

DIFFERENTIATION INDUCTION OF ACUTE PROMYELOCYTIC LEUKEMIA CELLS TOWARD MONOCYTIC/MACROPHAGIC LINEAGE

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(Accepted September 11, 1998)

To study the stability of lineage commitment of acute promyelocytic leukemia (APL) cells, we treated freshly isolated leukemic cells from the patients with APL with various differentiation-inducers. APL cells showed granulocytic differentiation when treated with all-trans retinoic acid as previously reported. We showed here that freshly isolated APL cells could be also induced to differentiation to monocytic/macrophagic lineage with inducers such as 1, 25 dihydroxyvitamin D₃, 12-O-tetradecanoylphorbol-13-acetate or tumor necrosis factor- α . These results suggest that APL cells have the capacity to differentiate toward not only granulocytic lineage but also monocytic/macrophagic lineage. It is not clear that these results indicate the lineage promiscuity of normal promyelocytes or lineage infidelity of APL cells. However, these data provide an important clinical implication, because a possible therapeutic efficacy of using differentiation-inducing agents toward monocytic/macrophagic lineage in the remission induction therapy for the patient with APL or salvage therapy for the resistant APL cases to all-trans retinoic acid therapy is suggested.

Key words: acute promyelocytic leukemia /differentiation induction

Acute promyelocytic leukemia (APL, M3 in the French-American-British classification) is characterized by the clonal proliferation of immature hematopoietic cells whose differentiation is blocked at a promyelocytic stage, and severe coagulopathy. The specific t(15; 17) chromosomal translocation results in the formation of PML-RAR- α fusion protein which blocks differentiation of the cells in a dominant-negative manner (1-4). The striking feature of APL cells is their susceptibility to be induced to differentiate by all-trans retinoic acid (ATRA). ATRA selectively induce granulocytic differentiation of APL cells (5,6) and has been shown to induce a high rate of complete remission in the patients with APL (7,8). However, resistance to ATRA which develops at the time of relapse has been reported and prognosis of these patients are quite poor (9).

Several myeloid leukemia cell lines have been shown to be able to differentiate along either granulocytic or monocytic/macrophagic lineages by various inducing agents (10-13). So, we examined whether APL cells can be induced to differentiate not only to granulocytic lineage but also monocytic/macrophagic lineage. We obtained APL cells from two newly diagnosed patients;

case 1 was 71-year-old man and case 2 was 42-year-old man. Both patients fulfilled the criteria for the French-American-British classification; M3, and had the t(15; 17) translocation, and they were complicated with disseminated intravascular coagulation (DIC). APL cells were freshly isolated from heparinized bone marrow blood of these patients by using Ficoll-Hypaque method. The isolated cells consisted of more than 90% of APL cells and were cultured in the RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum at 37°C in a humidified atmosphere containing 5% CO₂. We treated APL cells isolated from patient 1 with 10⁻⁷ M ATRA, 10⁻⁷ M 1, 25 dihydroxyvitamin D₃ (Vit D₃) or 10⁻⁸ M 12-O-tetradecanoylphorbol-13-acetate (TPA). In this study, we used ATRA as a granulocytic inducer, and Vit D₃ and TPA as a monocytic and macrophagic inducer. As shown in Fig 1, five days treatment of APL cells with ATRA resulted in a morphological change showing mature granulocyte-like cells. Whereas, Vit D₃ induced APL cells to monocytic differentiation. The cells had an indented nucleus and abundant cytoplasm containing vacuoles. Treatment with TPA also resulted in morphological changes showing characteristic of macrophage with an indented nucleus and large cytoplasm with marked vacuolization. The presence of Auer rods in the cytoplasm of these cells strongly indicates that these cells were differentiated from APL cells. Similar results could be obtained by using APL cells from patient 2 (Fig. 1). In addition, tumor necrosis factor- α (TNF- α) which is known as a monocytic inducer of HL-60 promyelocytic leukemia cells and U937 monoblastic leukemia cells (10,11) also induced monocytic differentiation in APL cells (Fig. 1). These results suggest that APL cells can be induced to differentiate not only to granulocytic lineage but also monocytic/macrophagic lineage and this lineage determination is an inducer-specific event.

There are a wide variety of agents capable of inducing leukemia cell differentiation (10-14). Among them, ATRA has been shown to induce granulocytic differentiation in HL-60 cells and freshly isolated APL cells in vitro system (5,6,10). Clinical trials have also demonstrated that ATRA is singularly efficacious in inducing complete remission through differentiation induction of leukemic cells in APL patients (7,8). However, relapses may occur when ATRA is prescribed as a maintenance therapy and resistance to a second ATRA-induction therapy is frequently observed (9). In such conditions, the salvage therapeutic approach including use of differentiation-inducing agents other than ATRA may be considerable. Bhatia and colleagues have recently shown that NB4 APL cell line could be induced to differentiate along with monocytic/macrophagic lineage by M-CSF and Vit D₃, and this effect was synergized by the

