Activin A induction of erythroid differentiation sensitizes K562 chronic myeloid leukemia cells to a subtoxic concentration of imatinib

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Huang YW, Lee WH, Tsai YH, Huang HM. Activin A induction of erythroid differentiation sensitizes K562 chronic myeloid leukemia cells to a subtoxic concentration of imatinib. Am J Physiol Cell Physiol 306: C37–C44, 2014. First published October 2, 2013; doi:10.1152/ajpcell.00130.2013.—Chronic myeloid leukemia (CML) is a hematopoietic stem/progenitor disorder in which Bcr-Abl oncoprotein inhibits cell differentiation. Differentiation induction is considered an alternative strategy for treating CML. Activin A, a member of the transforming growth factor-β superfamily, induces erythroid differentiation of CML cells through the p38 MAPK pathway. In this study, treatment of the K562 CML stem/progenitor cell line with activin A followed by a subtoxic concentration of the Bcr-Abl inhibitor imatinib strongly induced growth inhibition and apoptosis compared with simultaneous treatment with activin A and imatinib. Imatinib-induced growth inhibition and apoptosis following activin A pretreatment were dose- and time-dependent. Imatinib-induced growth inhibition and apoptosis were also dependent on the pretreatment dose of activin A. More than 90% of the activin A-induced increases in glycophorin A-positive cells were sensitive to imatinib. However, only some of original glycophorin A-positive cells in the activin A treatment group were sensitive to imatinib. Sequential treatment with activin A and imatinib decreased Bcr-Abl, procaspase-3, Mcl-1, and Bcl-xL and also induced cleavage of procaspase-3/poly(ADP-ribose)polymerase. The reduction of erythroid differentiation in p38 MAPK dominant-negative mutants or by short hairpin RNA knockdown of p38 MAPK suppressed increases in glycophorin A-positive cells were sensitive to imatinib. Furthermore, the same inhibition level of multidrug resistance 1 expression was observed in cells treated with activin A alone, treated sequentially with activin A and imatinib, or treated simultaneously with activin A and imatinib. The p38 MAPK inhibitor SB-203580 can restore activin A-inhibited multidrug resistance 1 expression. Taken together, our results suggest that a subtoxic concentration of imatinib could exhibit strong cytotoxicity against erythroid-differentiated K562 CML cells.

Imatinib; activin A; erythroid differentiation; p38 MAPK; K562 chronic myeloid leukemia cells

CHRONIC MYELOID LEUKEMIA (CML) is a cancer of hematopoietic stem/progenitor cells caused primarily by the Bcr-Abl fusion protein, which constitutively active tyrosine kinase produced as a result of translocation of chromosomes 9 and 22 (16). Bcr-Abl blocks cell differentiation and protects cells from apoptosis to allow the proliferation of undifferentiated stem cells in the absence of growth factors (24). During the chronic phase, CML progenitor cells undergo excess proliferation; however, these cells maintain the capacity to differentiate and function normally. Eventually, CML progresses from the chronic phase to the blast crisis phase, in which differentiation becomes arrested and undifferentiated CML stem/progenitor cells accumulate in bone marrow and peripheral blood (13, 35).

Imatinib (STI-571, Gleevec), a specific inhibitor of Bcr-Abl tyrosine kinase, is a highly effective, first-line treatment for CML (13, 35). However, CML patients become resistant to imatinib treatment in the blast crisis phase (31, 40). In the blast crisis phase, CML stem/progenitor cells were found to be drug-insensitive, and imatinib failed to remove these cells (21, 23, 28, 29). These CML stem/progenitor cells may contribute to the pathogenesis of disease relapse and therapeutic resistance (21, 29). Therefore, differentiation induction may be a promising therapeutic approach to treat CML.

Activin A, a member of the transforming growth factor-β superfamily, is vital in a variety of biological functions, such as cell proliferation, apoptosis, differentiation, development, tissue repair, and inflammation (17, 34, 36, 42, 44). We and others previously reported that activin A induced the erythroid differentiation of CML cells (10, 25). In addition, our previous study showed that activin A induces erythroid differentiation of CML cells through activation of the MAPK kinase 6 (MKK6)-p38 MAPK pathway (27).

