Tumor and Stem Cell Biology

eIF4B Phosphorylation by Pim Kinases Plays a Critical Role in Cellular Transformation by Abl Oncogenes

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Abstract

Alterations in translation occur in cancer cells, but the precise pathogenic processes and mechanistic underpinnings are not well understood. In this study, we report that interactions between Pim family kinases and the transformation initiation factor eIF4B are critical for Abl oncogenicity. Pim kinases, Pim-1 and Pim-2, both directly phosphorylated eIF4B on Ser406 and Ser422. Phosphorylation of eIF4B on Ser422 was highly sensitive to pharmacologic or RNA interference-mediated inhibition of Pim kinases. Expression and phosphorylation of eIF4B relied upon Abl kinase activity in both v-Abl- and Bcr-Abl–expressing leukemic cells based on their blockade by the Abl kinase inhibitor imatinib. Ectopic expression of phosphomimetic mutants of eIF4B conferred resistance to apoptosis by the Pim kinase inhibitor SMI-4a in Abl-transformed cells. In contrast, silencing eIF4B sensitized Abl-transformed cells to imatinib-induced apoptosis and also inhibited their growth as engrafted tumors in nude mice. Extending these observations, we found that primary bone marrow cells derived from eIF4B-knockdown transgenic mice were less susceptible to Abl transformation, relative to cells from wild-type mice. Taken together, our results identify eIF4B as a critical substrate of Pim kinases in mediating the activity of Abl oncogenes, and they highlight eIF4B as a candidate therapeutic target for treatment of Abl-induced cancers. Cancer Res; 73(15); 4898–4908. © 2013 AACR.

Introduction

Expression of Abl proteins is associated with a variety of hematopoietic malignancies (1, 2). In mice, the v-Abl oncogene of Abelson murine leukemia virus (A-MuLV) induces pre-B-cell transformation through constitutively activated JAK–STAT signaling (2, 3). In humans, chromosomal translocations result in the formation of Bcr-Abl hybrid gene that mediates the pathogenesis of chronic myelogenous leukemia (4). Despite extensive studies, the precise mechanisms by which Abl oncoproteins cause cancer are not fully understood.

Growing evidence suggests that the full transforming activity of Abl involves the activation of STAT/Pim pathway (2, 5–7). For example, previous studies have shown that v-Abl cannot efficiently transform bone marrow cells (BMC) derived from Pim-1−/−/Pim-2−/− mice (6), indicating that Pim-1 and Pim-2 are required for v-Abl–mediated tumorigenesis. A report from the other group also showed that the signal transmitted through Pim kinases is important for survival of hematopoietic cells transformed by Bcr-Abl (8). In addition, it has been revealed that Pim-1 expression was markedly upregulated following Bcr-Abl–dependent activation of STAT5 (9, 10). Together, these observations show that Pim pathway contributes to cellular transformation by v-Abl and Bcr-Abl. However, the precise roles of Pim kinases in Abl-mediated transformation remain elusive.

The Pim kinases are a family of 3 ubiquitously expressed serine/threonine (Ser/Thr) kinases (Pim-1, Pim-2, and Pim-3). Pim-1 was first identified as a common integration site in Moloney murine leukemia virus-induced T-cell lymphoma (11). Subsequent studies have shown that Pim kinases contribute to both cell proliferation and survival and have thus been implicated in the control of tumor formation (12). For example, Pim-1 and Pim-2 have been shown to be transcriptionally upregulated in various hematopoietic malignancies (13). In addition, a high frequency of Pim-1 somatic mutation has been found in diffuse large B-cell lymphoma cases (14). Recent studies have also revealed that enhanced Pim expression is associated with other tumors including human prostate, pancreatic, colon, and

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gastric cancers (11, 15, 16). These findings suggest that upregulation of Pim kinases or Pim mutation may provide a selective advantage during cellular transformation. On the other hand, an increasing number of substrates have been identified that are phosphorylated by Pim kinases. These substrates include transcriptional regulators, cell-cycle regulators, signaling intermediates, and apoptosis mediators (17–23). Interestingly, eukaryotic translation initiation factor 4B (eIF4B) has recently been identified as a novel Pim-2 substrate (17). Pim-2 kinase has also been shown to modulate protein translation regulator eIF4E-binding protein 1 (24–27).

