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IκB kinase β inhibition induces cell death in Imatinib-resistant and T315I Dasatinib-resistant BCR-ABL$^+$ cells

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Abstract

Chronic myelogenous leukemia is a malignant disease of the hematopoietic stem cell compartment, which is characterized by expression of the BCR-ABL fusion protein. Expression of BCR-ABL allows myeloid cells to grow in the absence of the growth factors interleukin-3 and granulocyte-macrophage colony-stimulating factor. The tyrosine kinase activity of BCR-ABL constitutively activates signaling pathways associated with Ras and its downstream effectors and with the Jak/STAT pathway. Additionally, we reported previously that BCR-ABL activates the transcription factor nuclear factor-κB (NF-κB) in a manner dependent on Ras and that inhibition of NF-κB by expression of a modified form of IκBα blocked BCR-ABL-driven tumor growth in a xenograft model. Here, we show that a highly specific inhibitor of IκB kinase β, a key upstream regulator of the NF-κB pathway, induces growth suppression and death in cells expressing wild-type, Imatinib-resistant, or the T315I Imatinib/Dasatinib-resistant forms of BCR-ABL. Cell cycle variables were not affected by this compound. These data indicate that blockage of BCR-ABL-induced NF-κB activation via IκB kinase β inhibition represents a potential new approach for treatment of Imatinib- or Dasatinib-resistant forms of chronic myelogenous leukemia. [Mol Cancer Ther 2008;7(2):391–7]

Introduction

The t(9;22)(q34;q11) chromosomal translocation is the most frequent cytogenetic abnormality found in human leukemias where it can be detected in ~95% of patients with chronic myelogenous leukemia (CML) and in 30% to 40% of pre-B and acute lymphoblastic leukemia (1–3). This translocation results in the fusion of the BCR and ABL genes, leading to the expression of a BCR-ABL fusion protein with constitutively active ABL tyrosine kinase activity (1, 4). BCR-ABL-induced signaling is known to activate Ras-dependent signaling, phosphatidylinositol-3-kinase/Akt, and the Jak/STAT pathway (5). Additionally, BCR-ABL activates the transcription factor nuclear factor-κB (NF-κB) at least partly in a manner dependent on Ras (6). Suppression of NF-κB activation by expression of the so-called superrepressor form of IκBα blocked BCR-ABL-dependent xenograft tumor formation (6). Others (7) have also observed that NF-κB is activated by BCR-ABL in manner dependent on Ras. Furthermore, that study reported constitutive NF-κB DNA-binding activity in CML blasts (7).

The NF-κB/Rel family of transcription factors is comprised of homodimers and heterodimers of p65/RelA, c-Rel, RelB, NF-κB1/p50, or NF-κB/p52 (8). NF-κB is negatively regulated by the IκB family of inhibitory proteins, which functions to promote cytoplasmic accumulation of NF-κB dimers and to prevent DNA-binding activity (8). NF-κB is activated downstream of signals elicited by inflammatory cytokines, such as tumor necrosis factor, and by bacterial products, such as lipopolysaccharide (8). The primary mechanism for activation of NF-κB involves the activation of the IκB kinase (IKK), which induces the phosphorylation and ubiquitination of IκB. This leads to the degradation of the inhibitory IκB proteins and the subsequent accumulation of NF-κB dimers in the nucleus (8). IKK is composed of two catalytic subunits, IKKα and IKKβ, and a regulatory subunit, IKKγ/NEMO. IKKβ is described as the dominant catalytic subunit in the classic pathway downstream of tumor necrosis factor, and IKKα is involved in a pathway associated with activation of the so-called alternative pathway that activates p52/RelB heterodimers (8). NF-κB is strongly associated with oncogenesis where it is often important for cell proliferation, suppression of apoptosis, and invasion/metastasis (9, 10). NF-κB has been shown to be activated in several hematologic malignancies, including CML (see above), acute myelogenous leukemia, acute lymphoblastic leukemia,

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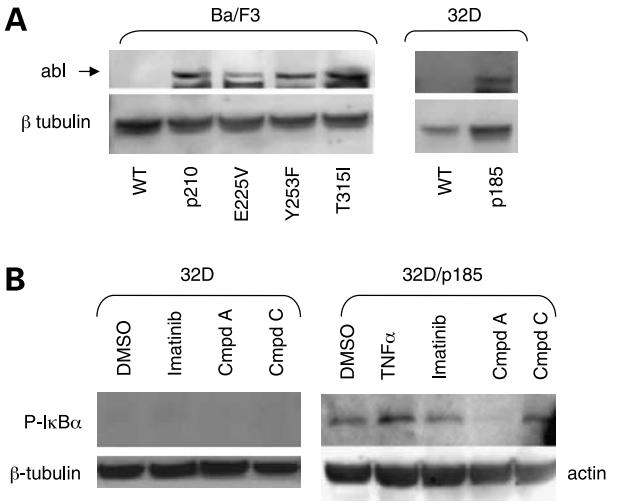

