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c-Jun Blocks Cell Differentiation but not Growth Inhibition or Apoptosis of Chronic Myelogenous Leukemia Cells Induced by STI571 and by Histone Deacetylase Inhibitors

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The constitutively active Bcr-Abl tyrosine kinase plays a crucial role in chronic myelogenous leukemia (CML) pathogenesis. The Bcr-Abl protein induces the upregulation of proto-oncogene c-Jun, which is involved in Bcr-Abl transforming activity in Bcr-Abl positive cells. Recent studies reported that c-Jun inhibited hemoglobin synthesis in human CML cell line K562. However, c-Jun also plays a critical role in cell proliferation and apoptosis. In this study, we investigated the physiological roles of c-Jun in cell proliferation, apoptosis and erythroid differentiation of K562 cells. Firstly, we generated K562 cell lines stably overexpressing c-Jun. These clones have the same proliferation rate as the parental cell line in general culture medium. Endogenous c-Jun expression was analyzed to determine the effective concentration of STI571 for inhibiting Bcr-Abl signaling. Western blots show that STI571 inhibited c-Jun expression in a dose-dependent manner, reaching a maximum inhibition at 1 μM. STI571 could inhibit c-Jun expression in K562 cells, but not in c-Jun-overexpression cells. c-Jun did not alter growth inhibition and apoptotic induction by STI571 treatment, but inhibited STI571-induced erythroid differentiation. Moreover, c-Jun did not alter growth inhibition and apoptotic induction by histone deacetylase (HDAC) inhibitors (apicidin, sodium butyrate, and MS275) treatment, but inhibited HDAC inhibitors-induced erythroid differentiation. These results suggest that c-Jun may modulate anticancer drugs-induced cell differentiation but not growth inhibition and apoptosis in CML cells.

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Chronic myelogenous leukemia (CML) is a cancer of the hematopoietic stem/progenitor cells caused by reciprocal translocation of chromosomes 9 and 21 in human, resulting in the formation of the Philadelphia (Ph) chromosome (Nowell and Hungerford, 1960; Rowley, 1973). The Ph chromosome produces an oncogenic Bcr-Abl fusion protein, which is a constitutive active tyrosine kinase and underlies the development of most CML cases (Shtivelman et al., 1986; Ben-Neriah et al., 1986). Bcr-Abl signaling allows cells to proliferate in the absence of growth factors, protects cells from apoptosis in the absence of external survival factors, and may block cell differentiation (Era, 2002; Steelman et al., 2004; Jagani et al., 2008). STI571 is a specific inhibitor of Abl tyrosine kinase (Buchdunger et al., 1996) that inhibits cell proliferation and induces apoptosis of Bcr-Abl-positive cells (Druker et al., 1996; Carroll et al., 1997). Significantly, treatment with STI571 has been proven to be effective for CML patients (Druker et al., 2001). It has been reported that STI571 induces the hemoglobin synthesis of human CML cell line K562 (Druker et al., 1996; Kohmura et al., 2004). The histone deacetylase (HDAC) inhibitors, apicidin, sodium butyrate and MS275, were also reported to induce erythroid differentiation and growth inhibition of K562 cells (Witt et al., 2003; Chen et al., 2008).

The proto-oncogene c-Jun is a major component of activating protein-1 (AP-1) transcription factor that forms homo- or hetero-dimers with other AP-1 family members. c-Jun is involved in many cellular processes including cell proliferation, apoptosis, differentiation and cell transformation (Karin et al., 1997). c-Jun is a downstream target gene of Bcr-Abl signaling and plays an important role in Bcr-Abl-mediated cell transformation (Raitano et al., 1995) and differentiation (Druker et al., 1996; Kohmura et al., 2004). However, the involvement of c-Jun in cell proliferation and in apoptosis of CML cells is not clear. In the present study, we have examined the roles of c-Jun in STI571-induced and HDAC inhibitors-induced growth inhibition, apoptotic induction and cell differentiation of K562 cells.

Materials and Methods

Cell culture, reagent, and antibodies

The human CML cell line K562 was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 4 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. STI571 was kindly provided by Novartis Pharma AG (Basel, Switzerland). Apicidin and MS275 were purchased from Calbiochem (La Jolla, CA). Sodium butyrate and benzidine were purchased from Sigma-Aldrich Co. (St. Louis, MO). Anti-c-Jun antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-α-tubulin monoclonal antibody, anti-mouse and anti-rabbit IgG-horseradish peroxidase (HRP) conjugated

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secondary antibodies were purchased from Amersham Pharmacia Biotech, Inc. (San Francisco, CA).

Transfection of K562 cells

For the establishment of stable K562 cell lines overexpressing c-Jun, K562 cells (2×10^6 cells) were cotransfected with plasmids pCMV-c-Jun (1.5 μg) (Alani et al., 1991; Brown et al., 1992) and pcDNA3 (0.5 μg, Invitrogen) using lipofectamine reagent (Invitrogen) according to the manufacturer's instructions. Because pCMV-c-Jun does not carry neomycin resistant gene, pcDNA3 was used as a G418 selection vector. Twenty-four hours after transfection, cells were diluted to about 0.5 cell/well in 96-well plates and selected with 800 μg/ml G418. Multiple monoclonal cultures were screened for c-Jun expression by Western blot analysis. pCMV-c-Jun was kindly provided by Dr. Michael J. Birrer (Alani et al., 1991; Brown et al., 1992).

