

Role of the tyrosine phosphatase SHP-1 in K562 cell differentiation

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The erythro-megakaryoblastic leukemia cell line K562 undergoes erythroid or myeloid differentiation in response to treatment with various inducing agents. We observed that expression of the SH2-containing protein tyrosine phosphatase SHP-1 was induced upon exposure of K562 cells to differentiating agents. Under the same conditions, expression of SHP-2, a close relative of SHP-1, and the more distantly related PTP-1B remained unchanged. Induction of SHP-1 expression correlates with dephosphorylation of a specific and limited set of tyrosyl phosphoproteins, suggesting that dephosphorylation of these proteins may be important for the differentiation process. Importantly, expression of exogenous SHP-1 inhibits K562 proliferation and alters the adhesion properties of these cells, indicating a more differentiated phenotype. Moreover, SHP-1 is found in a complex with both p210 Bcr-Abl and p190 Bcr-Abl, suggesting that it may regulate Bcr-Abl or Bcr-Abl-associated phosphotyrosyl proteins. Our results indicate that induction of SHP-1 expression is important for K562 differentiation in response to various inducers and raise the possibility that functional inactivation of SHP-1 may play a role in progression to blast crisis in chronic myelogenous leukemia. *Leukemia* (2001) 15, 1424–1432.

Keywords: tyrosine phosphatase; differentiation; leukemia; erythroid lineage; myeloid lineage

Introduction

The phosphorylation of proteins on tyrosyl residues is a critical mechanism for regulating multiple signal transduction pathways controlling cell growth, differentiation and development. The level of tyrosyl phosphorylation, and thus the strength and duration of signals transmitted, is governed by the coordinated actions of protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPs).^{1–3} Not surprisingly, abnormal regulation of PTKs can result in disease. For example, translocations involving several different PTK genes cause hematopoietic disorders such as myeloproliferative disease and leukemia.⁴ In such disorders, cellular PTPs cannot prevent the increased tyrosyl phosphorylation that results from the dysregulated PTK gene. Little is known about which specific PTPs antagonize the actions of abnormal PTKs, or about how these PTKs overcome negative regulation by such PTPs. Defining the specific PTPs that antagonize the actions of transforming PTKs should provide insight into pathogenesis and may suggest novel targets for therapeutic intervention.

The prototypical transforming PTK in hematopoietic cells is the Bcr-Abl fusion protein, which causes human chronic myelogenous leukemia (CML) and, less commonly, acute leukemias. Bcr-Abl is produced as a consequence of the Philadelphia chromosome translocation t(9;22) (q34;q11), which jux-

taposes the gene for *bcr*, a protein of unknown function that has multiple potential activities including rhoGAP and serine/threonine kinase domains,^{5–7} with the *c-abl* gene, which encodes a non-transmembrane PTK normally localized to the nucleus and focal adhesions.^{8,9} In the resultant fusion protein, the N-terminal region of *c-Abl* is replaced by *bcr*-encoded sequences. Depending on the breakpoint in the *bcr* gene, the fusion protein can include variable numbers of *bcr*-derived exons; consequently, Bcr-Abl fusion proteins of different molecular size (190 kDa, 210 kDa, or 230 kDa) can result. p210 Bcr-Abl is mostly associated with CML, whereas p190 Bcr-Abl is commonly found in acute leukemias.^{10–14} While the reason for this correlation had remained unclear for a long time, recently in an elegant study using a murine transduction/transplantation model, it has been clearly demonstrated that the two isoforms differ in their intrinsic potential to cause specific types of leukemias.¹⁵

How Bcr-Abl causes leukemia remains incompletely understood (reviewed in Ref. 16). All Bcr-Abl fusion proteins exhibit enhanced kinase activity compared to the normal *c-abl* gene, with p190 Bcr-Abl having the highest.¹⁵ Enhancement of Abl PTK activity is essential for transformation by Bcr-Abl *ex vivo* (ie in cell culture), and is believed to be important for pathogenesis *in vivo*, although this has not been shown rigorously. PTK activity is elevated further during progression of CML to blast crisis, by a mechanism(s) that remains unclear. In addition to increasing Abl kinase activity, fusion of Bcr sequences to Abl results in altered localization. Whereas *c-Abl* is predominantly nuclear, Bcr-Abl proteins are cytoplasmic, with a large fraction associated with the cytoskeleton.¹⁷ Several domains within Bcr-Abl (eg Y177 and the SH2 domain) are important for its ability to transform various cultured cell lines.^{4,18–20} The relevance of these observations for CML pathogenesis is not known, however, it is thought that Bcr-Abl fusion proteins cause disease by both activating and relocating the *c-Abl* PTK.

Specific PTPs that antagonize Bcr-Abl action are largely unknown. Recent work suggests that PTP-1B may directly dephosphorylate Bcr-Abl at Y177 and possibly other sites.²¹ PTP-1B expression is elevated upon transformation of hematopoietic and fibroblast cells with Bcr-Abl, consistent with a homeostatic role for PTP-1B in controlling Bcr-Abl phosphorylation. SHP-2 has been reported to form a complex with Bcr-Abl,²² although the stoichiometry of this complex is quite low and its functional significance remains unclear.²³ Two recent studies suggested that SHP-1 may also be present in a complex with Bcr-Abl.^{24,25} SHP-1, a non-transmembrane PTP containing two N-terminal Src homology 2 (SH2) domains, is predominantly expressed in hematopoietic cells, where it is implicated in the negative regulation of signaling pathways mediated by growth factor, cytokine and antigen receptors.^{3,26}

