consisting 197 cases of primary malignant lymphomas of 22 different histological categories and 10 cases of normal reactive lymphoid hyperplasia (RLH) samples were used for the malignant lymphoma tissue microarray. Core tissue biopsies (diameter, 0.8 mm) were taken from selected regions of individual paraffin-embedded malignant lymphoma specimens (donor block) and precisely arrayed into a new recipient paraffin block (50 mm $\times$ 23 mm) using a custom-built instrument. After the block construction was completed, 5 μ m sections of the resulting malignant lymphoma tissue microarray block were cut with a microtome. These specimens were used for hematoxylin-eosin staining, immunostaining, and *in situ* hybridization.

In Situ Hybridization

EBV-encoded small RNA-1 (EBER1) expression was detected by RNA *in situ* hybridization using a single-stranded 30-base FITC-labeled oligonucleotide complementary (antisense probe) or anticomplementary (sense; negative control probe) to a portion of the EBER1 gene. The sequence of the anti-sense probe was 5'AGACAC-CGTCCTCACCACCCGGGACTTGTA3'.¹⁶ The *in situ* hybridization was performed on routinely processed sections of the paraffin-embedded tumor tissues or tissue microarray using a DAKO *in situ* hybridization kit (DAKO Japan, Kyoto, Japan) as described previously.¹⁷ Briefly, after proteinase K digestion, the sections were post-fixed

with 4% paraformaldehyde and acetylated with triethanolamine and acetic anhydride to reduce nonspecific electrostatic hybridization. The hybridization was carried out at 37°C overnight. The substrate reaction was developed using an *in situ* detection kit (DAKO, K046) according to the manufacturer's instructions. Paraffin-embedded pellets of an EBV-infected cell line (B95-8) and EBV-positive non-Hodgkin's lymphoma tissues were used as the positive controls. Pellets of a non-EBV-infected T cell line (Jurkat) and reactive-non-neoplastic lymph nodes served as the negative controls. For the analysis of SHP1 mRNA *in situ* hybridization, we used digoxigenin-labeled sense and antisense SHP1 riboprobes, which were synthesized with T7 RNA polymerase using a labeling kit (Boehringer, Germany), and were hybridized without alkaline hydrolysis. *In situ* hybridization was performed at 50°C according to the method by Meyer et al.¹⁸

Western Blot Analysis

Western blot analysis was performed according to the method of Towbin et al.¹⁹ After 12.5% polyacrylamide gel electrophoresis of cellular protein lysate from 7.5×10^4 cells of each culture, banded proteins were electrophoretically transferred to polyvinylidene difluoride membrane (Immobilon; Millipore, Ltd., Bedford, MA) and then reacted with the antibody to SH-PTP1 (D11; mouse monoclonal antibody against the C-terminal of SHP1, C-19; rabbit polyclonal antibody against whole SHP1 protein, Santa Cruz Biotechnology Inc., Santa Cruz, CA) and monoclonal anti- β -actin (Sigma, St. Louis, MO). The immunoreactive bands were visualized with peroxidase-labeled goat anti-mouse or anti-rabbit immunoglobulin (Ig) (Amersham Co., Ltd., Tokyo, Japan) followed by reactions with the substrate of the enhanced chemiluminescence (ECL)-SuperSignal Western blotting system (Pierce, Rockford, IL) and exposed to x-ray film. The intensity of the spots was measured using Image Gauge Computer Software (Fujifilm Co., Tokyo, Japan).

Results

To investigate the molecular pathological mechanism of NK/T cell lymphoma, we comprehensively and systematically analyzed the expression profile of the human NK/T cell lines (NK-YS and NK-TY2) genome by means of a cDNA expression array (ATLAS Human Cancer 1.2 Array containing 1176 genes). The resulting profiles were compared to those of Jurkat cells, fresh human peripheral blood mononuclear cells (PBMCs), and fresh NK cells, which were enriched by the magnetic beads method using anti-CD56 antibody from PBMCs. We detected increased expression of CD56 in purified normal NK cells compared to PBMCs in cDNA expression array, indicating that cDNA expression array is working well and also purification of normal NK cell is successful. We detected significant changes in the gene expression of the NK-YS cell line: an increase in 18 genes and a decrease in 20 genes compared to normal NK cells. These genes were

Figure 1. cDNA expression array analysis of NK-YS, NK-TY2, Jurkat, freshly isolated human PBMCs, and human NK cells. Expression profile of NK-YS cells compared to that of fresh normal human NK cells (**a**), normal human PBMCs (**b**), and Jurkat cells (**g**). cDNA expression array profile of NK-TY2 were shown as the control of fresh normal human NK cells (**d**), PBMCs (**e**) NK-YS cells (**f**), and Jurkat cells (**h**). Expression profile of fresh NK cells compared to that of normal human PBMCs (**c**). Expression profile of Jurkat cells was shown as the control of PBMCs (**i**). **Arrows** indicate the green spots in **a**, **b**, and **g**, showing that SHP1 gene expression of NK-YS cells decreased compared with fresh NK cells (**a**), PBMCs (**b**), and Jurkat cells (**g**). **Arrows** indicate green spots in **d**, **e**, and **h** showing the reduced expression of SHP1 gene in NK-TY2 cells compared with fresh NK cells (**d**), PBMCs (**e**), and Jurkat cells (**h**). Yellow spots indicated by **arrows** in **c** and **i**, showing that SHP1 gene expression of fresh NK cells (**c**) and Jurkat cells (**i**) were comparable to that of normal human PBMCs. Black spot in **f** indicated by an **arrow**, shows that no expression of SHP1 gene in NK-TY2 cells is comparable to no expression of SHP1 gene in NK-YS cells.