The control of mRNA translation is preferentially exerted at the initiation phase, which is crucial for the specific expression of genes important for development, cell growth, proliferation, and survival. eIF4B is part of the protein complex involved in the ribosome recruitment to the 5' ends of eukaryotic mRNAs and is thought to stimulate eIF4F activity by potentiating the eIF4A RNA helicase activity in unwinding secondary structures in the 5' untranslated region (5'UTR) of the mRNA (28). Therefore, eIF4B is a key component in the regulation of eukaryotic translation initiation. eIF4B is a phosphorylated protein, and its phosphorylation is responsive to extracellular stimuli including serum, insulin, and phorbol esters (29, 30). In addition, it has been revealed that eIF4B is regulated by several proto-oncogenic signaling pathways including mitogen-activated protein kinase, PI3K/mTOR, and Akt (30–32). Moreover, eIF4B has been shown to regulate translation of proliferative and prosurvival mRNAs with structured 5'UTR and thus control cell proliferation and apoptosis (24, 33). For example, recent studies have shown that eIF4B is involved in regulating the expression of Cdc25C, c-Myc, Bcl-2, and XIAP. These observations suggest that eIF4B may contribute to cellular transformation.

In this study, we investigated the role of Pim kinases leading to eIF4B phosphorylation downstream of Abl signaling. Our data revealed that expression and phosphorylation of eIF4B are Abl kinase dependent in Abl transformants. Furthermore, we showed that Pim kinase–phosphorylated eIF4B, especially on Ser422, is required for efficient cellular transformation by Abl oncogenes. These results provide novel insights into complex mechanisms by which Abl oncoproteins cause hematopoietic malignancies. In addition, our experiments establish a key role for eIF4B phosphorylation and thus, eIF4B may be a potential target for treatment of Abl-induced cancers.

Materials and Methods
Cell lines and cell culture
Cancer cell lines K362, 293T, Jurkat, A549, and HeLa were purchased from American Type Culture Collection. Hepatocellular carcinoma cell line SMMC-7721 and breast carcinoma cell line MCF-7 were purchased from National Platform of Experimental Cell Resources for Sci-Tech (http://cellresource.cn). Cells were grown in Dulbecco’s Modified Eagle Medium or RPMI-1640 supplemented with 10% FBS (Gibco) and antibiotics (penicillin and streptomycin; Invitrogen) as previously described (7). The v-Abl–transformed mouse cell lines named as NS2 and W44 in this study were generated and cultured as described previously (6, 34). These cell lines were characterized in our laboratory as CD10+ /CD19+ pre-B cells. The 32D myeloid cells stably expressing wild-type (WT) Bcr-Abl or Bcr-Abl-T315I were kind gifts from Dr. Guangbiao Zhou (Institute of Zoology, Chinese Academy of Science, Beijing, China). Pim- or eIF4B-overexpressing Abl transformants (K562, N2, and W44) were generated by stably infecting the cells with retroviruses or lentiviruses encoding Pim or eIF4B using viral vectors pMSCV-GFP or pNL-GFP (Addgene) as previously described (35). Short hairpin RNA (shRNA)-expressing stable Abl transformants were generated by infection of the cells with lentiviruses expressing specific shRNA in pSH-H1-GFP vector (System Biosciences) as described previously (36). The shRNA sequences are described in Supplementary Materials and Methods.

Antibodies and reagents
The following antibodies were used in this study: anti-FLAG (Sigma F1804 M2); anti-Pim-1 (Santa Cruz sc-13513 12H8); anti-c-Myc (Santa Cruz sc-40 9E10); anti-Bcr (Santa Cruz sc-885 N-20); anti-Bcl-xl. (Cell Signaling 2764 54H6); anti-eIF4B (Cell Signaling 3592); anti-phospho-eIF4B (Ser422; Cell Signaling 5399); anti-phospho-eIF4B (Ser422; Cell Signaling 3591); anti-Bcl-2 (Cell Signaling 2870 50E3); anti-c-Abl (Merck Millipore OP19 19–110); anti-p70 S6K, and anti-phospho-p70S6K [Thr389]. (Cell Signaling 9202 and 9205). All other antibodies were obtained and used as described previously (7). Pim-1/Pim-2 kinase inhibitor SMI-4a was purchased from Calbiochem.

