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Activin A Induces Erythroid Gene Expressions and Inhibits Mitogenic Cytokine-Mediated K562 Colony Formation by Activating p38 MAPK

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Abstract Activin A, a member of the transforming growth factor (TGF)-β superfamily, is involved in the regulation of erythroid differentiation. Previous studies have shown that activin A inhibited the colony-forming activity of mouse Friend erythroleukemia cells, however, the mechanism remains unknown. First, we show herein that activin A induced the expression and activated the promoters of α-globin and ζ-globin in K562 cells, confirming that activin A induces erythroid differentiation in K562 cells. The p38 mitogen activated protein kinase (MAPK) inhibitor, SB203580, inhibited and the extracellular signal regulated kinase (ERK) inhibitor, PD98059, enhanced the expression and promoter activities of α-globin and ζ-globin by activin A, indicating that p38 MAPK and ERK are crucial for activin A-induced erythroid genes expression. Second, SB203580 inhibited the inhibitory effect of activin A on the colony-forming activity of K562 cells using the methylcellulose colony assay, indicating that activin A inhibits K562 colony formation by activating p38 MAPK. In addition, mitogenic cytokines SCF, IL-3, and GM-CSF induced colony formation of K562 cells that could be inhibited by PD98059 or enhanced by SB203580, respectively, indicating that these mitogenic cytokines induce K562 colony formation by activating ERK and inactivating p38 MAPK. Furthermore, activin A reduced the induction effect of these mitogenic cytokines on K562 colony formation in a dose-dependent manner. The inhibition of p38 MAPK reverted the inhibitory effect of activin A on mitogenic cytokine-mediated K562 colony formation. We conclude that activin A can regulate the same pathway via p38 MAPK to coordinate cell proliferation and differentiation of K562 cells. J. Cell. Biochem. 98: 789–797, 2006.

Key words: Activin A; SCF; IL-3; GM-CSF; globin gene; p38 MAPK; ERK

Activin A, a member of the transforming growth factor (TGF)-β superfamily, is a multifunctional cytokine that regulates cell proliferation, differentiation, and apoptosis in various cell types [Luisi et al., 2001; Chen et al., 2002; Shav-Tal and Zipori, 2002]. It has been reported that activin A induced erythroid differentiation in hematopoietic progenitor cells, therefore, activin A is also termed the erythroid differentiation factor (EDF) [Eto et al., 1987; Shiozaki et al., 1992; Maguer-Satta et al., 2003]. Activin A as a paracrine regulator modulates erythropoiesis in the bone marrow microenvironment [Yu and Dolter, 1997]. Recently, there has been a striking increase in interest in the role of mitogen-activated protein kinase (MAPK) pathway in governing erythroid differentiation in hematopoietic progenitor cells by a variety of stimulations [Nagata et al., 1998; Park et al., 2001; Witt et al., 2003]. The MAPK family, comprised of extracellular signal-regulated kinase 1/2 (ERK), c-jun *N*-terminal kinase (JNK), and p38 MAPK, are involved in a variety of cellular processes, including cell proliferation, differentiation, and apoptosis [Ono and Han, 2000; Chang and Karin, 2001]. Previously, we reported that activin A induced erythroid differentiation by activating p38 MAPK and deactivating ERK in human chronic myeloid leukemia (CML)-derived K562 cell line [Huang et al., 2004]. In addition, activin A was also reported to

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inhibit colony-forming activity of mouse Friend erythroleukemia cells [Eto et al., 1987]. However, the molecular signaling of these observations has not been defined. In hematopoiesis, stem cell factor (SCF), interleukin-3 (IL-3), and granulocyte-macrophage colony-stimulating factor (GM-CSF) act as mitogenic cytokines to stimulate the growth of hematopoietic progenitor cells [McNiece et al., 1989; Migliaccio et al., 1992; Zandstra et al., 1997]. These mitogenic cytokines were found to stimulate the colony-forming activity of K562 cells [Komatsu et al., 1995]; we, therefore, asked whether activin A inhibited the mitogenic cytokine-induced colony-forming activity of K562 cells. In this study, we show that activin A induced the expression and promoter activity of α-globin and ζ-globin genes by activating p38 MAPK and deactivating ERK in K562 cells. Besides, we found that the mitogenic cytokines SCF, IL-3, and GM-CSF induced the colony formation of K562 cells by activating ERK and deactivating p38 MAPK. Furthermore, activin A inhibited colony formation of K562 cells and reduced mitogenic cytokine-induced K562 colony formation by activating p38 MAPK.