Figure 1. IKKβ inhibitor compound A blocks phosphorylation of IκBα in BCR-ABL-expressing myeloid cells. **A,** whole-cell extracts were prepared from untreated Ba/F3 parental and BCR-ABL-expressing cells and 32D and 32D/p185 cells. Immunoblotting was done with an antibody to Abl. **B,** cytoplasmic extracts were prepared from 32D and 32D/p185 BCR-ABL cells treated with vehicle (DMSO), 1 μmol/L Imatinib, 1 μmol/L IKKβ inhibitor (compound A), or 1 μmol/L inactive related compound (compound C). As a control, 32D/p185 cells were treated with tumor necrosis factor (5 ng/mL). Immunoblot analysis of was carried out using an antibody that recognizes IκBα phosphorylated on Ser^{32}/Ser^{36}. β-Tubulin or actin antibodies were used to normalize loading. Representative of three experiments.

and Hodgkin's disease (11–15). Recently, inhibitors of IKKβ have been used to suppress growth of acute myelogenous leukemia cells (16) and a subset of diffuse large B-cell lymphoma (17).

The hypothesis that CML can be treated by selective inhibition of BCR-ABL has now been shown by the generation of Abl tyrosine kinase inhibitors with positive results in clinical trials of these compounds (18). Phase 2 studies on the use of Imatinib for the treatment of chronic and advanced-stage CML have shown dramatic responses, with more than 90% of patients with IFN-resistant chronic-stage CML achieving a complete hematologic response. Furthermore, ~75% of patients with newly diagnosed chronic-phase disease achieve cytogenetic remission (19–21). However, most of the patients in blast crisis who initially respond to Imatinib therapy ultimately relapse with disease that is resistant to Imatinib. Resistance to Imatinib in CML patients is caused primarily by point mutations in the kinase domain of BCR-ABL, which impair drug binding (22, 23). Dasatinib, a second-generation Abl inhibitor, is effective for most Imatinib-resistant CML (24, 25). However, Dasatinib resistance is generated from mutations occurring directly in the drug binding site on Abl (26). One of these mutations, T315I, generates resistance to Imatinib, Dasatinib, and Nilotinib (25, 27). Imatinib resistance can also be generated by poorly described mechanisms. In this regard, IKK inhibition blocked growth of Imatinib-resistant cells (K562 and KCL) that do not express BCR-ABL point mutants (28).

Here, we have tested whether inhibition of the NF-κB/IKK pathway is effective at suppressing growth of cells expressing point mutants in BCR-ABL that lead to Imatinib or Dasatinib resistance. Our results show that a highly selective IKKβ inhibitor, shown to be effective at blocking inflammatory progression *in vivo* (compound A; ref. 29),