included transcription factors, membrane-bound surface molecules or receptors and signal transducer proteins. Among 18 genes, which showed increasing expression in NK-YS cell line compared with normal NK cells, the expression of thirteen genes significantly increased in NK-YS cells than that in normal NK cells. However, no difference was detected in the gene expression level of these thirteen genes between NK-YS cells and PBMCs. The expression of five genes among these 18 genes increased in NK-YS cells compared with that in normal NK cells and PBMCs. Among 20 genes which showed reducing expression in NK-YS cells with the control of normal NK cells, the expression of four genes were significantly reduced in NK-YS cells than that in normal NK cells. On the other hand, a large difference was not detected in the expression of these four genes between NK-YS cells and PBMCs. The expression of 16 genes among these 20 genes were reduced in NK-YS cells compared with that in normal NK cells and PBMCs. Only SH-PTP1 (SHP1) gene among various kinds of phosphatase

genes, decreased dramatically in NK-YS cells of which expression was less than one-hundredth of that in normal NK cells (Figure 1 and Table 1). This decreased value of SHP1 gene expression (ratio: 0.0077) was highly significant, because the density of SHP1 spots in fresh NK cells, which was very strong and clearly higher than the background level, dropped down to almost the background level in NK-YS cells. Similar results were obtained with another NK/T lymphoma cell line (NK-TY2). The green spots in Figure 1 (d), (e), and (h), which were indicated by arrows, showing that the significant reduced expression of SHP1 gene in NK-TY2 cells compared with fresh NK cells (d), PBMCs (e), and Jurkat cells (h). It is similar to the results of green spots in Figure 1 (a), (b), and (g), showing the reduction of SHP1 gene expression in NK-YS cells compared with fresh NK cells (a), PBMCs (b), and Jurkat cells (g). On the other hand, SHP1 gene expression of fresh NK cells (c) and Jurkat cells (i) were comparable to that of normal human PBMCs, showing yellow spots indicated by arrows in Figure 1, c and i. No

Table 1. Comparison of Phosphatase Gene Expression between NK-YS and Normal Fresh NK Cells Revealed by cDNA Macroarray

Genes	GenBank accession no.	Fresh NK density	NK-YS density	Ratio
Hematopoietic cell protein-tyrosine phosphatase; SH-PTP1/SHP1	X62055	487.29	3.76	0.0077
Protein tyrosine phosphatase- σ	U35234	8.52	5.31	0.6229
Phosphatidic acid phosphatase 2B	AF017786	23.54	22.9	0.9729
Protein phosphatase 2C- γ	Y13936	15.23	18.08	1.1871
Dual-specificity protein phosphatase 2; PAC-1	L11329	3.08	3.69	1.1982
T cell protein-tyrosine phosphatase (TCPTP)	M25393	7.7	11.56	1.5003
Protein tyrosine phosphatase	D15049	5.62	9.2	1.6372
Protein phosphatase 2A B56- β (PP2A)	L42374	1.94	3.35	1.7258
Serine/threonine protein phosphatase 6 (PP6)	X92972	3.89	7.93	2.0387
Protein phosphatase with EF-hands-2 long form (PPEF2)	AF023456	2.45	5.44	2.2227
Putative protein-tyrosine phosphatase PTEN; mutated in multiple advanced cancers 1	U92436	1.58	4.43	2.7999
51C protein (homology with inositol polyphosphate phosphatases)	L36818	14.98	45.39	3.0299
Protein phosphatase PP2A 61-kd regulatory subunit epsilon	L76703	1.79	5.65	3.1496
Protein phosphatase 2A B56- α	L42373	2.02	7.26	3.5863
CDC25B; CDC25HU2; M-phase inducer phosphatase 2	M81934	16.7	67.25	4.0261
Protein phosphatase WIP1	U78305	1.08	4.41	4.0936
Protein tyrosine phosphatase- σ	Z48541	1.32	5.64	4.2606
Dual-specificity protein phosphatase 8; HVH5	U27193	1.56	6.72	4.2926
CDC25C; M-phase inducer phosphatase 3	M34065	3.27	14.65	4.4834
PTPCAAX1 nuclear tyrosine phosphatase (PRL-1)	U48296	5.03	22.85	4.5411
Serine/threonine protein phosphatase 2B catalytic subunit γ ; calcineurin A subunit γ	S46622	0.86	4.25	4.9407
Phospholipase D1 (PLD 1); choline phosphatase 1	U38545	2.28	16.29	7.152
Cell division cycle protein 25A (CDC25A); M-phase inducer phosphatase 1	M81933	3.98	29.94	7.5215
Protein phosphatase PP2A 65-kd regulatory subunit β (P65- β)	M65254	0.26	4.36	16.5528

significant expression of SHP1 gene in NK-TY2 cells is comparable to that of SHP1 gene in NK-YS cells, which was shown as a black spot in Figure 1f indicated by an arrow. This was confirmed by RT-PCR analysis: a clear band corresponding to SHP1 mRNA was detected in the Jurkat, KCA, SP53, BALL1, EDS, and ATL1K cell lines, as well as in normal human PBMCs and fresh human NK cells. On the other hand, no SHP1 mRNA band was detected in the NK-YS cell line, and only a faint band could be recognized in the K562 cell line (Figure 2).

