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Induction of Differentiation of the Human Promyelocytic Cell Line HL-60 by Activin/EDF

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A human promyelocytic cell line, HL-60, treated with activin/EDF was found to differentiate into monocyte/macrophage-like cells. This was shown not only by morphology but by the loss of myeloperoxidase granules and the appearance of non-specific esterase. Dose-dependent inhibition of the differentiation by follistatin, an activin-binding protein, confirmed that it was indeed caused by activin. Thus, activin/EDF exerts its effect on hematopoietic cells not only on erythroid differentiation but also on at least a part of myeloid cell differentiation.

Activin was first purified from ovarian fluid based on the activities to stimulate the secretion of follicle stimulating hormone (FSH) from pituitary gland (1-3). More recently, a factor was isolated from the THP-1 human monocytic leukemia cell line treated with 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) that induces differentiation of mouse friend leukemia cell and designated as erythroid differentiation factor (EDF) (4). EDF was found to be identical with activin by the analysis of amino acid sequences from purified protein. Activin belongs to the TGF-β family (5) that may play important roles in development and cell

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differentiation in various systems. Recently, activin is shown to participate in a number of cellular events, including mesoderm induction in early amphibian development, the neural differentiation of P19 mouse embryonal carcinoma cells and retinoic acid (RA)-dependent differentiation of osteoblastic cell line MC3T3-E1 (6-9).

HL-60 cells are a human promyelocytic cell line that was isolated from a patient with acute myeloid leukemia and differentiate *in vitro* to a variety of cell types (10,11). For example, all-trans-retinoic acid (RA), actinomycin D, prostaglandin E and dimethylsulfoxide (DMSO) induce HL-60 cells to differentiate into mature granulocytes. Other inducers such as TPA, vitamin D_3, differentiation-inducing factor (DIF), tumor necrosis factor (TNF) and interferon-γ (INF-γ) bring about differentiation into monocyte/macrophage-like cells. Although activin was shown to specifically bind to non-erythroid HL-60 cells (12), its effect on these cells was not so far reported. In this paper, we describe the identification of activin receptor expressed on HL-60 cells and the effect of activin as a differentiation inducer toward monocyte/macrophage-like cells.

MATERIALS AND METHODS

Cell Culture: HL-60 cell was supplied from Japanese Cancer Research Resources Bank (JCRB). Cells were incubated in α-minimal essential medium (GIBCO, USA) containing 10% fetal calf serum (KC Biologicals) at 37°C in a humidified atmosphere of 5% CO_2.

Peptide: Recombinant human follistatin and activinA were purified from CHO cell culture supernatant as described previously (13).

^{125}I-labeling of activinA: ActivinA was radioiodinated with chloramin-T as described (14). Specific radioactivity of labeled activinA was 10,000-15,000 cpm/ng. ^{125}I was purchased from NEN.

Scatchard plot analysis: The binding assay of ^{125}I-labeled activinA was described (15).

Activin treatment: HL-60 cells were treated with various concentrations of activinA. Medium was changed every 3 days. A series of staining for assessment of cell differentiation were applied onto activin-treated cells or nontreated cells at day 4-10.

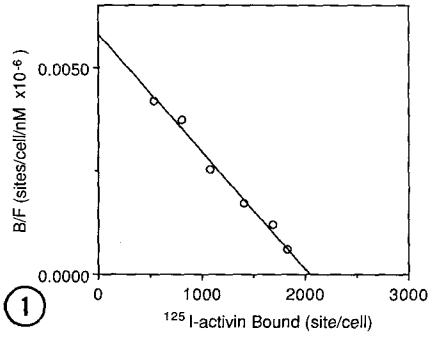
Cytochemical assays: The cells were stained with different reagents according to the manufacturer's protocols. Wright solution and Giemsa solution were obtained from Wako Pure Chemicals. Reagents for nonspecific esterase and myeloperoxidase staining were purchased from Muto Pure Chemicals.