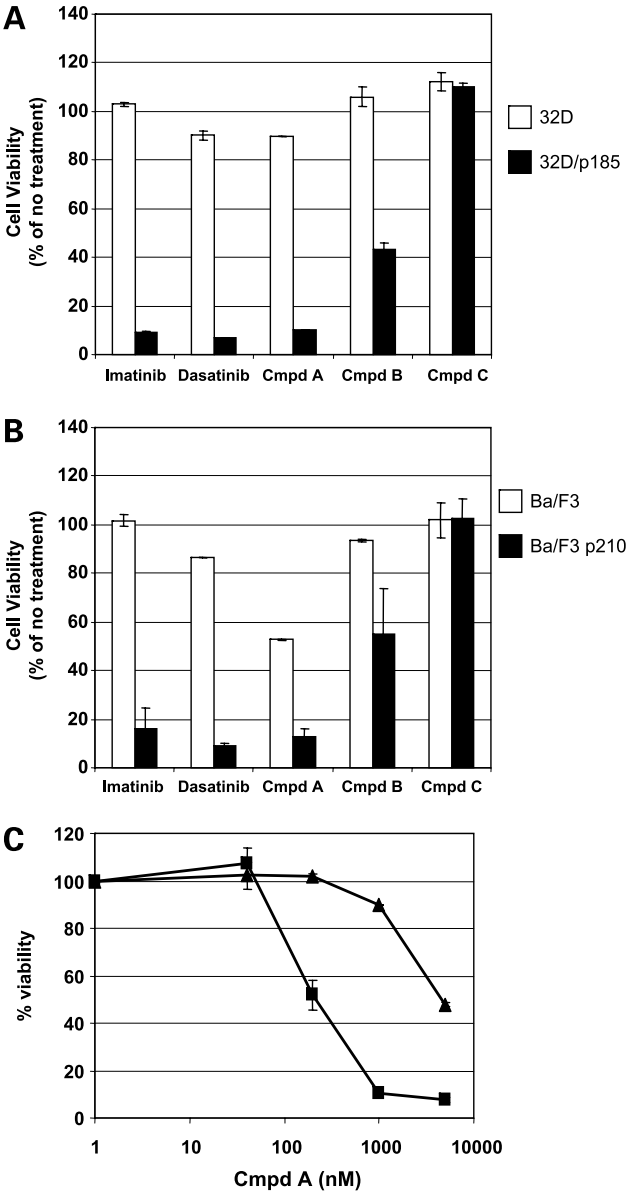

Figure 2. IKKβ inhibition suppresses growth/viability of BCR-ABL⁺ cells. **A,** 32D cells or 32D cells expressing p185 BCR-ABL were incubated for 20 h in the presence of 1 μmol/L IKKβ inhibitor (compound A), 1 μmol/L compound B or C, Imatinib (1 μmol/L), Dasatinib (5 nmol/L), or vehicle (DMSO). Cell viability was assessed by reduction of MTS. **B,** Ba/F3 cells and Ba/F3 cells expressing p210 BCR-ABL were treated as in **A**. *Points,* mean of biological replicates; *bars,* SD between replicates. Representative of experiments done in triplicate. **C,** 32D (*triangles*) and 32D expressing p185 BCR-ABL (*squares*) were treated with varying concentrations (as shown) of IKKβ inhibitor compound A for 20 h, and cell viability was measured as in **A**.

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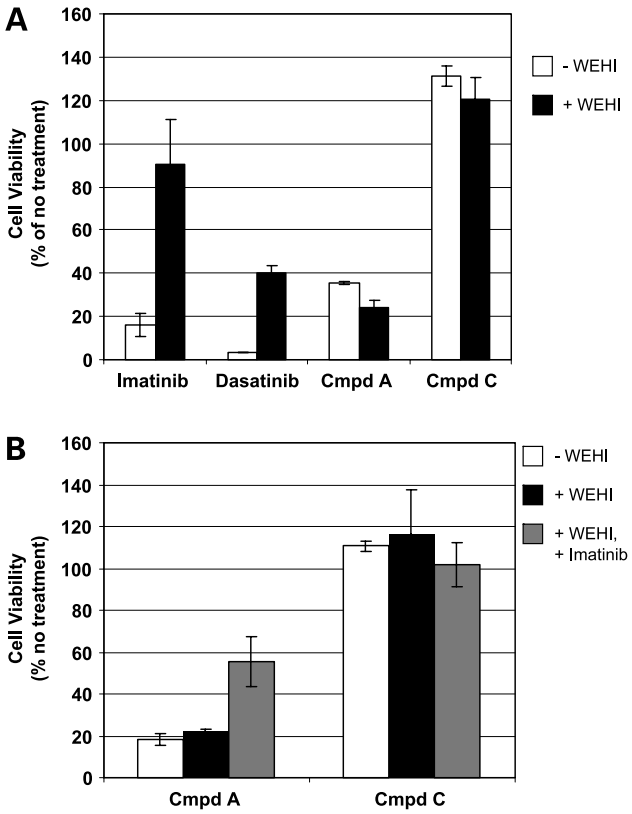

Figure 3. WeHi medium rescues BCR-ABL$^+$ cell susceptibility to tyrosine kinase inhibitors but not to IKKβ inhibition. Inhibition of BCR-ABL kinase activity promotes rescue by WeHi medium. **A,** 32D cells expressing p185 BCR-ABL cells were cultured in the presence (*black columns*) and absence (*white columns*) of WeHi medium for 24 h. The cells were subsequently incubated with 1 μmol/L Imatinib, 5 nmol/L Dasatinib, 1 μmol/L compound A, or 1 μmol/L compound C and cell growth/viability was assessed after 20 h of treatment as described in Fig. 2. *Columns,* average values from replicates; *bars,* SD. **B,** 32D cells expressing p185 BCR-ABL were cultured for 24 h in the absence of WeHi medium (*white columns*), the presence of WeHi medium (*black columns*), or the presence of WeHi medium and Imatinib (*gray columns*). The cells were subsequently incubated with 1 μmol/L compound A or C, and cell viability was assessed after 20 h of treatment. *Columns,* average values from biological replicates; *bars,* SD. Representative of experiments done in triplicate.

strongly suppresses growth/viability of cells expressing either wild-type or mutant versions of BCR-ABL, including the Dasatinib-resistant T315I mutation. Experimental results suggest that the involvement of NF-κB/IKK downstream of BCR-ABL is distinct from the NF-κB activation response shown to be associated with WeHi medium containing interleukin-3. Overall, the data indicate that inhibition of IKKβ represents a therapeutic strategy for tyrosine kinase inhibitor-resistant BCR-ABL-expressing CML as well as in acute leukemias that express BCR-ABL.

