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Induction of gp130 and LIF by differentiation inducers in human myeloid leukemia K562 cells

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Abstract

It has been previously shown that phorbol 12-myristate 13-acetate (PMA), a potent differentiation inducer, induced the expression of both interleukin-6 (IL-6) and IL-6 receptor α component (IL-6Rα) in K562 leukemia cells. In the present study, we examined the ability of several differentiation inducers to regulate the expression of the signal-transducing receptor component for IL-6, gp130, and cytokine leukemia inhibitory factor (LIF) in K562 cells. We found that the expression of gp130 was dramatically induced at both the mRNA and protein levels by the two megakaryocytic inducers sodium butyrate (NaBut) and PMA. In contrast, the mRNA expression of LIF was induced by the two erythroid inducers 1-β-D-arabinofuranosyl cytosine (Ara-C) and hemin. Furthermore, activation of the PMA-induced gp130 receptor by exogenous IL-6 potentiated the differentiating effects of PMA. Our findings suggest that IL-6/gp130 signaling may be involved in the regulation of the megakaryocytic differentiation of K562 cells.

Keywords: IL-6; gp130; LIF; Sodium butyrate; PMA; K562; Leukemia differentiation

1. Introduction

The human chronic myelogenous leukemia cell line K562 is a well-established model system for the study of leukemia differentiation [1]. K562 cell line was established from the pleural effusion of a patient with chronic myelogenous leukemia in blast crisis [2]. It has been extensively demonstrated that K562 cells can be induced to differentiate towards erythroid and megakaryocyte lineages by various differentiation inducers [3,4]. Hemin and 1-β-D-arabinofuranosyl cytosine (Ara-C) induced K562 cells to differentiate towards the erythroid lineage, while phorbol 12-myristate 13-acetate (PMA) induced K562 cells to differentiate towards the megakaryocyte lineage [5–8]. In contrast, sodium butyrate (NaBut) induced the characteristics of both the erythroid and megakaryocyte lineages in K562 cells [6,8–12]. Retinoic acid (RA) alone did not induce the obvious differentiation, but RA enhanced the hemin-induced erythroid differentiation in K562 cells [13]. Although the differentiating effects of various differentiation inducers on K562 cells have been well-documented, the molecular mechanisms underlying such differentiation induction are still unclear.

It has been previously demonstrated that PMA induced the expression of both IL-6 and IL-6Rα in K562 cells [14]. IL-6 is a pleiotropic cytokine acting on a variety of cells [15–17]. The pleiotropic functions of IL-6 are mediated through two membrane receptor components, a ligand-binding molecule IL-6Rα and a signal-transducing molecule gp130 [18]. Upon IL-6 binding, IL-6Rα associates with gp130 and signal is transduced through the dimerization and tyrosine phos-

Abbreviations: Ara-C, 1-β-D-arabinofuranosyl cytosine; PMA, phorbol 12-myristate 13-acetate; NaBut, sodium butyrate; RA, retinoic acid; IL-6, interleukin-6; IL-6Rα, interleukin-6 receptor α; LIF, leukemia inhibitory factor; OSM, oncostatin M; IL-11, interleukin-11; CNTF, ciliary neurotrophic factor; NT-4/5, neurotrophin-4/5; FACS, fluorescence-activated cell sorting..

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phorylation of gp130 [18]. Other cytokines of the IL-6 family, including leukemia inhibitory factor (LIF), oncostatin M (OSM), IL-11, cardiotrophin-1, and ciliary neurotrophic factor (CNTF), also utilize gp130 as a signal-transducing receptor component [17,19]. K562 cells similar to many other human leukemia cell lines with erythroid and/or megakaryocytic features, including CMK, HEL, MEG-01, Dami, M-07e and UT-7, have IL-6 and its transducing receptor gp130 [14,20,21]. To explore the potential involvement of gp130 and LIF in leukemia differentiation, we examined the ability of various differentiation inducers to regulate the expression of these two genes in K562 cells.

In this paper, we report that the expression of gp130 was dramatically induced at both the mRNA and protein levels by the two megakaryocytic inducers NaBut and PMA. In contrast, the mRNA expression of LIF was induced by the two erythroid inducers Ara-C and hemin. Furthermore, activation of the PMA-induced gp130 receptor by exogenous IL-6 potentiated the differentiating effects of PMA, as evidenced by the increase in the expression level of the megakaryocyte markers CD41a, CD61 and α-naphthyl acetate esterase. Our findings suggest that IL-6/gp130 signaling may be involved in the regulation of the megakaryocytic differentiation of K562 cells.