While screening a large number of hematopoietic cell lines for SHP-1 expression, we found that K562 cells fail to express SHP-1 protein. K562 cells are derived from a patient with erythro-megakaryoblastic leukemia arising during Ph⁺ CML

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blast crisis and express p210 Bcr-Abl.²⁷ These cells are multipotent and can differentiate into myeloid or erythroid cells upon exposure to appropriate differentiating agents. The absence of SHP-1, together with the known role of SHP-1 as a negative regulator of multiple hematopoietic signaling pathways, led us to ask whether there might be a functional connection between Bcr-Abl and SHP-1, such that lack of SHP-1 protein might enhance the effect of Bcr-Abl, thereby contributing to the differentiation block found in this cell line. Indeed, we found that upon exposure of K562 cells to various differentiation-inducing agents, SHP-1 expression is induced, whereas expression of other PTPs, such as SHP-2 and PTP1B, remains unchanged. Moreover, forced expression of SHP-1 causes a decrease in proliferation and morphological changes of K562 cells indicating a more differentiated phenotype. Our results raise the possibility that interference with SHP-1 function may play a role in the pathogenesis of CML blast crisis.

Materials and methods

Chemicals

DMSO, sodium butyrate, 12-o-tetradecanoylphorbol (TPA) and Benzidine were obtained from Sigma (St Louis, MO, USA).

Cell culture and induction of differentiation

K562, HL60 and HEL cells were obtained from the American Type Culture Collection (Manassas, VA, USA). BaF3 and 32D cell lines stably transformed by p210 Bcr-Abl or p190 Bcr-Abl have been described previously.⁵ All cells were passaged routinely in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 10 units/ml penicillin, 10 µg/ml streptomycin. Cell counts were performed in duplicate every 24 h using a Coulter Beckman counter (Coulter Electronics, Fullerton, CA, USA). To induce K562 differentiation, cells at a density of 10⁵ cells/ml were treated with DMSO (1.5%), sodium butyrate (1.5 mM) or TPA (5 nM), as indicated.

Antibodies

Affinity-purified antibodies directed against full length SHP-1 (FL antibodies), which predominantly recognize N-terminal determinants, or against the SHP-1 C-terminus (CTM antibodies), have been described previously.^{28,29} The following antibodies were purchased: polyclonal antibodies against a C-terminal peptide in SHP-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), monoclonal anti-SHP-1 antibodies used for immunoblotting (Transduction Laboratories, Lexington, KY, USA), monoclonal anti-phosphotyrosine (anti-pTyr) antibodies (4G10) (Upstate Biotechnology, Lake Placid, NY, USA), monoclonal anti-Abl antibodies and monoclonal anti-PTP1B antibodies (Oncogene Research/Calbiochem, San Diego, CA, USA) and secondary horseradish peroxidase-conjugated antibodies (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

Immunoprecipitation and immunoblotting

Immunoprecipitations were performed as previously described.²⁸ Briefly, cells (10⁷) were lysed in 600 µl RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% deoxycholate (DOC), 0.1% sodium dodecyl sulfate (SDS), and 50 mM Tris, pH 7.4) containing phosphatase and protease inhibitors. Protein concentrations of clarified cell lysates were measured using the bicinchoninic acid assay (Pierce, Rockford, IL, USA) and cell lysates were equalized accordingly. Immunoprecipitations were carried out by adding the indicated antibodies and 40 µl of protein A-Sepharose beads for 2–3 h at 4°C. Upon washing of the immune complexes four times with RIPA buffer, they were resolved by 6 or 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred on to Immobilon membranes (Millipore, Bedford, MA, USA) for immunoblotting.²⁸ Before reprobing of the immunoblots with additional antibodies, blots were stripped by incubation in 100 mM 2-mercaptoethanol/62.5 mM Tris (pH 6.7)/2% SDS for 1 h at 70°C.

For metabolic labeling experiments, cells (1.5 × 10⁷ at 10⁶/ml) were pre-incubated in methionine-free RPMI 1640 medium supplemented with 10% dialyzed FCS for 90 min before the addition of 0.1 mCi/ml of Trans ³⁵S-label (ICN; Costa Mesa, CA, USA) for 2 h. After resolution by SDS-PAGE, radio-labeled immunoprecipitates were analyzed by fluorography using 1M sodium salicylate.

Northern blotting

RNA was isolated by the guanidine hydrochloride method, and Northern blotting was performed as described previously.³⁰ Briefly, total RNA (20 µg) was resolved on 1% agarose gels in the presence of formaldehyde, transferred on to Nitrocellulose (Nitroplus, purchased from MSI, Westborough, MA, USA) and hybridized³¹ with human SHP-1 cDNA which had been labeled with ³²P-dCTP by the random primer technique to a specific radioactivity of 10⁹ c.p.m./µg.³²

Expression constructs and transfection assays

K562 cells (10⁷) were washed twice in serum-free RPMI 1640, incubated with 20 mg of plasmid DNA for 10 min at 4°C, and subjected to electroporation using a Gene Pulser TM (Bio-Rad, Hercules, CA, USA) at 300 V, 960 µF. Thirty-six hours post-electroporation, the cells were collected and plated in 96 wells at a concentration of 10⁴ cells/well. Cell clones were grown under G418 (1 mg/ml) selection and examined for SHP-1 expression by immunoblotting. To direct expression of SHP-1 into K562 cells, the vector pMHneo-SHP-1 was used, which expresses SHP-1 under the control of the SFFV promoter and confers G418 resistance. pMHneo-SHP-1 was generated by subcloning of human SHP-1 cDNA into the XbaI (5') and XhoI (3') sites of pMHneo.³³ As a control the parental expression vector was used. Numbers of clones were counted daily to survey growth patterns of the transfected cells.