GST pull-down experiment and in vitro kinase assay
Glutathione S-transferase (GST) fusion proteins were expressed and purified as previously described (6, 35). Pull-down assays were conducted by incubating aliquots of purified GST fusion protein beads with cell lysates at 4°C overnight. Beads were washed and examined by Western blotting. For in vitro kinase assay, purified GST fusion proteins were mixed with each other in the kinase buffer incubated at 30°C for 30 minutes (37). Samples were separated by electrophoresis and probed with the indicated antibodies.

Immunoprecipitation, immunoblotting, and apoptosis assay
Preparation of cell extracts, immunoprecipitation, and immunoblotting were conducted as previously described (6, 36). Where indicated, immunoblot signals were quantified by densitometry. For apoptosis assay, cells were treated with 10 μmol/L imatinib or 50 μmol/L SMI-4a. Cells were then stained with propidium iodide/Annexin V–FITC and analyzed by fluorescence-activated cell sorter (BD Biosciences).

Examination of tumorigenicity using xenograft model in nude mice
Nude-mouse injection was carried out as described previously (35). Tumor growth was monitored and measured in volume (length × height × width) at the indicated time points after inoculation. Bioluminescent imaging was used to detect tumor growth from GFP-expressing cells. Images were quantified as photons/s using the indigo software (Berthold Technologies).
Generation of eIF4B-knockdown transgenic mice

eIF4B-knockdown transgenic mice were generated by the microinjection method as previously described (38). Western blotting using anti-eIF4B antibody was conducted to determine the interference efficiency. The transgenic founders with high interference efficiency were selected and maintained on a C57BL/6j genetic background.

Primary murine bone marrow transformation assay

BMcs were freshly isolated from 5- to 6-week old mice. v-Abl- and Bcr-Abl–mediated bone marrow transformation was conducted as previously described (6, 35). Transformation efficiency was scored by counting the number of the wells containing the transformed cell clones showing cytokine-independent growth 3 weeks after infection.

DNA construction and mass spectrometry

DNA construction and mass spectrometry (MS) analysis are described in the Supplementary Materials and Methods.

Results
eIF4B was found to be a potential target of Pim-1 kinase in v-Abl transformants

Substrates of Pim kinases in Abl transformants are poorly characterized. To identify novel Pim targets in Abl transformants, we used v-Abl–transformed pre-B-cell lines derived from mice (6). The v-Abl transformants were metabolically labeled with [32P]orthophosphate. Radiolabeled Pim-1 was then isolated by immunoprecipitation, and phosphorylation was examined by autoradiography. Several phosphorylated proteins were detected in the precipitated protein complex (Fig. 1A). The identities of Pim-1 and SOCS-1 were confirmed by Western blotting. As expected, when expressed in cells, Pim-1 became constitutively autophosphorylated. The observed phosphorylation of SOCS-1 is consistent with the previous studies (6, 39).

Interestingly, we observed a highly phosphorylated protein at Ser406 and Ser422 by several kinases, and phosphorylation of these residues is essential for optimal translational activity of eIF4B (17, 30, 31). Also, previous experiments have revealed the substrate consensus sequence of Pim kinases (R-X-R/H-X-S/T; ref. 17). Using an in silico search, we found that Ser406 and Ser422 in the arginine-rich motif region of eIF4B are potential Pim phosphorylation sites. To test this possibility, 293T cells were cotransfected with eIF4B and Pim kinase, and eIF4B phosphorylation status was then determined by

Pim-1 and Pim-2 can directly phosphorylate eIF4B on Ser406 and Ser422

Previous studies have shown that eIF4B is serine phosphorylated on Ser406 and Ser422 by several kinases, and phosphorylation of these residues is essential for optimal translational

Figure 1. Pim-1 interacts with eIF4B in v-Abl–transformed cells and 293T cells. A, phosphoamino acid(s) in v-Abl–transformed cells was metabolically labeled by adding 500 μCi (18.5 MBq) of H3-32P04 for 2 hours. [32P]-labeled Pim-1 protein was isolated by immunoprecipitation, and phosphorylation was detected by autoradiography ([32P]). Pim-1 and SOCS-1 were examined by Western blotting (WB) and Coomassie Blue staining (CBS). eIF4B was identified by MS. B, 293T cells were transfected with Myc–Pim-1 or Myc–Pim-1-KD (Pim-1K67M). Lysates were incubated with purified GST, GST–eIF4B, GST–eIF4BS406A, or GST–eIF4BS422A coupling beads. The bound proteins were identified by WB using anti-Myc antibody. C, 293T cells were transfected with Flag–eIF4B, Flag–eIF4BS406A, or Flag–eIF4BS422A. Pull-down assay using purified GST, GST–Pim-1, or GST–Pim-1-KD coupling beads was conducted as described in B.