RESULTS AND DISCUSSION

First, we examined the presence of high affinity binding sites for activinA on promyelocytic cell line HL-60 cells by Scatchard plot analysis (Fig.1). Although the existence of specific binding of activinA on HL-60 cells was reported by comparison with other cells, detailed data on the binding affinity and the number of receptor per cell were lacking. Fig.1 shows that HL-60 cells have about 2000 sites/cell for activinA specific binding with the Kd value of 0.34 nM. HL-60 cells expressed only the high affinity type receptor in contrast to P19 embryonic carcinoma cells having the high and the low affinity receptors. The presence of activin specific binding sites on cell surface suggests some physiological role of activin on HL-60 cells. We therefore investigated the effect of activin on cell growth and differentiation in HL-60.

Fig.2 shows that activin inhibited the growth of HL-60 cells by 20% at 1nM and by 35% at 10nM when examined at day 4.

Microscopic examination showed a decrease of azurophile granules, a characteristic structure of HL-60 cells recognized by Wright-Giemsa staining. Since the HL-60 cells differentiate into different cell types with specific biochemical characteristics, we



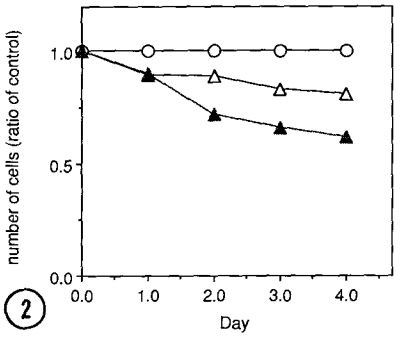


Fig. 1. Scatchard plot analysis of ^{125}I-labeled activinA binding to human promyelocytic HL-60 cells. Binding affinity of receptor can be compared directly from the slope. The ratio of vertical to horizontal axis is identical for all plots.

Fig. 2. Growth of HL-60 cells in the absence (○) or the presence (1nM: △), (10nM: ▲) of activinA. Cells were seeded at $3x10^4$/ml and cell number was counted every day.

assayed various enzyme activities by histological staining. HL-60 cells are known to have a high myeloperoxidase activity which decreases with differentiation. The cells were grown in the medium with or without activinA and were stained for this enzyme at day 7. As shown in Fig.3, HL-60 cells without activin treatment were stained brightly, whereas activin treated cells did not show any myeloperoxidase activity.

Non-specific esterases are among other markers of HL-60 differentiation. HL-60 cells are positive for naphthol AS-D chloroacetate esterase (CAE) as an indicator of granulocytic lineage, but almost negative for α-naphthyl butyrate esterase (ANBE) as an indicator of the monocytic lineage. The results of this test suggested that HL-60 cells differentiated into monocytic cells by treatment with 0.5nM activinA (data not shown). To confirm this,