Results

Treatment of K562 cells with differentiating agents induces SHP-1 expression

In the course of screening many hematopoietic cell lines for SHP-1 expression, we noticed that one of these lines, K562,

was devoid of SHP-1 expression at both the protein and RNA levels (Figure 1). Southern analysis of genomic DNA digested with several restriction enzymes revealed no differences in SHP-1-specific bands between K562 cells and normal human DNA (data not shown), suggesting that the regulation of SHP-1 expression was altered in K562 cells. K562 is a multipotent erythro-megakaryoblastic leukemia cell line, which consists of a mixed population that can be further differentiated into more mature erythroid or myeloid cells.²⁷ Since SHP-1 is expressed in most hematopoietic cell lines,^{34–36} we suspected that lack of SHP-1 expression might be related to the undifferentiated status of K562 cells. Therefore, we examined SHP-1 expression in K562 cells treated with agents known to induce their differentiation, such as sodium butyrate, DMSO, or TPA. Previous studies established that sodium butyrate promotes K562 differentiation along the erythroid pathway, whereas DMSO and TPA induce myeloid differentiation.³⁷ Treatment with any of the inducers led to enhanced SHP-1 expression, as revealed by immunoblotting (Figure 2a–c). Northern blotting confirmed that SHP-1 RNA levels increased following inducer treatment (Figure 2d). Following treatment with each of the agents, SHP-1 protein was first detected within 24 h and increased further over the time course. Sodium butyrate reproducibly induced the largest increase in SHP-1 expression, followed by DMSO and TPA treatment, respectively. Treatment with differentiation inducers did not evoke generalized increases in PTP expression, as neither SHP-2 (Figure 2a–c) nor PTP1B (Figure 4) expression were affected.

Several lines of evidence confirmed that inducer treatment led to K562 cell differentiation. Butyrate-treated cells exhibited a more differentiated morphology (eg denser nuclei, increased vacuolization and decreased nuclear-cytoplasmic ratio), and produced hemoglobin, as visualized by Benzidine staining³⁸ (data not shown). Likewise, cells treated with TPA became adherent and generated pseudopodia, consistent with the myeloid differentiation-inducing properties of this agent.

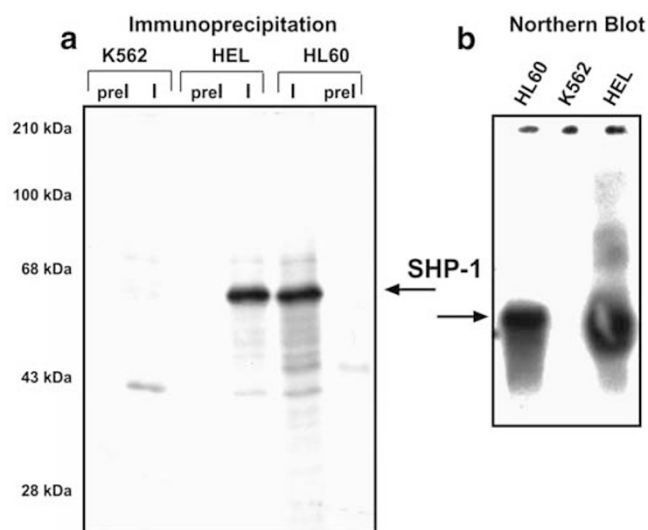


Figure 1 SHP-1 is not expressed in K562 cells. (a) Immunoprecipitations of lysates of ³⁵S-methionine-labeled K562, HEL, and HL60 cells (5×10^6 cells each) with polyclonal anti-SHP-1 antibodies (I) or matched pre-immune IgG (prel). Immunoprecipitates were resolved by 8% SDS-PAGE. (b) Northern blot analysis of total RNA (20 µg) isolated from the indicated cell lines using ³²P-labeled human SHP-1 cDNA as the probe.

As expected, treated K562 cells displayed a decrease in proliferation compared to untreated cells (Figure 3). Flow cytometric analysis confirmed that, as reported previously,³⁹ all three inducers caused a marked increase of cells in the G1 phase of the cycle and a corresponding decrease in S phase cells, consistent with G1/S arrest (data not shown). Interestingly, TPA was the most effective inducer of growth arrest, causing a complete block of proliferation after 3 days, yet it was a relatively inefficient inducer of SHP-1 expression. This

