v-Abl protein tyrosine kinase encoded by Abelson murine leukemia virus (Ab-MLV) transforms pre-B cells. Transformation requires the phosphatidylinositol 3-kinase (PI3K) pathway. This pathway is antagonized by SH2-containing inositol 5′-phosphatase (SHIP), raising the possibility that v-Abl modulates PI3K signaling through SHIP. Consistent with this, we show that v-Abl expression reduces levels of full-length p145 SHIP in a v-Abl kinase activity-dependent fashion. This event requires signals from the Abl SH2 domain but not the carboxyl terminus. Forced expression of full-length SHIP significantly reduces Ab-MLV pre-B-cell transformation. Therefore, reduction of SHIP protein by v-Abl is a critical component in Ab-MLV transformation.

Abelson murine leukemia virus (Ab-MLV) originated from recombination between Moloney murine leukemia virus (Mo-MuLV) and the cellular proto-oncogene c-abl (32). The resulting oncprotein, v-Abl, is a constitutively active nonreceptor protein tyrosine kinase that includes the Gag region of Mo-MuLV, the SH2 domain, the tyrosine kinase domain, and the carboxyl terminus of c-abl. The expression of SHIP is restricted to hematopoietic cells, and its loss is associated with myeloproliferative disease and various leukemias, including breakpoint cluster region/Abelson (BCR/ABL)-mediated chronic myelogenous leukemia (CML) (5, 13–15, 21, 22, 25–27). In vitro studies have revealed that the kinase activity of BCR/ABL suppresses SHIP protein levels, suggesting that SHIP plays a negative role in transformation by oncogenic Abl proteins (33–35). In the current study, we investigated the regulation of SHIP by v-Abl and directly tested the role of SHIP during Ab-MLV transformation.

To determine if v-Abl regulates SHIP protein levels, Ab-MLV-transformed pre-B cells (11) expressing P120 v-Abl were treated with the Abl tyrosine kinase inhibitor imatinib mesylate (imatinib) (8) for 2 h and analyzed by Western blotting as described in reference 2 by using an antibody that recognizes the full-length p145 SHIP isoform (see the supplemental material for additional methodology). Increased doses of imatinib resulted in an accumulation of p145 SHIP protein (Fig. 1A, left), which coincided with reduced total cellular phosphotyrosine (Fig. 1A, left). Furthermore, treatment of Ab-MLV-transformed pre-B cells with 1 μM imatinib for 16 h, followed by removal of the drug, restored SHIP levels to those seen in untreated cells within 4.5 h (Fig. 1A, right). These data demonstrate that the kinase activity of v-Abl reduces total SHIP protein levels in a manner similar to that reported for BCR/ABL (35).

SHIP is expressed as multiple isoforms that may be generated by calpain-mediated carboxyl-terminal proteolytic cleavage of full-length p145 SHIP (7). In particular, the p110 isoform expressed in normal hematopoietic cells (7, 9) is not detected by the antibody used for the analyses shown in Fig. 1A, raising the possibility that v-Abl reduces SHIP protein levels by promoting carboxyl-terminal proteolytic cleavage. To test this idea, Ab-MLV-transformed pre-B cells were treated with imatinib for 16 h, and lysates were analyzed by Western blotting with a SHIP antibody that recognizes the p110 isoform. In untreated cells, the 145-kDa band was the most prominent form, while a faint 110-kDa band was also present (Fig. 1B). Imatinib treatment resulted in a similar dose-dependent increase in both p145 and p110 (Fig. 1B), demonstrating that v-Abl kinase activity reduces both forms of SHIP and does not promote carboxyl-terminal proteolytic cleavage of p145 SHIP to reduce SHIP protein levels. Because the normal CD43+ B220+ target cells that give rise to Ab-MLV transformants (M. Gunthart and N. Rosenberg, unpublished data; see the supplemental material for the methods used) predominantly express the p110 isoform (Fig. 1B), these data suggest that Ab-MLV transformation alters the pattern of SHIP isoforms expressed by the cells. Although the function of the different isoforms has yet to be clarified, differences in association with Shc, a protein prominently involved in v-Abl-mediated signal-
were elevated in cultures expressing both P120/S2 and P70/S2 expression of SHIP by Western blotting. Levels of p145 SHIP pre-B cells expressing P120 or the mutants were tested for these modifications affect SHIP protein levels, transformed and display reductions in Ras activation (41). To determine if P70/S2 are compromised in Shc/Grb2/Sos complex formation for SHIP expression (29, 41). Cells expressing P120/S2 or in the absence of the carboxyl terminus (P70/S2) were analyzed in the presence of a full-length carboxyl terminus (P120/S2) or which the v-Src SH2 domain replaces the v-Abl SH2 domain expression, cells transformed with v-Abl mutants (Fig. 2A) in

