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IL-6 enhanced the retinoic acid-induced differentiation of human acute promyelocytic leukemia cells

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Abstract

It has been shown that retinoic acid (RA) induced the expression of interleukin-6 (IL-6) in human acute promyelocytic leukemia HL-60 cells. In the present study, we examined the ability of RA to induce the expression of gp130, the signal-transducing receptor component for IL-6, in HL-60 and a RA-supersensitive cell line HL-60/S4. We found that RA induced the expression of gp130, at both the mRNA and protein levels, in HL-60 and HL-60/S4 cells. Interestingly, the induction of gp130 expression observed in the RA-supersensitive HL-60/S4 cells was much more pronounced than that observed in HL-60 cells. Furthermore, activation of the RA-induced gp130 by exogenous IL-6 potentiated the differentiating effects of RA. The synergistic effects observed for IL-6 and RA was also much stronger in HL-60/S4 cells than in HL-60 cells. Our findings suggest that the differentiating effects of RA may partially be mediated by the up-regulation of IL-6/gp130 signaling in HL-60 and HL-60/S4 cells.

Keywords: IL-6; gp130; Retinoic acid; Leukemia differentiation; HL-60; Cytokines

1. Introduction

Retinoic acid (RA), a metabolite of vitamin A, is currently used as a standard treatment for acute promyelocytic leukemia with a successful rate of about 90%, and thus has been recognized as the most successful model for differentiation therapy [1,2]. The human acute promyelocytic leukemia cell line HL-60 is a well-characterized model system for the study of RA-induced leukemia differentiation [3,4]. RA induces HL-60 cells to differentiate towards the granulocyte lineage [5]. We have previously established a RA-supersensitive cell line, HL-60/S4, derived from HL-60 [6]. However, the underlying molecular mechanism by which RA induces the differentiation in HL-60 cells and the much more pronounced differentiation observed in HL-60/S4 cells still remains unclear.

It has been shown that RA induced the mRNA expression as well as protein secretion of interleukin-6 (IL-6) in HL-60 cells [7]. IL-6 is a pleiotropic cytokine acting on a variety of cell types [8–10]. The pleiotropic functions of IL-6 are mediated by two membrane receptor components, a ligand-binding molecule IL-6 receptor α (IL-6Rα) and a signal-transducing molecule gp130 [11]. Upon IL-6 binding, IL-6Rα associates with gp130 and signal is transduced through the dimerization and tyrosine phosphoryla-

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tion of gp130 [11]. 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 [10,12].

To explore the potential involvement of gp130 in the RA-induced leukemia differentiation, we examined the ability of RA to regulate the expression of gp130 in HL-60 and HL-60/S4 cells. We found that RA significantly induced the expression of gp130 in these cells at both the mRNA and protein levels. Interestingly, the induction of gp130 expression observed in the RA-supersensitive HL-60/S4 cells was much more pronounced than that observed in HL-60 cells. Furthermore, activation of RA-induced gp130 by exogenous IL-6 potentiated the differentiating effects of RA, and the synergistic effects observed for IL-6 and RA was much stronger in HL-60/S4 cells than in HL-60 cells.

2. Materials and methods

2.1. Cell culture and treatment

HL-60 cells (human acute promyelocytic leukemia, (PML) initially provided by Dr R.C. Gallo, National Cancer Institute, MD) and HL-60/S4 cells (a RA-supersensitive cell line derived from HL-60 [6]) 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 (ethanol), 1 μM all-*trans* RA (Sigma, St. Louis, MO), 1 μM 13-*cis* RA (Sigma), or 40 ng/ml human recombinant IL-6 (Pepro Tech, Inc., Rocky Hill, NJ) for indicated days.