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Western blotting with the specific antibodies. We found that expression of Pim-1 or Pim-2 resulted in marked increase in Ser422 phosphorylation and slight increase in Ser406 phosphorylation of eIF4B (Fig. 2A–D). Importantly, expression of Pim-1-KD or Pim-2-KD mutant abolished the phosphorylation, indicating that phosphorylation of eIF4B is Pim kinase dependent in the cells (Fig. 2A–D). However, expression of Pim-3 had little effect on eIF4B phosphorylation (Supplementary Fig. S1). We further conducted in vitro kinase assay by incubating the purified recombinant GST-Pim kinases with eIF4B protein. Indeed, Pim-1 or Pim-2 was able to phosphorylate eIF4B at Ser406 and Ser422, and mutation of these sites to Ala abolished the phosphorylation (Fig. 2E–H). In addition, kinase-dead mutation of Pim kinases failed to phosphorylate eIF4B at Ser406 and Ser422. These experiments show that Pim-1 and Pim-2 directly phosphorylate eIF4B on Ser406 and Ser422.

**eIF4B is highly expressed and phosphorylated in v-Abl and Bcr-Abl transformants in an Abl kinase-dependent manner**

It is believed that deregulated translational control plays an important role in oncogenic transformation (40). To explore the contribution of eIF4B in Abl-induced cellular transformation, we investigated the eIF4B expression and phosphorylation status in Abl transformants as compared with other cell lines and normal controls. Interestingly, we observed that eIF4B was overexpressed in all 3 Abl-transformed cell lines analyzed (v-Abl–transformed cell lines W44 and NS2, and Bcr-Abl–expressing K562 leukemic cell; Fig. 3A and Supplementary Fig. S2A). Strikingly, eIF4B was also highly phosphorylated in Abl transformed cells, especially on Ser422 residue (Fig. 3A). To examine whether eIF4B is expressed and phosphorylated downstream of Abl signaling, Abl transformants were treated in a time course with imatinib (Fig. 3B–D and Supplementary...
The results showed that the endogenous protein levels of eIF4B decreased after treatment with imatinib, suggesting that expression of eIF4B is Abl kinase dependent. Similar to the situation seen with eIF4B expression, the phosphorylation of eIF4B on Ser406 displayed a similar decrease after imatinib treatment. However, the eIF4B phosphorylation on Ser422 was more sensitive to imatinib treatment as indicated by rapidly reduced phosphorylation levels after exposure to the compound (Fig. 3C and Supplementary Fig. S2B and S2C). To confirm that expression and phosphorylation of eIF4B is Abl kinase dependent, we used Bcr-Abl T315I mutant to test the effect of imatinib. As expected, imatinib treatment had little effect on eIF4B expression and phosphorylation in cells expressing the imatinib-resistant mutant (Fig. 3D). These results reveal that eIF4B is expressed and activated by Abl signaling, and Ser422 is a critical residue in eIF4B that can be regulated by this signaling.