For the luciferase reporter assay, transient transfections of K562 cells and stable clones (1×10^6 cells) were also performed by lipofection. Reporter plasmids containing pHS40-α590 Luc or pHS40-ζ597 Luc (1 μg, Huang et al., 2006) and pRL-TK internal control vector (0.05 μg) were cotransfected with or without pCMV-c-Jun (1 μg), pCMV-TAM-67 (1 μg) or its control vector (1 μg). For STI571 treatment, 1 μM STI571 was added to the culture medium at 5 h post-transfection. The cells were then incubated for 24 h then harvested. The luciferase activities were measured by using the Dual Luciferase Reporter Assay System (Promega, Madison, WI). The luciferase activity was adjusted for transfection efficiency by normalizing firefly luciferase activity to the renilla luciferase activity generated by pRL-TK (Promega). Human c-Jun mutant cDNA had been generated by deletion of the transactivation domain (transactivation domain mutant TAM-67, Grant et al., 1996). pCMV-TAM-67 was kindly provided by Dr. Michael J. Birrer (Grant et al., 1996).

Western blot analysis

Total cell extracts were prepared as described (Huang et al., 2006). Protein lysate (50 μg) was resolved using SDS–polyacrylamide gel electrophoresis (PAGE), transferred to PVDF membranes (Millipore, Bedford, MA), then probed with primary antibodies. After binding with HRP-conjugated secondary antibodies, the blots were visualized with an enhanced chemiluminescence (ECL) detection system (PerkinElmer Life and Analytical Sciences, Waltham, MA). The protein expression levels were quantified with the Quantity One software (Bio-Rad Laboratories, Hercules, CA).

Trypan blue exclusion assay

Cells were cultured at a starting density of 1×10^5 cells/ml and treated with or without 1 μM STI571, 0.1 μM apicidin, 0.3 mM sodium butyrate or 0.1 μM MS275. Viable cells were stained with 0.2% trypan blue (Sigma-Aldrich Co.). The numbers of viable cells were counted under microscope at different time points.

Analysis of DNA fragmentation

The DNA fragmentation was analyzed as previously described (Huang et al., 2005). In brief, 1×10^6 cells were cultured for 72 h in the indicated medium, then washed, resuspended in 50 μl of Williams lysis buffer (50 mM Tris–HCl, pH 8.0, 10 mM EDTA, 0.5% Sarkosyl, and 500 μg/ml proteinase K), and incubated at 50°C for 3 h. The samples were incubated for 1 h at 37°C after addition of 10 μl of RNase A (2 mg/ml). After addition of 1 μl of ethidium bromide (10 mg/ml), the samples were extracted with an equal volume of phenol/chloroform (1:1), and stored at 4°C after the addition of 10 μl of 1% low melting agarose solution containing 10 mM EDTA (pH 8.0). Samples were melted at 70°C and allowed to solidify inside the wells of agarose gel before electrophoresis was initiated.

Annexin V/propidium iodide (PI) staining and flow cytometry

The level of cell apoptosis was measured by Annexin V-FITC and PI staining. Cells were cultured in the indicated medium for 72 h, collected by centrifugation, and washed with PBS. The cells were stained with Annexin V-FITC and PI (Apoptosis Kit, MBL Medical and Biological Laboratories, Nagoya, Japan) and incubated for 15 min at room temperature in the dark. Samples were acquired on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA) and analyzed with Cellquest software (Becton Dickinson); 10,000 cells were analyzed. Results are shown as the percentage of early apoptotic cells (Annexin V^+ PI^-) and late apoptotic cells (Annexin V^+ PI^+).

Benzidine staining assay

Cell differentiation was determined by hemoglobin synthesis in K562 cells using the benzidine staining assay as previously described (Huang et al., 2004). Cells were cultured in the indicated medium at a density of 1×10^5 cells/ml for 72 h. Cells were suspended in a staining solution of 49:1 ratio of benzidine solution (0.2% benzidine in 0.5% acetic acid) to 30% H_2O_2, and then subjected to cytospin centrifugation after 10 min of incubation at room temperature. The black benzidine-stained hemoglobin-positive cells were determined microscopically.

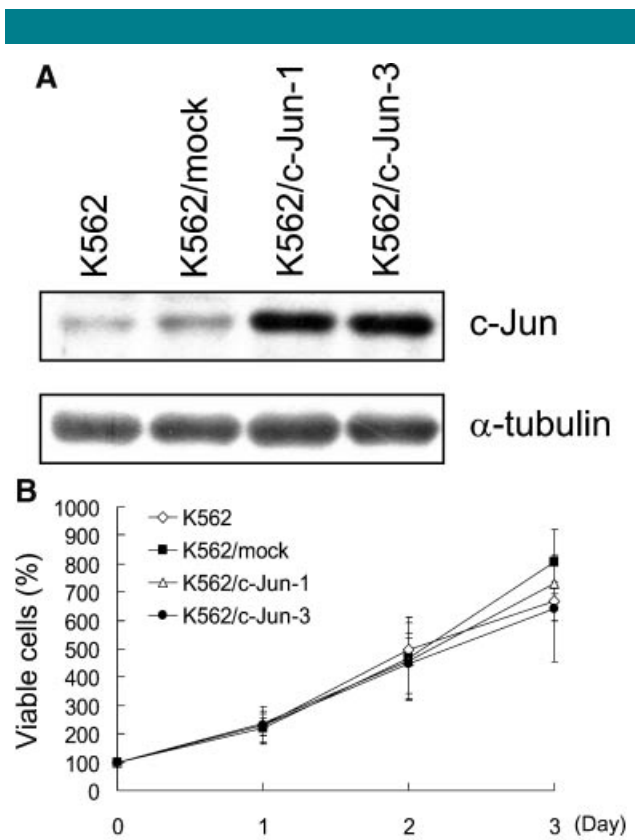

Fig. 1. c-Jun overexpression did not affect cell proliferation of K562 cells. A: c-Jun was overexpressed in stable clones. K562 cells were transfected with empty vector or c-Jun cDNA, followed by G418 selection. Single clones were then picked and maintained in the culture medium containing 200 μg/ml of G418 for c-Jun expression by Western blot. α-tubulin was used as loading control. B: K562, K562/mock, K562/c-Jun-1 and K562/c-Jun-3 cells were maintained in general culture medium. Viable cells were determined by trypan blue exclusion assay every day, cell numbers were normalized to day 0 (100%). Values are mean ± SEM from four experiments.