To clarify whether the decreased mRNA expression of SHP1 in NK/T cell lymphoma cell lines *in vitro* is also observed in NK/T cell lymphoma patient specimens and to determine whether this phenomenon is specific to NK/T cell lymphoma or common among various types of lymphomas, we analyzed the protein expression of SHP1 in about 200 lymphoma specimens using the tissue microarray method (Figure 3). All NK/T cell lymphoma specimens were clearly

negative for the expression of SHP1 protein. In reactive lymphoid hyperplasia (RLH), nearly all mantle-zone B cells and a part of interfollicular lymphoid cells expressed SHP1. In contrast, germinal center cells were only faintly immunostained (Figure 3, Table 2). As for immunoreactivity with malignant lymphomas, more than 95% of malignant lymphoma/leukemia specimens, including those of diffuse large B cell lymphoma (DLL), follicular lymphoma (FL), Hodgkin's disease (HD), mantle cell lymphoma (MCL), peripheral T cell lymphoma (PT), adult T cell lymphoma/leukemia (ATLL) and plasmacytoma were negative in SHP1 immunohistochemistry. About 60% of marginal zone B cell lymphoma (MZL) and MALToma specimens were also negative for SHP1 immunostaining. To confirm these immunohistochemical data, SHP1 mRNA *in situ* hybridization was performed, showing the same the results as the immunohistochemistry. (Figure 3, e and f) Epstein-Barr virus infections were detected by *in situ* hybridization of EBV1 mRNA in 91.1% of NK/T cell lymphoma, 50.0% of Hodgkin's disease, 18.2% of DLL and 7.7% of plasmacytoma specimens. These results were further confirmed by standard full-tissue immunostaining. The tissue microarray and standard immunostaining data are summarized in Table 2.

We next investigated the expression of SHP1 protein in various types of hematopoietic cell lines by Western blotting using both monoclonal and polyclonal antibodies (Figure 4). Like NK-YS cells, another NK/T cell lymphoma cell line NK-TY2 also showed no expression of SHP1 protein. SHP1 protein was not detected in ATLL tumor cell lines: EDS and ATL1K cells. This finding was in obvious contrast to the strong positive SHP1 protein band in the IWA1 cell line, which was a virus producing cell line freshly immortalized *in vitro* by co-cultivation with an HTLV-I producer cell line and carrying the normal human karyotype. Cell lines of T cell chronic lymphocytic leuke-

Figure 2. RT-PCR analysis of SHP1 gene in hematopoietic cells. cDNAs of SHP1 and β -actin in freshly isolated human NK cells and PBMCs in addition to several cell lines were amplified by hot start RT-PCR, then separated by 1.2% agarose gel electrophoresis. NC indicates the controls, which did not contain cDNA.

A

B

Figure 3. Tissue microarray analysis of SHP1 immunostaining and SHP1 mRNA *in situ* hybridization. **A:** Hematoxylin-eosin (HE) staining and EBER1 mRNA *in situ* hybridization. An overview of a tissue microarray section containing 207 tissue samples (**a**); HE staining of tissue microarray in higher magnification (**b**, **c**); EBER1 mRNA *in situ* hybridization showing positive (left) and negative (right) reaction (**d**); higher magnification of EBER1-positive sample (**e**). **B:** Immunohistochemistry of SHP1 protein, showing reactive lymphocyte hyperplasia (RLH) (**a**); NK/T cell lymphoma (**b**); Hodgkin's disease (**c**) and mantle cell lymphoma (**d**). SHP1 gene mRNA *in situ* hybridization of reactive lymphocyte hyperplasia (**e**) and NK/T lymphoma (**f**).

Table 2. Expression of SHP1 Protein and EBER1 mRNA in Various Malignant Lymphomas Analyzed by Tissue Microarray and Standard Immunohistochemistry