**Pim-1 and Pim-2 regulate eIF4B phosphorylation in v-Abl and Bcr-Abl transformants**

To determine whether Pim kinases are able to regulate eIF4B phosphorylation in Abl-transformed cells, SM-4a, a novel small-molecule inhibitor of Pim-1 and Pim-2 kinases (41), was used. We found that eIF4B protein level and phosphorylation on Ser406 slightly decreased in Abl transformsants treated in a time course with SM-4a (Fig. 4A and B). Interestingly, treatment with SM-4a led to a more rapid reduction in the phosphorylation of eIF4B on Ser422 (Fig. 4A and B and Supplementary Fig. S3A and S3B), similar to the pattern observed in imatinib treatment (Fig. 3B and C). To determine the responsible kinases that phosphorylate eIF4B in Abl transformants, we generated shRNA-based Pim-1, Pim-2, or S6K knockdown cells (Fig. 4C–E and Supplementary Fig. S3C–S3E). Phosphorylation of eIF4B on Ser422 was greatly suppressed by silencing the Pim-1 and clearly decreased after Pim-2 depletion (Fig. 4C and data not shown). In contrast, eIF4B is still phosphorylated in Abl transformsants upon silencing S6K (Fig. 4D and E). Similar results were obtained from experiments using rapamycin (Supplementary Fig. S3F and S3G). These data suggest that the ability of Abl oncoproteins to regulate eIF4B phosphorylation, especially on Ser422 residue, is mainly mediated by their downstream effectors Pim kinases.

To further test this possibility, we generated v-Abl- and Bcr-Abl-transformed cell lines stably expressing either Pim WT, Pim KD mutants, or GFP control using bicistronic retroviruses (Supplementary Fig. S3H). Although the endogenous Pim expression was inhibited by imatinib treatment, the ectopic expression of Pim-1 and Pim-2 caused a marked increase in eIF4B phosphorylation on Ser422 compared with the GFP control (Fig. 4F and G and Supplementary Fig. S3I). As expected,
the effect of Pim kinases was dependent on the catalytic activity because the cells expressing Pim KD mutants recapitulated that of the control. These results confirm that phosphorylation of eIF4B on Ser422 is regulated by Pim-1 and Pim-2 kinases in Abl transformants.

**eIF4B plays a critical role in regulating survival of Abl transformants and functions downstream of Pim signaling in these cells**

Because eIF4B was found to be regulated by Abl kinase-dependent oncogenic Pim signaling, and eIF4B is involved in control of prosurvival mRNA translation, we hypothesized that eIF4B might play an important role in regulating survival of Abl transformants. To examine this possibility, we generated stable v-Abl- and Bcr-Abl–transformed cell lines expressing eIF4B-specific shRNAs (Fig. 5A and B and Supplementary Fig. S4A). v-Abl transformants initiated apoptosis following imatinib treatment, and approximately 51% of WT cells remained viable after incubation with this inhibitor for 15 hours under our culture condition. In contrast, only approximately 28% eIF4B silencing cells were viable under the same conditions (Fig. 5C). Similarly, approximately 48% of Bcr-Abl–positive K562 cells expressing control shRNA were viable after treatment with imatinib for 32 hours. Strikingly, only 27% of K562 cells expressing eIF4B-specific shRNA exhibited viable following the same treatment (Fig. 5D). These experiments show that silencing eIF4B sensitizes Abl transformants to undergo apoptosis induced by imatinib, although there is no significant difference in the survival of untreated cells expressing control or eIF4B shRNA (Supplementary Fig. S4B and S4C).

Furthermore, we asked whether eIF4B functions downstream of Pim signaling in Abl transformants. To address this issue, we generated Abl transformants stably expressing either GFP control, eIF4B WT, or its phosphomimetic mutants (Supplementary Fig. S4D). These cells were treated in a time course with Pim inhibitor SM-4a and analyzed for cell survival. Inhibition of Pim kinases with SM-4a for 20 hours reduced cell viability to approximately 25% in the control v-Abl cells (pNL), to approximately 40% in the cells expressing eIF4B WT or eIF4B S406E, and 52% to 54% in the cells expressing eIF4B S422E or eIF4B S406E/S422E (Fig. 5E). Similar results were observed in K562 cells (Fig. 5F). These data indicate that expression of eIF4B WT and its phosphomimetic mutants conferred various resistance to apoptosis induced by the Pim inhibitor. It seemed that the cells expressing S422E or S406E/S422E were more resistant to SM-4a–induced apoptosis than those expressing WT or S406E. Because it has been suggested that Janus-activated kinase (Jak)-2 and Stat5 are key upstream factors that regulate Pim expression, we further tested the effects of silencing these factors on eIF4B phosphorylation. Indeed, Pim-1 expression and phosphorylation of eIF4B on Ser422 were markedly reduced upon depletion of Jak2 and Stat5 (Supplementary Fig. S4E and S4F). Together, these results
reveal that Pim kinase-dependent eIF4B phosphorylation is important for the viability of Abl-transformed cells, and Ser422 in eIF4B is a critical phosphorylation site that is regulated by Abl-Jak-Stat-Pim signaling.