K562 is a hematopoietic stem/progenitor cell line established from a human CML patient in the blast crisis phase (33). K562 cells have the ability to proliferate to an unlimited degree and an inability to proceed with differentiation (19). Here, K562 cells were used as a model for the in vitro study of differentiation therapy to evaluate the interactive effects of activin A and imatinib on cell growth and apoptosis. Our results show that differentiated K562 cells induced by the activin A-mediated p38 MAPK pathway become more sensitive to a subtoxic concentration of imatinib.

MATERIALS AND METHODS

Reagents. Recombinant human activin A was purchased from R & D Systems (Minneapolis, MN) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Sigma (St. Louis, MO). Imatinib was kindly provided by Novartis Pharma (Basel, Switzerland). Antibodies for Western blotting, including procaspase-3, cleaved caspase-3, poly(ADP-ribose)polymerase (PARP), and Bcl-xL, were obtained from Cell Signaling Technology (Danvers, MA). Antibodies specific for c-Abl, Mcl-1, and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Glycophorin A (GPA)-FITC and IgG-FITC monoclonal antibodies were obtained from Dako.

Cell culture. The human CML cell line K562 was purchased from the Bioresource Collection and Research Center (Taiwan). Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine

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serum, 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin in a 5% CO₂ incubator at 37°C. K562/p38α(AF)1 and K562/p38β(AF)1 cells stably expressing dominant-negative (AF) forms of p38α and p38β, respectively, in K562 cells are described elsewhere (27). The medium used to maintain K562/p38α(AF)1 and K562/p38β(AF)1 cells was the same as that used to maintain parental K562 cells, except for inclusion of 200 μg/ml G418 in the medium.

**MTT assay.** Cells were seeded at a density of 1.25 × 10⁶ cells/ml and treated as shown in Fig. 1A. After 3 or 6 days of incubation, MTT (final concentration 0.5 mg/ml) was added to each well, and cells were incubated for 3–4 h. The MTT solution was removed from the wells by aspiration, and the formazan crystals were dissolved in DMSO. Absorbance was measured at 570 nm using a microplate reader (model 450, Bio-Rad Laboratories).

**Annexin V/propidium iodide staining and flow cytometry.** The level of cell apoptosis was measured by annexin V-FITC and propidium iodide (PI) staining, as previously described (26). Results are shown of cell apoptosis was measured by annexin V-FITC and propidium iodide (PI) staining, as previously described (26).

**Benzidine staining assay.** Erythroid differentiation was determined by hemoglobin synthesis in K562 cells using the benzidine staining assay, as previously described (26).

**Flow cytometric analysis and cell purification.** Cell surface expression of CD235α was analyzed by flow cytometry. Cells were mixed with FITC-conjugated anti-CD45 and FITC-conjugated IgG, incubated for 50 min at 4°C, and then washed three times in ice-cold PBS. Fluorescence intensity was analyzed by a FACScan flow cytometer (BD Biosciences, San Jose, CA) and CellQuest software (BD Biosciences), GPA-positive cells were purified on a FACSaria cell sorter (BD Biosciences). Purity of the populations for imatinib treatment was >95%.

**Western blot analysis.** Cells were lysed at 4°C in lysis buffer [1% Triton X-100, 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM PMSF, 1 μg/ml leupeptin, and 1 mM Na₃VO₄]. Protein lysates (30 μg) were resolved using SDS-PAGE, the protein bands were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA), and the membranes were probed with primary antibodies. After binding with horseradish peroxidase-conjugated secondary antibodies, the blots were visualized with an enhanced chemiluminescence detection system (ECL, PerkinElmer Life and Analytical Sciences, Waltham, MA).

**Knockdown of p38α and p38β by short hairpin RNAs.** K562 cells (1 × 10⁶) were mixed with 100 μl of Nucleofector solution V (Amaxa Biosystems) and 4 μg of SureSilencing short hairpin RNA (shRNA) plasmids. Transfection was carried out using the Nucleofector apparatus (Amaxa Biosystems) and the T-16 program. SureSilencing shRNA plasmids for human p38α (5'-CAAGGTCTCTGAGGAAGCT-3') and p38β (5'-CACTGAGATCATGCTCAACT-3') and a negative control shRNA plasmid (catalog no. KH01361) were purchased from SABioscience (Frederick, MD).