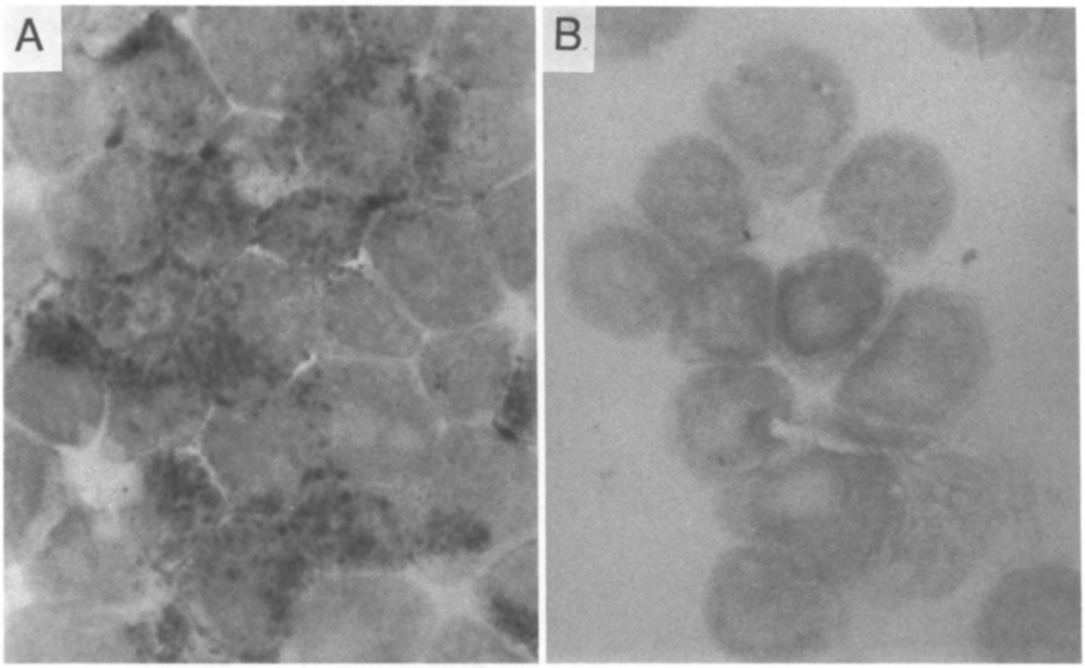


Fig. 3. Effect of activin A treatment on myeloperoxidase activity determined by the cytochemical method. HL-60 cells in the absence (A) or the presence (1nM:B) of activinA. Photomicrographs are taken at day 7. Dark stained cells in A are myeloperoxidase positive.

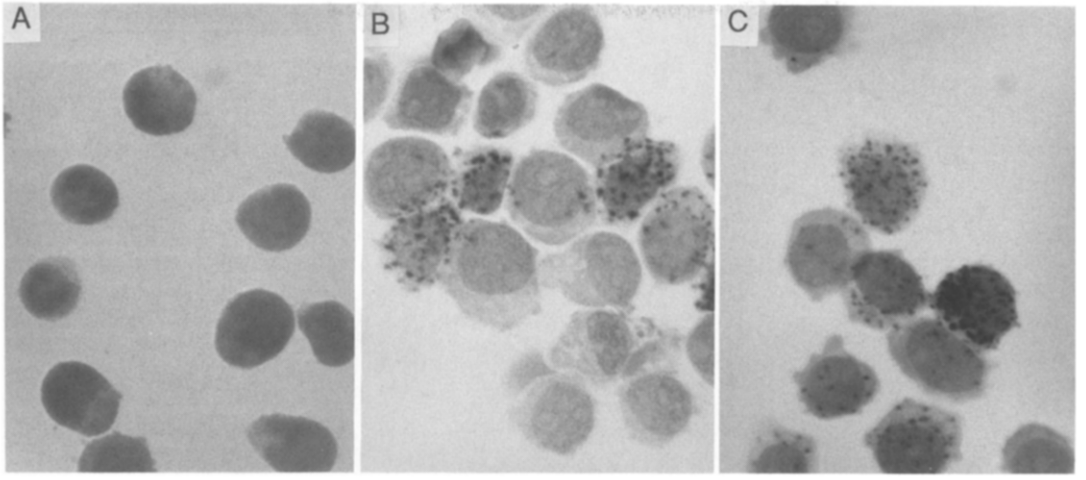


Fig. 4. Induction of a-naphthyl butyrate (non specific) esterase in HL-60 cells by activin. HL-60 cells were cultured in the absence (A) or the presence (1nM: B, C) of activinA. Esterase positive granules are seen in (B) and (C).