Materials and Methods

Cells and Reagents

Ba/F3 cell lines expressing BCR-ABL mutants were developed in the Sawyers laboratory (see ref. 30). Ba/F3 and 32D parental cells were maintained in RPMI with 10% fetal bovine serum, 10% conditioned WeHi medium (as a source of interleukin-3), 100 μg/mL penicillin, and 100 μg/mL streptomycin (Sigma Chemical). Ba/F3 and 32D cells expressing BCR-ABL oncoproteins (see ref. 6; gift of Dr. A.M. Pendergast) were maintained without conditioned WeHi medium. All cell lines were grown at 5% CO_2 at 37°C. Antibodies for c-Abl and cleaved caspase-3 were obtained from Cell Signaling. Antibodies to proliferating cell nuclear antigen and β-tubulin were obtained from Santa Cruz.

Stimulation of Ba/F3 and 32D Cells for Flow Cytometry

To examine apoptosis using flow cytometry, cells were stimulated in the following manner. Cells (0.5×10^6) were added to 10 mL growth medium and either DMSO, Imatinib, Dasatinib, the IKKβ inhibitor compound A, or compound B (less active), or compound C (an inactive structural analog of compound A) were added for 24 h at concentrations shown in the figures or figure legends. The cells were processed for flow cytometry as described below.

Cell Growth/Viability

Cell viability after treatment was measured by spectrophotometric monitoring of MTS reduction using the CytoquantAQ assay system (Promega). After optimization of cell number, an equal number of cells were seeded in a 96-well plate. Inhibitors or DMSO (as indicated in the figures) was added to each well and the cells were incubated at 37°C overnight. CytoquantAQ reagent (20 μL) was added to each well, including the blank wells, and the mixture was incubated for 1 h at 37°C. Absorbance was read at 490 nm.

Apoptosis Assay

Apoptosis and cell death were assessed by staining with propidium iodide (PI; BD Biosciences) and APC-labeled Annexin V (BD Biosciences) according to the manufacturer's protocol. After stimulation with the inhibitors described above, the cells were washed twice in ice-cold 1× PBS. They were then resuspended in 100 μL of 1× binding buffer (BD Biosciences) and 2.5 μL PI and Annexin V were added to the cells. The cells were incubated at room temperature for 15 min, and 250 μL of 1× binding buffer was subsequently added to stop the reaction. The cells were analyzed immediately on a Cyan flow cytometer (Dako-Cytomation). Cell death was characterized by the level of Annexin V and PI staining where apoptotic cells are Annexin V$^+$, PI$^-$ and dead cells are Annexin V$^+$, PI$^+$.

Cell Cycle Analysis

Cell cycle analysis was done by staining with PI (BD Biosciences) according to the manufacturer's protocol. After stimulation for 24 h with the inhibitors described above, 32D and 32D/p185 cells were fixed in ice-cold 70% ethanol (added dropwise while vortexing) for 1 h at 4°C. The cells were then centrifuged ($400 \times g$ for 5 min) and washed once in staining buffer (2% fetal bovine serum, 1× PBS). After centrifugation, the cells were resuspended in 100 μL staining buffer. The cells were then treated with RNase A (Sigma; 1 μL/sample) and incubated for 30 min at 37°C. After RNase A incubation, the cells were stained for 30 min at room temperature (protected from light) with 10 μg PI in a final volume of 0.5 mL staining buffer. The samples were then

