Depletion of Pleckstrin Homology Domain Leucine-rich Repeat Protein Phosphatases 1 and 2 by Bcr-Abl Promotes Chronic Myelogenous Leukemia Cell Proliferation through Continuous Phosphorylation of Akt Isoforms*

Received for publication, October 24, 2008, and in revised form, March 4, 2009 Published, JBC Papers in Press, March 4, 2009, DOI 10.1074/jbc.M808182200

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The constitutive activation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway commonly occurs in cancers and is a crucial event in tumorigenesis. Chronic myelogenous leukemia (CML) is characterized by a reciprocal chromosomal translocation (9;22) that generates the Bcr-Abl fusion gene. The PI3K/Akt pathway is activated by Bcr-Abl chimeric protein and mediates the leukemogenesis in CML. However, the mechanism by which Bcr-Abl activates the PI3K/Akt pathway is not completely understood. In the present study, we found that pleckstrin homology domain leucine-rich repeat protein phosphatase (PHLPP)1 and PHLPP2 weakened the Abl kinase inhibitor-mediated inhibition of colony formation activity by the Abl kinase inhibitors in Bcr-Abl

Continuous Phosphorylation of Akt Isoforms*

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2 The abbreviations used are: CML, chronic myelogenous leukemia; PHLPP, pleckstrin homology domain leucine-rich repeat protein phosphatase; CFU-GEMM, colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte; CFU-GM, colony-forming unit-granulocyte, macrophage; BFU-E, burst-forming unit-erythroid; PP2A, the serine/threonine protein phosphatase 2A; PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; siRNA, short interfering RNA; AML, acute myeloblastic leukemia; ALDH, aldehyde dehydrogenase; RT-PCR, real time PCR; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; E3, ubiquitin-protein isopeptide ligase.

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homology domain with a region of leucine-rich repeats, a PP2C phosphatase domain, and a C-terminal PDZ ligand (13). The genes encoding PHLPP1 and PHLPP2 were frequently lost in various cancers such as colon (16), breast (17), and ovarian cancers (18), Wilms tumors (19), prostate cancer (20), and hepatocellular carcinomas (21). PHLPP1 and PHLPP2 are present in the cytosolic, nuclear, and membrane fraction of cells and are expressed in cell lines including brain, breast, lung, prostate, and ovarian cancer cell lines (15). PHLPP1 and PHLPP2 decrease activity of Akt and increase apoptosis and inhibition of cell proliferation through the dephosphorylation of the hydrophobic motif (Ser-473) in Akt. Depletion of either PHLPP1 or PHLPP2 causes a 30-fold increase in Akt phosphorylation after EGF stimulation in a normal breast cell line (22). Knockdown studies have revealed that PHLPP1 influences the phosphorylation state of Akt2 and Akt3, whereas PHLPP2 affects the phosphorylation state of Akt1 and Akt3.

In the PI3K/Akt signaling pathway, defective PTEN activates Akt signaling by preventing conversion of phosphatidylinositol 3,4,5-trisphosphate back to phosphatidylinositol 4,5-bisphosphate and contributes to retaining Akt phosphorylation. Nonetheless there are many examples of elevated Akt phosphorylation in cancer cells that have intact PTEN expression. PHLPP levels are markedly reduced in some cancer cell lines that have elevated Akt phosphorylation, and the reintroduction of PHLPP reduces cell growth (23). However, in Bcr-Abl-associated leukemogenesis, the role of PLHPP remains unclear.

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In the present study, we found that Bcr-Abl activates PHLPP expression in CML cells. Depletion of PHLPP1 and PHLPP2 in CML cells leads to the reduction of Bcr-Abl kinase activity. These results suggest that PHLPP1 and PHLPP2 may be potential targets for the treatment of Bcr-Abl-positive leukemia.

Experimental Procedures

Reagents—Imatinib mesylate (STI571) and AMN107 were kindly provided by Novartis Pharma (Basel, Switzerland). BMS354825 was kindly provided by Bristol-Myers Squibb Co. Each compound was prepared as a 10 mM stock solution in dimethyl sulfoxide and stored at -80 °C. Experiments were performed with 1000-fold dilutions of the stock solutions into reaction mixtures.