MATERIALS AND METHODS

Cell Culture and Materials

Human K562 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). Recombinant activin A, SCF, IL-3, and GM-CSF were purchased from R&D (Minneapolis, MN). PD98059 and SB203580 were purchased from Calbiochem (La Jolla, CA). Antibodies specific for phospho-ERK and ERK were purchased from New England Biolabs, Inc. (Beverly, MA).

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from K562 cells using the Trizol reagent (Gibco BRL, Life Technology, Inc., Grand Island, NY) according to the manufacturer's instructions. After purification, 1 μg of RNA was reverse-transcribed at 42°C for 60 min with primer oligo dT_{18} followed by enzyme inactivation at 70°C for 15 min. The resulting cDNA samples were amplified by PCR, using the following primers: α-globin sense strand 5′-GACAAGACCAACGTCAAG GCCGCC-3′ and antisense strand 5′-CAGGA ACTTGTCCAGGGAGGC-3′ generated a ~370 bp product; ζ-globin sense strand 5′-GCCACCC-GCAGACCAAGACC-3′ and antisense strand 5′-TAGGCGTGCAGCTCGCTCAG-3′ generated a ~160 bp product; β-actin sense strand 5′-GC-ATCCCCCAAAGTTCACAA-3′ and antisense strand 5′-AGGACTGGGCCATTCTCCTT-3′ generated a ~150 bp product as internal control. The PCR products were electrophoresed on 2% agarose gels, stained with ethidium bromide, and photographed under ultraviolet light.

Western Blot Analysis

Total cell extracts were prepared as described previously [Huang et al., 2004]. Protein concentration of samples was determined using Bio-Rad's Bradford protein assay. Equal quantities of protein were electrophoresed on 10% SDS–polyacrylamide gels and transferred onto PVDF membranes (Millipore, Bedford, MA), then probed with antibodies. After binding with horseradish peroxidase-conjugated secondary antibodies, the blots were visualized with an enhanced chemiluminescence (ECL) detection system (MEN Life Science Products, Inc., Boston, MA).

In vitro Kinase Assay

The p38 MAPK in vitro kinase assay was performed using the p38 MAPK kinase assay kit (New England BioLabs). Briefly, total cell extracts were prepared as described [Huang et al., 2004], and p38 MAP kinases were immunoprecipitated with the immobilized phospho-p38 MAPK monoclonal antibody. After washing twice with lysis buffer and twice with kinase buffer (25 mM Tris-HCl, pH7.5, 5 mM β-glycerolphosphate, 2 mM DTT, 0.1 mM Na_3VO_4, 10 mM $MgCl_2$), the immunoprecipitates were assayed for p38 MAPK kinase activity in kinase buffer with 200 mM ATP and 2 μg ATF-2 fusion protein per reaction. The reaction was stopped with SDS sample buffer and analyzed by Western blotting with specific anti-phospho-ATF-2 antibody.

DNA Transfection and Luciferase Reporter Assay

Transient transfections of K562 cells were performed by lipofection. For each transfection, 3×10^6 K562 cells were cotransfected with 6 μg DNA consisting of 5.4 μg pHS40-α590 Luc (or pHS40-ζ597 Luc) [Liu et al., 2003] and 0.6 μg pRL-TK internal control vector, along with 12 μl lipofectamine 2000 (Invitrogen,

Carlsbad, CA) according to the manufacturer's instructions. For cytokine induction, 50 ng/ml of recombinant activin A was added to the culture medium at 6 h post-transfection. The cells were then incubated for 24 h and 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 the firefly luciferase activity to the Renilla luciferase activity generated by pRL-TK (Promega). pHS40-α590 Luc and pHS40-ζ597 Luc were kindly provided by Dr. C.-K. James Shen [Liu et al., 2003].