Statistics

Qualitative data are presented as the mean and the standard error of the mean (SEM). Statistically significant differences between groups were analyzed with Student's *t*-test. A *P*-value of <0.05 was considered significant.

Results

c-Jun overexpression did not alter cell proliferation of K562 cells

To investigate the role of c-Jun in CML cells, we generated stable clones overexpressing c-Jun in K562 cells (Fig. 1A). c-Jun protein level in the stable clones were analyzed by Western blot. The results showed that the c-Jun stable clones (K562/c-Jun-1 and K562/c-Jun-3) had four- to fivefold higher c-Jun expression compared with parental K562 cells and empty vector-transfected cells (K562/mock) (Fig. 1A). The influence of c-Jun on cell proliferation of K562 cells was studied. Ectopic expression of c-Jun did not alter cell proliferation under general culture condition, as measured by trypan blue exclusion assay (Fig. 1B).

c-Jun overexpression did not alter STI571-induced growth inhibition and apoptosis

To determine the effective concentration of STI571 for inhibiting c-Jun expression in K562 cells, cells were exposed to STI571 at various concentrations for 72 h, and then cell extracts were subjected to Western blot analysis. As shown in Figure 2A, STI571 acted in a dose-dependent manner to inhibit the protein expression of c-Jun, reaching maximum inhibition at 1 μM (left part). The 1 μM STI571 treatment did not inhibit the protein expression of c-Jun in K562/c-Jun-1 and K562/c-Jun-3 cells compared with K562/mock cells (right part). We used 1 μM STI571 to perform the subsequent experiments; this concentration also seems to be clinically relevant and has been previously shown to inhibit growth of Bcr-Abl-positive cells (Druker et al., 2001). To study the roles of c-Jun in STI571-mediated growth inhibition, cells were cultured in 1 μM

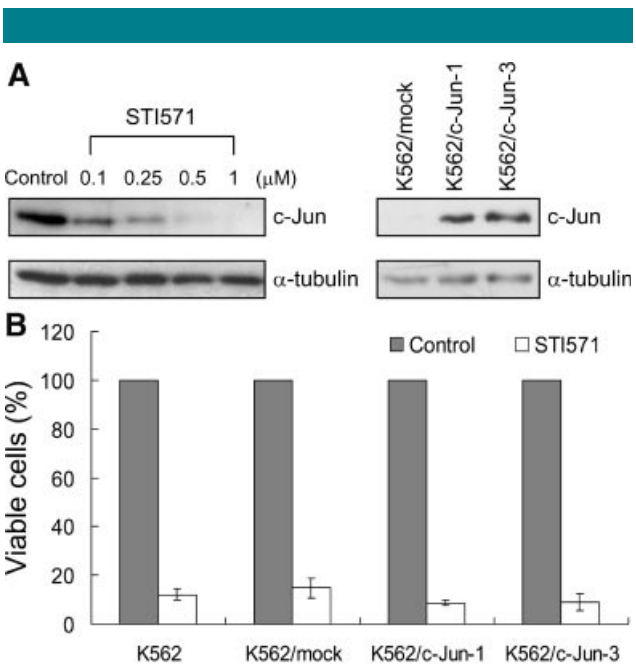

Fig. 2. Effect of c-Jun on STI571-induced growth inhibition. A: K562 cells were treated without (control) or with STI571 at various concentrations for 72 h (left part). K562/mock, K562/c-Jun-1, and K562/c-Jun-3 cells were treated with 1 μM STI571 for 72 h (right part). Prepared cell lysates were subjected to Western blot analysis for the c-Jun using anti-c-Jun antibody, and then reprobed with anti-α-tubulin antibody as loading controls. B: K562, K562/mock, K562/c-Jun-1, and K562/c-Jun-3 cells were treated without (control) or with 1 μM STI571 for 72 h. Viable cells were determined by trypan blue exclusion assay, cell numbers were normalized to control (100%). Values are mean ± SEM from four experiments.