2. Materials and methods

2.1. Cell culture and treatment

Human chronic myelogenous leukemia K562 cells (obtained from American Type Culture Collection, ATCC, MD) were cultured in RPMI 1640 medium (GIBCO-BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, GIBCO-BRL). The cell cultures were incubated in humidified air with 5% CO_2 at 37°C. Cells were treated with control solvent, 1 μM all-*trans* RA (Sigma, St. Louis, MO), 1 μM 9-*cis* RA (Sigma), 1 μM Ara-C (Sigma), 5 μM hemin (Sigma), 1 mM NaBut (Sigma), 10 nM PMA (Sigma), or 40 ng/ml IL-6 (Pepro Tech, Inc., Rocky Hill, NJ).

2.2. Northern blot analysis

Total cellular RNA was prepared according to the method of Chomczynski and Sacchi [22] at day 3 and 6 after treatment. Equal amounts of RNA (20 μg) were separated by gel electrophoresis using 1% agarose-formaldehyde gel. The RNA was transferred onto a nylon membrane (Micron Separations Inc., Westborough, MA) and crosslinked by UV irradiation (Stratalinker, Stratagene, CA). The RNA blots were hybridized with ^{32}P-labelled cDNA probes, including full-length human gp130 cDNA (a 3.0 kb EcoRI-Spel fragment [23]), LIF and neurotrophin-4/5 (NT-4/5) [23,24]. The cDNA probes was gel-purified by using GENECLEAN II Kit (BIO 101, La Jolla, CA), and labelled by random priming using Megaprime Labelling Kit (Amersham, England). Hybridization was carried out in 0.5 M sodium phosphate buffer (PBS, pH7.4) containing 7% SDS, 1% BSA, 1 mM EDTA, and 40 μg/ml salmon sperm DNA at 65°C. The hybridized membranes were washed in $2 \times$ SSC/0.1% SDS at 65°C for 3×30 min, then exposed to X-ray films (Kodak, Eastman Kodak Company, Rochester, NY, USA) with intensifying screens (C.B.S. Scientific Co., CA) at -80°C.

2.3. Fluorescence-activated cell sorting analysis

Leukemia cells (1×10^6 cells/sample) were collected and washed with PBS. Cells were stained with an anti-gp130 monoclonal antibody (with 1:100 dilution, PharMingen, San Diego, CA), washed with PBS, then stained with a fluorescein-5-isothiocyanate (FITC)-conjugated goat anti-mouse IgG antibody (Cappel, Durham, NC). For the immunophenotyping analysis of hematopoietic markers, K562 cells (1×10^6 cells/sample) were collected and washed with PBS twice, then stained with phycoerythin (PE)- or FITC-conjugated antibodies (with 1:10 dilution) against specific cell surface markers at room temperature for 30 min. The cell surface markers examined include the myeloid lineage marker CD15 (Becton Dickinson, San Jose, CA), the megakaryocyte lineage markers CD41a and CD61 (PharMingen), and the erythroid lineage markers CD71 and Glycophorin A (PharMingen). The stained cells were subsequently washed with PBS, fixed with ice-cold 1% paraformaldehyde and stored at 4°C. The fluorescence signal of the stained cells was recorded by using a flow cytometer (Becton Dickinson). The mean of fluorescence intensity and the percentage of positively stained cells were analyzed by using Lysis II software (Becton Dickinson).

2.4. α-Naphthyl acetate esterase staining analysis

The cytoplasmic α-naphthyl acetate esterases were detected by staining with an α-Naphthyl Acetate Esterase Kit (Sigma) according to the manufacturer's procedure. Briefly, leukemia cells were collected onto a glass slide using a Cytospin II (Shandon Scientific Limited, England) centrifuge, then fixed in Fixative Solution. The fixed cells were subsequently stained in α-naphthyl acetate esterase staining solution at 37°C for 30 min. The stained slides were washed thoroughly in deionized water, air-dried and mounted with Airvol mounting media (Air Products and Chemicals, Inc., Allentown, PA). The morphology of the stained cells was examined by using Zeiss Axiophot microscope (Zeiss, Germany) equipped with a $40 \times$ objective.