In an attempt to provide insights into the mechanism by which eIF4B regulates survival of Abl transformants, we evaluated the expression of Pim kinase target, c-Myc, and several Bcl-2 family members that are the critical regulators of apoptosis. Our results indicate that silencing eIF4B did not alter protein expression of Bcl-XL, but obviously reduced protein expression of Bcl-2 and c-Myc (Fig. 5G).

Silencing eIF4B significantly inhibits tumor formation induced by K562 leukemic cells in xenograft mouse model

In an attempt to gain a better understanding of the role of eIF4B in Abl-mediated tumorigenesis, we investigated the effect of silencing eIF4B on the tumor formation of Abl-transformed cells using xenograft model in nude mice. Each mouse was inoculated subcutaneously with K562 cells stably expressing shRNA targeting either eIF4B or luciferase control. Remarkably, we found that the tumors formed by cells expressing luciferase-specific shRNA grew much faster than those formed...
BMCs was measured as previously described (6, 35). eIF4B WT equal titer of A-MuLV. The capacity of the virus to transform knockdown mice or their WT littermates were infected with ciency were selected (Fig. 7A). BMCs derived from eIF4B- ref. 38). The transgenic founders with high interference ef transcription, we wished to establish a more physiologic model system for analysis of eIF4B involvement in the Abl transfor-
mation. For this, we generated eIF4B-knockdown transgenic mice as previously described (Supplementary Fig. S5A and S5B; transgenic mice shows a requirement for eIF4B in -cient cellular transformation by Abl oncogenes. Together, these results suggest that eIF4B is required for Abl-dependent tumor growth.

Primary bone marrow transformation assay using transgenic mice shows a requirement for eIF4B in efficient cellular transformation by Abl oncogenes

To further define the role of eIF4B in Abl-mediated tumorigenesis, we wished to establish a more physiologic model system for analysis of eIF4B involvement in the Abl transformation. For this, we generated eIF4B-knockdown transgenic mice as previously described (Supplementary Fig. S5A and S5B; ref. 38). The transgenic founders with high interference efficiency were selected (Fig. 7A). BMCs derived from eIF4B-knockdown mice or their WT littermates were infected with equal titer of A-MuLV. The capacity of the virus to transform BMCs was measured as previously described (6, 35). eIF4B WT cells infected with the A-MuLV displayed v-Abl transformation with average results of 14 wells showing cytokine-independent growth of cell clones per 96-well plate (Fig. 7B). However, silencing eIF4B significantly decreased v-Abl transformation efficiency. This study shows that disrupting the eIF4B expression has profound effects on the v-Abl–mediated bone marrow transformation.

To confirm this observation, we generated bicistronic retroviruses encoding the v-Abl or Bcr-Abl and either eIF4B, eIF4B-S422A, or GFP control (Supplementary Fig. S5C and S5D). BMCs derived from eIF4B-knockdown or WT mice were infected with these retroviruses. As expected, GFP and eIF4B-S422A failed to complement eIF4B deficiency for v-Abl- or Bcr-Abl–mediated transformation (Fig. 7C and D). In contrast, significantly increased transformants were found in cells infected with the retroviruses encoding Abl and eIF4B WT proteins. To show the involvement of Pim kinase in the cellular transformation, we generated bicistronic retroviruses encoding the v-Abl or Bcr-Abl and either Pim-1, Pim-1-KD mutant, or GFP control (Supplementary Fig. S5E and S5F) and conducted the bone marrow transformation. We observed that overexpression of Pim-1 resulted in significantly increased Abl transformation efficiency in WT mice but not in eIF4B-knockdown mice (Fig. 7E and F), suggesting that Pim promotes Abl transformation through phosphorylating eIF4B. Together, these results suggest that
v-Abl- and Bcr-Abl-mediated cellular transformation critically requires activated eIF4B that is regulated by Pim-dependent phosphorylation mainly on Ser422.