**RT, PCR, and quantitative PCR.** Total RNA was extracted with TRizol reagent (Invitrogen, Carlsbad, CA). cDNA was then synthesized using the SuperScript First-Strand Synthesis System (Invitrogen) with the primer oligo(dT)₁₈. The resulting cDNA samples were amplified by PCR or quantitative PCR using the following primers: p38α [5'-GTGCCCGAGCGGTTACCAGCCAC-3' (sense) and 5'-CTG- TAAGCTTCTGACTTCTC-3' (antisense)], p38β [5'-CACCGAGC- CCAAGTTCT-3' (sense) and 5'-AAATCCACACCCGAGG-3' (antisense)], and mult dr us resistance [MDR1; 5'-GGAGGAGAAGGT- GCTGCTGAGT-3' (sense) and 5'-CTCCTTTGGCCCTCA- CAATC-3' (antisense)]. β-Actin [5'-GCATCCCCAAAAATTCA- CAA-3' (sense) and 5'-AGGACTGGCGGACTTCTC-3' (antisense)] was used as the internal control. Quantitative PCR was performed using a QuantiFast SYBR Green PCR kit (Qiagen, Valencia, CA) on a Rotergene Q real-time PCR machine (Qiagen). Normalized expression of p38α or p38β was calculated relative to β-actin for all samples. The PCR products were electrophoresed on 2% agarose gels that were stained with ethidium bromide and photographed under ultraviolet light.

**Statistics.** Quantitative data are presented as means ± SE. Statistically significant differences between groups were analyzed with Student’s t-test. P < 0.05 was considered significant.

**RESULTS**

**Induction of erythroid differentiation by activin A sensitized K562 cells to a subtoxic concentration of imatinib.** K562 cells can be induced to differentiate toward an erythroid lineage after exposure to the differentiation inducers (3, 7, 9, 10, 25). To investigate whether activin A-induced differentiation can increase the sensitivity of K562 cells to imatinib, cells were treated under the following conditions: 1) sequential treatment with activin A and imatinib, 2) cotreatment with activin A and imatinib, 3) treatment with activin A alone, and 4) treatment with imatinib alone (Fig. 1A). In activin A-treated cells, the percentage of benzidine-positive cells increased significantly (25–30% positive cells at 72 h) for all the following experiments (data not shown) (10, 25). Treatment with imatinib only (200 nM) or activin A only (50 ng/ml) slightly inhibited cell viability (Fig. 1B). Imatinib alone slightly induced apoptosis, but activin A alone did not (Fig. 2, C and D). Simultaneous treatment with 50 ng/ml activin A and 200 nM imatinib reduced cell viability (72 ± 6.8%; Fig. 1B) and increased cell apoptosis.
activin A pretreatment increased sensitivity of K562 cells to imatinib.

Sequential treatment with activin A and imatinib decreased Bcr-Abl, procaspase-3, Mcl-1, and Bcl-XL and induced cleavage of procaspase-3/PARP. To resolve the mechanism for apoptosis induction by sequential treatment of K562 cells with activin A and imatinib, we analyzed the level of Bcr-Abl protein by Western blotting. No significant reduction in the protein level of Bcr-Abl was observed following treatment with activin A alone or imatinib alone compared with untreated control cells (Fig. 6A). In contrast, simultaneous treatment with 50 ng/ml activin A and 200 nM imatinib produced a mild increase in GPA-positive cells (GPA^+/activin A) was induced by activin A, and 58% of the GPA-positive cells (original GPA^+/activin A) were isolated from the activin A-treated group. As shown in Fig. 5, B and C, the apoptotic cell population increased from 6% in untreated control cells to 93% following 72 h of treatment with imatinib in GPA^+/activin A cells. The original GPA^-/control cells were not sensitive to imatinib; however, 56% of the original GPA^+/activin A cells were sensitive to imatinib (Fig. 5, B and C). These results suggest that the erythroid-differentiated cells (GPA^+/activin A) and some of the erythroid precursors (original GPA^+/activin A) were sensitive to imatinib after activin A treatment.