Cells and Cell Cultures—Human CML cell lines, K562 and Meg01, and human acute myeloblastic leukemia (AML) cell lines, U937 and HL60, were purchased from American Type Culture Collection (Manassas, VA). We established SHG3 cells from the bone marrow of a patient with AML M5b (French-American-British classification). These cells were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 2 mM l-glutamine, 100 μg/ml streptomycin, and 200 units/ml penicillin (Invitrogen) and maintained in a humidified 5% CO₂ atmosphere at 37 °C.

Bone Marrow Samples—Prior to participation in the study, patients gave informed consent according to the Declaration of Helsinki. Samples of normal bone marrow were obtained from three healthy volunteers. Bone marrow was also obtained from four patients with CML in the first chronic phase. Mononuclear cells were isolated from bone marrow samples by Ficol-Hypaque density gradient centrifugation. CML cells were obtained from patients before they began treatment with Abl kinase inhibitors.

Cell Purification by Aldehyde Dehydrogenase (ALDH) Activity—Mononuclear cells were further fractionated according to ALDH activity by staining with Aldefluor reagent (StemCo Biomedical, Durham, NC) according to the manufacturer’s specifications. Aldefluor substrate (0.625 μg/ml) was added to 2–7 × 10⁶ cells/ml suspended in Aldefluor assay buffer and incubated for 20–30 min at 37 °C to allow the conversion of Aldefluor substrate to a fluorescent product that is retained within the cell because of its negative charge. The amount of intracellular fluorescence was measured by flow cytometry, and ALDHhi cells were selected by a fluorescence-activated cell sorter (BD Biosciences).

Real Time PCR and Quantitative RT-PCR to Detect PHLPP1 and PHLPP2 in Leukemia Cells—Total RNA was extracted from leukemia cells using an RNeasy kit. Reverse transcription was performed by using a DNA thermal synthesis kit (Roche Applied Science). PCR was performed with a DNA thermal cycler (model PTC 200; MJ Research, Watertown, MA). Oligonucleotide sequences for each primer were as follows: PHLPP1: sense, 5'-GGCAGCTACTGACGATCC-3'; antisense, 5'-TCTGAGCATCTCTTC-3'; PHLPP2: sense, 5'-GATCTAAGGTTGAACGTAA-3'; antisense, 5'-AGGGCTACCCAGGCTTTCA-3'; Bcr-Abl: sense, 5'-GCATAAAAAAGCAGAGTTCAGAAGCCCTAG-3'; antisense, 5'-CACCAGTATTTGGAGGAGG-3'; and G3PDH: sense, 5'-GAACGGGAAGCTCACTGGCAT-3'; antisense, 5'-GAGCTTCACCTGCTGACAT-3'. PCR products were electrophoresed in a 1.5% agarose gel containing 50 μg/l ethidium bromide and visualized with UV light. In each experiment, RT-PCR was performed in duplicate. We used the PHLPP1α splice variant. The quantitative real time PCR was performed by using SYBR Green dye on an ABI PRISM 7700 Sequence detector (PerkinElmer Life Sciences/Applied Biosystems, Foster City, CA). For real time PCR using SYBR Green, a dissociation curve was obtained for melting curve analysis to confirm PCR product specificity.

RNA Interference—The vectors for RNA interference specific to human PHLPP and Bcr-Abl were constructed based on the pGEME PUR hU6 vector (IGENE Therapeutics, Tsukuba, Japan) according to the manufacturer’s instructions. We used the following targeting sequences and oligonucleotides: PHLPP1, 5'-GATCTAAGGTTGAACGTA-3'; PHLPP2, 5'-GAAAAGCCACGCTGGATA-3'; Bcr-Abl sense, 5'-CACCAGTATTTGGAGGAGG-3'; and Bcr-Abl antisense, 5'-GCATAAAAAAGCAGAGTTCAGAAGCCCTAC-3'. The green fluorescent protein control oligonucleotide sequence was
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CAAGCUGAACCUGAAGUUCdTdT. The vector was transfected into cells by using the Lipofectamine 2000 kit (Invitrogen) according to the manufacturer's instructions. After 12 h, the same transfection procedure was repeated, and cells were harvested 48 and 72 h after the initial transfection. Transfection efficiency was consistently 50–60% as determined by the RT-PCR measurement of PHLPP1, PHLPP2, and Bcr-Abl mRNA.