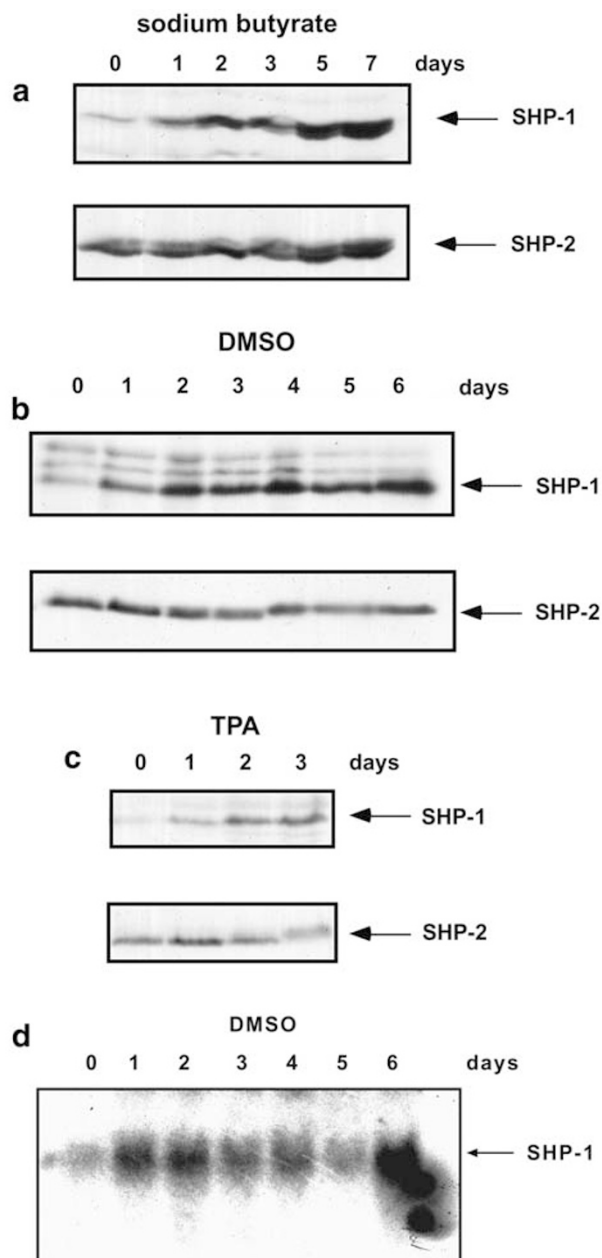


Figure 2 SHP-1 expression is induced upon K562 cell differentiation. Undifferentiated K562 cells were treated with (a) sodium butyrate (1.5 mM), (b) DMSO (1.5%) or (c) TPA (5 nM) for the indicated times and total cell lysates (100 µg) were analyzed by 8% SDS-PAGE and anti-SHP-1 immunoblotting. To control for loading, the same blot was reprobed with anti-SHP-2 antibodies. (d) Northern blot analysis of total RNA (20 µg) isolated from the K562 cells that were treated for the indicated times with DMSO (1.5%). SHP-1 mRNA was detected using ³²P-labeled human SHP-1 cDNA as the probe.

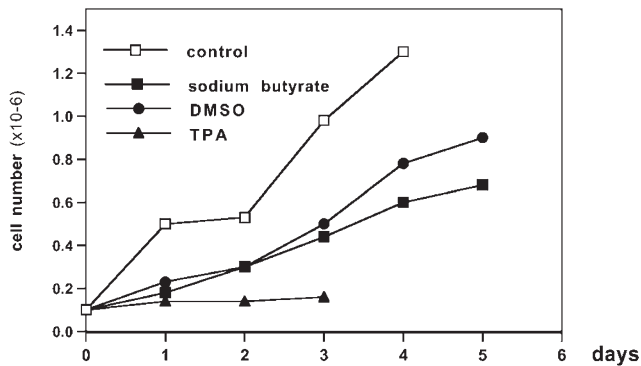


Figure 3 Effect of inducer treatment on K562 cell proliferation. Shown are cell counts of K562 cells treated with the indicated inducers. The result shown is representative of several independent experiments.

result suggests that induction of SHP-1 expression is not a mere consequence of the decreased proliferative rate that accompanies differentiation. Induction of SHP-1 expression correlated in time with the onset of differentiation, raising the possibility of a causal relationship between these events (see below).

To investigate whether the increased SHP-1 expression we observed upon induction of differentiation correlated with a change in tyrosyl phosphorylation of the cells, we analyzed

the total protein phosphotyrosyl levels in undifferentiated and differentiated K562 cells. Notably, treatment with either sodium butyrate (Figure 4a) or DMSO (Figure 4b) did not cause global changes in total cellular tyrosyl phosphoproteins. However, inducer treatment did show marked tyrosyl dephosphorylation of selected proteins (Figure 4a and b, see arrows). These data are consistent with a role for SHP-1 in promoting dephosphorylation of these proteins, although further work will be required to determine whether some or all of these proteins are direct SHP-1 targets.

Expression of exogenous SHP-1 induces differentiated phenotype in K562 cells

Our data indicated a correlation between induction of SHP-1 expression and K562 cell differentiation, suggesting that lack of SHP-1 expression might contribute to the differentiation block in this leukemic cell line. To determine whether forced expression of SHP-1 could direct some or all aspects of K562 cell differentiation, we stably transfected K562 cells with an expression vector directing SHP-1 expression or the expression vector alone.

SHP-1 expressing and mock-transfected control clones exhibited remarkable differences in growth pattern and morphology (Figures 5 and 6, and Table 1). In multiple independent transfection experiments performed with different DNA preparations, we obtained about five-fold fewer clones from SHP-1-transfected compared with vector control trans-