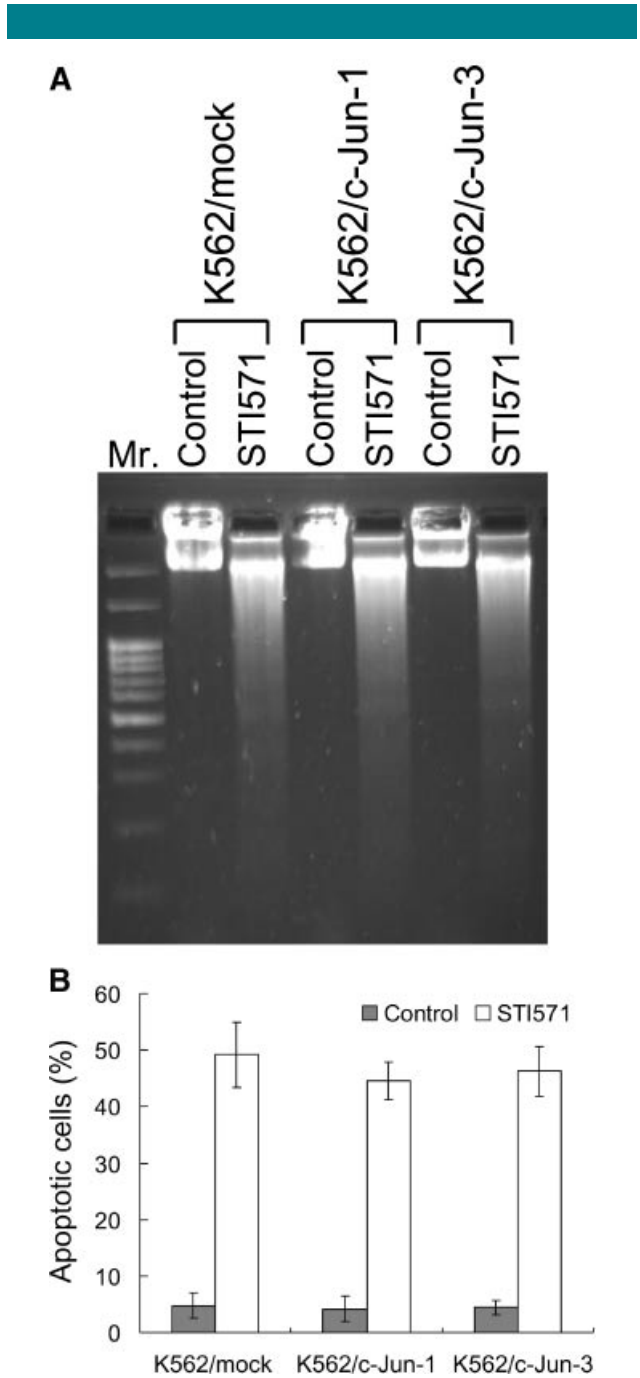

Fig. 3. Effect of c-Jun on STI571-induced cell apoptosis. A: K562/mock, K562/c-Jun-1, and K562/c-Jun-3 cells were treated without (control) or with 1 μM STI571 for 72 h. DNA fragmentation was examined by gel electrophoresis. B: K562/mock, K562/c-Jun-1, and K562/c-Jun-3 cells were treated without (control) or with 1 μM STI571 for 72 h. Cells were stained with annexin V-FITC and PI and analyzed by flow cytometry. Values are mean ± SEM from four experiments.

STI571 for 72 h. The cell viability of K562, K562/mock, K562/c-Jun-1 and K562/c-Jun-3 cells were inhibited by STI571, indicating that c-Jun overexpression does not affect STI571 inhibition of cell proliferation (Fig. 2B).

It has been reported that c-Jun overexpression may induce or inhibit apoptosis by different apoptotic stimuli in murine erythroleukemia cells (Poindessous-Jazat et al., 2002). We next examined whether c-Jun overexpression is involved in STI571-induced CML cell apoptosis. As shown in Figure 3A, DNA fragmentation of K562, K562/mock, K562/c-Jun-1, and K562/c-Jun-3 cells were observed after 72 h incubation with STI571. The rate of apoptosis was quantified by flow cytometry after staining with annexin V and PI. Exposure of these cells to STI571 resulted in increased number (percentage) of apoptotic cells, the ectopic expression of c-Jun failed to induce or inhibit apoptosis induced by STI571 (Fig. 3B). These results indicated that c-Jun overexpression did not alter STI571-induced apoptosis.

c-Jun blocked STI571-induced erythroid differentiation

Several studies have demonstrated that c-Jun may regulate the differentiation of hematopoietic progenitor cells (Gaynor et al., 1991; Rosson and O'Brien, 1998). STI571 has been reported to induce hemoglobin synthesis in K562 cells (Druker et al., 1996). We investigated the erythroid differentiation-inducing effect of STI571 on K562, K562/mock, K562/c-Jun-1 and K562/c-Jun-3 cells; benzidine staining was used after 72 h of incubation with the drug. Benzidine-positive cells induced under STI571 treatment were referenced to benzidine-positive cells under untreated control (normalized as 1). STI571 induced the appearance of about 9- to 10-fold benzidine-positive K562 and K562/mock cells versus untreated control. c-Jun overexpression resulted in about 3.5-fold increase of benzidine-positive K562/c-Jun-1 and K562/c-Jun-3 cells versus untreated control (Fig. 4A,B). These results show that K562/c-Jun-1 and K562/c-Jun-3 cells expressed lower levels of benzidine-positive cells than those of K562 and K562/mock cells, indicating that c-Jun blocked STI571-induced hemoglobin synthesis.

c-Jun inhibited STI571-induced activation of α-globin and ζ-globin promoters

To study the effect of c-Jun on STI571-induced erythroid differentiation, the erythroid genes, α-globin and ζ-globin, were analyzed. To examine the transcriptional activity of α-globin and ζ-globin genes, we used transient transfection of K562 cells with reporter constructs expressing the luciferase gene under the control of α-globin or ζ-globin promoters, pHS40-α590Luc or pHS40-ζ597Luc (Huang et al., 2006). The relative luciferase activities of the reporters activated under STI571 treatment were referenced to the reporter activity under untreated control (normalized as 1). Results in Figure 5A,B show that a 24-h exposure to STI571 significantly induced promoter activities of α-globin and ζ-globin genes in K562 and K562/mock cells. c-Jun overexpression in K562/c-Jun-1 and K562/c-Jun-3 cells resulted in the inhibition of promoter activities of α-globin and ζ-globin genes by STI571 compared with K562 and K562/mock cells ($P < 0.05$) (Fig. 5A,B). Additionally, transient cotransfection analysis shows that the inhibition of c-Jun transcriptional activity by c-Jun dominant-negative mutant, TAM-67, resulted in the enhancement of promoter activities of α-globin and ζ-globin genes in K562 cells by STI571 compared with vector transfected cells (data not shown). These results suggest that c-Jun is involved in blocking STI571-induced promoter activation of α-globin and ζ-globin genes.