Methylcellulose Colony Assay

K562 cells (1×10^3 cells) were washed once with phosphate-buffered saline (PBS, pH 7.4) and plated in each well of a 24-well dish of a semi-solid methylcellulose culture system. The methylcellulose culture medium consists of 1ml of RPMI 1640 containing 0.8% methylcellulose (Sigma Chemical Company, St. Louis, MO) and 2.75% FBS. K562 cells formed colonies with plating efficiency of 1–2% in the absence of mitogenic cytokines under these conditions. K562 cells were cultured in this methylcellulose culture system with 10% FBS; colonies form with plating efficiency of 50–60%. Duplicate cultures were supplemented with or without 50 ng/ml of different cytokines. After a 7-day incubation, the number of colonies was counted using an inverted microscope. Duplicated cultures with no added cytokine were plated as negative controls.

Statistical Analysis

Data are expressed as the average ± SE. Statistical analyses were performed by the Student's *t*-test between two groups and analysis of variance (ANOVA) among multiple groups to determine whether there were significant differences between conditions. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Roles of p38 MAPK and ERK in Activin A-Induced Expression of α-Globin and ζ-Globin

We have reported that activin A induced hemoglobin (Hb) synthesis by activating p38 MAPK and deactivating ERK [Huang et al., 2004]. To determine whether activin A induces the expression of α-globin and ζ-globin through these pathways, K562 cells were induced to undergo erythroid differentiation in 3-day cultures using 50 ng/ml activin A treatment first. RT-PCR amplification using α-globin and ζ-globin-specific primers revealed that activin A induced the expression of α-globin and ζ-globin (Fig. 1A). These results are consistent with a previous report using Northern blot analysis [Frigon et al., 1992]. To examine the contribution of p38 MAPK and ERK, K562 cells were incubated in the presence or absence of p38 MAPK inhibitor (SB203580) or MEK/ERK inhibitor (PD98059) alone, or in combination with activin A for 3 days. Results in Figure 1A show that the inhibition of p38 MAPK signaling by SB203580 inhibited and the inhibition of ERK signaling by PD98059 enhanced activin A-induced α-globin and ζ-globin expressions compared with activin A treatment alone (Fig. 1A). These results suggest that p38 MAPK activation and ERK inactivation are involved in activin A-induced expression of α-globin and ζ-globin. To confirm the specificity of these kinase inhibitors, the activities of ERK and p38 MAPK after treatment with inhibitors were measured. Results from Western blot analyses with phospho-ERK revealed that the phosphorylation of ERK by their upstream kinases were dramatically inhibited by PD98059 (Fig. 1B). The p38 MAPK kinase activity was measured by in vitro kinase assay (Fig. 1B). A known p38 MAPK substrate, ATF-2, was included in the p38 MAPK kinase reaction and its phosphorylation was detected with phospho-ATF-2 specific antibody. The results of the in vitro kinase reaction suggest that SB203580 has a very potent inhibitory effect on p38 MAPK (Fig. 1B).

Roles of p38 MAPK and ERK in Activin A-Induced Activation of α-Globin and ζ-Globin Gene Promoters

To examine the effect of activin A on the transcriptional activity of α-globin and ζ-globin genes, we used transient transfection of K562 cells by reporter constructs expressing the luciferase gene under the control of α-globin or ζ-globin promoters, pHS40-α590Luc or pHS40-ζ597Luc [Liu et al., 2003]. The relative luciferase activities of the reporters activated under various conditions were referenced to the reporter activity under untreated condition (normalized as 1). Results in Figure 2A and B show that a 24-h exposure to activin A significantly induced