2.2. Northern blot analysis

Total cellular RNA was prepared according to the method of Chomczynski and Sacchi [13] at day 0.25, 0.5, 1, 3, and 6 after treatment. Equal amounts of RNA (20 μg) were separated by gel electrophoresis using 1% agarose–formaldehyde gels. 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 full-length human gp130 cDNA probe (~ 3.0 kb) [14]. The cDNA probe was gel-purified by using GENECLEAN II Kit (BIO 101, La Jolla, CA), and labeled by random priming using Megaprime Labelling Kit (Amersham, UK). Hybridization was carried out in 0.5 M sodium phosphate buffer (pH 7.4) containing 7% sodium dodecyl sulphate (SDS), 1% bovine serum albumin (BSA), 1 mM EDTA, and 40 μg/ml salmon sperm DNA at 65°C. The hybridized membranes were washed in 2× SSC/0.1% SDS at 65°C for 3 × 30 min, then exposed to X-ray films (Kodak, Eastman Kodak Company, Rochester, NY) with intensifying screens (CBS 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 phosphate-buffered saline (PBS). For the analysis of transmembrane gp130 protein, 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 granulocyte markers, cells were stained with phycoerythin (PE)-conjugated anti-CD11b (Becton Dickinson, San Jose, CA) or PE-conjugated anti-CD11c (Becton Dickinson) antibodies at room temperature for 30 min. The stained cells were washed with PBS, then fixed with ice-cold 1% paraformaldehyde and stored at 4°C. The fluorescence signal of stained cells was recorded by using a flow cytometer (Becton Dickinson). The mean of fluorescence intensity was analyzed by using Lysis II software (Becton Dickinson).

3. Results and discussion

In this study, we explored the potential involvement of gp130, the signal-transducing receptor component for the IL-6 family cytokines, in the RA-induced differentiation in acute promyelocytic leukemia cells. We initially examined the ability of RA to regulate the mRNA expression of gp130 by Northern blot analysis using full-length human gp130 cDNA as probe. We found that the mRNA expression of

gp130 was significantly induced in both HL-60 and HL-60/S4 cells after treatment with 1 μM all-*trans* RA or 13-*cis* RA (Fig. 1). The induction of gp130 mRNA (7 kb) was detected at day 3 after exposure to either all-*trans* RA or 13-*cis* RA, gradually increased up to day 6. Interestingly, the induction of gp130 mRNA observed in the RA-supersensitive cell line HL-60/S4 (about 8-fold of the control level) was much more pronounced than that observed in its parental cell line HL-60 (about 3.5-fold of the control level). Whether RA induced the expression of gp130 mRNA directly or indirectly remains to be elucidated.

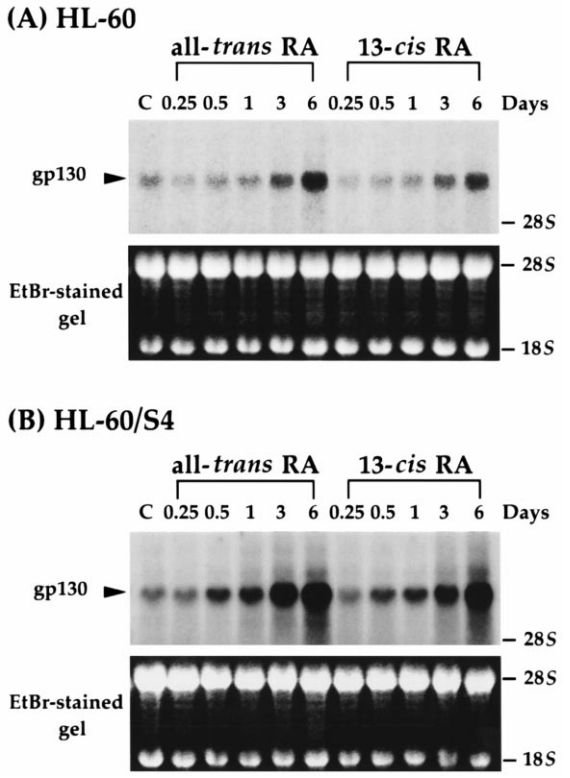

Fig. 1. The expression of gp130 mRNA in HL-60 and HL-60/S4 cells following treatment with RA. HL-60 and HL-60/S4 cells were grown in RPMI 1640 medium containing 10% FBS in the presence of control solvent (C), 1 μM all-*trans* RA, or 1 μM 13-*cis* RA. RNA samples were prepared at indicated time points, from 0.25 to 6 days. The mRNA expression for gp130 was examined by Northern blot analysis using full-length human gp130 cDNA as probe. Ethidium bromide (EtBr)-stained gel was shown to indicate that equal amount of RNA was loaded (20 μg per sample). Ribosomal RNA bands (18S and 28S) are indicated on the right, and the gp130 transcript is indicated on the left.