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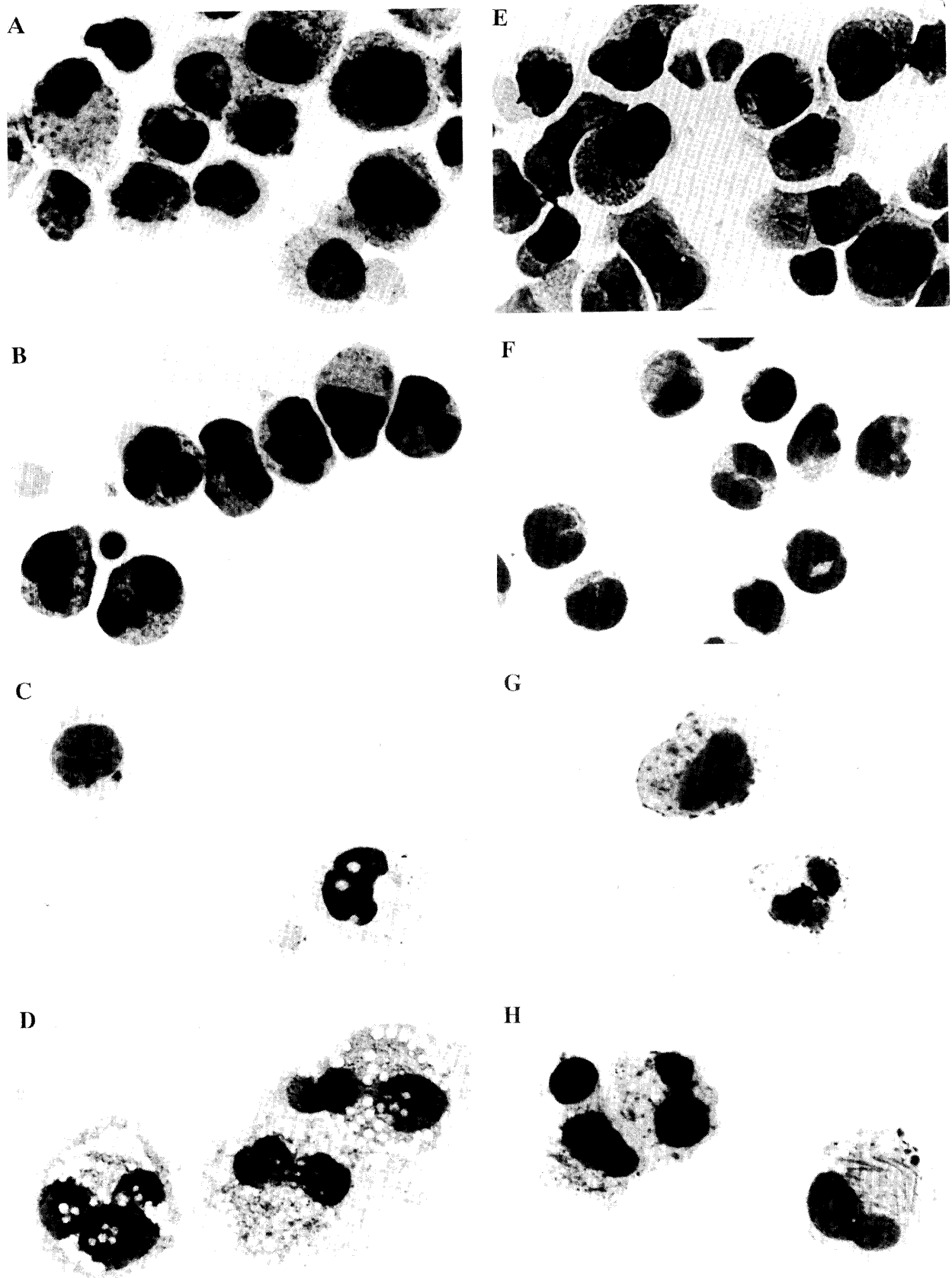


Fig. 1. Differentiation induction of freshly isolated APL cells. APL cells obtained from patent 1 (A-D) or patent 2 (E-H) were treated with various inducers for 5 or 3 days, respectively; (A, E) untreated, (B, F) 10^{-7} M ATRA, (C) 10^{-7} M Vit D₃, (G) 100U/ml TNF- α , (D, H) 10^{-6} M TPA. Photomicrograph shows morphology of May-Grüwald-Giemsa-stained cytocentrifuge preparations ($\times 1000$).

addition of TPA (15). We showed here that freshly isolated APL cells are also able to be induced to differentiate to monocytic/macrophagic lineage in primary culture. It has been postulated that commitment to a single lineage is maintained in leukemic blasts which arise from a clonal expansion of precursor cell after its transformation and arrest at a specific stage of differentiation (16). Thus, APL cells could be recognized as the transformed promyelocytes whose further differentiation along with granulocytic pathway were blocked. However, the recent reports of mixed lineage leukemia have provided the evidences that some leukemia cells might be transformed from a progenitor cell capable of differentiating into more than one lineage (lineage promiscuity) (17), or these cells might result from aberrant gene expression due to the abnormal assembly of differentiation program (lineage infidelity) (18). So we think that our cases can be interpreted as the indication of either the lineage promiscuity of normal promyelocytes or lineage infidelity of APL cells. The molecular mechanism of the induction of monocytic/macrophagic differentiation in APL cells requires further study, especially with special reference to PML-RAR- α fusion protein. However, our results provide an important clinical implication and strongly suggest a possible therapeutic efficacy of using monocyte/macrophage-differentiation inducing agents such as Vit D3 in the remission induction therapy for the patients with APL or the salvage therapy for ATRA-resistant APL cases.

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