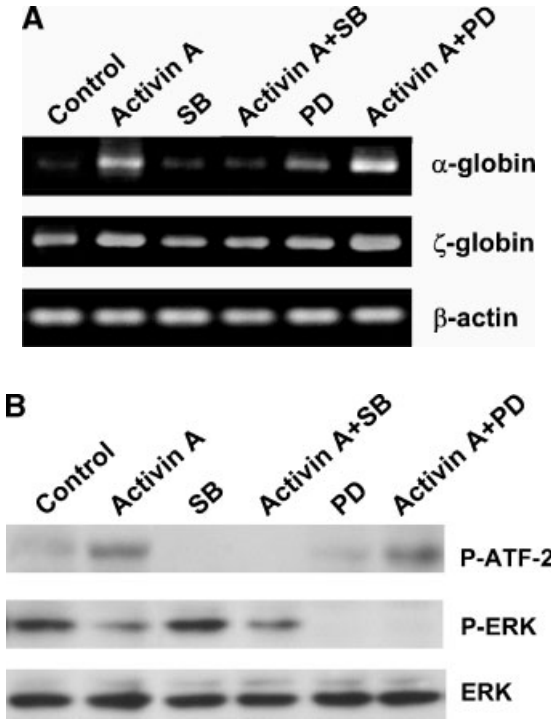

Fig. 1. Roles of p38 MAPK and ERK in activin A-induced expression of α-globin and ζ-globin in K562 cells. **A**: K562 cells were treated with or without (control) 50 ng/ml activin A, 10 μM SB203580 (SB), 10 μM PD98059 (PD), activin A plus SB203580, or activin A plus PD98059 for 3 days. RNA was isolated from these distinct cultured cells and submitted to RT-PCR analysis as described in Materials and Methods, followed by electrophoresis on 2% agarose gel. **B**: K562 cells were treated as described in panel A. Cell lysates were prepared and subjected to Western blot analysis for the activation of ERK using specific anti-phospho-ERK (P-ERK) antibody, then reprobed with the anti-ERK antibody as loading controls. Cell lysates were immunoprecipitated with anti-phospho-p38 MAPK antibody. The immunoprecipitates were then subjected to in vitro kinase assay for p38 MAPK described in ''Materials and Methods.'' Phospho-ATF-2 (P-ATF-2) is the product of the kinase reaction of p38 MAP kinase. DMSO was used as the solvent and was included as a negative control (lane 1).

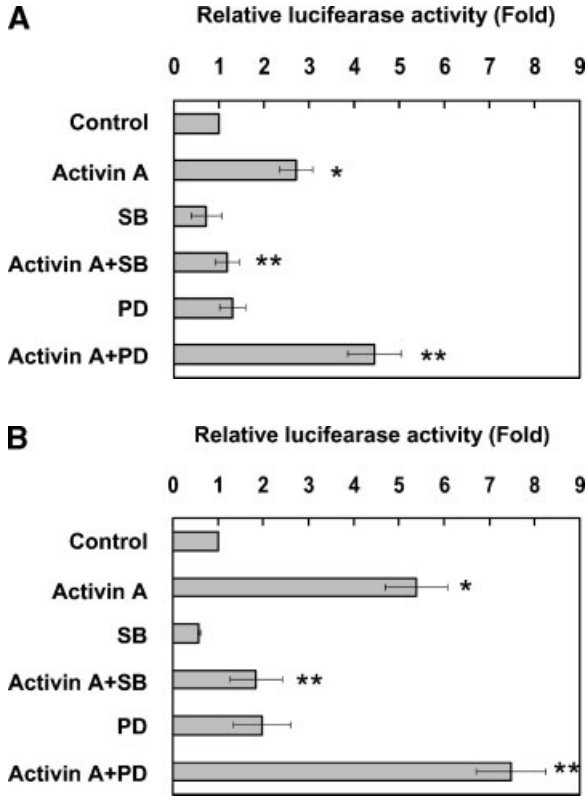

Fig. 2. Roles of p38 MAPK and ERK in activin A-mediated promoter activation of α-globin and ζ-globin genes in K562 cells. **A**: K562 cells were cotransfected with plasmids pHS40-α590 Luc and pRL-TK (as an internal control of transfection). **B**: K562 cells were cotransfected with plasmids pHS40-ζ597 Luc and pRL-TK. After 6 h of transfection, cells were subsequently treated with or without (control) 50 ng/ml activin A, 10 μM SB203580 (SB), 10 μM PD98059 (PD), activin A plus SB203580, or activin A plus PD98059. Luciferase activity was measured 24-h after transfection and was normalized to RL expression. The values were expressed relative to the activity of the control value (normalized as 1). Data from four independent experiments are shown as the average ± the standard error (SE). *, $P < 0.05$ versus untreated, **, $P < 0.05$ versus activin A treated, by Student's *t*-test.

promoter activities of α-globin (a 2.7 ± 0.4-fold increase, $P < 0.05$) and ζ-globin (a 5.4 ± 0.7-fold increase, $P < 0.05$). The inhibition of p38 MAPK signaling by SB203580 resulted in the inhibition of promoter activities of α-globin (1.2 ± 0.3 vs. 2.7 ± 0.4 fold, $P < 0.05$) and ζ-globin (1.8 ± 0.6 vs. 5.4 ± 0.7 fold, $P < 0.05$) by activin A. The inhibition of ERK signaling by PD98059 resulted in the enhancement of promoter activities of α-globin (4.5 ± 0.6 vs. 2.7 ± 0.4 fold, $P < 0.05$) and ζ-globin (7.5 ± 0.8 vs. 5.4 ± 0.7 fold, $P < 0.05$) by activin A. These results suggest that p38 MAPK activation and ERK inactivation are involved in activin A-induced promoter activation of α-globin and ζ-globin genes.