	SHP1 (%) [*]					EBER1 (%) [†]	
	+++	++	+	+/-	-	+++	-
NK/T	0/11 (0)	0/11 (0)	0/11 (0)	0/11 (0)	11/11 (100)	11/12 (91.1)	1/12 (8.9)
DLL	0/26 (0)	1/26 (3.8)	0/26 (0)	0/26 (0)	25/26 (96.2)	4/22 (18.2)	18/22 (81.8)
FL	0/20 (0)	0/20 (0)	0/20 (0)	0/20 (0)	20/20 (100)	0/18 (0)	18/18 (100)
HD	0/22 (0)	0/22 (0)	0/22 (0)	0/22 (0)	22/22 (100)	6/12 (50.0)	6/12 (50.0)
PT	1/20 (5)	0/20 (0)	0/20 (0)	0/20 (0)	19/20 (95)	0/11 (0)	11/11 (100)
MCL	1/15 (6.7)	0/15 (0)	0/15 (0)	0/15 (0)	14/15 (93.3)	0/4 (0)	4/4 (100)
MZL	0/13 (0)	3/13 (23.1)	0/13 (0)	2/13 (15.4)	8/13 (61.5)	0/3 (0)	3/3 (100)
ATLL	0/9 (0)	0/9 (0)	0/9 (0)	0/9 (0)	9/9 (100)	0/7 (0)	7/7 (100)
Plasmacytoma	0/17 (0)	0/17 (0)	0/17 (0)	0/17 (0)	17/17 (100)	1/13 (7.7)	12/13 (92.3)
MALToma	3/22 (13.6)	0/22 (0)	3/22 (13.6)	2/22 (9.2)	14/22 (63.6)	0/22 (0)	22/22 (100)
RLH							
Germinal center	0/10 (0)	0/10 (0)	0/10 (0)	8/10 (80)	2/10 (20)	0/10 (0)	10/10 (100)
Mantle zone	9/10 (90)	1/10 (10)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	10/10 (100)
Interfollicular	0/10 (0)	6/10 (60)	4/10 (40)	0/10 (0)	0/10 (0)	0/10 (0)	10/10 (100)

^{*}Immunohistochemical staining.

[†]RNA *in situ* hybridization.

+++ , positive more than 50% cells; ++ , positive between 10 to 50% cells; + , positive between 5 to 10% cells.

mia (T-CLL) and Sézary syndrome (SS) were negative for SHP1. Two-thirds of the cell lines of Hodgkin's disease and multiple myeloma and both of the two Burkitt's lymphoma cell lines were also negative for SHP1.

To investigate whether there exists a genetic disorder or modification that related to lack of expression of SHP1 protein in NK/T cell lines, we examined the ability to induce SHP1 expression by TPA treatment (Figure 5).

Figure 4. Western blot analysis of SHP1 protein expression in various hematopoietic cell lines. Cell lysates, which were extracted from the 10^5 cells in the standard culture condition, were loaded to the SDS-PAGE and blotted membranes were probed with anti-SHP1 (D11; mouse monoclonal antibody against C-terminal of SHP1, C19; rabbit polyclonal against whole SHP1) or anti- β actin antibodies. Results of Western blotting of SHP1 protein were summarized in the Table of this figure. The strength of the SHP1 bands was indicated according to the intensity from +++, ++ to +. -: completely negative for SHP1 band.

Figure 5. Induction of SHP1 protein synthesis by treatment of TPA. **A:** NK-YS, NK-TY2, Ramos, K562, KG1 cell lines, and PBMCs were treated with 20 ng/ml of TPA for 72 hours at 37°C in a CO_2 incubator. After the addition of TPA, the sample cells were harvested at 24-h intervals and cellular protein lysates from 7.5×10^4 cells of each culture were used for the ECL-Western blot analysis. **B:** SHP1 protein bands detected on x-ray film were measured with Image Gauge Software (Fujifilm Co., Ltd.). □, TPA-treated; ◆, control culture.

Burkitt's lymphoma cell lines Daudi and Ramos could be induced SHP1 expression by TPA treatment from no expression of SHP1 protein in the standard culture condition. KG1 cells, which showed weak but stable expression of SHP1, were strongly induced to express SHP1 by TPA. On the other hand, NK-YS, NK-TY2, and K562 were completely negative for the SHP1 expression both under the control culture and TPA stimulation condition. Normal human PBMCs that were stimulated by TPA showed a transient increase in SHP1 protein expression followed by a decrease during cultivation. Mitogens such as PHA-P or PWM also showed the same effect on PBMCs (data not shown).

Discussion

We previously established the first cell line of NK cell lymphoma cells, NK-YS, from a typical case of EBV-associated nasal angiocentric NK cell leukemia/lymphoma.¹⁵ In the present investigation we analyzed the genes involved in NK cell lymphomagenesis. We detected several specific gene expression changes in NK-YS and NK-TY2 cells compared with normal PBMCs, normal human NK cells, or Jurkat cells using the cDNA expression array method. Among these genes, we found a strong decrease in hematopoietic cell specific protein tyrosine phosphatase SH-PTP1(SHP1) mRNA by cDNA-expression array and RT-PCR. Among various kinds of phosphatase genes, only SHP1 gene exhibited prominent and specific decrease of mRNA expression (Table 1). In addition, SHP1 immunohistochemical staining of several cases of reactive lymphoid hyperplasia using tissue microarray and standard tissue specimens revealed that most of the mantle zone and some interfollicular zone lymphocytes in reactive lymphoid hyperplasia showed strongly positive staining of SHP1 antigen, indicating that resting B cells highly express SHP1 protein. This is in line with the present result that freshly isolated resting PBMCs robustly express SHP1. On the other hand, germinal center lymphocytes showed decreased staining of SHP1, which may be characteristic for centroblast cells. Western blotting using rabbit polyclonal anti-SHP1 antibody in Figure 4, shows that polyclonal antibody also detected essentially the same results of decreased expression of SHP1 protein in malignant lymphoma/leukemia cells as the results obtained with D11 monoclonal anti-SHP1 antibody. It clearly eliminates the possibility that the failure to detect SHP-1 expression in the lymphomas/leukemias represents augmented modification of C-terminal region of SHP1 and may therefore mask the D11 monoclonal antibody epitope in these samples. Those findings are consistent with the previous observation that SHP1 expression was down-regulated in Burkitt's lymphomas and germinal center B lymphocytes.²⁰ As germinal center B cells have developmentally regulated low thresholds for cellular activation, there is a possibility that the observed low level or lack of SHP1 expression in the malignant lymphomas/leukemias might have resulted from physiological regulation associated with the immaturity of the neoplastic cells. Another possibility is that the low level or