3. Results

3.1. Induction of the expression of gp130 and LIF mRNA by differentiation inducers

Total cellular RNA was extracted from K562 cells after treatment with various differentiation inducers for 3 and 6 days. The mRNA expression of gp130, LIF and NT-4/5 was determined by Northern blot analysis. The mRNA expression of gp130 was dramatically induced by 1 μM all-*trans* RA, 1 μM 9-*cis* RA, 1 mM NaBut, or 10 nM PMA, but not significantly changed by 1 μM Ara-C or 5 μM hemin. In contrast, the expression of LIF was induced by 1 μM Ara-C or 5 μM hemin, but not significantly increased by 1 μM all-*trans* RA, 1 μM 9-*cis* RA, 1 mM NaBut, or 10 nM PMA. Interestingly, there appears to be less LIF mRNA induction after 6 rather than 3 days exposure to Ara-C and hemin. The mRNA expression of NT-4/5 was not significantly induced by any differentiation inducer examined in this study (Fig. 1).

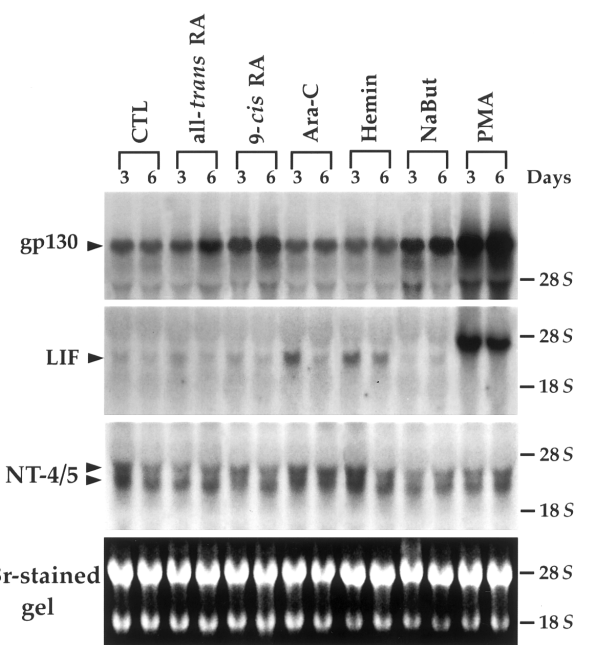

Fig. 1. Induction of the expression of gp130 and LIF mRNA by differentiation inducers in K562 cells. K562 cells were treated with control solvent (CTL), 1 μM all-*trans* RA, 1 μM 9-*cis* RA, 1 μM Ara-C, 5 μM hemin, 1 mM NaBut, or 10 nM PMA. RNA samples were prepared on day 3 and day 6 after treatment. The mRNA expression for gp130, LIF and NT-4/5 was examined by Northern blot analysis using specific cDNA probes. Ethidium bromide (EtBr)-stained gel was shown to indicate that equal amount of RNA was loaded (20 μg per sample). Ribosomal RNA bands (18*S* and 28*S*) are indicated on the right, and the probes used are indicated on the left.

3.2. Induction of the expression of transmembrane gp130 receptor by NaBut and PMA

To determine whether the up-regulated gp130 mRNA in K562 cells was translated as transmembrane gp130 receptor, FACS analysis was performed. Cells were stained with an anti-gp130 monoclonal antibody and a FITC-conjugated secondary antibody, then analyzed by a flow cytometer. The expression level of transmembrane gp130 protein was significantly increased by treatment with 1 mM NaBut or 10 nM PMA in K562 cells (Fig. 2).

3.3. Synergistic effects of IL-6 and PMA on K562 differentiation

The effects of 40 ng/ml IL-6 in combination with 1 mM NaBut, or 0.5 nM PMA on cellular differentiation in K562 cells were tested by immunophenotyping analysis after treatment for 5 days. No significant combined effect of IL-6 and 1 mM NaBut on cellular differentiation was detected in K562 cells (data not shown).