Proteasome-dependent downregulation of proteins has been observed in Abl-expressing cells (6, 10). To determine if v-Abl reduces levels of SHIP protein via proteasome degradation, Ab-MLV-transformed pre-B cells (11) were treated with 10 μM proteasome inhibitor MG132 and analyzed by Western blotting. Levels of SHIP protein increased by 1 h following MG132 treatment (Fig. 1C), similar to treatment of cells with imatinib, suggesting that SHIP downregulation by v-Abl involves proteasomal degradation in a manner similar to that recently reported for BCR/ABL (33). These findings contrast to the marked stability of SHIP isoforms in normal cells, where half-lives of approximately 10 h have been reported (7). Although one previous report indicated that BCR/ABL does not promote proteasomal degradation of SHIP (34), variations in MG132 treatment length and concentration could explain these differences.

The v-Abl SH2 domain and carboxyl-terminal domains are critical for the formation of signaling complexes that influence v-Abl-mediated transformation, including those involving Shc, a protein known to associate with SHIP (16, 19, 20). To determine if these domains are important for effects on SHIP expression, cells transformed with v-Abl mutants (Fig. 2A) in which the v-Src SH2 domain replaces the v-Abl SH2 domain in the presence of a full-length carboxyl terminus (P120/S2) or in the absence of the carboxyl terminus (P70/S2) were analyzed for SHIP expression (29, 41). Cells expressing P120/S2 or P70/S2 are compromised in Shc/Grb2/Sos complex formation and display reductions in Ras activation (41). To determine if these modifications affect SHIP protein levels, transformed pre-B cells expressing P120 or the mutants were tested for expression of SHIP by Western blotting. Levels of p145 SHIP were elevated in cultures expressing both P120/S2 and P70/S2 compared to cultures expressing P120 or P70 v-Abl (Fig. 2B); imatinib treatment of a P120/S2-transformed pre-B cell line did not further modulate SHIP protein levels (data not shown), indicating that a functional v-Abl SH2 domain is required to regulate SHIP protein levels. Although the specific signals from the v-Abl SH2 domain required for SHIP proteasome degradation have not been elucidated, activation of Ras or PI3K pathways could possibly influence SHIP protein stability in a manner similar to the requirement for PI3K in proteasome-dependent degradation of p27Kip by BCR/ABL (10).

To determine if v-Abl variants, such as P120/S2 and P70/S2, that fail to reduce SHIP protein levels transform pre-B cells poorly compared to cells expressing P120 or P70, the kinetics of bone marrow transformation by these variants (Fig. 2A) were examined using liquid transformation assays that monitor both the time to transformation and transformation frequency (2). Bone marrow cells were infected with matched titer stocks of the different viruses, plated in liquid cultures, and monitored for transformation for 30 days. This assay allows quantitation of transformation by weakly transforming mutants that do not stimulate all of the pathways involved in transformation.
by wild-type virus (2, 40). Such mutants transform fewer cells, thereby affecting the kinetics with which transformation occurs. No transformants arise in this assay in the absence of virus infection (31). The kinetics of P120/S2 transformation were delayed compared to those of P120 transformation, and similarly, kinetics of P70/S2 transformation were delayed compared to those of P70 transformation (log rank test P120 versus P120/S2, P < 0.0001; P70 versus P70/S2, P = 0.0096) (Fig. 2C), suggesting a correlation between elevated expression of SHIP and reduced transformation. This pattern contrasts to the modest enhancement of Ab-MLV transformation previously observed for bone marrow cells from SHIP−/− mice (27). Because these mice have reduced numbers of pre-B cells (13), the effect of SHIP on Ab-MLV transformation in SHIP−/− mice is difficult to assess.

Taken together, these results directly demonstrate that the v-Abl SH2 domain is required for v-Abl-mediated down-modulation of SHIP and that expression of elevated levels of SHIP significantly reduces Ab-MLV transformation (log rank test, P < 0.0001) (Fig. 3C). Despite the marked reduction in transformation efficiency, a small number of v-Abl/SHIP-infected cultures became transformed similarly to wild-type (WT) cultures; it is possible that these transformants develop secondary mutations, signal through alternative pathways that bypass the suppressive effects of SHIP, or no longer express exogenous SHIP. The dramatic reduction in transformation by P120/SHIP contrasts to the modest enhancement of Ab-MLV transformation observed for bone marrow cells from SHIP−/− mice (27). Because these mice have reduced numbers of pre-B cells (13), the effect of SHIP on Ab-MLV transformation in SHIP−/− mice is difficult to assess.

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