lack of SHP1 expression in malignant lymphomas/leukemias is associated with tumorigenesis itself rather than the associated immature phenotype of neoplastic cells. Present observation that a wide range of malignant lymphomas/leukemias, including well differentiated tumors such as plasmacytoma, consistently showed the decreased expression of SHP1 with the clear contrast of high expression in normal reactive lymphocyte hyperplasia, supports the latter possibility. However, it remains to be elucidated with further experiments.

SHP1, which is also called HCP, SHPTP1, and PTP1C, is a 68-kd, non-transmembrane protein-tyrosine phosphatase (PTP) containing two tandem Src homology (SH2) domains, a catalytic domain, and a C-terminal tail of about 100 amino acid residues. SHP1 is expressed primarily in hematopoietic cells and usually functions as a negative regulator in signal transduction. The consensus sequence (S/L/I/V)XYXX(L/V), based on sequences originally deduced from several receptors known to bind to the C-terminal SH2 domain of SHP1, defines all immunoreceptor tyrosine-based inhibitory motifs (ITIMs), including NK cell, B cell, and monocyte and dendritic cell inhibitory receptors.²¹⁻²⁴ SHP1 is known to associate with multiple signaling molecules, including ZAP70,²⁵ CD3 ϵ ,^{25,26} CD5²⁶ and interleukin-2R²⁷ in T cells; interleukin-3 receptor β chain^{28,29} and erythropoietin receptor³⁰ in hematopoietic cells; CD22,^{31,32} B cell receptor,³³ SLP76^{34,35} and CD72^{36,37} in B cells; and the killer cell inhibitory receptor^{38,39} in natural killer cells. These interactions appear to exert primarily inhibitory effects on their signaling cascades. Motheaten mice (me/me) and viable motheaten mice (me^v/me^v) are natural genetic models of mammals lacking the expression of functional SHP1. Motheaten mice display an increase in the phosphorylation state of Src family protein tyrosine kinases on T cell receptor (TCR) stimulation in T cells.⁴⁰ Transducing polypeptides bearing immunoreceptor tyrosine-based activation motifs (ITAMs) such as CD3 ζ and CD3, are also hyperphosphorylated in motheaten mice. Similarly, the adapter protein linker for activation of T cells (LAT) is hyperphosphorylated in motheaten mice, and can be directly dephosphorylated by SHP-1 *in vitro*. This dephosphorylation induces the dissociation of LAT and PLC γ in NK cells *in vivo*.⁴¹ Besides, the SLP-76 adapter protein is also a target of SHP1 in T and NK cells. Moreover, SHP1 can dephosphorylate ZAP70 and Syk.⁴² All this evidence suggests that, in T and NK cells, inhibitory killer cell immunoglobulin (Ig)-like receptors (KIRs) recruit SHP1 which, in turn, may dephosphorylate ITAM polypeptides, Src family kinases, ZAP-70/Syk, and adapter proteins responsible for the recruitment of downstream signaling molecules such as LAT and SLP76.⁴³ Therefore, SHP1 might be capable of terminating activating signals by dephosphorylating molecules involved early in signal transduction. These molecular events may cause motheaten phenotypes such as suffering from chronic macrophage and neutrophil activation, abnormal B-cell development, T- and B-cell depletion and dysfunction, and marked B cell enhancement in the induction of proliferation, intracellular calcium mobilization, and antigen activated protein kinase activation following B cell receptor

ligation.^{44,45} Present observation that SHP1 expression in centroblast cells are down-regulated in the germinal center of reactive lymphoid hyperplasia, are possibly reflecting a requirement at this stage of B cells for a lower threshold for signaling through certain cytokine receptors and membrane immunoglobulin. Low SHP1 expression may facilitate the signal transduction required for germinal center clonal expansion, isotype switching, hypermutation, and selection for high affinity memory B cells. SHP1 expression in freshly isolated normal PBMCs decreased gradually in the standard culture condition and reached a steady low state (Figure 5), suggesting that this behavior may be a physiological response after removal of growth inhibitory soluble factors in the serum and the expression of inhibitory signaling molecule: SHP1 was down-regulated.