Sequential treatment with activin A and imatinib strongly induced apoptosis in a dose-dependent manner (Fig. 4A). Pre-treatment with activin A followed by imatinib strongly decreased cell viability (24.2 ± 5.8%; Fig. 1B) and strongly increased apoptosis (68.5 ± 5.7%; Fig. 2, C and D). These results suggest that erythroid-differentiated K562 cells are sensitive to a minimally toxic concentration of imatinib.

Although sequential treatment with 50 ng/ml activin A and imatinib (100–1,000 nM) increased induction of cell apoptosis in a dose-dependent manner, reaching a maximum at 500 nM imatinib (100–1,000 nM) increased induction of cell apoptosis (Fig. 3B). Activin A induced hemoglobin synthesis in a dose-dependent manner (Fig. 4A) but had no significant effect on the level of apoptosis (Fig. 4B). Pretreatment with 12.5–100 ng/ml activin A followed by 200 nM imatinib induced apoptosis in a dose-dependent manner (Fig. 4B). Together, these findings indicate that sequential treatment with activin A and a subtoxic concentration of imatinib resulted in dramatic growth inhibition and apoptosis induction in K562 cells.

In Fig. 4, activin A (50 ng/ml) induced a benzidine-positive response in only 30% of K562 cells, while imatinib subsequently induced apoptosis in 60% of cells. To explore whether, after activin A treatment, no further differentiated K562 cells were sensitive to imatinib, we used GPA antibody to isolate GPA-positive cells. GPA is a glycoprotein expressed on the cell surface of erythroid precursors and mature erythrocytes. K562 cells grown in the presence of activin A showed an increase in the surface expression of GPA erythroid marker at day 3 (Fig. 5A), and three types of cells were isolated. Original GPA-positive cells were isolated from the untreated control (original GPA^+/control) cells. Twenty-three percent of the increase in GPA-positive cells (GPA^+/activin A) was induced by activin A, and 58% of the GPA-positive cells (original GPA^+/activin A) were isolated from the activin A-treated group. As shown in Fig. 5, B and C, the apoptotic cell population increased from 6% in untreated control cells to 93% following 72 h of treatment with imatinib in GPA^+/activin A cells. The original GPA^-/control cells were not sensitive to imatinib; however, 56% of the original GPA^+/activin A cells were sensitive to imatinib (Fig. 5, B and C). These results suggest that the erythroid-differentiated cells (GPA^+/activin A) and some of the erythroid precursors (original GPA^+/activin A) were sensitive to imatinib after activin A treatment.

Sequential treatment with activin A and imatinib strongly induced apoptosis in K562 cells (1.25 × 10^6 cells/ml) were seeded in a 6-well plate (3 ml per well) and treated as described in Fig. 1A. Apoptotic cells were stained with annexin V-FITC and propidium iodide (PI) and analyzed by flow cytometry. Values are means ± SE from 4 experiments. *P < 0.05, **P < 0.01, ***P < 0.005 vs. (untreated) control. ###P < 0.005 vs. control. **P < 0.01, ***P < 0.005 vs. IM.

Fig. 1. Activin A pretreatment increased sensitivity of K562 cells to imatinib effects in a dose- and time-dependent manner. A: cells were treated as described in Fig. 1A with 0–1,000 nM imatinib to determine dose dependence. Values are means ± SE from 4 experiments. *P < 0.05, **P < 0.01, ***P < 0.005 vs. IM. #P < 0.05, ##P < 0.01. B: cells were treated for 1–3 days with imatinib as described in Fig. 1A to determine time dependence. Cells were stained with annexin V-FITC and PI and analyzed by flow cytometry. Values are means ± SE from 4 experiments. ***P < 0.005 vs. (untreated) control.
activin A-sensitized K562 CML cells to imatinib