IL-6 alone could not significantly induce the expression of the two megakaryocyte markers CD41a and CD61 in K562 cells. However, when administered together with 0.5 nM PMA, IL-6 significantly further increased the expression level of CD41a and CD61 induced by 0.5 nM PMA alone. The expression level of CD61 induced by IL-6 in combination with 0.5 nM PMA was almost the same as that induced by 1 nM PMA alone. IL-6 alone could not inhibit the expression of the two erythroid markers CD71 and Glycophorin A. However, when added in combination with 0.5 nM PMA, IL-6 caused a further decrease in the expression level of CD71 and Glycophorin A than 0.5 nM PMA alone. In contrast, IL-6 did not significantly affect the expression of the myeloid marker CD15 (Fig. 3).

The effects of 40 ng/ml IL-6 in combination with 0.5 nM PMA on cellular differentiation in K562 cells were also examined by α-naphthyl acetate esterase staining. IL-6 alone could not significantly induce the expression of α-naphthyl acetate esterase in K562 cells. However, when administered together with 0.5 nM PMA, IL-6 significantly further increased the intensity of α-naphthyl acetate esterase induced by 0.5 nM PMA alone. The intensity of α-naphthyl acetate esterase induced by IL-6 in combination with 0.5 nM PMA was almost the same as that induced by 1 nM PMA alone (Fig. 4).

4. Discussion

In this study, we explored the potential involvement of the signal-transducing receptor component for the IL-6 family cytokines, gp130, and cytokine LIF in the differentiation of human chronic myelogenous leukemia

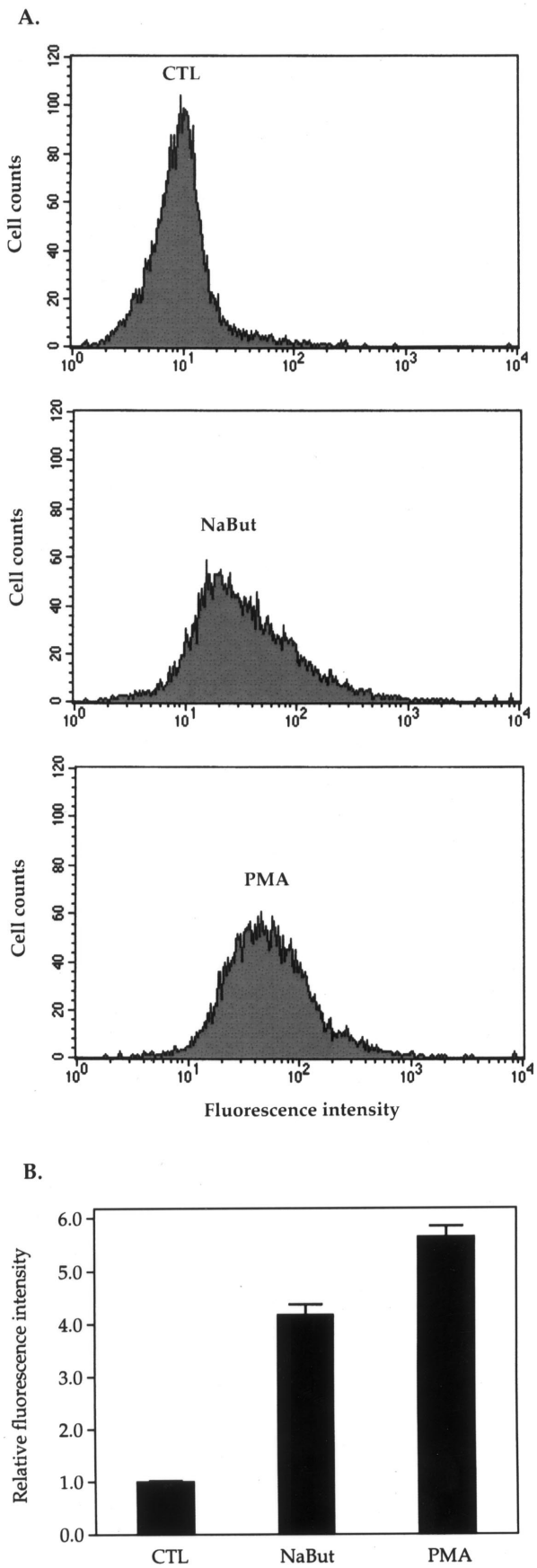

Fig. 2.