The present finding that a wide range of malignant lymphomas/leukemias, including NK/T cell lymphoma, diffuse large cell lymphoma (DLL), follicle center lymphoma (FL), Hodgkin's disease (HD), mantle cell lymphoma (MCL), peripheral T cell lymphoma (PL), adult T cell lymphoma/leukemia (ATLL), and plasmacytoma showed a reduction in SHP1 protein expression in more than 93% of cases suggests that SHP1 plays an important role in the molecular pathogenesis of these lymphomas. In particular, such highly aggressive lymphomas as NK/T, ATLL, and Burkitt's lymphoma were completely negative for SHP1 protein expression. On the other hand, low-grade malignant lymphomas such as MZL and MAL-Toma showed a low percentage of negativity in SHP1 expression, suggesting that there is some correlation between loss of SHP1 expression and aggressiveness. As SHP1 is one of a key molecule in the hematopoietic cell signal transduction, genetic defects in functional domain of SHP1 gene or continuous suppression of SHP1 gene expression may cause a failure in termination of activating signals by dephosphorylating molecules early involved in signal transduction. This loss of SHP1 expression may have contributed to the abnormal cell growth and viability of these malignant cells as a result of abrogation of the inhibitory signaling function of SHP1 on both cell growth and apoptosis.⁴⁶ These results coincide well with the previous observation that mice heterozygous for the *me* or *me*^v mutations, which are loss of function mutations in the SHP1 gene, are unusually susceptible to B-cell lymphomas.⁴⁷

Interestingly, freshly immortalized human T cell lines with HTLV-I: IWA-I strongly expressed SHP1, which is in evident contrast to the lack of SHP1 expression in ATLL tumor cell lines, EDS, and ATL1K, implying that SHP1 may be related to the progression of malignant transformation from the virus carrier state to the lymphoma/leukemia stage. EBV infection had no correlation to the loss of SHP1 expression, judging from the evidence that 96.2% of diffuse large lymphomas (DLL) were negative for SHP1 protein immunostaining, whereas 18.2% of DLL carried EBV. The observation that both terminally differentiated plasmacytoma and immature type lymphoma showed negative expression of SHP1 indicates that extinction of SHP1 protein in various types of lymphoma is independent of the differentiation state. Evidence that

both NK/T cell lymphoma cell lines failed to induce SHP1 expression with TPA treatment suggests that the regulatory region of the SHP1 gene, including the enhancer and promoter region, may have genetic defects such as mutation, deletion, or some kind of DNA modification. Genomic Southern hybridization of the entire region of the SHP1 gene showed no gross changes in NK/T cell lines (data not shown). This result indicates that NK/T lymphoma cells may be carrying some minor, if any, genetic changes, such as point mutations or small deletions, or some other genetic modification in the SHP1 gene locus, such as methylation. The percentage of SHP1 protein negative cell lines was lower than that of lymphoma patient specimens (Table 2 and Figure 4), suggesting that suppression of SHP1 is more likely to be induced by genetic modification such as methylation rather than genetic changes. Recently, Zhang et al reported that lack of SHP1 expression in malignant T cell lymphoma was induced by methylation of the SHP1 promoter region.⁴⁸ In the present cases of other malignant lymphomas/leukemias it is possible that methylation of SHP1 promoter/enhancer region caused reduced or lack of SHP1 gene expression, which should be elucidated by further analysis. This evidence strongly suggests that the SHP1 gene plays an important role in multistep tumorigenesis not only in NK/T cell lymphomas but also in a wide range of lymphomas/leukemias and other hematopoietic malignancies, possibly as an anti-oncogene molecule.

The large-scale cDNA microarray or DNA chip methods are tremendously powerful and promising for the systematic and comprehensive analysis of gene expression profiles of known and unknown genes, genetic linkage studies with single nucleotide polymorphism (SNP), mutation detection and analysis with oligo arrays, analysis of disease genes and so on, if the problem of high cost could be overcome. At this moment, combination of the low-cost middle-scale cDNA macroarray and tissue microarray is more realistic and also useful and informative. This strategy may have a great advantage especially in rapid and easy identification of the disease-responsible or closely related genes among known genes with quick screening of multiple genes and multiple tumor types in the same condition, thereby leading to a more unbiased reliable analysis. This strategy may be very useful for identification of genes from various types of diseases where a particular molecular alteration is very important.