Lentivirus Construction and Production—The full-length b324 Bcr-Abl cDNA (a kind gift from Dr. J. Y. Wang, University of California, San Diego, CA) was cloned upstream from the internal ribosomal entry site of replication-deficient, self-inactivating lentiviral vectors, pRRLsin-IRES-EGFP. The Bcr-Abl-containing vector was termed LV-Bcr-Abl, and the control vector was termed LV-Con. All vector particles pseudotyped with the vesicular stomatitis virus G glycoprotein were produced by using a three-plasmid expression system. Briefly the human 293T cells were transfected with two plasmids, one that encoded the defective packaging construct, pCMVΔ8.91- and a human immunodeficiency virus, type 1-based vector construct and 10 μg of pMD.G plasmids were co-transfected into subconfluent 293T cells by using the calcium phosphate precipitation method. 293T cells were seeded into 10-cm-diameter plates (Corning, Inc., Corning, NY) 24–48 h prior to transfection in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (Omega Scientific, Inc., Irvine, CA), 100 μg/ml streptomycin (Invitrogen), and 100 units/ml penicillin (Invitrogen). The medium was changed after 24 h with modified Eagle’s medium (Invitrogen). Cells were transferred to a 10-cm-diameter plate. After serum starvation of cells for 24 h, transfection medium was harvested and filtered through a 0.45-μm filter (Millipore, Bedford, MA) containing two virus particles, LV-Bcr-Abl and LV-Con, was concentrated by ultracentrifugation at 28,000 rpm for 2 h at 10°C. The virus vector titer of the self-inactivating human immunodeficiency virus, type 1-derived lentivirus vector was calculated by the formula ([P] = 4.2 × 10⁵ and 4.6 × 10⁶ gene-transducing units/ml, respectively.

Immunoprecipitation and Western Blot Analysis—For immunoblotting, cells were incubated with STI571 (10 μM), AMN107 (10 μM), or BMS354825 (10 nM) at 37°C for 4 h and then harvested. Protein concentrations were determined by the bicinchoninic acid protein assay (Pierce). Samples containing 50 μg of protein were added to SDS-PAGE loading buffer with 5% 2-mercaptoethanol, heated to 100°C for 2 min, and loaded onto 10% polyacrylamide gels. Proteins were then transferred to polyvinylidene difluoride membranes (Millipore). The membranes were blocked with 0.5% milk in phosphate-buffered saline for 1 h at room temperature. After being washed in Tris-buffered saline with Tween, the membranes were incubated for 1 h at room temperature with an appropriate dilution of rabbit anti-PHLPP antibody (PHLPP1) and rabbit anti-PHLPP-like antibody (PHLPP2) (Bethyl Laboratories, Montgomery, TX). To assure equal protein loading, similar experiments were performed with a mouse monoclonal anti-actin antibody (C-4; ICN, Aurora, OH) as an internal control. After being washed in Tris-buffered saline with Tween, the blots were incubated with horseradish peroxidase-conjugated goat anti-mouse IgG or anti-rabbit IgG (Amersham Biosciences) for 1 h at room temperature. After being washed in Tris-buffered saline with Tween, the blots were incubated with horseradish peroxidase-conjugated goat anti-mouse IgG or anti-rabbit IgG (Amersham Biosciences) for 1 h and exposed to x-ray film at room temperature. The signal was detected by chemiluminescence and the detection kit (Amersham Biosciences). All blots were then stripped and reblotted with PHLPP1 and PHLPP2 siRNA. Depletion of PHLPP1 and -2 by Bcr-Abl continues Phospho-Akt phosphorylations, which was 4.2 × 10⁵ and 4.6 × 10⁶ gene-transducing units/ml, respectively.
buffered saline three times, cells were resolved by 0.2% Triton X-100 for 15 min at room temperature and then permeabilized in 0.5% Triton X-100 for 1 h at room temperature. The cells were incubated with diluted anti-c-Abl rabbit polyclonal antibody (Santa Cruz Biotechnology) for 1 h at room temperature and then washed and incubated with phycoerythrin-conjugated anti-rabbit immunoglobulin G (Santa Cruz Biotechnology) for 1 h in the dark. The cells were viewed by phase-contrast and fluorescence microscopy (IMT-2; Olympus, Tokyo, Japan).