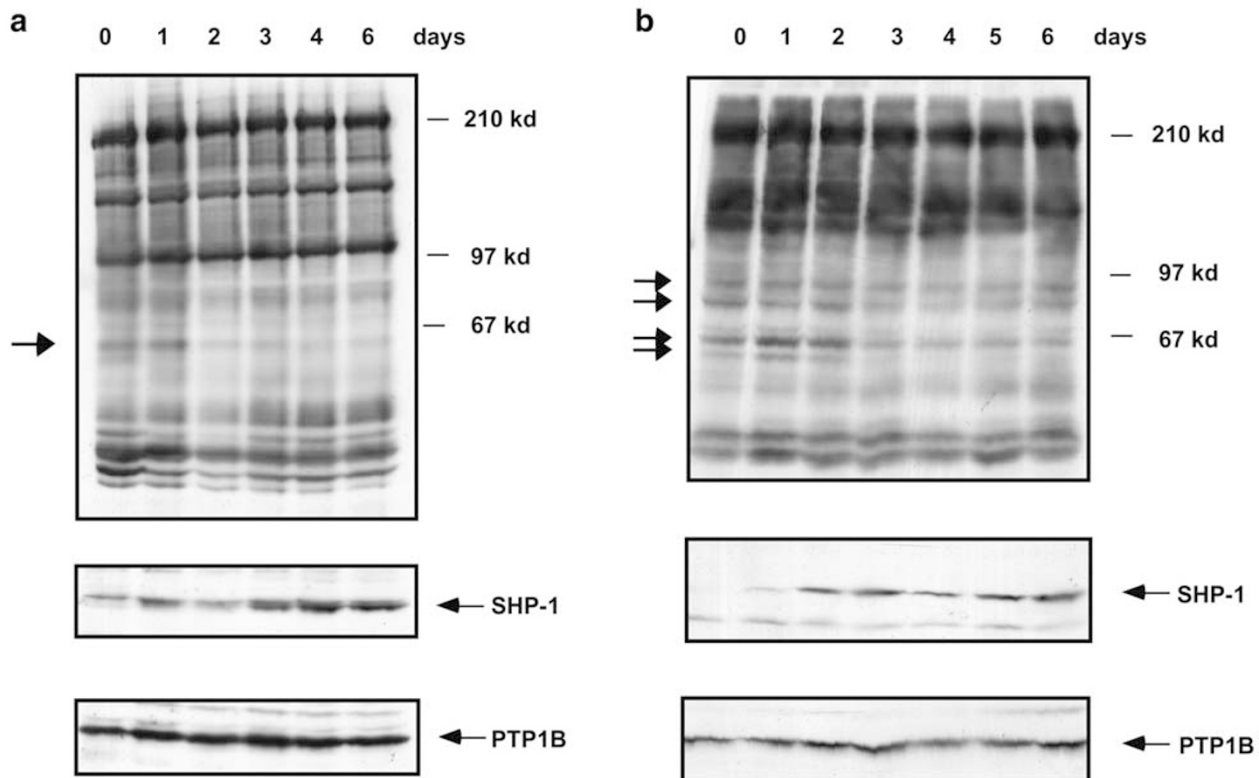


Figure 4 Effect of inducer treatment on K562 cell tyrosyl phosphorylation. K562 cells were treated with sodium butyrate (a) or DMSO (b) for the indicated times, and total cell lysates (50 μ g) were resolved by 8% SDS-PAGE followed by anti-pTyr (top panel), anti-SHP-1 (middle panel), and anti-PTP1B (lower panel) immunoblotting. Specific pTyr proteins that undergo dephosphorylation following inducer treatment are indicated by arrowheads.

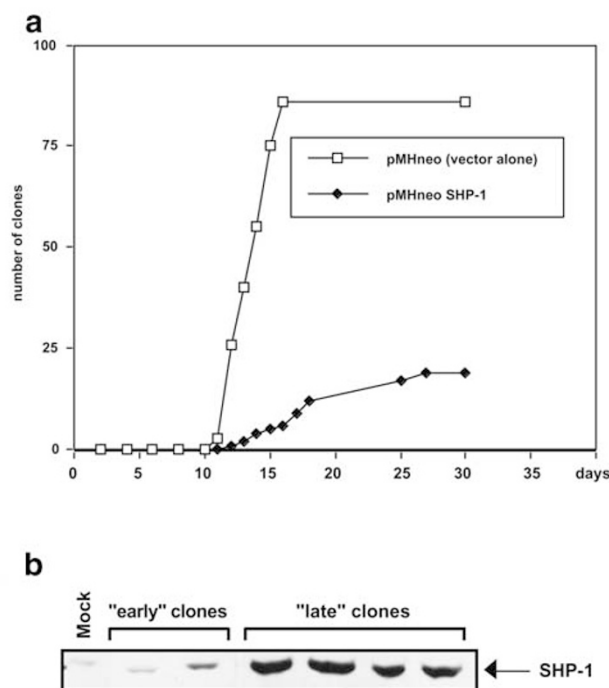


Figure 5 Reconstitution of SHP-1 expression in K562 cells. K562 cells were transfected with pHNeo SHP-1 or pHNeo alone and G418-resistant clones were selected. (a) The graph depicts the number of G418 resistant colonies obtained at various times following electroporation of pHNeo-SHP-1 or pHNeo. Shown is a representative experiment from five independent transfections. (b) Anti-SHP-1 immunoblots from representative clones arising early (within 14 days post-transfection) or late (after 21 days post-transfection) after electroporation. Note that SHP-1 expression level is much higher in the later, more slowly growing clones.

fectured populations. Most SHP-1-transfected clones appeared much later than vector alone-transfected cells, with a delay of about 10–14 days (Figure 5a). Interestingly, 'late' clones exhibited substantially higher levels of SHP-1 expression than 'early' clones (Figure 5b). Consistent with the longer time required for clonal outgrowth, SHP-1-transfected clones initially grew more slowly than either K562 cells transfected with parental expression vector or untransfected K562 cells (Table 1). However, when attempts were made to expand SHP-1-expressing cells to mass culture, they reproducibly exhibited an accelerated growth rate, such that by the time enough cells could be obtained for more detailed biochemical analyses, SHP-1-transfected and control-transfected cells grew at comparable rates. These data are most consistent with a growth inhibitory effect of SHP-1 followed by selection for clones that resist this growth-suppressive action.