HL-60 cells were treated with 1nM activinA and stained for ANBE positive cells from day 4 through day 10. ANBE positive cells increased gradually as shown in Fig.4 and reached more than 50% by 10 days after activin treatment. Untreated control or those treated by other inducers such as RA or DMSO did not show this activity at all (data not shown). The ANBE positive staining was inhibited by fluoride, a strong inhibitor of this enzyme. Also, the addition of follistatin, activin binding inhibitory protein, to the culture medium dose-dependently inhibited the appearance of myeloperoxidase positive cells (Fig.5). This confirms that the effect of the recombinant activinA is caused by activin itself. We conclude from these results that activin acts as an differentiation inducer on HL-60 cells particularly towards monocytic lineage.

Previously, Okabe-Kado et al.(16) reported that activin had no effect on HL-60 cells but without shown data. The discrepancy between their and our conclusions may be due to subtle differences in experimental procedures including culture conditions or to the relatively mild activin effect on HL-60 cell differentiation. To exclude the possible change in the phenotypes during long term

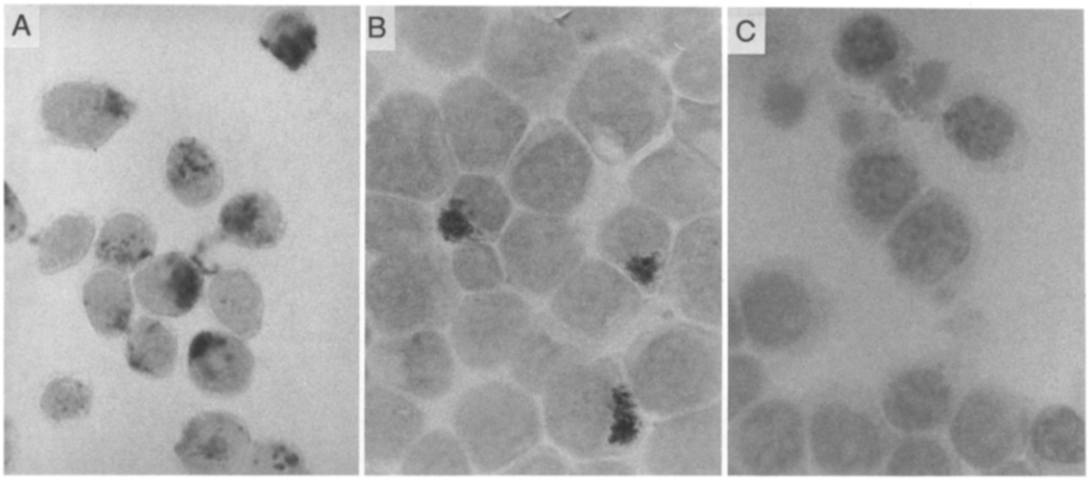


Fig. 5. Suppressive effect of follistatin on the differentiation of HL-60 cells by activinA. The concentration of activinA was 1nM each and those of follistatin were 0.1nM (A), 1nM (B) and 10nM (C). Cells were harvested at day 7 and stained for myeloperoxidase. No myeloperoxidase positive cells are seen in (C), while 18% and 95% myeloperoxidase positive cells are seen in (B) and (A), respectively.

culture, we repeated the same experiment with another HL-60 cell line freshly obtain from the cell bank of JCRB (see Materials and Methods). The results were the same.

It was reported that HL-60 cells produced the activin themselves by TPA treatment(17) that induced differentiation to monocyte/ macrophage-like cell. It is interesting that activin induces HL-60 differentiation to the same direction as TPA. A possible mechanism may be that a part of the effect of TPA induction into monocytic cells is bourne out by autocrine activin.

Recently, activin has been shown to influence not only FSH production (and secretion) and erythroid differentiation, but also neural differentiation, osteoblastic differentiation (6,7) and other developmental systems. In this report we have shown that it can act on the differentiation of promyelocyte into monocyte/macrophage-like cell, another system of non-erythroid lineage. Thus, activin is widely used for a number of phenomenon *in vivo* in early as well as late development and may act as a key molecule for the decision of cell fate in differentiation.

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