response, and sequential treatment with activin A and imatinib resulted in a striking decrease in the protein level of Bcr-Abl. These sequential treatment events were accompanied by a marked decrease in the precursor form of caspase-3 (pro-caspase-3) and an increase in caspase-3 cleavage and PARP degradation (Fig. 6A). Although caspase-3 cleavage and PARP degradation were slightly affected by imatinib alone, they were mildly increased by simultaneous administration of activin A and imatinib. Since sequential treatment with activin A and imatinib significantly increased apoptosis and caspase-3 cleavage, we analyzed levels of the antiapoptotic proteins Mcl-1 and Bcl-xL in K562 cells. Simultaneous treatment with activin A and imatinib induced a small decrease in levels of the antiapoptotic proteins Mcl-1 and Bcl-xL, whereas sequential treatment with activin A and imatinib resulted in a further decrease in Mcl-1 and Bcl-xL levels (Fig. 6B). These results demonstrate that pretreatment of K562 cells with activin A followed by a subtoxic concentration of imatinib results in a marked decrease in the levels of Bcr-Abl and antiapoptotic proteins and an increase in the processing of caspase-3.

Activin A sensitized K562 cells to imatinib through the p38 MAPK pathway. Our previous studies showed that activin A induced erythroid differentiation through p38α and p38β (27). K562/p38α(AF)1 and K562/p38β(AF)1 clones stably expressing dominant-negative p38α and p38β, respectively, which we previously established and characterized (27), were used for our next study. To examine whether inhibition of the p38 MAPK pathway could rescue K562 cells from activin A-mediated sensitization to a subtoxic concentration of imatinib, K562/p38α(AF)1 and K562/p38β(AF)1 cells were subjected to activin A-mediated differentiation followed by imatinib treatment. Growth inhibition (Fig. 7A) and apoptosis induction (Fig. 7, B and C) were reduced in K562/p38α(AF)1 and K562/p38β(AF)1 cells sequentially treated with activin A and imatinib compared with K562/mock cells. Moreover, p38α or p38β knockdown was performed in K562 cells using shRNA plasmids. After transfection with shRNA plasmids for 3 days, the shRNAs successfully decreased p38α and p38β mRNA levels by ∼40–50% (Fig. 8A). Knockdown of p38α or p38β reduced activin A-induced erythroid differentiation compared with control shRNA cells (Fig. 8B). The shRNA-mediated knockdown of p38α and p38β significantly reduced growth inhibition (Fig. 8C) and apoptosis (Fig. 8D) induced by sequential treatment with activin A and imatinib. These results suggest that activin A/p38 MAPK pathway-mediated erythroid

Fig. 4. K562 cell sensitivity to imatinib was dependent on pretreatment dose of activin A. A: cells were grown in the absence or presence of 0–100 ng/ml activin A and stained with benzidine to determine hemoglobin synthesis at 72 h. Values are means ± SE from 4 experiments. **P < 0.01, ***P < 0.005 vs. (0 ng/ml) control. B: cells were treated with 0–100 ng/ml activin A as described in Fig. 1A. stained with annexin V-FITC and PI, and analyzed by flow cytometry. Values are means ± SE from 4 experiments. *P < 0.05, ***P < 0.005 vs. ActA.