SHP-1 expression also resulted in cells that were more adherent to each other and to the tissue culture dish (Figure 6). Again, this increased adherence phenotype was most prominent in 'late' clones (ie those arising after about 3 weeks) (Table 1). Taken together, these results support the hypothesis that expression of SHP-1 in the leukemic K562 cell line not only correlates with, but can cause decreased proliferation and changes in morphology, characteristics associated with a more differentiated phenotype.

SHP-1 is part of a complex containing Bcr-Abl

Since SHP-1 can modulate the biological effects of Bcr-Abl, and since SHP-1's relative SHP-2 is found in a complex with Bcr-Abl, we asked whether SHP-1 also co-immunoprecipitated with Bcr-Abl. Indeed, SHP-1 was found in complex with p210 Bcr-Abl in BaF3 and 32D cells stably transfected with this oncoprotein (Figure 7), as well as in p210 Bcr-Abl expressing Mo-7e cells (data not shown). SHP-1 also forms a complex with p190 Bcr-Abl (Figure 7), and is constitutively tyrosyl phosphorylated in cells expressing either Bcr-Abl fusion protein. Experiments with GST-SH2 domain fusion proteins and recombinant p210 Bcr-Abl produced in SF9 insect cells strongly suggest that at least part of the Bcr-Abl/SHP-1 interaction is mediated through the SH2 domain of SHP-1 (data not shown). However, we cannot exclude contributions of other parts of the SHP-1 protein, nor can we exclude the involvement of other proteins serving as linkers between Bcr-Abl and SHP-1 in mammalian cells (although there is a direct interaction in insect cells, see above). Similar results demonstrating an association between SHP-1 and p210 Bcr-Abl have been reported recently by Tauchi *et al*²⁵ and Liedtke *et al*.²⁴

Discussion

In this study we show that SHP-1 is involved in the regulation of proliferation and differentiation of the K562 leukemic cell line. Although nearly all hematopoietic cell lines express SHP-1, parental K562 cells exhibit no SHP-1 RNA or protein. However, upon differentiation of these multipotent erythro-megakaryoblasts towards either the erythroid or myeloid lineage, SHP-1 expression is dramatically induced. This kinetics of induction coincide with acquisition of the differentiated phenotype, which raised the possibility that absence of SHP-1 expression might play a causal role in the defective differentiation of the K562 cell line. Indeed, forcing expression of SHP-1 in K562 cells results in a reduced proliferation rate and altered morphology, suggesting that SHP-1 expression can drive K562 cells into a more differentiated stage and, by inference, that lack of SHP-1 expression in parental K562 cells may help explain the failure of these cells to differentiate. Consistent with a role for SHP-1 in regulating Bcr-Abl function, these two proteins are found in a complex in multiple Bcr-Abl-expressing cell lines, and induction of SHP-1 expression (and differentiation) correlates with decreased phosphorylation of a limited and specific set of phosphotyrosyl proteins in K562 cells.

The mechanism by which SHP-1 expression is inhibited in K562 cells is unclear. The fact that various differentiation inducers induce high levels of SHP-1 expression, along with the absence of gross rearrangements of the SHP-1 gene, argues that the SHP-1 gene likely is intact. Since both SHP-1 RNA and protein are absent in these cells, presumably, transcriptional or post-transcriptional control is responsible. Interestingly, the down-regulation of another regulator of hematopoiesis, the inositol-5'-phosphatase SHIP, has recently been shown to be directly linked to Bcr-Abl expression.⁴⁰ In contrast, based on our data, Bcr-Abl does not affect expression of SHP-1. The reason why different inducers differentially induce SHP-1 expression remains to be determined, although our data (Figures 2 and 3) clearly show that the ability to induce SHP-1 expression is not correlated with the ability of a given inducer to cause cell cycle arrest/growth inhibition. Notably, other PTPs are not induced upon differentiation of K562 cells;