K562 cells. We firstly examined the ability of various differentiation inducers to regulate the expression of gp130 and LIF mRNA by Northern blot analysis. Our results demonstrated that the mRNA expression for gp130 was induced by the two megakaryocyte lineage inducers NaBut and PMA, but not by the two erythroid lineage inducers Ara-C and hemin. In contrast, the mRNA expression for LIF was induced by the two erythroid lineage inducers Ara-C and hemin, but not by the two megakaryocyte lineage inducers NaBut and PMA. Therefore, the induction of gp130 was an event specifically associated with the megakaryocytic differentiation, while the induction of LIF was an event specifically associated with the erythroid differentiation in K562 cells. Our findings suggested that the induction of gp130 or LIF seems to be a lineage-specific phenomenon in K562 cells.

It has been shown that the gp130 mRNA could be translated and processed into transmembrane form as well as several soluble forms of gp130 protein, and the soluble forms of gp130 could inhibit signal-transducing through the membrane-anchored gp130 [25,26]. To determine whether the induced gp130 mRNA was translated as functional receptor, we examined the expression of transmembrane gp130 protein by FACS analysis. Our results indicated that both NaBut- and PMA- induced gp130 mRNA was translated as functional transmembrane gp130 proteins in K562 cells.

To explore the functional roles of the up-regulated gp130 receptor, we examined the effects of exogenous IL-6 alone or in combination with PMA or NaBut on the cellular differentiation of K562 cells. IL-6 in combination with PMA synergistically induced the expression of the megakaryocyte lineage markers CD41a and CD61 as well as α-naphthyl acetate esterases. Previous studies demonstrated that PMA induced the expression of both IL-6 and IL-6Rα in K562 cells and a human megakaryocytic leukemia cell line CMK cells [14,27,28]. Increasing evidence indicated that IL-6 could stimulate both normal and leukemic megakaryocytopoiesis [20,29]. In addition, it has been shown that IL-6 stimulates the differentiation of megakaryocytes in vivo [29–31]. Our findings together with these previous observations suggested that the megakaryocytic differentiating effects of PMA in K562 cells may partially be mediated through the activation of the up-regulated

Fig. 2. Induction of the expression of transmembrane gp130 receptor by NaBut and PMA in K562 cells. K562 cells were grown in RPMI 1640 medium containing 10% FBS in the presence of control solvent (CTL), 1 mM NaBut (NaBut), or 10 nM PMA (PMA). The FACS analysis was performed on day 5 after treatment. A. Representative FACS data. B. Relative fluorescence intensity. The mean of fluorescence intensity was analyzed by using Lysis II software (Becton Dickinson). Data represents the mean of three experiments ± SEM. In each experiment, each sample had three independent replicates.

IL-6Rα and gp130 by endogenous IL-6 which was also up-regulated by PMA. However, IL-6 in combination with NaBut could not synergistically induce the megakaryocytic differentiation of K562 cells, although NaBut also dramatically induced the expression of gp130 in these cells. One possibility is that multiple growth factors are required for the megakaryocytic differentiation in K562 cells. In the PMA-induced signaling systems, IL-6 level may be a limiting factor, while in the NaBut-induced signaling systems, the expression level of some other growth factors may be the limiting factor. Thus, the lack of combined effects of exogenous IL-6 and NaBut in K562 cells may be due to the existence of sufficient endogenous IL-6.

K562 cells express both erythroid and megakaryocytic-specific genes in the uninduced state and have the capacity to differentiate along the erythroid or megakaryocytic lineage [3,4,7,32,33]. Commitment toward mgakaryocyte development requires obligatory silencing of erythroid-specific genes. In the present study, we found that IL-6, when added in combination with 0.5 nM PMA, caused a further inhibition of the protein expression of the two erythroid-specific genes CD71 and Glycophorin A. It has been demonstrated that IL-6 also inhibited the mRNA and protein biosynthesis of the erythroid-specific gene γ globin in K562 cells, and decreased the γ and β globin mRNA expression in primary erythroid progenitor cells [34]. The IL-6 mRNA level was decreased by the erythroid differentiation inducer hemin in K562 cells [34]. Furthermore, IL-6 inhibited the production of erythropoietin in perfused rat kidneys [35]. Collectively, our results and previous findings suggest that IL-6 may serve as a positive regulatory signal in megakaryocytic differentiation, but play a negative regulatory role in erythroid differentiation.