Fig. 5. Purified glycophorin A (GPA)-positive cells from activin A-treated group were sensitive to imatinib. A: K562 cells were treated without (control) or with activin A (50 ng/ml) for 72 h, stained with FITC-conjugated IgG isotype control antibody (solid line) or FITC-conjugated anti-GPA antibody (dashed line), and analyzed by flow cytometry. B: original GPA-positive cells were isolated from untreated control (original GPA+/control). Original GPA-positive cells (original GPA+/activin A) and GPA intensity-increased cells (GPA+/activin A) were isolated from the activin A-treated group. Cells were then treated with 200 nM imatinib for 72 h. Apoptotic cells were stained with annexin V-FITC and PI and analyzed by flow cytometry. Values are means ± SE from 3 experiments. *P < 0.05 vs. control. #P < 0.05. C: flow cytometry data showing representative results from 1 of 3 independent experiments.
differentiation is required for efficient sensitization of K562 cells to imatinib. Previous studies revealed P-glycoprotein (MDR1) overexpression in imatinib-resistant K562 cells, suggesting the involvement of MDR1 in the development of resistance to imatinib (6, 43). We further explore whether activin A reduces MDR1 expression, resulting in sensitivity of K562 cells to imatinib. Our RT-PCR and real-time PCR results show that activin A alone can decrease the mRNA level of MDR1 compared with an untreated control (Fig. 9A). In addition, the level of MDR1 expression also decreased similarly in K562 cells treated sequentially or simultaneously with activin A and imatinib (Fig. 9A). The p38 MAPK inhibitor SB-203580 eliminated activin A-decreased MDR1 mRNA expression (Fig. 9B). These results suggest that activin A inhibited MDR1 expression through the p38 MAPK pathway, which may be partially involved in the sensitivity of K562 cells to imatinib.

**DISCUSSION**

In this report, we demonstrate that erythroid-differentiated K562 cells induced by activin A become more sensitive to a subtoxic concentration of imatinib, resulting in growth inhibition and apoptosis. These effects are associated with the decrease of Bcr-Abl, Mcl-1, and Bcl-xL and the induction of caspase-3 cleavage and PARP degradation.

In cancer, activin A inhibits cell proliferation and tumorigenicity and induces apoptosis in various human tumor cell types (8, 12). In addition, it has been reported that activin A blocks angiogenesis in neuroblastoma (37) and gastric cancer (20). In the present study, we showed that activin A induction of erythroid differentiation sensitized K562 cells to imatinib in sequential treatment. These results suggest that activin A is a potential anticancer agent for therapeutic intervention in these cancer cells via growth inhibition, apoptosis, and differentiation.

The primitive CML cells have been found to be drug-insensitive, and imatinib fails to eliminate these CML stem/progenitor cells (21). Cancer differentiation therapy provides an alternative treatment for these cells. Here, the CML cell line K562 was used as a model to evaluate the differentiation induction treatment scheme. It has been reported that K562 CML cells are not responsive to apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (18), but once the erythroid differentiation is induced, cells become sensitive to TRAIL-induced apoptosis (22). In addition, astro-
cytotic differentiation sensitizes C6 glioblastoma cells to interferon-γ or taxol-induced apoptosis in sequential treatment (14). Neuronal differentiation sensitizes SH-SY5Y neuroblastoma cells to flavonoids to increase the amount of apoptosis (15). The finding of the present study was similar to findings of previous studies. Taken together, these results suggest that differentiation induction has the potential to sensitize cancer stem/progenitor cells to anticancer agents.

In this study we demonstrated that sequential treatment with activin A and 200 nM imatinib induced cell death equal to that in K562 cells treated with 1,000 nM imatinib alone (Fig. 3A). It has been shown that 1,000 nM imatinib seems to be clinically relevant, and 1,000 nM imatinib has been previously shown to induce the growth inhibition and apoptosis of Bcr-Abl-positive cells (20). Thus imatinib concentrations could be reduced fivefold when cells are pretreated with activin A.

K562 cells are erythroid precursors that can be stained with the erythroid precursor marker GPA. We found that treatment with imatinib strongly increased apoptosis by 56% and 93% in 58% of original GPA-positive cells (original GPA+/activin A) and 23% of GPA intensity-increased cells (GPA+/activin A). These results show that sequential treatment with activin A and imatinib induced apoptosis in 32.48% (58 × 56%) of original GPA+/activin A cells and 21.39% (23 × 93%) of GPA+/activin A cells. This could explain why the percentage of apoptotic cells induced by sequential treatment with activin A and imatinib was higher than the percentage of benzidine-positive cells stimulated by activin A pretreatment.