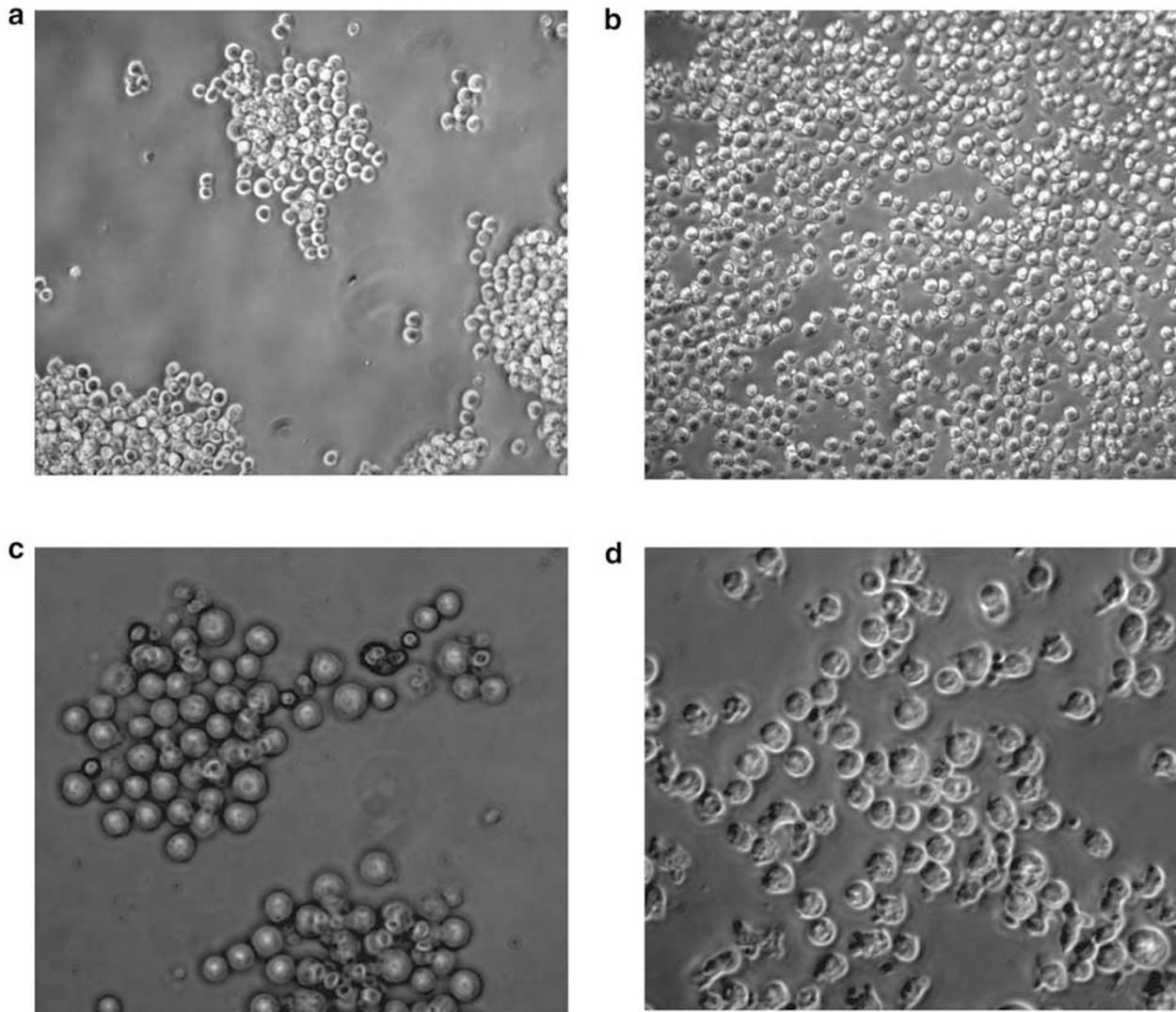


Figure 6 Reconstituting SHP-1 expression alters morphology of K562 cells. The different morphologies of clones expressing SHP-1 (a and c) and non-expressing clones (b and d) are shown (a and b: 10-fold original magnification; c and d: 40-fold original magnification).

Table 1 Reconstitution of SHP-1 in K562 cells

| Cell line | Adherent growth | Growth rate |
|-----------------|-----------------|-------------|
| parental K562 | – | +++ |
| pMHneo 1 | – | +++ |
| pMHneo 2 | – | +++ |
| pMHneo SHP-1 1* | – | ++ |
| pMHneo SHP-1 2* | – | + |
| pMHneo SHP-1 3 | + | + |
| pMHneo SHP-1 4 | +++ | +(+) |
| pMHneo SHP-1 5 | +++ | + |
| pMHneo SHP-1 6 | +++ | +(+) |
| pMHneo SHP-1 7 | +++ | + |

K562 cells were stably transfected with pMHneo SHP-1 or pMHneo. The clones were examined for growth morphology (adherent growth) and growth rate. 'Early' clones (arisen within 14 days post-transfection) are marked (*). The symbol (+++) stands for a doubling time of about 24 h, whereas (++) stands for an about 1.5-fold longer and (+) for an about 2-fold longer doubling time.

eg the level of expression of SHP-2, the close relative of SHP-1, and the more distantly related PTP1B remain unchanged.

Previous studies revealed a correlation between increases in PTP activity and/or levels of specific PTPs and leukemic cell differentiation. TPA-induced differentiation of the HL60 promyelocytic leukemia cell lines leads to an approximately 11-fold increase in PTP activity, which correlates with decreased total cellular tyrosyl phosphorylation.⁴¹ Induction of SHP-1 RNA and protein levels occurs upon TPA treatment of HL60 cells, suggesting that SHP-1 accounts for at least part of this increase.^{42,43} Interestingly in HL60 cells, no⁴² or very limited⁴⁴ SHP-1 induction was observed upon treatment with DMSO, which induces myeloid differentiation. In contrast, in K562 cells, DMSO clearly induces SHP-1 expression (Figure 2b). Taken together, these results suggest that induction of SHP-1 expression in different hematopoietic cell lines is likely to reflect the ability of a given inducer to access a pre-existing differentiation program in that cell line, rather than differences in the intrinsic properties of various inducers.