However, based on the time course of induction observed in our study (started from day 3, gradually increased to day 6), it is more likely that RA induced the expression of gp130 mRNA via an indirect mechanism. It is known that RA acts through two types of nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Both all-*trans* RA and 13-*cis* RA are high affinity ligands for RARs [15,16]. It would be interesting to dissect whether the induction of gp130 is a pan-RA receptor effect or only specific to RARs, and application of specific agonists and antagonists for RARs or RXRs should be helpful in addressing this issue.

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 [17,18]. To determine whether the RA-induced gp130 mRNA was translated as functional transmembrane receptor, we examined the expression of transmembrane gp130 protein by FACS analysis. Cells were stained with an anti-gp130 monoclonal antibody followed by a FITC-conjugated secondary antibody, and then analyzed by a flow cytometer. Our results demonstrated that the expression level of transmembrane gp130 protein was significantly induced by treatment with 1 μM all-*trans* RA in both HL-60 and HL-60/S4 cells (Fig. 2). Together with the previous study which has shown that RA also induced the secretion of IL-6 in HL-60 cells [7], our findings suggest that the RA-induced gp130 receptor and endogenous IL-6 may work in an autocrine manner in these leukemia cells. Examination of the effects of an IL-6 inhibitor, dexamethasone, as well as neutralizing antibodies against IL-6, IL-6Rα or gp130 would be helpful in further elucidating the existence of such autocrine loop [19–22].

To explore the functional roles of the up-regulated gp130 receptor in leukemia cells, we examined the effects of exogenous IL-6 alone or in combination with RA on the cellular proliferation and differentiation. While no significant effect of IL-6 on cellular proliferation was detected (data not shown), we found that IL-6 potentiated the differentiating effects of RA in both HL-60 and HL-60/S4 cells, as evidenced by the increase in the expression level of the two granulocyte markers CD11b and CD11c (Fig.

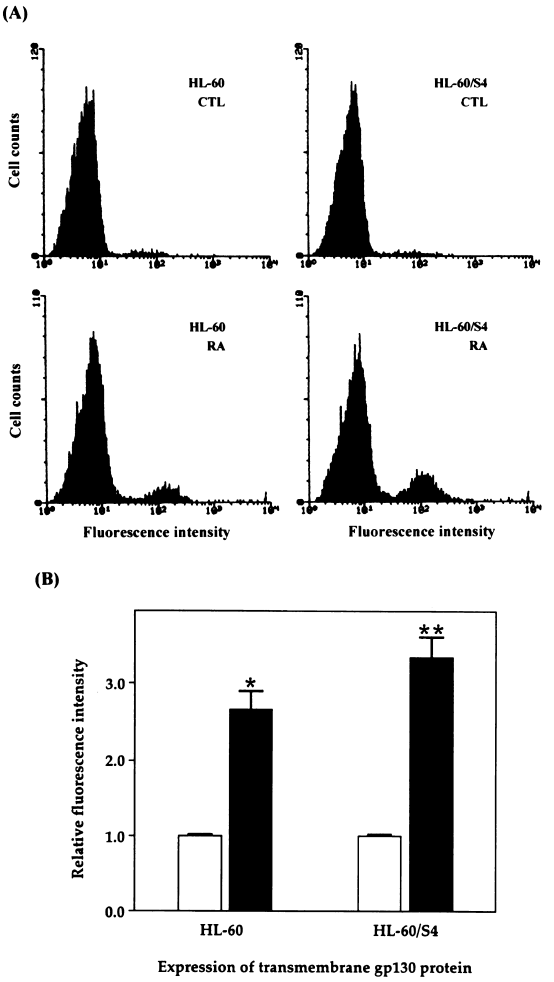

Fig. 2. Induction of the expression of transmembrane gp130 protein by RA in HL-60 and HL-60/S4 cells. HL-60 and HL-60/S4 cells were grown in RPMI 1640 medium containing 10% FBS in the presence of control solvent (CTL, □), or 1 μM all-*trans* RA (RA, ■). The FACS analysis was performed on day 5 after 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. *, significantly different from control (*t*-test, $P < 0.001$); **, significantly different from * data (*t*-test, $P < 0.001$).