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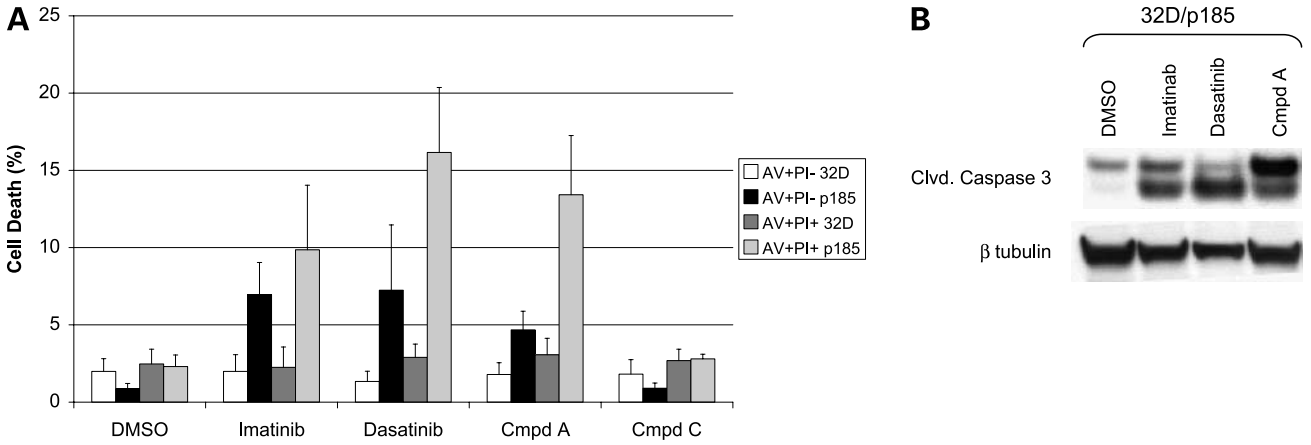

Figure 4. BCR-ABL$^+$ cells undergo cell death in response to tyrosine kinase inhibitors or IKKβ inhibition. **A,** 32D and 32D-p185 cells were stimulated for 24 h with either DMSO, Imatinib, Dasatinib, compound A, or compound C (as described above). Cells were stained with Annexin V and PI, and cell death was measured by flow cytometry on a Cyan flow cytometer. Apoptotic cells are characterized as Annexin V$^+$, PI$^-$ and dead cells are Annexin V$^+$, PI$^+$. *Bars,* *Bars,* SD. Representative of three experiments. **B,** whole-cell extracts from the experiment in **A** were analyzed by immunoblotting for cleaved caspase-3. β-Tubulin antibody is used to normalize loading. Representative of three different experiments.

analyzed by flow cytometry at a flow rate <400 events/s. The percentage of cells in G_0-G_1, S, and G_2-M were calculated using ModFit (Verity Software House).

Western Blot Analysis

Western blot analysis was done after fractionation of cellular contents into cytosolic and nuclear fractions or whole-cell lysis as indicated. Proteins (20 μg/lane) from the extracts were separated using NuPAGE Novex 4% to 12% bis-Tris gels (Invitrogen) followed by immunoblotting with antibodies as indicated.

Results and Discussion

IKKβ Inhibition Decreases IκBα Phosphorylation Levels in BCR-ABL-Expressing Cells

Ba/F3 cells stably expressing p210 BCR-ABL as well as drug-resistant variants, and 32D cells expressing p185 BCR-ABL, were developed previously (see refs. 6, 30). Immunoblotting confirms the expression of BCR-ABL in the different cell lines (Fig. 1A). As a measure of NF-κB activation downstream of BCR-ABL, we analyzed phosphorylation of IκBα at Ser^{32}/Ser^{36}. Notably, phosphorylation of IκBα is significantly higher in 32D/p185 cells compared with the parental 32D cells (Fig. 1B), consistent with reports showing that BCR-ABL induces NF-κB activation (6, 7). Additionally, this result shows that IKK is activated downstream of BCR-ABL because IKK phosphorylates IκBα at Ser^{32} and Ser^{36} (see ref. 8). To analyze the effects of Imatinib and a recently described inhibitor of IKKβ (compound A; see ref. 29) on IκBα phosphorylation, 32D cells and those expressing the p185 form of BCR-ABL were treated with DMSO, Imatinib, the IKK inhibitor compound A, or a structurally inactive form of compound A (compound C). Treatment with Imatinib partly reduced,