We also observed that the mRNA expression of cytokine LIF was induced by the two erythroid inducers Ara-C and hemin. The functional roles of the induced LIF in erythroid differentiation remain to be determined. There appears to be less LIF mRNA induction after 6 rather than 3 days exposure to Ara-C and hemin. It is possible that LIF may be involved in the early stage of erythroid differentiation of K562 cells. In contrast, another erythroid inducer tiazofurin increased IL-6 production, but reduced the percentage of K562 cells with the IL-6Rα [36]. The functional significance of the increased IL-6 production in tiazofurin-induced erythroid differentiation is still unknown. Further investigation and comparison of the molecular mechanisms of Ara-C, hemin and tiazofurin will provide a better understanding toward the regulatory roles of LIF and IL-6 in the erythroid differentiation of K562 cells.

Our results have demonstrated that activation of the PMA-induced gp130 receptors by exogenous IL-6 could

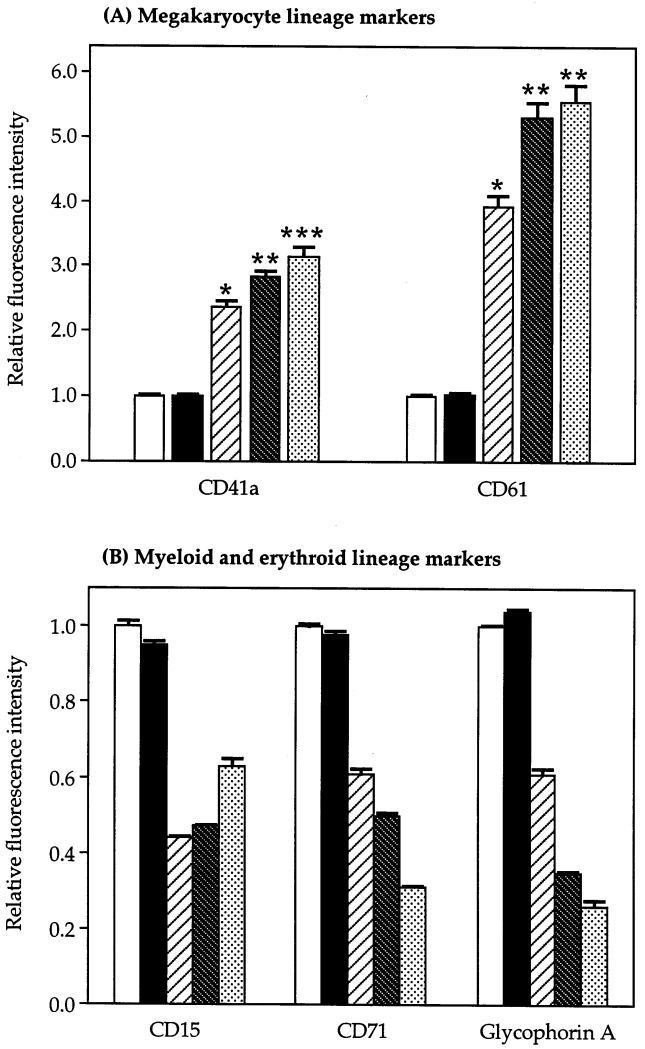

Fig. 3. Synergistic effects of IL-6 and PMA on K562 differentiation. K562 cells were grown in RPMI 1640 medium containing 5% FBS in the presence of control solvent (□), 40 ng/ml IL-6 (■), 0.5 nM PMA (▨), 40 ng/ml IL-6 in combination with 0.5 nM PMA (▧), or 1 nM PMA (▩). The immunophenotyping analysis was performed on day 5 after treatment. The mean of fluorescence intensity was analyzed by using Lysis II software (Becton Dickinson). Data represents the mean of three experiments ± SEM. In each experiment, each sample had three independent replicates. * significantly different from control (*t*-test, $P < 0.001$); ** significantly different from the * data (*t*-test, $P < 0.001$); *** significantly different from ** data (*t*-test, $P < 0.001$).

contribute to regulate the differentiation of K562 leukemia cells. However, since gp130 is the shared signaling component of several cytokines including IL-6, IL-11, LIF, OSM and CNTF [17,19], it remains possible that other cytokine(s), such as IL-11 or OSM, may also activate the induced gp130 receptors in K562 cells. Interestingly, during PMA-induced monocytic differentiation of human monocytoid cell line U937, the IL-6 binding receptor (IL-Rα) but not the IL-6 transducing receptor (gp130) was inhibited [37]. Therefore, the expression of IL-6Rα and gp130 could be regulated by the same differentiation inducer PMA in a coupled

manner or separately in different leukemia cells. This phenomenon further implied that gp130 could transduce the signal for multiple cytokines. In addition, IL-6Rα expression could be up-regulated or down-regulated by PMA in different leukemia cells [14,27,28,37]. It is conceivable that IL-6 may play different roles in leukemia differentiation along different hematopoietic lineages. This is consistent with the notion that IL-6 is a pleiotropic cytokine [15–17].