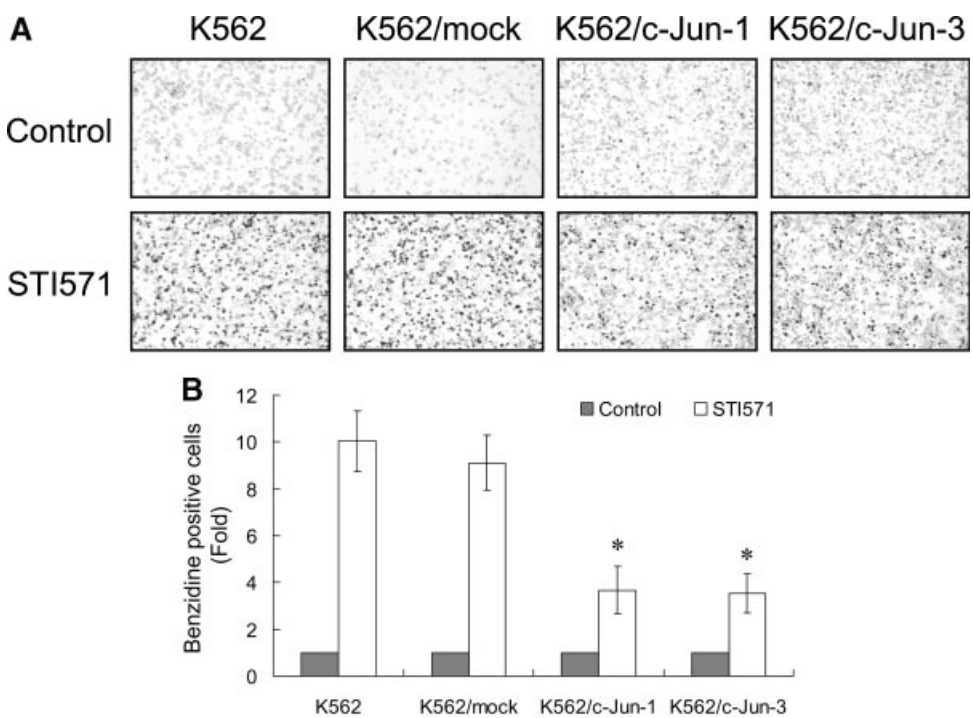

Fig. 4. Effect of c-Jun on STI571-induced erythroid differentiation. A: K562, K562/mock, K562/c-Jun-1 and K562/c-Jun-3 cells were treated without (control) or with 1 μM STI571 for 72 h. Intracellular hemoglobin was detected by benzidine staining. The black benzidine-stained hemoglobin-positive cells were determined microscopically. B: The percentage of benzidine-positive cells is shown. The values were expressed relative to the control value (normalized as 1). Values are mean ± SEM from four experiments. *$P < 0.05$ comparing K562/c-Jun-1 + STI571 or K562/c-Jun-3 + STI571 to K562 + STI571 or K562/mock + STI571.

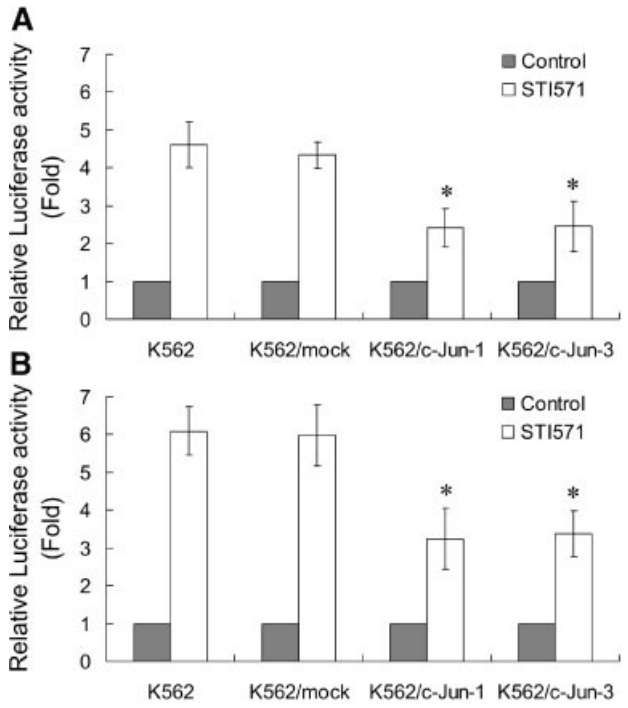

Fig. 5. Effects of c-Jun on STI571-induced promoter activation of the erythroid genes. A: K562, K562/mock, K562/c-Jun-1, and K562/c-Jun-3 cells were transfected with plasmid pHS40-α590 Luc. B: K562, K562/mock, K562/c-Jun-1, and K562/c-Jun-3 cells were transfected with plasmid pHS40-ζ597 Luc. pRL-TK plasmid was included in each transfection as an internal control of transfection. After 5 h of transfection, cells were subsequently treated without (control) or with 1 μM STI571. Luciferase activity was measured 24-h after STI571 addition and was normalized to RL expression. The values were expressed relative to the activity of the control value (normalized as 1). Values are mean ± SEM from four experiments. *$P < 0.05$ comparing K562/c-Jun-1 + STI571 or K562/c-Jun-3 + STI571 to K562 + STI571 or K562/mock + STI571 (A,B).