An effective strategy to reduce Bcr-Abl activity may be inhibition of the Bcr-Abl expression level (5, 11, 38). Our studies have demonstrated that sequential treatment with activin A and imatinib decreases the protein level of Bcr-Abl and induces apoptosis. These findings imply that a decrease in Bcr-Abl may be involved in the inhibitory effect of sequential treatment with activin A and imatinib in K562 cells. In addition to decreasing Bcr-Abl, sequential treatment with activin A and imatinib effectively decreased Mcl-1 and Bcl-xL levels in K562 cells. Mcl-1 and Bcl-xL are antiapoptotic members of the Bcl-2 family. Mcl-1 has been identified as a Bcr-Abl-dependent survival factor in CML cells (2, 32), and its upregulation has been shown to play an important role in resistance to apoptosis (4). Other studies reported that downregulation of Bcl-xL is induced in apoptosis of K562 cells (41). Thus cytotoxicity of K562 cells induced by sequential treatment with activin A and imatinib is associated with the decrease of
Bcr-Abl, Mcl-1, and Bcl-xL. Moreover, the decrease of procaspase-3 and the cleavage of procaspase-3/PARP indicated caspase-3 processing during apoptosis induced by sequential treatment with activin A and imatinib in K562 cells.

Previous studies demonstrated that the p38 MAPK signal pathway is important for the induction of erythroid differentiation in CML cells (10, 25, 27). Our previous results showed that inhibition of the p38 MAPK isoforms p38α and p38β by overexpression of p38α and p38β dominant-negative mutants significantly inhibited activin A-induced erythroid differentiation (27). The studies presented here further clarify the role of p38α or p38β in K562 cell sensitivity to imatinib. We inhibited p38α or p38β kinase activity (data not shown) using dominant-negative mutants and knocked down p38α or p38β expression with shRNAs to decrease activin A-mediated erythroid differentiation and found a reduction of sequential treatment-mediated growth inhibition and apoptosis induction in K562 cells. The results suggest that the loss of differentiation may be reduced to sensitization of K562 cells to imatinib effects. Erythroid differentiation mediated by each of the p38 MAPK isoforms, p38α and p38β, contributed to the cytotoxicity mediated by sequential treatment with activin A and imatinib, but each isoform had only a partial effect. Previous studies revealed high levels of MDR1 in imatinib-resistant K562 cells (6, 43). It might be that the sensitivity of K562 cells to imatinib is linked to the lower level of MDR1. Comparison between treatment with activin A alone, sequential treatment with activin A and imatinib, and simultaneous treatment with activin A and imatinib showed the same inhibition of the MDR1 expression. However, sequential treatment with activin A and imatinib induced a higher percentage of apoptotic cells than simultaneous treatment with activin A and imatinib. The implication is that the MDR1 expression inhibited by activin A is associated with the sensitivity to imatinib in K562 cells treated simultaneously with activin A and imatinib but is only partially effective in cells treated sequentially with activin A and imatinib. In addition, activin A inhibited MDR1 expression and induced erythroid differentiation through the same pathway, p38 MAPK. Thus further study is needed to determine whether other genes induced by activin A/p38 MAPK during erythroid differentiation can enhance the sensitivity of K562 cells to imatinib.

Clinical treatment has focused on differentiation therapy for the treatment of acute promyelocytic leukemia. Treatment with all-trans-retinoic acid (a differentiation-inducing agent) and arsenic trioxide (an antitumour drug) can effectively induce the differentiation and apoptosis of leukemic stem/progenitor cells and reduce disease recurrence in acute promyelocytic leukemia patients (39). Moreover, bryostatin 1 induced the differentiation of chronic lymphocytic leukemia cells, which were then treated with the purine analog 2-chlorodeoxyadenosine, resulting in cell apoptosis and disease remission in a chronic lymphocytic leukemia patient (1). The successful clinical application of differentiation therapy implies that sequential treatment with activin A and imatinib can be used in CML. The present study suggests that this sequential treatment might be used as a research tool and in clinical application to eradicate the pool of CML stem cells in human patients.

In conclusion, activin A induction of erythroid differentiation sensitizes K562 CML cells to imatinib. Our results point to a potential route of action by which differentiation induction might have an impact on increasing a subtoxic concentration of imatinib sensitivity for treatment of CML cells using differentiation therapy approaches.

REFERENCES
ACTIVIN A SENSITIZES K562 CML CELLS TO IMATINIB