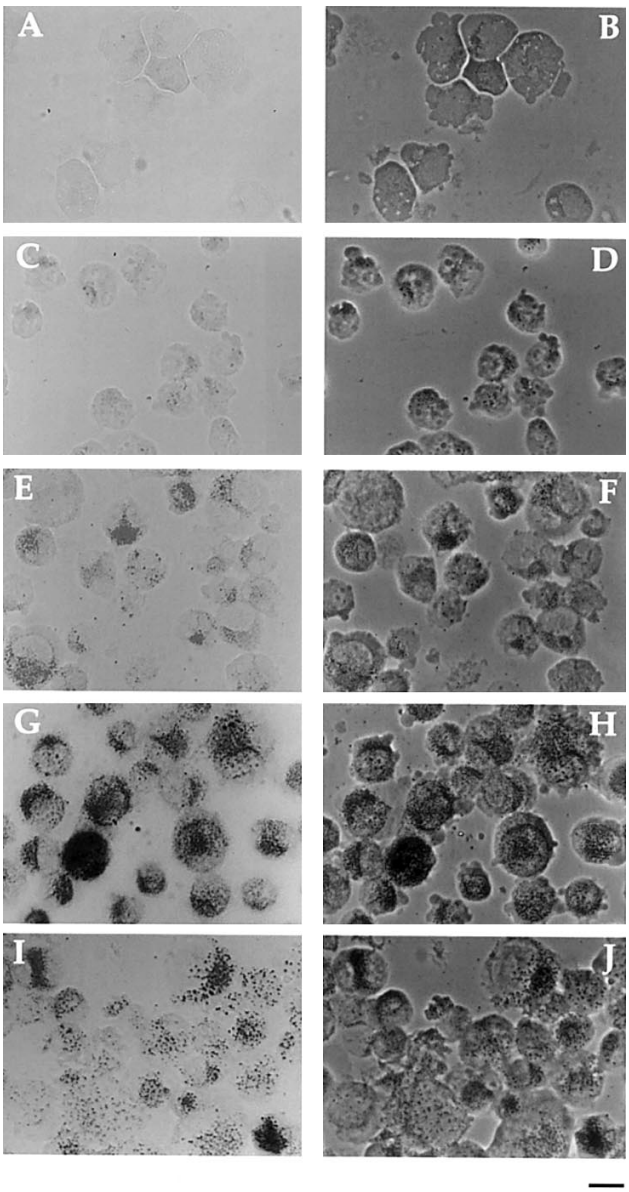

Fig. 4. Synergistic effects of IL-6 and PMA on the expression of α-naphthyl acetate esterase in K562 cells. K562 cells were grown in RPMI 1640 medium containing 5% FBS in the presence of control solvent (A and B), 40 ng/ml IL-6 (C and D), 0.5 nM PMA (E and F), 40 ng/ml IL-6 in combination with 0.5 nM PMA (G and H), or 1 nM PMA (I and J). The cytoplasmic expression of α-naphthyl acetate esterase was visualized by α-naphthyl acetate esterase staining after treatment for 5 days. The left panel (A, C, E, G, and I) are bright-field micrographs showing the positively stained granules (black particles in the cytoplasm), while the right panel (B, D, F, H and J) are phase-contrast micrographs showing the morphology of the cells. Bar, 20 μm.

In summary, the present study provided the systematic examination of the regulation of gp130 and LIF expression by various differentiation inducers in human chronic myelogenous leukemia K562 cells. Our results demonstrated that the expression of gp130 was dramatically induced at both the mRNA and protein levels by the two megakaryocytic inducers NaBut and PMA. In contrast, the mRNA expression of LIF was induced by the two erythroid inducers Ara-C and hemin. Furthermore, activation of the induced gp130 by exogenous IL-6 promoted the megakaryocytic differentiation of K562 cells induced by PMA. Our findings suggest that IL-6/gp130 signaling may be involved in the regulation of the megakaryocytic differentiation of K562 cells.

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