3). All-trans RA alone significantly induced the expression of CD11b and CD11c in a dose-dependent manner, while IL-6 alone did not significantly induce the expression of these two markers in both HL-60 and HL-60/S4 cells. However, IL-6 in combination with RA dramatically further increased the expression level of CD11b and CD11c induced by 1 μM all-*trans* RA alone. The expression level of CD11b and CD11c induced by IL-6 in combination with 1 μM all-*trans* RA was even much higher than that induced by 10 μM all-*trans* RA alone in both cell lines. It is interesting that the synergistic effects between IL-6 and all-*trans* RA observed in HL-60/S4 cells were much stronger than that observed in HL-60 cells. This observation is consistent with the notion that the supersensitive responses to RA may partially be mediated through the enhanced induction of gp130 receptor by RA in HL-60/S4 cells.

Since gp130 is the shared signaling receptor component for several cytokines including IL-6, IL-11, LIF, OSM and CNTF [10,12], it remains possible that other cytokine(s), such as LIF, IL-11 or OSM, may also activate the RA-induced gp130 receptor in these leukemia cells. Furthermore, previous studies have shown that the expression of IL-1β, tumor necrosis factor α (TNFα), transforming growth factor α (TGFα), TGFβ and its receptor was also induced by RA in HL-60 cells [7,23–25]. It is conceivable that the differentiating effects of RA in HL-60 cells may be mediated by the sequential and collaborative actions of these growth factors and cytokines, in a way analogous to that observed in normal hematopoiesis [26,27].

Our results have shown that both all-*trans* RA and 13-*cis* RA induced the expression of gp130, however, whether the expression of IL-6Rα is also induced by RA still awaits for further investigation. In other cell types such as some myeloma cell lines, RA reduced the expression of both IL-6Rα and gp130; in contrast, in some other myeloma cell lines, RA reduced the expression of IL-6Rα but gp130 expression was unaffected [28–30]. Therefore, the expression of IL-6Rα and gp130 could be regulated by the same differentiation inducer RA in a coupled manner or separately in different myeloma cell lines. Such differential regulation of IL-6Rα and gp130 by RA may reflect the different constitutive expression level of IL-6Rα and gp130 as well as the relative ratios of IL-6Rα to gp130 in different myeloma cell lines [31,32]. Alternatively, this phenomenon may further imply that gp130 could transduce the signal for multiple cytokines [10,12].

We have recently reported that in addition to RA, several other differentiation inducers including