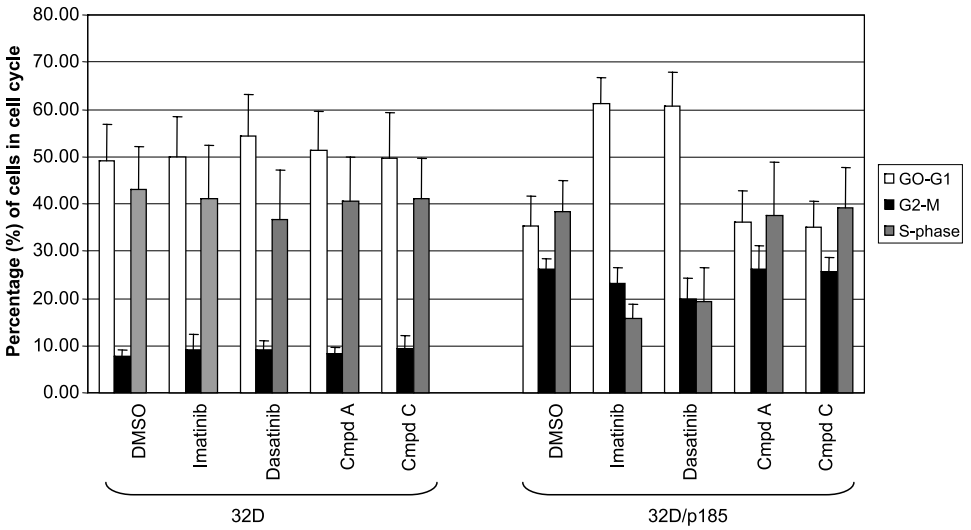

Figure 5. BCR-ABL$^+$ cells exhibit G_0-G_1 accumulation in response to Imatinib or Dasatinib treatment but not to IKKβ inhibition. 32D and 32D-p185 cells were stimulated for 24 h with either DMSO, Imatinib, Dasatinib, compound A, or compound C (inactive form). Cells were fixed, treated with RNase A, and stained with PI. Cell cycle progression was measured by flow cytometry on a Cyan flow cytometer. The percentage of cells in G_0-G_1, S, and G_2-M were calculated using ModFit. *Bars,* SD. Representative of three experiments.

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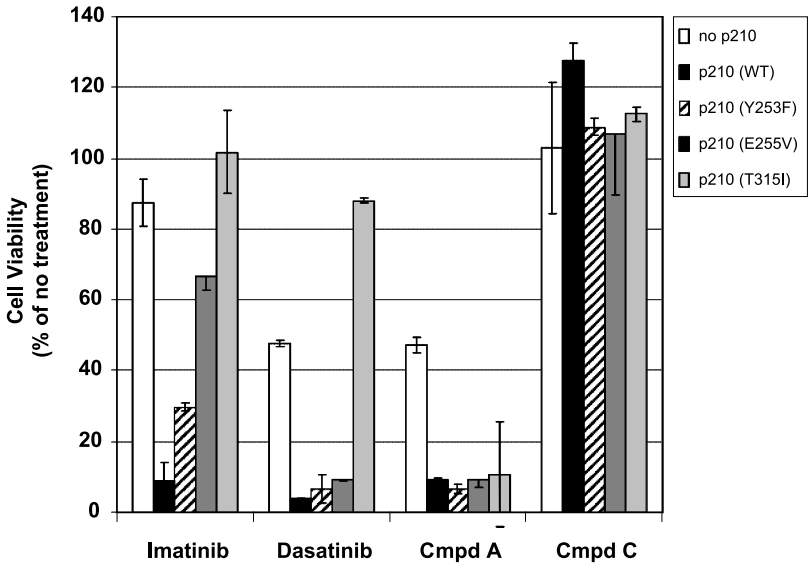

Figure 6. Cells expressing tyrosine kinase inhibitor resistant BCR-ABL mutants are susceptible to IKKβ inhibition. Ba/F3 cells expressing p210 BCR-ABL (*WT,* wild-type) or kinase inhibitor-resistant variants were treated with tyrosine kinase or IKKβ inhibitors and then analyzed for viability. Cells expressing p210 BCR-ABL WT (*white*), Y253H, E255V, and T315I were treated for 18 h with 1 μmol/L Imatinib, compound A, or compound C, or 5 nmol/L Dasatinib, and cell viability was assessed by reduction of MTS. *Columns,* average values of triplicates; *bars,* SD. Representative of three experiments.

whereas compound A but not compound C, significantly reduced IκBα phosphorylation in 32D/p185 cells (Fig. 1B), indicating that BCR-ABL activates IKK, which induces IκBα phosphorylation. Additionally, these results show that compound A is an effective inhibitor of IKK/NF-κB activation downstream of BCR-ABL-induced signaling.