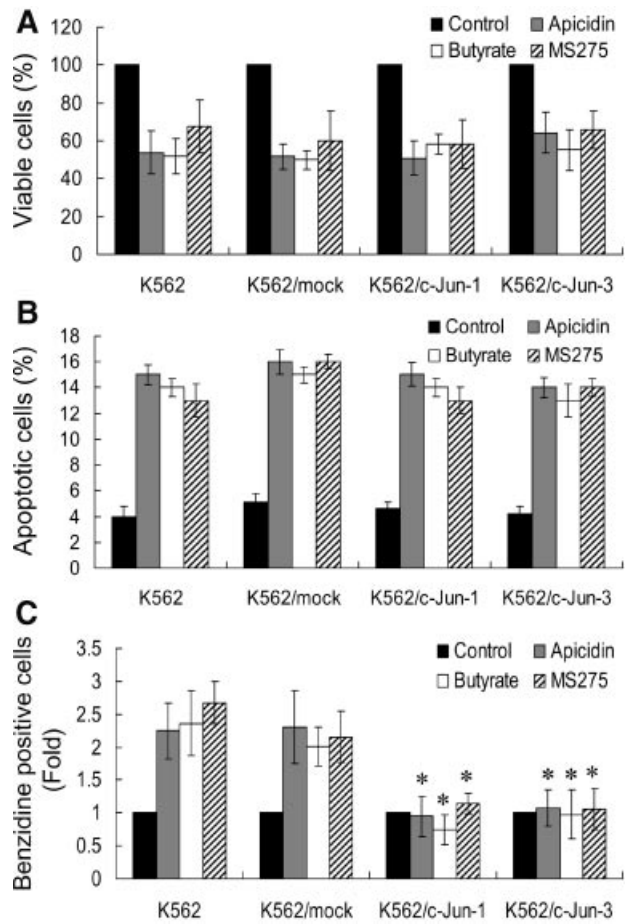

Fig. 6. Effect of c-Jun on HDAC inhibitors-induced growth inhibition, apoptosis and cell differentiation. K562, K562/mock, K562/c-Jun-1, and K562/c-Jun-3 cells were treated without (control) or with 0.1 μM apicidin, 0.3 mM sodium butyrate (butyrate) or 0.1 μM MS275 for 72 h. A: Viable cells were determined by trypan blue exclusion assay, cell numbers were normalized to control (100%). B: Cells were stained with annexin V-FITC and PI and analyzed by flow cytometry. C: Intracellular hemoglobin was detected by benzidine staining. The values were expressed relative to the control value (normalized as 1). Values are mean ± SEM from four experiments. *$P < 0.05$ comparing K562/c-Jun-1 + HDAC inhibitor or K562/c-Jun-3 + HDAC inhibitor to K562 + HDAC inhibitor or K562/mock + HDAC inhibitor.

c-Jun blocked HDAC inhibitors-induced erythroid differentiation but not growth inhibition and apoptosis

Our and other studies have demonstrated that HDAC inhibitors, apicidin, sodium butyrate and MS275, could induced growth inhibition and hemoglobin synthesis in K562 cells (Witt et al., 2003; Chen et al., 2008). To examine the roles of c-Jun in HDAC inhibitors-modulated cell proliferation, apoptosis and differentiation of K562 cells, K562, K562/mock, K562/c-Jun-1, and K562/c-Jun-3 cells were treated with apicidin, sodium butyrate or MS275 for 72 h. Results in Figure 6 show that c-Jun overexpression did not alter HDAC inhibitors-induced growth inhibition and cell apoptosis (Fig. 6A,B). The relative benzidine-positive cells induced under HDAC inhibitor treatments were referenced to the benzidine-positive cells under untreated control (normalized as 1). These results show that c-Jun overexpression significantly blocked HDAC inhibitors-induced hemoglobin synthesis (Fig. 6C).

Discussion

In this study, we seek to clarify the role played by c-Jun overexpression in cell proliferation, apoptosis and differentiation of CML cells. We show that c-Jun may block STI571-induced and HDAC inhibitors-induced erythroid differentiation but not growth inhibition and apoptosis of K562 cells.

The process of differentiation of progenitor cells into more specialized cells involves inhibiting their proliferative capacity. We found that c-Jun overexpression blocked erythroid differentiation but did not simultaneously interrupt growth inhibition caused by STI571 or HDAC inhibitors, suggesting that cell proliferation and differentiation of CML cells can be separately modulated. Consistent with these findings, c-Jun blocked hemoglobin synthesis of DMSO-treated murine erythroleukemia cells but not growth inhibition (Francastel et al., 1994; Poindessous-Jazat et al., 2002). Of interest, STI571 induces not only erythroid differentiation but also myeloid differentiation in K562 cells (Kohmura et al., 2004). It will be interesting to know whether c-Jun can inhibit myeloid differentiation in CML cells. In addition to acting as an inhibitor of erythroid differentiation, c-Jun also acts as an inducer of differentiation of hematopoietic progenitor cells. Notably, c-Jun level increases during macrophage differentiation of

HL-60 cells (Gaynor et al., 1991), and during monocytic differentiation of M1 cells (Lord et al., 1993); furthermore, exogenous c-Jun induces partial monocytic differentiation in M1 and U937 cells (Lord et al., 1993; Szabo et al., 1994). A recent report (Choi et al., 2004) showed that c-Jun induces monocytic differentiation by orphan nuclear receptor SHP. c-Jun inhibits erythroid differentiation through HERP2 to repress key erythroid transcription factor GATA-1 (Elagib et al., 2004). These results suggest that c-Jun transcription factor regulates the different target genes to modulate the cell differentiation of specific hematopoietic lineages. In addition to HERP2, the other downstream target genes of c-Jun will be screened and studied further to decipher how c-Jun inhibits the erythroid differentiation of hematopoietic progenitor cells.

c-Jun has been shown to be not necessary for apoptosis to occur in a variety of cell types in vivo (Roffler-Tarlov et al., 1996; Herzog et al., 1999). We observed similar results and showed that STI571 and HDAC inhibitors induced apoptosis of K562 cells but that this induction could not be increased or decreased by c-Jun overexpression. On the other hands, several evidences implicate c-Jun as an apoptotic inducer of leukemia cells (Qian et al., 1997; Kondo et al., 2000; Poindessous-Jazat et al., 2002). For example, the studies by Poindessous-Jazat et al. (2002) revealed that c-Jun overexpression induced and c-Jun knockdown with antisense construct inhibited anticancer drugs-induced apoptosis in murine erythroleukemia cells. Contrary to those observations, Poindessous-Jazat et al. (2002) also showed that c-Jun overexpression inhibited serum deprivation-induced apoptosis in these cells. In addition, the studies of apoptosis induced in other cell types have implicated c-Jun protects cells against serum deprivation-induced or anticancer drug-induced cell death (Huang et al., 2000; Duan et al., 2007). These results suggest that c-Jun does not affect apoptosis or may function to modulate apoptosis either positively or negatively, depending on the microenvironment and the cell type.