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References

- Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, Delsol G, De Wolf-Peters C, Falini B, Gatter KC: A revised European-American classification of lymphoid neoplasm: a proposal from the International Lymphoma Study Group. *Blood* 1994, 84:1361-1392
- Jaffe ES, Chan JKC, Su IJ, Firizzer G, Mori S, Feller AC, Ho EC: Report of the workshop on the nasal and related extranodal angio-centric T/natural killer cell lymphoma. *Am J Surg Pathol* 1996, 20:103-111
- Magrath I: The pathogenesis of Burkitt's lymphoma. *Adv Cancer Res* 1990, 55:133-270
- Ye BH, Chaganti S, Chang CC, Niu H, Corradini P, Chaganti RS, Dalla-Favera R: Chromosomal translocations cause deregulated BCL6 expression by promoter substitution in B cell lymphoma. *EMBO J* 1995, 14:6209-6217
- Poetsh M, Weber-Matthiesen K, Plendl HJ, Grote W, Schlegelberger B: Detection of the t(14;18) chromosomal translocation by interphase cytogenetics with yeast-artificial-chromosome probes on follicular lymphoma and nonneoplastic lymphoproliferation. *J Clin Oncol* 1996, 14:963-969
- Komatsu H, Yoshida K, Seto M, Iida S, Aikawa T, Ueda R, Mikuni C: Overexpression of PRAD1 in a mantle zone lymphoma patient with a t(11;22)(q13;q11) translocation. *Br J Haematol* 1993, 85:2 427-429
- Chesi M, Nardini E, Brents LA, Schrock E, Ried T, Kuehl WM, Bergsagel PL: Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. *Nat Genet* 1997, 16:260-264
- Zhang L, Zhou W, Velculescu VE, Kern SE, Hruban RH, Hamilton SR, Vogelstein B, Kinzler KW: Gene expression profiles in normal and cancer cells. *Science* 1997, 276:1268-1272
- Wang K, Gan L, Jeffery E, Gayle M, Gown AM, Skelly M, Nelson PS, Ng WW, Schummer M: Monitoring gene expression profile changes in ovarian carcinomas using cDNA microarray. *Gene* 1999, 229:101-108
- Alizadeh AA, Eisen MB, Izidore DR, Lossos S, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JI, Yang L, Marti GE, Moore T, Hudson J, Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Levy R, Wilson W, Grever MR, Byrd JC, Botstein D, Brown PO, Staudt LM: Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000, 403:503-511
- Duggan DJ, Bittner M, Chen Y, Meltzer P, Trent JM: Expression profiling using cDNA microarrays. *Nat Genet* 1999, 21:10-14
- Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G: Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 1998, 4:844-847
- Schraml P, Kononen J, Burbendorf L, Moch H, Bissig H, Nocito A, Mihatsch MJ, Kallioniemi OP, Sauter G: Tissue microarrays for gene amplification surveys in many different tumor types. *Clin Cancer Res* 1999, 5:1966-1975
- Moch H, Schraml P, Burbendorf L, Mirlacher M, Kononen, J, Gasser T, Mihatsch MJ, Kallioniemi OP, Sauter G: High-throughput tissue microarray analysis to evaluate genes uncovered by cDNA microarray screening in renal cell carcinoma. *Am J Pathol* 1999, 154:981-986
- Tsuchiya J, Yoshino T, Mori M, Kondoh E, Oka T, Akagi T, Hiraki A, Nakayama H, Shibuya A, Ma Y, Kawabata T, Okada S, Harada M: Characterization of a novel natural killer-cell line (NK-YS) established from natural killer cell lymphoma/leukemia associated with Epstein-Barr virus infection. *Blood* 1998, 92:1374-1383
- Chang KL, Chen YY, Shibata D, Weiss LM: Description of an in situ hybridization methodology for detection of Epstein-Barr virus RNA in paraffin-embedded tissues with a survey of normal and neoplastic tissues. *Diagn Mol Pathol* 1998, 1:246-255
- Hayashi K, Koirala T, Ino H, Chen HL, Ohara N, Teramoto N, Yoshino T, Takahashi K, Yamada M, Nii S, Miyamoto K, Fujimoto K, Yoshikawa Y, Akagi T: Malignant lymphoma induction in rabbits by intravenous inoculation of Epstein-Barr-virus related herpesvirus from HTLV-II-transformed cynomolgus leukocyte cell line (Si-IIA). *Int J Cancer* 1995, 63:872-880
- Meyer D, Yamaai T, Garratt A, Sonnenberg-Riethmacher E, Kramer R., Theil LE, Birchmeier C: Isoform-specific expression and function of neuregulin. *Development* 1997, 124:3575-3586
- Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979, 76:4350-4354
- Delibrias CC, Floettmann JE, Rowe M, Fearon DT: Downregulated expression of SHP-1 in Burkitt lymphomas and germinal center B lymphocytes. *J Exp Med* 1997, 186:1575-1583
- Long EO, Burshtyn DN, Clark WP, Peruzzi M, Rajagopalan S, Rojo S, Wagtmann N, Winter CC: Killer cell inhibitory receptors: diversity, specificity, and function. *Immunol Rev* 1997, 155:135-144
- Siminovitch KA, Neel BG: Regulation of B cell signal transduction by SH2-containing protein-tyrosine phosphatase. *Semin Immunol* 1998, 10:329-347
- Healy JI, Goodnow CC: Positive versus negative signaling by lymphocyte antigen receptors. *Annu Rev Immunol* 1998, 16:645-670
- Long EO: Regulation of immune responses through inhibitory receptors. *Annu Rev Immunol* 1999, 17:875-904
- Brockdorff J, Williams S, Couture C, Mustelin T: Dephosphorylation of ZAP70 and inhibition of T cell activation by activated SHP1. *Eur J Immunol* 1999, 29:2539-2550
- Pani G, Fischer KD, Mlinaric-Rascan I, Siminovitch KA: Signaling capacity of the T cell antigen receptor is negatively regulated by the PTP1C tyrosine phosphatase. *J Exp Med* 1996, 184:839-852
- Migone TS, Cacalano NA, Taylor N, Yi T, Waldmann TA, Johnston JA: Recruitment of SH2-containing protein tyrosine phosphatase SHP-1 to the interleukin 2 receptor: loss of SHP-1 expression in human T-lymphotropic virus type I-transformed T cells. *Proc Natl Acad Sci USA* 1998, 95:3845-3850
- Yang W, Tabrizi M, Berrada K, Yi T: SHP-1 phosphatase C-terminus interacts with novel substrates p32/p30 during erythropoietin and interleukin-3 mitogenic responses. *Blood* 1998, 91:3746-3755
- Bone H, Dechert U, Jirik F, Schrader JW, Welham MJ: SHP1 and SHP2 protein-tyrosine phosphatases associate with betac after interleukin-3-induced receptor tyrosine phosphorylation: identification of potential binding sites and substrates. *J Biol Chem* 1997, 272:14470-14476
- Bittorf T, Seiler J, Zhang Z, Jaster R, Brock J: SHP1 protein tyrosine phosphatase negatively modulates erythroid differentiation and suppression of apoptosis in J2E erythroleukemic cells. *Biol Chem* 1999, 380:1201-1209
- Blasioli J, Paust S, Thomas ML: Definition of the sites of interaction between the protein tyrosine phosphatase SHP-1 and CD22. *J Biol Chem* 1999, 274:2303-2307
- Cornall RJ, Cyster JG, Hibbs ML, Dunn AR, Otipoby KL, Clark EA, Goodnow CC: Polygenic autoimmune traits: lyn, CD22, and SHP-1 are limiting elements of a biochemical pathway regulating BCR signaling and selection. *Immunity* 1998, 8:497-508
- Cornall RJ, Goodnow CC, Cyster JG: Regulation of B cell antigen receptor signaling by the Lyn/CD22/SHP1 pathway. *Curr Top Microbiol Immunol* 1999, 244:57-68
- Binstadt BA, Billadeau DD, Jevremovi D, Williams BL, Fang N, Yi T, Koretzky GA, Abraham RT, Leibson PJ: SLP-76 is a direct substrate of SHP-1 recruited to killer cell inhibitory receptors. *J Biol Chem* 1998, 273:27518-27523
- Mizuno K, Katagiri T, Hasegawa K, Ogimoto M, Yakura H: Hematopoietic cell phosphatase, SHP-1, is constitutively associated with the SH2 domain-containing leukocyte protein, SLP-76, in B cells. *J Exp Med* 1996, 184:457-463
- Wu Y, Nadler MJ, Brennan LA, Gish GD, Timms JF, Fusaki N, Jongstra-Bilen J, Tada N, Pawson T, Wither J, Neel BG, Hozumi N: The B-cell transmembrane protein CD72 binds to and is an in vivo substrate of the protein tyrosine phosphatase SHP-1. *Curr Biol* 1998, 8:1009-1017
- Fusaki N, Tomita S, Wu Y, Okamoto N, Goitsuka R, Kitamura D, Hozumi N: BLNK is associated with the CD72/SHP-1/Grb2 complex in