Activin A Inhibits the Colony-Forming Activity of K562 Cells by p38 MAPK Pathway

Activin A was reported to inhibit the colony-forming activity of mouse Friend erythroleukemia cells in soft agar [Eto et al., 1987], however, the mechanism remains unknown. We observed that p38 MAPK activation and ERK inactivation are involved in activin A-mediated α-globin and ζ-globin expressions (Fig. 1) and Hb synthesis [Huang et al., 2004]. Therefore, we studied the roles of p38 MAPK and ERK pathways in the influence of activin A on colony-forming activity of K562 cells by using a methylcellulose culture system. The colony formation ability of K562

cells in the presence and absence (control) of activin A was comparable with the combination of activin A and inhibitor (SB203580 or PD98059)-containing cultures. Results in Figure 3 show that a 7-day exposure to activin A significantly inhibited by threefold the colony-forming activity of K562 cells compared to the control cultures ($P < 0.05$). The inhibition of p38 MAPK signaling by SB203580 blocked the inhibitory effect of activin A on colony-forming activity of K562 cells ($P < 0.05$), indicating that the p38 MAPK activation is crucial for activin A-inhibited colony formation of K562 cells (Fig. 3). PD98059 treatment alone inhibited colony-forming activity of K562 cells, indicating that ERK activation is required for colony formation of K562 cells (Fig. 3).

Roles of ERK and p38 MAPK in Mitogenic Cytokine-Induced Colony-Forming Activity of K562 Cells

Mitogenic cytokines SCF, IL-3, and GM-CSF have been shown to induce the colony formation of K562 cells [Komatsu et al., 1995]. To study the roles of ERK and p38 MAPK pathways on the effect of mitogenic cytokines on the colony-forming activity of K562 cells, the cells were incubated with or without different mitogenic cytokines or inhibitors (PD98059 or SB203580) alone or in combinations for 7 days in semi-solid methylcellulose culture. We found that the inhibition of ERK by PD98059 inhibited and the inhibition of p38 MAPK by SB203580 enhanced all mitogenic cytokines-induced colony formation of K562 cells (Fig. 4). These results indicate that mitogenic cytokines SCF, IL-3, and GM-CSF induced the colony-forming activity of K562 cells by activating ERK and deactivating p38 MAPK. ANOVA statistics revealed significant differences between groups (Fig. 4).

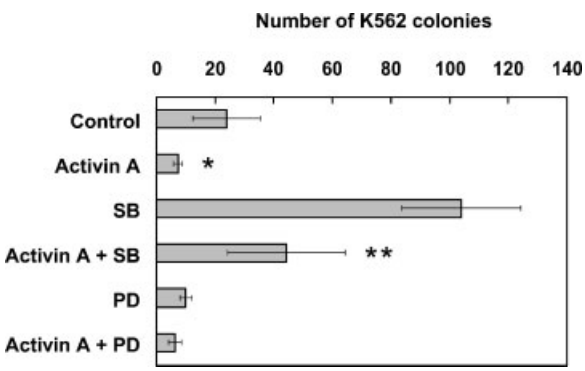

Fig. 3. Effect of p38 MAPK and ERK activity on activin A-inhibited colony formation of K562 cells. Graph shows net number of K562 colonies in methylecellulose culture in the presence or absence of 50 ng/ml activin A, 10 μM SB203580 (SB), 10 μM PD98059 (PD), activin A plus SB203580, or activin A plus PD98059 after 7 days. Colony formation of K562 cells was determined from three independent experiments, and the results are presented as average ± SE. *, $P < 0.05$ versus untreated, **, $P < 0.05$ versus activin A treated, by Student's *t*-test.