STI571 is an effective therapy for Bcr-Abl-positive leukemia, but many patients are resistant to STI571 treatment. The major mechanisms of STI571 resistance are either by increased expression of the Bcr-Abl protein through gene amplification (Gorre et al., 2001) or by Bcr-Abl mutation, especially mutation sites located on ATP binding site where STI571 compete for binding (Shah et al., 2002; Branford et al., 2003). Hence, Bcr-Abl still upregulate and activate c-Jun in STI571-resistant CML cells treated with STI571. The K562 cells was sensitive to STI571, and its growth inhibition was accompanied by the down-regulation of c-Jun expression (Fig. 2) that provided for understanding the role of c-Jun in CML cells with STI571 treatment. When overexpressed in K562 cells, c-Jun has been determined to be an inhibitor of the erythroid differentiation, including inhibition of hemoglobin synthesis and erythroid gene promoter activities in STI571 treatment, whereas STI571-induced growth inhibition and apoptosis were not influenced. These results probably reflect the fact that c-Jun plays a role in both cell transformation (Raitano et al., 1995) and differentiation arrest in CML cells, including STI571-resistant CML cells.

Histone deacetylase (HDAC) inhibitors cause cell differentiation, cell-cycle arrest, and apopotic effects on different cells and are being explored for use as anticancer drugs. Previous studies showed that different structural classes of HDAC-inhibitors (apicidin, sodium butyrate and MS275) exhibited erythroid differentiation inducing activity and growth inhibition in K562 cells (Witt et al., 2003; Chen et al., 2008). We also studied the effects of these inhibitors on c-Jun overexpressing K562 cells and found that c-Jun inhibited HDAC inhibitors-induced erythroid differentiation but not growth inhibition and apoptosis. These results are consistence with studies using STI571 treatment. HDAC inhibitors acted as inducers of acetylation of histones, which is different from the actions of STI571. However, apicidin and sodium butyrate have been reported to downregulate the expression of Bcr-Abl (Nishimura et al., 1988; Cheong et al., 2003). So far, we do not know whether the MS275 also downregulate Bcr-Abl. In addition, our and other studies have demonstrated that STI571 and HDAC inhibitors (apicidin, sodium butyrate and MS275) induce erythroid differentiation and growth inhibition in K562 cells by deactivating ERK pathway and activating p38 MAPK pathway (Kohmura et al., 2004; Chen et al., 2008). Thus, that is why STI571 and HDAC inhibitors have the similar effect on CML K562 cells.

In summary, the physiological function of c-Jun is to block cell differentiation but do not affect cell proliferation and apoptosis in CML cells. Thus, our findings raise the possibility that differentiation arrest, one of c-Jun functions, may play an important role in CML progression.

Acknowledgments

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Literature Cited

Alani R, Brown P, Binétruy B, Dosaka H, Rosenberg R, Angel P, Karin M, Birrer MJ. 1991. The transactivating domain of the c-Jun proto-oncoprotein is required for cotransformation of rat embryo cells. Mol Cell Biol 11:6286–6295.

Ben-Neriah Y, Daley GQ, Mes-Masson AM, Witte ON, Baltimore D. 1986. The chronic myelogenous leukemia-specific p210 protein is the product of the bcr-abl hybrid gene. Science 233:212–216.

Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J, Taylor K, Herrmann R, Seymour JF, Arthur C, Joske D, Lynch K, Hughes T. 2003. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood 102:276–283.

Brown PH, Alani R, Preis LH, Szabo E, Birrer MJ. 1992. Suppression of oncogene-induced transformation by a deletion mutant of c-jun. Oncogene 8:877–886.

Buchdunger E, Zimmermann J, Mett H, Meyer T, Müller M, Druker BJ, Lydon NB. 1996. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res 56:100–104.

Carroll M, Ohno-Jones S, Tamura S, Buchdunger E, Zimmermann J, Lydon NB, Gilliland DG, Druker BJ. 1997. CGP 57148, a tyrosine kinase inhibitor, inhibits the growth of cells expressing BCR-ABL, TEL-ABL, and TEL-PDGFR fusion proteins. Blood 90:4947–4952.

Chen CH, Lin JY, Liu FH, Chang JL, Huang HM. 2008. Basic fibroblast growth factor inhibits p38-mediated cell differentiation and growth inhibition by activin A but not by histone deacetylase inhibitors in CML cells. Ann Hematol 87:175–182.

Cheong JW, Chong SY, Kim JY, Eom JI, Jeung HK, Maeng HY, Lee ST, Min YH. 2003. Induction of apoptosis by apicidin, a histone deacetylase inhibitor, via the activation of mitochondria-dependent caspase cascades in human Bcr-Abl-positive leukemia cells. Clin Cancer Res 9:5018–5027.

Choi YH, Park MJ, Kim KW, Lee HC, Choi YH, Cheong J. 2004. The orphan nuclear receptor SHP is involved in monocytic differentiation, and its expression is increased by c-Jun. J Leukoc Biol 76:1082–1088.

Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB. 1996. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 2:561–566.

Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, Talpaz M. 2001. Activity of a specific inhibitor of BCRABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with Philadelphia chromosome. N Engl J Med 344:1038–1042.

Duan L, Sterba K, Kolomeichuk S, Kim H, Brown PH, Chambers TC. 2007. Inducible overexpression of c-Jun in MCF7 cells causes resistance to vinblastine via inhibition of drug-induced apoptosis and senescence at a step subsequent to mitotic arrest. Biochem Pharmacol 73:481–490.

Elagib KE, Xiao M, Hussaini IM, Delehanty LL, Palmer LA, Racke FK, Birrer MJ, Shanmugasundaram G, McDevitt MA, Goldfarb AN. 2004. Jun blockade of erythropoiesis: Role for repression of GATA-1 by HERP2. Mol Cell Biol 24:7779–7794.