BCR-ABL Expression Induces Susceptibility to IKKβ Inhibition

Previously (6), we have shown that BCR-ABL induces strong NF-κB-dependent transcriptional activity and that inhibition of NF-κB via IκBα expression blocked tumor growth driven by BCR-ABL in a xenograft model. Based on these results, we asked whether IKKβ inhibitor compound A (1 μmol/L) would affect the growth/viability of 32D cells or 32D/p185 cells as measured in a MTT assay. The effects of IKKβ inhibitor compound A were compared with the effects of Imatinib (1 μmol/L) and Dasatinib (5 nmol/L), both with well-established activity against BCR-ABL-expressing cells. Additionally, we included an IKKβ inhibitor (compound B) with weaker activity than IKKβ inhibitor compound A and an inactive, related compound (compound C). The results (Fig. 2) show that IKKβ inhibitor compound A, at a dose of 1 μmol/L, is effective at suppressing cell growth/viability of 32D/p185 cells with minimal effects on the parental 32D cells. As expected, Imatinib and Dasatinib each reduced cell growth/viability of 32D/p185 cells. IKKβ inhibitor compound B reduced viability of 32D/p185 cells but less effectively than IKKβ inhibitor compound A, consistent with its lower effectiveness as an IKKβ inhibitor. The inactive IKKβ inhibitor compound did not affect cell growth/viability. Similar results were observed with the pro-B Ba/F3 cell line expressing p210 BCR-ABL (Fig. 2*B*), although the IKKβ inhibitor reduced cell growth/viability of the parental pro-B cells, presumably indicating the involvement of NF-κB at some level in control of proliferation or survival of these cells. Titration of IKKβ inhibitor compound A showed observable cell growth/viability effects around 100 nmol/L, with an ~10-folder stronger effect at that concentration, and at 1 and 10 μmol/L, in 32D/p185 cells compared with the 32D parental cells (Fig. 2*C*).

WEHI medium does not overcome the inhibitory effect of IKKβ blockade on 32D/p185 cell viability. WEHI-controlled growth/survival does not require IKKβ in myeloid cells where BCR-ABL is inactive.

Expression of BCR-ABL generates growth factor–independent growth of 32D myeloid cells (5). Addition of WEHI medium (as a source of interleukin-3 and granulocyte-macrophage colony-stimulating factor) to BCR-ABL-expressing cells overcomes the inhibitory block on cell viability induced by Imatinib or Dasatinib, essentially recreating a myeloid cell without BCR-ABL activity (Fig. 3*A*). However, IKKβ inhibitor-treated 32D/p185 cells cannot be rescued with WEHI medium (Fig. 3*A*). This result suggests one of the following possibilities: (*a*) WEHI medium/interleukin-3 requires IKK/NF-κB to promote cell growth/survival, (*b*) BCR-ABL-activated signaling where IKKβ is inhibited interferes with the ability of interleukin-3 to induce cell survival, or (*c*) NF-κB activity promoted by growth factor support, which involves Stat5 (see ref. 31), is not sufficient to replace the NF-κB/IKK activity suppressed by IKKβ inhibition in the BCR-ABL-expressing cells. This latter hypothesis may be explained by different forms of NF-κB activated by growth factors versus BCR-ABL, by different modifications to NF-κB in response to growth factors or to BCR-ABL expression leading to distinct gene responses, or by a function of IKK controlled by BCR-ABL that is not regulated by growth factors. We then asked if 32D/p185 cells exposed to IKKβ inhibitor could be rescued with WEHI medium or with Imatinib and WeHi medium. 32D/p185 cells were incubated in the presence or absence of WEHI and/or Imatinib for 24 h. Subsequently, IKKβ inhibitor compound A (or C) was added for an additional 20 h and cell viability was assessed. The results of this

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experiment (Fig. 3*B*) reveal that WEHI alone does not rescue the cells when IKK is inhibited, consistent with the results shown in Fig. 3*A*. However, WEHI medium plus Imatinib rescues cell viability in 32D/p185 cells exposed to IKKβ inhibitor. This result shows that growth factor–induced growth/survival of 32D cells (where BCR-ABL is inactivated or not expressed) does not require IKKβ activity. Thus, the effect of IKKβ inhibition on blocking rescue of growth/survival by exogenous growth factors is mediated through BCR-ABL-controlled suppression of the growth factor response under these conditions and/or through inhibition of an IKK/NF-κB function in BCR-ABL⁺ cells that cannot be recapitulated by growth factors.

IKKβ Inhibition Induces Cell Death in BCR-ABL-Expressing Cells but Does Not Affect Cell Cycle Variables