Colony Forming Cell Assay—Human clonogenic progenitor assays were performed by plating purified populations of cells at concentrations ranging from $2 \times 10^2$ to $2 \times 10^3$ (ALDHhi) into methylcellulose medium (MethoCult H4435; Stem Cell Technologies). Colonies were evaluated for morphologic characteristics and enumerated under light microscopy (Zeiss, Muenchen, Germany) following incubation at 37 °C in 5% CO2 for 14–17 days.

Isolation of Progenitor Cells and Quantitative RT-PCR in Progenitor Cells—After the colony forming assays, each colony (CFU-GEMM, CFU-GM, and BFU-E) was harvested by glass syringe, pooled, and washed. An RNeasy system was used to extract total RNA from 7 x 10^4 cells from each colony.

FIGURE 1. PHLPP1 and PHLPP2 mRNA expression in leukemia cells. A, CML cell lines (K562, Meg01, and SHG3) and AML cell lines (U937, HL60, and YRK2) were untreated or treated with STI571 (10^6 M), ANM107 (10^6 M), BMS354825 (10 nM), or control siRNA or Bcr-Abl siRNA. CML cells were harvested 5 days after transfection with control siRNA or Bcr-Abl siRNA. U937 cells were harvested after 24-h treatment with STI571 (10^6 M) and ANM107 (10^6 M), or treatment with BMS354825 (10 nM). Bcr-Abl mRNA expression were normalized to the relative ratio of the expression of 36B4 mRNA. The results were expressed relative to untreated control. The graphs show the mean of S.D. of three independent experiments. *p < 0.01 compared with untreated control. FITC, fluorescein isothiocyanate.
Statistical Analysis—Data are representative of at least three experiments with essentially similar results. These results are expressed as the means ± S.D. from three independent experiments. The means were compared by using the Student’s t test. p values less than 0.05 were considered statistically significant.

RESULTS

The Abl Kinase Inhibitors Induce PHLPP1 and PHLPP2 mRNA Expression in CML Cell Lines—PHLPP1 and PHLPP2 mRNAs were constitutively expressed in CML cell lines (K562, Meg01, and SHG3 cells) and AML cell lines (U937, HL60, and YRK2 cells) (Fig. 1A). In CML cell lines, the expression of these mRNAs was inhibited as compared with AML cell lines. Interestingly we found that the mRNA expression of PHLPP1 and PHLPP2 increased in the three CML cell lines treated with Abl kinase inhibitors (STI571, AMN107, or BMS354825) for 24 h as compared with untreated cells. However, the Abl kinase inhibitors did not affect the PHLIPP1 and PHLPP2 mRNA expression in the AML cell lines, which did not express Bcr-Abl mRNA. Moreover in the CML cell lines transfected with Bcr-Abl siRNA, the PHLPP1 and PHLPP2 mRNA expression was significantly increased as compared with control cells. In the AML cell lines transfected with LV-Bcr-Abl, the PHLPP1 and

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PHLPP2 mRNA expression was reduced as compared with control cells (Fig. 1B). Thus, we found that Bcr-Abl expression regulates the PHLPP1 and PHLPP2 mRNA expression.

Next we examined the PHLPP1 and PHLPP2 mRNA expression in clinical specimens from CML patients. We isolated the ALDH<sup>hi</sup> cells from the bone marrow of CML patients (n = 4) by using the gated region E (2.3 ± 0.4%) (Fig. 1C, left upper panel). In purified ALDH<sup>hi</sup> cells that were treated with Abl kinase inhibitors for 24 h, the expression of PHLPP1 and PHLPP2 mRNA was strongly induced, and the expression of Bcr-Abl mRNA was reduced. Representative data from one of the CML samples is shown in Fig. 1C (left bottom panels). The effects of the Abl kinase inhibitors on PHLPP1 and PHLPP2 mRNA expression were assessed by RT-PCR in ALDH<sup>hi</sup> cells from CML patients (n = 4) (Fig. 1C, right panels). In all clinical specimens, the expression of PHLPP1 and PHLPP2 mRNA was noticeably induced by the Abl kinase inhibitors compared with control cells. There were no significant differences in the induction of PHLPP1 and PHLPP2 mRNA among the Abl kinase inhibitors.