Era T. 2002. Bcr-Abl is a "molecular switch" for the decision for growth and differentiation in hematopoietic stem cells. Int J Hematol 76:35–43.

Francastel C, Groisman R, Pfarr CM, Robert- Lézénès J. 1994. Antisense c-*jun* overcomes a differentiation block in a murine erythroleukemia cell line. Oncogene 9:1957–1964.

Gaynor R, Simon K, Koeffler P. 1991. Expression of c-jun during macrophage differentiation of HL-60 cells. Blood 77:2618–2623.

Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL. 2001. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 293:876–880.

Grant SA, Freemerman J, Birrer MJ, Martin HA, Turner AJ, Szabo E, Chelliah J, Jarvis WD. 1996. Effect of 1-b-D-arabinofuranosylcytosine on apoptosis and differentiation in human monocytic leukemia cells (U937) expressing c-Jun dominant-negative mutant protein (TAM67). Cell Growth Differ 7:603–610.

Herzog KH, Chen SC, Morgan JI. 1999. c-jun is dispensable for developmental cell death and axogenesis in the retina. J Neurosci 19:4349–4359.

Huang Y, Hutter D, Liu Y, Wang X, Sheikh MS, Chan AM, Holbrook NJ. 2000. Transforming growth factor-beta 1 suppresses serum deprivation-induced death of A549 cells through differential effects on c-Jun and JNK activities. J Biol Chem 275:18234–18242.

Huang HM, Chang TW, Liu JC. 2004. Basic fibroblast growth factor antagonizes activin A-mediated growth inhibition and hemoglobin synthesis in K562 cells by activating ERK1/2 and deactivating p38 MAP kinase. Biochem Biophys Res Commun 320:1247–1252.

Huang HM, Lin YL, Chen CH, Chang TW. 2005 Simultaneous activation of JAK1 and JAK2 confers IL-3 independent growth on Ba/F3 pro-B cells. J Cell Biochem 96:361–375.

Huang HM, Chiou HY, Chang JL. 2006. Activin A induces erythroid gene expressions and inhibits mitogenic cytokine-mediated K562 colony formation by activating p38 MAPK. J Cell Biochem 98:789–797.

Jagani Z, Singh A, Khosravi-Far R. 2008. FoxO tumor suppressors and BCR-ABL-induced leukemia: A matter of evasion of apoptosis. Biochim Biophys Acta 1785:63–84.

Karin M, Liu Z, Zandi E. 1997. AP-1 function and regulation. Curr Opin Cell Biol 9:240–246.

Kohmura K, Miyakawa Y, Kawai Y, Ikeda Y, Kizaki M. 2004. Different roles of p38 MAPK and ERK in STI571-induced multi-lineage differentiation of K562 cells. J Cell Physiol 198:370–376.

Kondo T, Matsuda T, Kitano T, Takahashi A, Tashima M, Ishikura H, Umehara H, Domae N, Uchiyama T, Okazaki T. 2000. Role of c-jun expression increased by heat shock- and ceramide-activated caspase-3 in HL-60 cell apoptosis. Possible involvement of ceramide in heat shock-induced apoptosis. J Biol Chem 275:7668–7676.

Lord KA, Abdollahi A, Hoffman-Liebermann B, Leibermann DA. 1993. Proto-oncogenes of the fos/jun family of transcription factors are positive regulators of myeloid differentiation. Mol Cell Biol 13:841–851.

Nishimura J, Takahira H, Shibata K, Muta K, Yamamoto M, Ideguchi H, Umemura T, Nawata H. 1988. Regulation of biosynthesis and phosphorylation of P210bcr/abl protein during differentiation induction of K 562 cells. Leuk Res 12:875–885.

Nowell PC, Hungerford DA. 1960. A minute chromosome in human chronic granulocytic leukemia. Science 132:1497–1499.

Poindessous-Jazat V, Augery-Bourget Y, Robert-Lézénès J. 2002. C-Jun modulates apoptosis but not terminal cell differentiation in murine erythroleukemia cells. Leukemia 16:233–243.

Qian M, Kralova J, Yu W, Bose HR, Dvorak M, Sanders BG, Kline K. 1997. c-Jun involvement in vitamin E succinate induced apoptosis of reticuloendotheliosis virus transformed avian lymphoid cells. Oncogene 15:223–230.

Raitano AB, Halpern JR, Hambuch TM, Sawyers CL. 1995. The Bcr-Abl leukemia oncogene activates Jun kinase and requires Jun for transformation. Proc Natl Acad Sci USA 92: 11746–11750.

Roffler-Tarlov S, Brown JJ, Tarlov E, Stolarov J, Chapman DL, Alexiou M, Papaioannou VE. 1996. Programmed cell death in the absence of c-Fos and c-Jun. Development 122: 1–9.

Rosson D, O'Brien TG. 1998. AP-1 activity affects the levels of induced erythroid and megakaryocytic differentiation of K562 cells. Arch Biochem Biophys 352:298–305.

Rowley JD. 1973. A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature 243:290–293.

Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, Sawyers CL. 2002. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2:117–125.

Shtivelman E, Lifshitz B, Gale RB, Roe BA, Canaani E. 1986. Alternative splicing of RNAs transcribed from the human cell gene and from the bcr/abl fused gene. Cell 47: 272–284.

Steelman LS, Pohnert SC, Shelton JG, Franklin RA, Bertrand FE, McCubrey JA. 2004. JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. Leukemia 18:189–218.

Szabo E, Preis LH, Birrer MJ. 1994. Constitutive cJun expression induces partial macrophage differentiation in U-937 cells. Cell Growth Differ 5:439–446.

Witt O, Monkemeyer S, Rönndahl G, Erdlenbruch B, Reinhardt D, Kanbach K, Pekrun A. 2003. Induction of fetal hemoglobin expression by the histone deacetylase inhibitor apicidin. Blood 101:2001–2007.