Our results extend the previous studies of PTPs in leukemic cells by showing that expression of SHP-1 alone evokes sev-

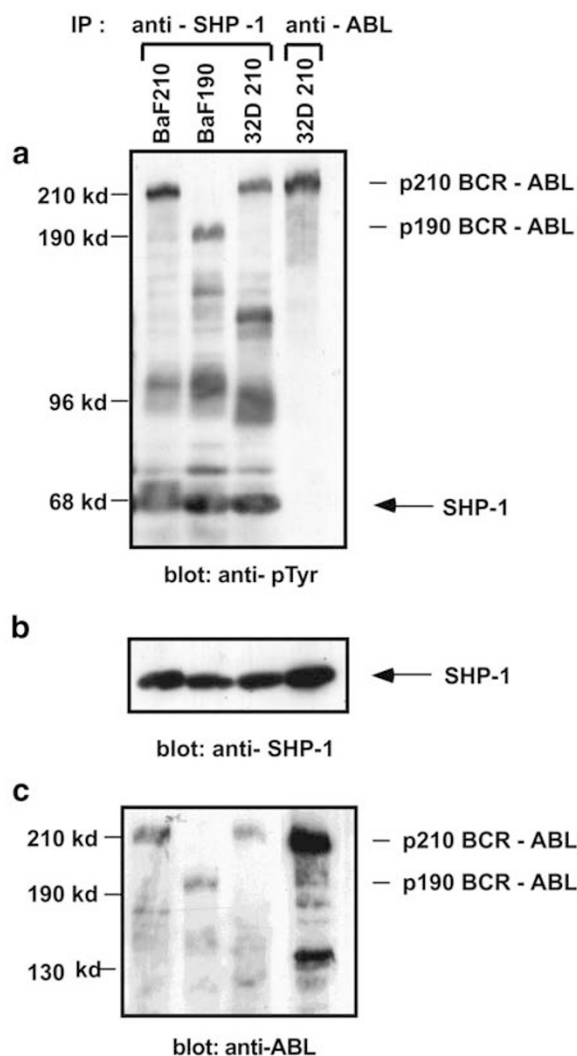


Figure 7 SHP-1 is in a complex with Bcr-Abl. (a) BaF3 and 32D cells, stably transfected with p210 or p190 Bcr-Abl, were lysed and immunoprecipitated with either polyclonal anti-SHP-1 (C-terminal peptide) antibodies or monoclonal anti-Abl antibody. Immunoprecipitates were resolved by 6% SDS-PAGE and subjected by anti-pTyr immunoblotting. The positions of SHP-1 and the respective Bcr-Abl fusion proteins are shown. After stripping, the blot was subjected to anti-SHP-1 (b) and anti-Abl (c) immunoblotting.

eral aspects of the differentiated phenotype (decreased growth rate, increased adherence) in the absence of inducer treatment. Previous workers showed that orthovanadate treatment blocked HL60 differentiation⁴³ arguing that (at least one) PTP activity is important for differentiation in this system. However, since vanadate is a general PTP inhibitor, the previous work could not implicate a specific PTP in the differentiation process. In contrast, our data show that SHP-1 expression is clearly induced during differentiation and moreover, forced expression of SHP-1 promotes a differentiated phenotype in K562 cells. In this regard, our results are analogous to recent studies in which SHP-1 expression was restored to Burkitt's lymphoma cells and increased B lymphocytic differentiation was observed.⁴⁵ However, it is important to note that expressing SHP-1 in K562 cells does not induce all aspects of the differentiated phenotype; for example, although transfected cells exhibit the increased adherence characteristic of more

mature myeloid cells, they do not exhibit the typical morphology of these cells (as is seen following TPA induction). It will be important to determine in future work whether SHP-1 expression lowers the threshold for differentiation in response to myeloid inducers such as TPA. The precise mechanism by which SHP-1 promotes K562 differentiation will require further study.

SHP-1 is present in a complex with Bcr-Abl, suggesting that it could act on Bcr-Abl itself or Bcr-Abl-associated phosphotyrosyl proteins. Indeed, since previous work showed that Bcr-Abl activity decreases upon K562 differentiation,^{39,46} it is tempting to speculate that SHP-1 acts directly on Bcr-Abl to promote differentiation. Unfortunately, we have been unable to directly test this hypothesis, because during propagation of SHP-1-transfected cells to mass culture, the initially slow growth rate of these cells dramatically increases, indicating that secondary events occur which overcome the effects of SHP-1 in these lines. Thus, although we detect no overall change in Bcr-Abl phosphorylation in these cultures, we cannot be confident that SHP-1 did not have strong effects on Bcr-Abl, which were lost during expansion of these clones. Studies in which SHP-1 is expressed under the control of an inducible promoter or in which SHP-1 expression is restored transiently at high efficiency (eg using retroviral vectors) will be required to resolve this issue. Regardless, induction of SHP-1 expression (Figure 4) correlates with the dephosphorylation of a specific set of phosphotyrosyl proteins. It will be important to determine whether one or more of these proteins is a direct target of SHP-1.

Recently, it has been reported that PTP-1B expression increases upon Bcr-Abl transformation, and PTP-1B directly targets Bcr-Abl for dephosphorylation, at least at Y177 of the Bcr-Abl fusion protein.²¹ Our results, together with those of Tauchi *et al*²⁵ and Liedtke *et al*²⁴ suggest that another PTP, SHP-1, also helps antagonize the effects of Bcr-Abl in hematopoietic cells. Together, these data suggest that inactivation of PTPs by genetic or epigenetic means may contribute to progression to blast crisis. This hypothesis is consistent with previous work, which suggested that high levels of PTP activity maintain Bcr-Abl tyrosyl phosphorylation at low levels during the chronic phase of CML.⁴⁷ Further work is required to determine if, in fact, PTP inactivation is part of the pathogenesis of worsening CML in humans, and if so to identify the relevant PTP(s) and the inactivation mechanism.

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