Discussion

Abl-induced tumorigenesis is a complicated process, involving the activation of signaling pathways that regulate cell survival and proliferation (2, 42). It has been shown that constitutive activation of STAT signaling endows the Abl-transformed cells with cytokine-independent growth (43–45). Importantly, activation of STAT signaling strongly induces the expression of Pim kinases in response to Abl oncogenic signaling. Pim-1 and Pim-2 have been shown to be critically required for malignant transformation of hematopoietic cells by Abl oncogenes (6, 8). Despite these progresses, the mechanisms underlying Pim action in Abl transformation and substrates of Pim kinases in Abl transformants are poorly characterized. Here, we, for the first time, show that Pim-1 and Pim-2 phosphorylate eIF4B mainly on Ser422 and to a lesser extent, on Ser406 in Abl-transformed cells, revealing a novel Pim kinase-dependent eIF4B activation that plays a crucial role in Abl-mediated cellular transformation (Supplementary Fig. S5G). However, Pim-3 has little effect on eIF4B phosphorylation. This is consistent with the previous observation that Pim-3 is not required for v-Abl transformation and it might have different functions in these cells (6).

Pim kinases are able to phosphorylate multiple targets and have therefore been implicated in diverse biologic processes. For example, there is compelling evidence showing that Pim
kinases are of great importance in cellular transformation due to their oncogenic activities that exert influence in regulation of cell survival signaling by phosphorylating BAD and regulation of cell-cycle progression through phosphorylation of p21^{CDPI}, p27^{KIP1}, Cdc25A, and Cdc25C, as well as regulation of c-Myc activity (25). In addition, Pim-1 and Pim-2 kinases are involved in regulating phosphorylation of SOCS-1, and thereby alter the inhibitory effects of SOCS-1 (34, 39). On the basis of their oncogenic potential, the Pim kinase family is emerging as an important new target for drug discovery (46). However, malignant transformation of lymphoid and myeloid cells involves the dysregulation or mutation of genes including PI3K/Akt pathway and Pim family (14, 47). The Akt and Pim kinases produce parallel oncogenic signals and share many molecular targets normally involved in regulating the cell proliferation and survival. Therefore, resistance to targeted Pim or PI3K/Akt therapeutics may become a major clinical problem because the redundancy of oncogenic signaling pathways provides back-up mechanisms that allow cancer cells to escape (26, 48).

In this study, our results represent the first report of Pim kinases-mediated phosphorylation of eIF4B in Abl transformants, suggesting that Pim kinases regulate cap-dependant protein translation in these cells. We found that expression of eIF4B S422E phosphomimetic mutant was able to complement inhibition of Pim kinases for Abl transformant survival, implying that phosphorylation of this site plays a key role in eIF4B activity. These data indicate that eIF4B Ser422 phosphorylation is critically required for efficient cellular transformation by Abl oncogenes. In addition, the results reveal that eIF4B mediates some of the effects of the Pim kinases on hematopoietic cell transformation. Previous studies from other groups have shown that activity of eIF4B can be regulated through phosphorylation by Akt (30). These experiments reveal that ability of Akt to phosphorylate eIF4B is potentially critical for the transforming capacity of this kinase. Our results presented in this study provide strong evidence that eIF4B can also be phosphorylated by Pim kinases. Together, these data suggest that the function of eIF4B in regulating cell survival and proliferation is mediated by several proto-oncogenic signaling pathways. These findings indicate that eIF4B represents one of the focal points whereby Akt, Pim, and other oncogenic signaling converge to exert their effects on malignant transformation. Therefore, targeting the convergence of oncogenic survival signals on eIF4B is an attractive alternative for development of anticancer therapies.

Our study has also begun to address the mechanism by which eIF4B may affect Abl transformation. We observed that depletion of eIF4B led to a significant reduction in the expression of Bcl-2 and c-Myc. This is consistent with the previous experiments showing that eIF4B regulates translation initiation of mRNAs harboring strong to moderate secondary structures in their 5’UTRs such as Bcl-2 and c-Myc (40). It has long been suggested that resistance to apoptosis in Abl-transformed cells is associated with increased expression of antiapoptotic proteins including Bcl-2 (49, 50). Importantly, our observation indicates that Abl kinase-dependent expression and phosphorylation of eIF4B are responsible for control of the antiapoptotic protein expression. Further research is required to address whether this regulation occurs in human leukemia patients and has any prognostic significance in the leukemia.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: J.-L. Chen
Development of methodology: J. Yang, G. Guo
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Chen, D. Whitten
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Yang, J. Wang, D. Whitten
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