Activin A Reduces Mitogenic Cytokine-Induced Colony-Forming Activity of K562 Cells Through the p38 MAPK Pathway

Since we observed that activin A inhibited and mitogenic cytokines induced the colony-forming activity of K562 cells by activating p38 MAPK and deactivating p38 MAPK, respectively (Figs. 3 and 4), we sought to determine the consequences on the colony-forming activity of K562 cells under simultaneous influence of activin A with different mitogenic cytokines. K562 cells were incubated with activin A and different mitogenic cytokines, either separately or in combination for 7 days in the methylcellulose colony assay. Results in Figure 5 show that SCF-, IL-3-, or GM-CSF-induced K562 colony formation were reduced by activin A in a dose-dependent manner. Co-treatments of cells with

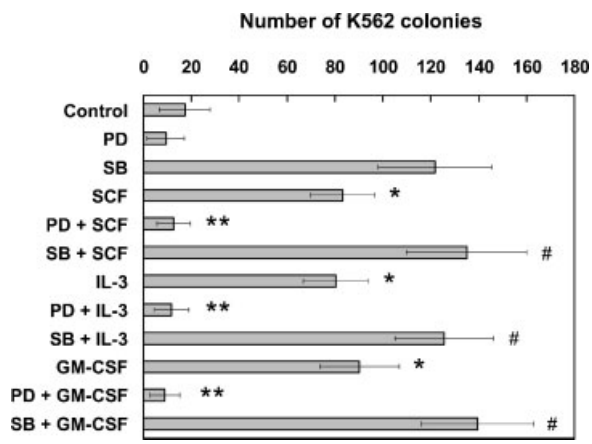

Fig. 4. Effect of ERK and p38 MAPK activity on mitogenic cytokine-induced colony formation of K562 cells. Graph shows net number of K562 colonies in methylcellulose culture in the presence or absence of 10 μM PD98059 inhibiotor (PD), 10 μM SB203580 inhibitor (SB), 50 ng/ml different mitogenic cytokines (SCF, IL-3, or GM-CSF), or different mitogenic cytokines plus inhibitor after 7 days. Colony formation of K562 cells was determined from four independent experiments and results are presented as average ± SE. In all mitogenic cytokines treatment fold increases reached significance (*, $P < 0.001$ by ANOVA). The difference between cytokine treatment alone and cytokine combined with PD98059 was statistically significant (**, $P < 0.001$ by ANOVA). The difference between cytokine treatment alone and cytokine combined with SB203580 was statistically significant (#, $P < 0.05$ by ANOVA).

SB203580 led to significant blockade of the inhibitory effect of activin A on the different mitogenic cytokines-induced colony formation of K562 cells (Fig. 6). These results suggested that activin A reduced mitogenic cytokines-induced colony-forming activity of K562 cells via the p38 MAPK pathway. ANOVA statistics revealed significant differences between groups (Figs. 5 and 6).

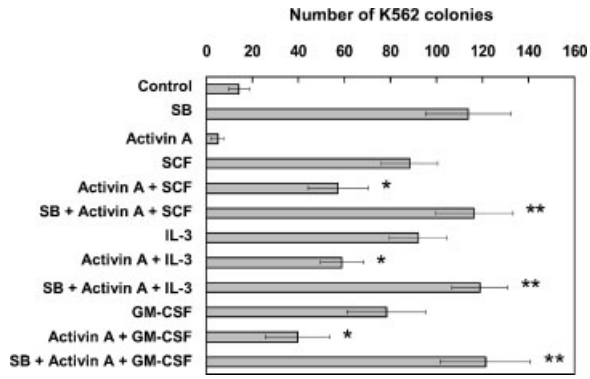

Fig. 6. Activin A reduced SCF-, IL-3-, or GM-CSF-stimulated K562 colony formation through p38 MAPK pathway. Graph shows net number of K562 colonies in the indicated methylecellulose culture. After 7 days, the number of K562 colony formation was determined from at least three independent experiments, and is shown as average ± SE. *, $P < 0.05$ versus mitogenic cytokine treated alone, by ANOVA. **, $P < 0.05$ versus mitogenic cytokine plus activin A treated by ANOVA.