### Regulation of PHLPP1, PHLPP2, and Phosphorylated Akt Expression in CML Cells

As shown in Fig. 2A, treatment with Abl kinase inhibitors increased the protein levels of PHLPP1 and PHLPP2 in K562 and Meg01 cells. Moreover, the protein levels of both were increased in K562 and Meg01 cells transfected with Bcr-Abl siRNA. Thus, Abl kinase inhibitors or the knockdown of Bcr-Abl protein induced PHLPP1 and PHLPP2 expression. When treated with the Abl kinase inhibitors or transfected with Bcr-Abl siRNA, the relative amounts of Bcr-Abl mRNA were significantly reduced in K562 (Fig. 2A, left bottom panel) and Meg01 (Fig. 2A, right bottom panel) cells. Next we examined whether the Abl kinase inhibitors induced the dephosphorylation...
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Effects of PHLPP1 or PHLPP2 Knockdown on CML Cell Proliferation—PHLPP1 and PHLPP2 were induced by the Abl kinase inhibitors or the knockdown of Bcr-Abl in CML cells. To examine the functional importance of PHLPP1 and PHLPP2 expression, we transfected K562 cells with PHLPP1 and/or PHLPP2 siRNA and assessed the effects of PHLPP knockdown on proliferation by MTT assays (Fig. 3). When K562 cells were transfected with PHLPP1 or PHLPP2 siRNA, the rate of cell proliferation was slightly increased compared with untreated cells (data not shown). Whereas Abl kinase inhibitors (STI571, AMN107, or BMS354825) significantly reduced the rate of proliferation in untransfected K562 cells, these inhibitors moderately reduced the rate of proliferation in PHLPP1- and/or PHLPP2 siRNA-transfected K562 cells. Knockdown of both PHLPP1 and PHLPP2 reduced the rate of K562 cell proliferation more than that of either alone in effects of the Abl kinase inhibitors (Fig. 3, B and C). These results show that PHLPP1 and -2 play an important role in the inhibition of cell proliferation in CML cell lines by Abl kinase inhibition.

Colony Forming Activity in CML Patients—Hematopoietic progenitor cells from the bone marrow of CML patients represented 2.34 ± 0.38 of ALDHhi cells, which included CD34+ cells, and 95% of CD34- cells were selected according to the side scatter and fluorescein isothiocyanate properties. The ALDHhi cells in CML patients represented 2.34 ± 0.38 of ALDHhi cells, which included CD34+ hematopoietic progenitor cells from the bone marrow. Immunofluorescent staining in K562 cells revealed that the Bcr-Abl protein was constitutively present in the cytoplasm, and Bcr-Abl siRNA transfection reduced the expression of Bcr-Abl protein compared with control siRNA (Fig. 4A, right upper panels). We examined the effect of Abl kinase inhibitors or knockdown of Bcr-Abl on the colony formation of ALDHhi hematopoietic progenitor cells from pretreatment CML patients (Fig. 4A, bottom panel). The numbers of CFU-GEMM, CFU-GM, and BFU-E were remarkably reduced when the cells were cultured with STI571, AMN107, or BMS354825 and transfected with Bcr-Abl siRNA. The difference between the effects of Abl kinase inhibitors and the knockdown of Bcr-Abl was not significant in progenitor cells. Moreover in each progenitor cell treated with the Abl kinase inhibitors or transfected with Bcr-Abl siRNA, the expression of both PHLPP1 and PHLPP2 mRNAs was increased compared with untreated cells. These results demonstrate that in the process of proliferation of progenitor cells derived from CML patients the inhibition of Bcr-Abl expression induced the PHLPP1 and PHLPP2 expression (Fig. 4B).

PHLPP1 and PHLPP2 Inhibited the Colony Formation in CML Progenitor Cells—To assess the function of PHLPP expression on colony formation of the ALDHhi progenitor cells from CML patients, we investigated whether the reduction of PHLPP1 and PHLPP2 expression increased the activity of colony formation in CFU-GEMM, BFU-E, and CFU-GM. The ALDHhi cells were transfected with PHLPP1 siRNA and/or PHLPP2 siRNA. As shown in Fig. 5, the depletion of PHLPP1 or PHLPP2 expression significantly increased the rate of colony formation of hematopoietic progenitor cells derived from CML patients the inhibition of Bcr-Abl expression induced the PHLPP1 and PHLPP2 expression (Fig. 4B).