To analyze effects on 32D/p185 cell proliferation/viability induced by IKKβ inhibition, cells were treated with Imatinib, Dasatinib, or IKKβ inhibitor compound A (or inactive compound C) and were then assessed for cell death by staining with Annexin V and PI. The parental 32D cells exhibited no induction of apoptosis (Annexin V^+, PI^-) with the various drug treatments. However, a measurable but relatively modest level early apoptosis was induced in the 32D/p185 BCR-ABL-expressing cells with tyrosine kinase inhibitors or with IKKβ inhibitor compound A (Fig. 4*A*). These results are consistent with known effects of BCR-ABL on blocking apoptosis in the transformation process (see ref. 32). The inactive IKKβ inhibitor (compound C) exhibited no effects on apoptosis and showed essentially the same profile as DMSO treatment. Correspondingly, the drug treatments that induced apoptosis also exhibited an increase in late apoptosis/necrosis (Annexin V^+, PI^+). To further examine an apoptotic response, immunoblotting was carried out to examine the level of cleaved caspase-3 in these cells following inhibitor treatment. Figure 4*B* shows that 32D/p185 cells treated with Imatinib, Dasatinib, or IKKβ inhibitor exhibit increased levels of cleaved caspase-3 relative to the DMSO control. This result suggests that the increased cell death observed in these cells on drug treatment is mediated at least partly via apoptosis. We note that IKKβ inhibition induces less early apoptosis at the 24-h time point compared with BCR-ABL inhibition but relatively equivalent levels of overall cell death (Annexin V^+, PI^+). Although there are elevated levels of apoptosis/cell death present in 32D/p185 cells treated with the drugs listed above, it is also possible that these drugs are exhibiting effects via a cell cycle block. Therefore, cell cycle analysis was done. It was observed that 32D/p185 cells exhibit higher levels of G_2-M- and S-phase compartments compared with parental 32D cells (Fig. 5). Experiments revealed that treatment with Imatinib or Dasatinib inhibits cell cycle progression in 32D/p185 cells, with enhanced G_0-G_1 accumulation and reduced numbers in S phase compared with DMSO control. Treatment with IKKβ inhibitor compound A, or with inactive compound C, did not significantly alter cell cycle variables. Importantly, it was shown that cell cycle progression is not abrogated in 32D cells treated with these compounds (see Fig. 5). Overall, these studies indicate that IKKβ inhibition of BCR-ABL-expressing myeloid cells induces a cell death response that can be partly attributed to apoptosis but does not induce a cell cycle block in a specific cell cycle compartment. Future studies will be required to determine if overall cell growth is blocked by IKKβ inhibition and whether nonapoptotic forms of cell death contribute to the IKKβ inhibition response in BCR-ABL⁺ cells.

IKKβ Inhibition Blocks Viability in Cells Expressing Wild-type, Imatinib-Resistant, or the T315I Dasatinib-Resistant Forms of BCR-ABL

Point mutations in the kinase domain of BCR-ABL produce resistance to Imatinib and promote oncogenicity (22–27). We analyzed Ba/F3 cells expressing wild-type p210 or variants that are either Imatinib resistant (Y253F and E255V) or both Imatinib and Dasatinib resistant (T315I). Cells were exposed to Imatinib, Dasatinib, IKKβ inhibitor (compound A), or the inactive structural variant of the IKKβ inhibitor compound (compound C). Consistent with experiments shown above (Fig. 2), each of the inhibitors strongly reduced the viability of cells expressing wild-type BCR-ABL (Fig. 6). As expected, cells expressing mutant variants of BCR-ABL (E255V, Y253H, and T315I) were variably resistant to Imatinib, with the T315I mutant exhibiting complete resistance to Imatinib. Those cells expressing the T315I mutation were also resistant to growth inhibition by Dasatinib (Fig. 6), as expected. Interestingly, each of the mutant cell lines, including the T315I line, was susceptible to IKKβ inhibition. The inactive IKKβ inhibitor (compound C) in DMSO showed no inhibitory effects on any of the cells. These studies show that blocking NF-κB/IKK suppresses viability of cells expressing all forms of BCR-ABL, including wild-type and Imatinib- and Dasatinib-resistant variants, indicating that IKK/NF-κB is required for growth/survival of BCR-ABL⁺ cells.

Summary

The dramatic success of targeting BCR-ABL in CML has been dampened by the emergence of Imatinib- and Dasatinib-resistant variants in the kinase domain. An alternative or adjuvant approach is to target essential downstream regulators of BCR-ABL. Here, we have addressed the potential that inhibition of NF-κB and/or its upstream regulator IKK will function to block growth/viability of cells expressing wild-type BCR-ABL or Imatinib- or Dasatinib-resistant variants. Our results show that IKKβ inhibition functions similarly to Imatinib and to Dasatinib in suppressing growth of myeloid or pro-B cells expressing wild-type BCR-ABL or Imatinib- or Dasatinib-resistant forms of BCR-ABL. These results indicate that NF-κB/IKK is essential for BCR-ABL-induced cell growth and survival. The growth inhibitory response associated with IKKβ inhibition is controlled, at least partly, through the induction of cell death for which apoptosis is a contributing component. Further studies are required to understand the effects of IKKβ inhibition on BCR-ABL⁺ cells

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relative to growth and survival. The data indicate the potential for the use of IKKβ inhibitors as stand-alone therapies for CML or in combination with existing therapies. The results also indicate that IKKβ inhibition may be therapeutic in Imatinib- and Dasatinib-resistant disease.

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