DISCUSSION

Hematopoiesis is regulated by many cytokines that direct the cell fate decisions of hematopoietic progenitors in the bone marrow microenvironment [Lotem and Sachs, 2002]. It has been demonstrated that the in vitro proliferation and erythroid differentiation of hematopoietic progenitor cells are dependent on the presence of specific cytokines. Activin A acts as a differentiation cytokine that induce the erythroid differentiation of hematopoietic progenitor cells, but many mitogenic cytokines, such as SCF, IL-3, and GM-CSF stimulate the growth of hematopoietic progenitor cells in the bone marrow. Therefore, we were interested in how the erythroid differentiation cytokine and the mitogenic cytokines affect hematopoietic progenitor cells within the in vitro semi-solid methylcellulose culture.

In this report, we explored two different parts to understand the molecular signaling of proliferation and differentiation by activin A and the mitogenic cytokines in K562 cells. First, we observed that activin A induced the expression and activated the promoters of erythroid genes α-globin and ζ-globin, through activating p38 MAPK and deactivating ERK. Our previous report showed that activin A acted through the

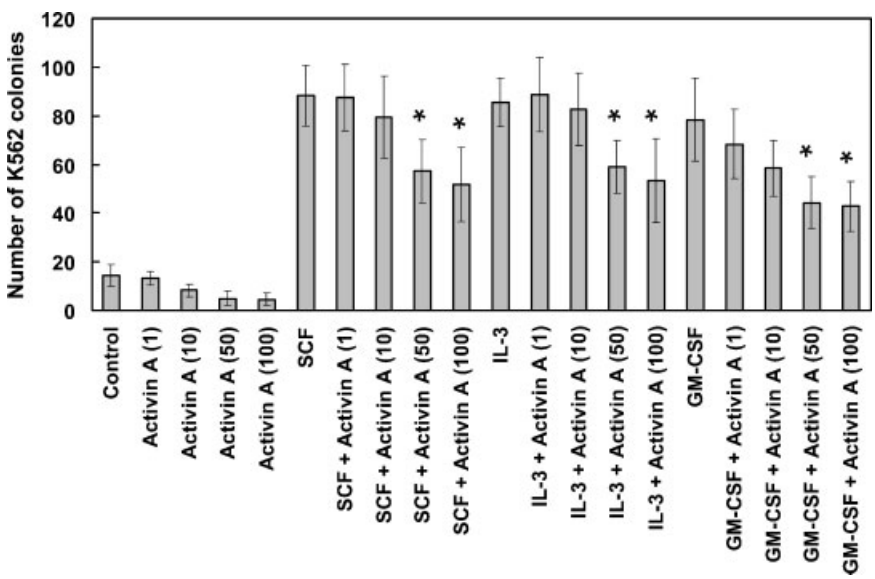

Fig. 5. The relationship between Activin A and different mitogenic cytokines on colony formation of K562 cells. Graph shows net number of K562 colonies in methylecellulose culture with various 50 ng/ml mitogenic cytokines (SCF, IL-3, or GM-CSF) in the presence or absence of increasing concentrations of activin A (0, 1, 10, 50, and 100 ng/ml). After 7 days, colony formation of K562 cells was determined from at least three independent experiments and are presented as average ± SE. *, $P < 0.05$ versus mitogenic cytokine treated alone, by ANOVA.