Antiproliferative effects of PHLPP1 and PHLPP2 in K562 cells. A, B, and C, K562 cells were transfected with PHLPP1 siRNA (∆) and/or PHLPP2 (∆) siRNA. After 48 h, the cells were treated with STI571 (10 μM) (A, upper panel), AMN107 (10 μM) (B, upper panel), and BMS354825 (10 nM) (C, upper panel) for 72 h. Viability was determined by the MTT assays. The rate of cell survival is expressed as the percentage of the corresponding control. Results are presented as the means ± S.D. from three independent experiments. The PHLPP1 and/or PHLPP2 siRNA-transfected or untreated K562 cells were collected at the indicated times after the Abl kinase inhibitors (A, STI571; B, AMN107; C, BMS354825). For the analysis of PHLPP1 (lower left) and PHLPP2 (lower right) expression, quantitative RT-PCR was performed relative to the G3PDH gene. Each bar represents the mean of S.D. of three independent experiments.

FIGURE 3. Antiproliferative effects of PHLPP1 and PHLPP2 in K562 cells. A, B, and C, K562 cells were transfected with PHLPP1 siRNA (∆) and/or PHLPP2 (∆) siRNA. After 48 h, the cells were treated with STI571 (10 μM) (A, upper panel), AMN107 (10 μM) (B, upper panel), and BMS354825 (10 nM) (C, upper panel) for 72 h. Viability was determined by the MTT assays. The rate of cell survival is expressed as the percentage of the corresponding control. Results are presented as the means ± S.D. from three independent experiments. The PHLPP1 and/or PHLPP2 siRNA-transfected or untreated K562 cells were collected at the indicated times after the Abl kinase inhibitors (A, STI571; B, AMN107; C, BMS354825). For the analysis of PHLPP1 (lower left) and PHLPP2 (lower right) expression, quantitative RT-PCR was performed relative to the G3PDH gene. Each bar represents the mean of S.D. of three independent experiments.
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are activated by phosphoinositide-dependent kinase 1 phosphorylation of their activation loop (Thr-308) and phosphoinositide-dependent kinase 2 phosphorylation of their hydrophobic motif (Ser-473). Akt1 is ubiquitously expressed; controls organism size, adipogenesis, and skeletal muscle differentiation; and inhibits cell motility.

**FIGURE 4.** PHLPP1 and PHLPP2 expression were induced by Abl kinase inhibitor or knockdown of Bcr-Abl in CML progenitor cells. A, the purified ALDHhi cells from one CML patient were isolated and cultured in semisolid methylcellulose medium (MethoCult H4435) as described under “Experimental Procedures,” and viewed under a confocal microscope (upper left). The ALDHhi cells, which were untransfected or transfected with Bcr-Abl siRNA, were treated with Abl kinase inhibitors for 14–17 days. The numbers of CFU-GEMM, CFU-GM, and BFU-E were counted. The rate of the progenitor cells was evaluated as the percentage of the corresponding control (bottom panel). Each bar represents the mean of S.D. of three independent experiments.

B, the relative expression levels of PHLPP1 and PHLPP2 mRNA of CFU-GEMM, CFU-GM, and BFU-E derived from Bcr-Abl siRNA-transfected ALDHhi cells were assessed after 14–17 days of treatment with the Abl kinase inhibitors. For the analysis of PHLPP1 and PHLPP2 expression, quantitative RT-PCR was performed relative to the G3PDH gene. Each bar represents the mean of S.D. of three independent experiments. Fourteen days after transfection with Bcr-Abl siRNA, the mRNA expression of Bcr-Abl was assessed by RT-PCR in CFU-GEMM, CFU-GM, and BFU-E (bottom panels). RT-PCR results are representative of three independent experiments.