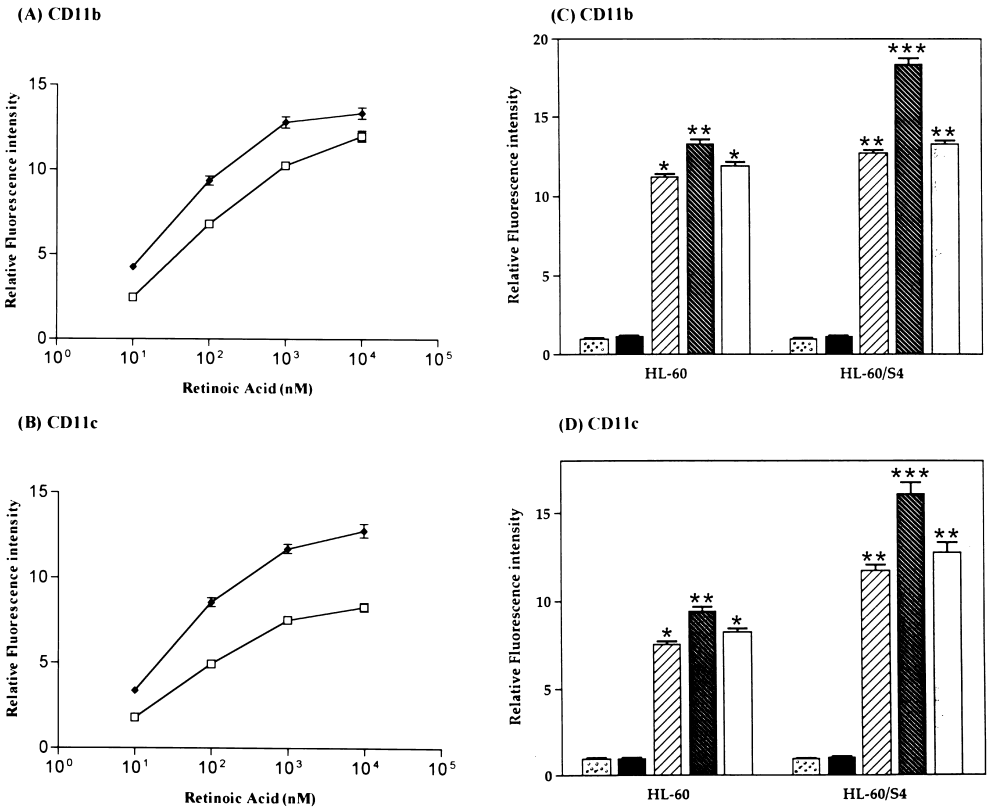

Fig. 3. Effects of RA and IL-6 on HL-60 and HL-60/S4 differentiation. (A and B), Effects of various dosages of RA. HL-60 (□) and HL-60/S4 (◆) cells were grown in RPMI 1640 medium containing 10% FBS in the presence of control solvent, various dosages of all-*trans* RA (10 nM, 100 nM, 1 and 10 μM). (C and D), The combined effects of IL-6 and RA. HL-60 and HL-60/S4 cells were grown in RPMI 1640 medium containing 10% FBS in the presence of control solvent (▩), 40 ng/ml IL-6 (■), 1 μM all-*trans* RA (▨), 40 ng/ml IL-6 in combination with 1 μM all-*trans* RA (▧), or 10 μM all-*trans* RA (□). The immunophenotyping analysis was performed on day 5 after treatment. Cell surface markers examined included the granulocyte lineage markers CD11b (A,C) and CD11c (B,D). 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 * data (*t*-test, $P < 0.001$); ***, significantly different from ** data (*t*-test, $P < 0.001$).

sodium butyrate and phorbol 12-myristate 13-acetate also dramatically induced the expression of gp130 at both the mRNA and protein levels in human chronic myelogenous leukemia K562 cells [33]. Although RA significantly induced the expression of gp130, it did not induce obvious differentiation in K562 cells. Therefore, the induction of gp130 is not a RA-specific phenomenon in K562 cells, and the RA-increased expression of gp130 was not sufficient to lead to differentiation of K562 cells. In this context, it would be interesting to investigate whether other differentiating agents could also induce the expression of gp130 in HL-60 cells and whether other agents which induce the expression of gp130 necessarily lead to differentiation of HL-60 cells.

In summary, the present study demonstrated that RA induced the expression of gp130 at both the mRNA and protein levels in human acute promyelocytic leukemia HL-60 and HL-60/S4 cells. Interestingly, the induction of gp130 expression observed in HL-60/S4 cells, the RA-supersensitive cell line, was much more pronounced than that observed in HL-60 cells. Furthermore, activation of the RA-induced gp130 receptor by exogenous IL-6 potentiated the

differentiating effects of RA, and the synergistic effects between IL-6 and RA observed in HL-60/S4 cells was much stronger than that observed in HL-60 cells. Our results are consistent with the notion that the differentiating effects of RA may partially be mediated through the up-regulated IL-6/gp130 signaling in HL-60 cells, while the supersensitive responses to RA may partially be mediated through the enhanced induction of gp130 signal transducer by RA in HL-60/S4 cells. Our findings suggest that IL-6, when administered together with RA, may have therapeutic potential in the treatment of acute promyelocytic leukemia.

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