- the WEHI231 cell line after membrane IgM cross-linking. *Eur J Immunol* 2000, 30:1326–1330
38. Wang LL, Blasioli J, Plas DR, Thomas ML, Yokoyama WM: Specificity of the SH2 domains of SHP-1 in the interaction with the immunoreceptor tyrosine-based inhibitory motif-bearing receptor gp49B. *J Immunol* 1999, 162:1318–1322
39. Lorenz U, Ravichandran KS, Burakoff SJ, Neel BG: Lack of SHPTP1 results in src-family kinase hyperactivation and thymocyte hyperresponsiveness. *Proc Natl Acad Sci USA* 1996, 93:9624–9629
40. Pani G, Fischer KD, Mlinaric-Rascan I, Siminovitch KA: Signaling capacity of the T cell antigen receptor is negatively regulated by the PTP1C tyrosine phosphatase. *J Exp Med* 1996, 184:839–852
41. Valiante NM, Phillips JH, Lanier LL, Parham P: Killer cell inhibitory receptor recognition of human leukocyte antigen (HLA) class I blocks formation of a pp36/PLC-gamma signaling complex in human natural killer (NK) cells. *J Exp Med* 1996, 184:2243–2250
42. Dustin LB, Plas DR, Wong J, Hu YT, Soto C, Chan AC, Thomas ML: Expression of dominant-negative src-homology domain 2-containing protein tyrosine phosphatase-1 results in increased Syk tyrosine kinase activity and B cell activation. *J Immunol* 1999, 162:2717–2724
43. Blery M, Olcese L, Vivier E: Early signaling via inhibitory and activating NK receptors. *Hum Immunol* 2000, 61:51–64
44. Shultz LD, Schweitzer PA, Rajan TV, Yi T, Ihle JN, Matthews RJ, Thomas ML, Beier DR: Mutations at the murine motheaten locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. *Cell* 1993, 73:1445–1454
45. Bignon JS, Siminovitch KA: Identification of PTP1C mutation as the genetic defect in motheaten and viable motheaten mice: a step toward defining the roles of protein tyrosine phosphatases in the regulation of hemopoietic cell differentiation and function. *Clin Immunol Immunopathol* 1994, 73:168–179
46. Bittorf T, Seiler J, Zhang Z, Jaster R, Brock J: SHP1 protein tyrosine phosphatase negatively modulates erythroid differentiation and suppression of apoptosis in J2E erythroleukemic cells. *Biol Chem* 1999, 380:1201–1209
47. Siminovitch KA, Lamhonwah AM, Somani AK, Cardiff R, Mills GB: Involvement of the SHP1 tyrosine phosphatase in regulating B lymphocyte antigen receptor signaling, proliferation, and transformation. *Curr Top Microbiol Immunol* 1999, 246:291–297
48. Zhang Q, Raghunath PN, Vonderheid E, Odum N, Wasik MA: Lack of phosphotyrosine phosphatase SHP-1 expression in malignant T-cell lymphoma cells results from methylation of the SHP-1 promoter. *Am J Pathol* 2000, 157:1137–1146