**Depletion of PHLPP1 and -2 by Bcr-Abl Continues Phospho-Akt**

**FIGURE 5.** Reduction of PHLPP1 and PHLPP2 expression on ALDHhi hematopoietic progenitor cells derived from CML patients. A, ALDHhi cells were transfected with PHLPP1 siRNA and/or PHLPP2 siRNA and then treated with STI571 (10 μM) for 14–17 days. The numbers of CFU-GEMM, CFU-GM, and BFU-E were counted. The rate of the progenitor cells was evaluated as the percentage of the corresponding control. Each bar represents the mean of S.D. of three independent experiments. B, the relative expression levels of PHLPP1 and PHLPP2 mRNA of CFU-GEMM, CFU-GM, and BFU-E derived from PHLPP1 and/or PHLPP2 siRNA-transfected ALDHhi cells were assessed after 14–17 days of treatment with STI571 (10 μM). For the analysis of PHLPP1 and PHLPP2 expression, quantitative RT-PCR was performed relative to the G3PDH gene. Each bar represents the mean of S.D. of three independent experiments. Fourteen days after transfection with PHLPP1 and/or PHLPP2 siRNA, the cells from CFU-GEMM, CFU-GM, and BFU-E were harvested. To detect PHLPP1 and PHLPP2, RT-PCR was performed. G3PDH is shown as an internal control. The RT-PCR results are representative of three independent experiments.

FITC, fluorescein isothiocyanate.
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We investigated the effects of depletion of PHLPP1 and PHLPP2 in CML cells. In both K562 and Meg01 cells, the cell proliferation was remarkably inhibited by treatment with STI571, AMN107, and BMS354825, whereas cell proliferation was moderately inhibited when these cells transfected with PHLPP1 and/or PHLPP2 siRNA were treated with STI571, AMN107, and BMS354825. These results reveal that PHLPP1 and PHLPP2 have an important role in the inhibition of CML cell proliferation by the Abl kinase inhibitors.

The CFU-GEMM, CFU-GM, and BFU-E derived from normal progenitor cells were moderately reduced when they were treated with STI571, AMN107, or BNM354825 (data not shown). In contrast, the CFU-GEMM, CFU-GM, and BFU-E derived from CML progenitor cells were significantly reduced when they were treated with STI571, AMN107, and BMS354825 or transfected with Bcr-Abl siRNA. Moreover the relative expression of PHLPP1 and PHLPP2 mRNA increased in CFU-GEMM, CFU-GM, and BFU-E treated with the Abl kinase inhibitors or transfected with Bcr-Abl siRNA, respectively. In CFU-GEMM, CFU-GM, and BFU-E transfected with PHLPP1 and PHLPP2 siRNA, the inhibition effects of CFU by the Abl kinase inhibitors were weakened. These results indicate that PHLPP1 and PHLPP2 synergize with Abl kinase inhibitors to greatly inhibit the growth of CML progenitor cells. These findings demonstrate that the expression of PHLPP1 and PHLPP2 is increased by phosphorylated Bcr-Abl in CML cells and that these increased expression of PHLPP1 and PHLPP2 inhibit CML cell growth in vitro and in vivo.

The PI3K pathway is deregulated in many human cancers and is considered an attractive target for the development of novel chemotherapeutic agents. PI3K contributes to transformation by promoting cell cycle progression and it synergizes with Abl kinase inhibitors to reduce the apoptosis of cells from chronic myeloid leukemia (CML) patients (22). The PI3K signaling pathway has an important role in cell growth, survival, and degradation by activated Abl kinase.

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Depletion of PHLPP1 and -2 by Bcr-Abl Continues Phospho-Akt

Evolutionary trace-based peptides identify a novel asymmetric interaction that mediates oligomerization in nuclear receptors.

Peili Gu, Daniel H. Morgan, Minawar Sattar, Xueping Xu, Ryan Wagner, Michele Raviscioni, Olivier Lichtarge, and Austin J. Cooney

Upon subsequent review of the source data for the figures in this article, it was noticed that portions of the figures assembled by the lead author were not faithful representations of their original source data. The authors are therefore submitting this erratum. The amended Figs. 1–7, which are correct representations of the original data, are available online only. The alterations do not change the conclusions drawn from this study, and the authors express their deep regret to the scientific community.

Depletion of pleckstrin homology domain leucine-rich repeat protein phosphatases 1 and 2 by Bcr-Abl promotes chronic myelogenous leukemia cell proliferation through continuous phosphorylation of Akt isoforms.

Isao Hirano, Satoki Nakamura, Daisuke Yokota, Takaaki Ono, Kazuyuki Shigeno, Shinya Fujisawa, Kaori Shinjo, and Kazunori Ohnishi

This article has been retracted by the Publisher.
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doi: 10.1074/jbc.M808182200 originally published online March 4, 2009

Access the most updated version of this article at doi: 10.1074/jbc.M808182200

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