activation of p38 MAPK and the inactivation of ERK to induce Hb synthesis in K562 cells [Huang et al., 2004]. In the current report, we further investigated the roles of p38 MAPK and ERK pathways on activin A-regulated erythroid genes. Our results indicate that activin A induces the expression of α-globin and ζ-globin genes by regulating their promoter activities. These results are consistent with the previous report in which p38 MAPK is involved in apicidin-induced activation of the Aγ-globin promoter [Witt et al., 2003]; apicidin is a histone deacetylase inhibitor. The p38 MAPK pathway was also reported to be involved in erythropoietin-induced erythroid differentiation of erythroleukemia cells [Nagata and Todokoro, 1999]. In addition, a specific inhibitor of Abl tyrosine kinase, STI571, used for clinical treatment of CML patients, has also been found to induce Hb synthesis by activating p38 MAPK and inactivating ERK in K562 cells [Kohmura et al., 2004]. The expression level of ζ-globin mRNA in Figure 1 is not as obvious as α-globin mRNA. However, the effect of activin A on the promoter activity of ζ-globin is greater than the promoter activity of α-globin (Fig. 2A and B). Two possible variations come to mind for the differences between the results, treatment time and promoter region. The RNA samples and the cell lysates of reporter assay were prepared from 3-day cell cultures and 24-h cell incubation, respectively, suggesting that may be mRNA stability and/or translational activation attributed to these results. Whether the expression level of α-globin and ζ-globin genes is similar to their promoter activities after 24-h induction needs to be proven by real-time PCR. The promoter constructs of α-globin and ζ-globin, pHS40-α590Luc (from −574 to +41) and pHS40-ζ597Luc (from −559 to +38), do not contain a full length promoter sequence [Liu et al., 2003]. Thus, an alternate explanation of our results would be that the ζ-globin promoter (pHS40-ζ597Luc) contains more activin A response elements than the α-globin promoter (pHS40-α590Luc). Second, activin A inhibited the colony-forming activity of K562 cells by activating p38 MAPK, but mitogenic cytokines SCF, IL-3, and GM-CSF induced the colony-forming activity of K562 cells by activating ERK and deactivating p38 MAPK. Thus, we suggest that activin A reduces these mitogenic cytokines-induced colony-forming activity of K562 cells by activating p38 MAPK. These results are in agreement with previous studies investigating ERK inhibition which suppressed cell growth [Pages et al., 1993] and induced cell differentiation [Kang et al., 1999]. On the other hand, p38 MAPK has been shown to be involved in cell differentiation [Ono and Han, 2000] and cell growth inhibition [Cocolakis et al., 2001]. These results suggest that the ERK pathway regulates cell growth and differentiation inhibition and the p38 MAPK pathway regulates cell differentiation and growth inhibition in erythroid differentiation. Therefore, pharmacological inhibitors of ERK and pharmacological activators of p38 MAPK may be a useful class of drugs to treat CML patients. Recently, an important feature in the inverse relationship between p38 MAPK and ERK activities in the regulation of keratinocyte differentiation was reported; p38 MAPK directly interacted with ERK to inhibit ERK activity [Efimova et al., 2003]. It is not clear whether activin A-mediated p38 MAPK activation and ERK inactivation in K562 cells are by direct interactions between p38 MAPK and ERK. Our previous results indicated that basic fibroblast growth factor (bFGF) antagonized activin A-mediated erythroid differentiation in K562 cells by activating ERK and deactivating p38 pathways [Huang et al., 2004]. Whether the antagonistic activity of colony formation on K562 cells by mitogenic cytokines SCF, IL-3, and GM-CSF, and activin A will convert into erythroid differentiation response needs to be proven.

K562 is a hematopoietic progenitor cell line established from a human CML patient. K562 cells possess ability for unlimited proliferation and an inability to proceed with differentiation. However, K562 cells can be differentiated into erythroid cells by activin A or other chemical agents [Leppa et al., 1997; Park et al., 2001; Witt et al., 2003], or into megakaryocytic cells by TPA [Leary et al., 1987]. It has been shown that K562 cells not only expressed the receptors for mitogenic cytokines, such as SCF, IL-3, etc. [McGuckin et al., 1996], but also expressed activin A receptors [Hilden et al., 1994, 1999]. Our results show that the activin A inhibition of mitogenic cytokine-mediated colony formation of K562 cells is largely dependent on concentration. These results are consistent with previous observations in other studies on the effect of activin A on erythropoiesis in vivo. The in vivo studies showed that the elevation of activin A increased erythroid progenitors [Shiozaki et al.,

1989], circulating red blood cells [Schwall et al., 1989], and reticulocyte release [Broxmeyer et al., 1991]; and the neutralization of endogenous activin A by follistatin (a specific binding protein for activin A) decreased erythroid progenitors [Shiozaki et al., 1992]. It was reported that activin A can inhibit IL-3-mediated granulocyte-macrophage colony-forming unit [Mizuguchi et al., 1993] and activin A can maintain the undifferentiated state of human embryonic stem cells [Beattie et al., 2005; James et al., 2005]. Therefore, activin A have different functions on different cell types, may be due to the expression of activin A receptors and the specific signaling mediators that provide particular cells with unique responsiveness to activin A. In addition, once erythroid progenitor cells expressed the receptor for erythroid differentiation cytokines, their cell fate decisions may be regulated by the concentration of cytokines in the microenvironment.

In summary, our study suggests that the opposite modulation of p38 MAPK and ERK by activin A and by mitogenic cytokines is critical for proliferation versus differentiation decision of hematopoietic progenitor cells.

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