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Mechanisms for Induction of Differentiation in the Human Promyelocytic Cell Line HL-60

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Acute myeloid leukemia (AML) is characterized by a block in cell differentiation. Leukemic cell lines which grow continuously in vitro can be used to explore regulation of differentiation in leukemia. The promyelocytic HL-60 line [3] is induced to mature into granulocytes by incubation with agents such as dimethylsulfoxide (DMSO) [4] and retinoic acid (RA) [2]. The mechanisms by which these and other agents induce differentiation are unknown. Knowledge of them could improve understanding of the defects in terminal differentiation characteristic of AML. We have concentrated on mechanisms of action of RA, DIF (see below), and cAMP-inducing agents [1] because these act at low concentrations and most of them are regarded as physiological. Nitroblue tetrazolium (NBT) reduction has been used as the parameter of induced differentiation as it is a reliable measure of functional maturation in HL-60.

Mitogen-stimulated human mononuclear blood cells release polypeptide factors called differentiation-inducing factors (DIFs), which induce HL-60 cells to mature into phagocytizing cells with the morphological characteristics of granulopoietic or myelomonocytic cells [5]. The T-lymphocyte line HUT-102 is a reliable constitutive producer of DIF [8]. HUT-102 supernatant was used for partial purification of DIF employing chromatography on DEAE-Sepharose, blue Sepharose, and Sephadex G-75 followed by electrophoresis in polyacrylamide gels. The HUT-102 produced DIF is a polypeptide with an apparent molecular weight of 58,000. An activity which cochromatographs with DIF acts synergistically with RA to induce maturation not only of HL-60 but also of the monoblast-like cell line U-937 (measured as percentage of cells reducing NBT). Thus, the combination of 10 n*M* RA (which alone gives 20% maturtion of HL-60) and DIF (at a concentration which alone is inactive) induced the maturation of 70%–80% of HL- 60 cells. The combination of 100 n*M* RA (which alone give 5% maturation of U-937) and DIF (which alone is inactive) induced the maturation of 50% of U-937 cells. The synergistic effect between RA and DIF indicates that these agents act by different mechanisms to induce differentiation.

The effect of RA on both HL-60 [1] and U-937 [6] is potentiated not only by DIF but also by agents which increase the intracellular level of cAMP such as prostaglandin E (PGE) and choleratoxin. We found that cells can be primed for differentiation by pretreatment for approximately 1 day with RA followed by exposure to a cAMP-inducing agent or DIF [6]. The reverse sequence was ineffective. Thus, HL-60 could be primed by incubation for 15–20 h with 10 n*M* RA to respond by maturation to the addition of 10 m*M* PGE_2 or 1 n*M* choleratoxin while 10 n*M* RA alone was almost inactive [7]. RA-primed HL-60 also responded to DIF, which alone was inactive at the concentration used in these experiments. U-937 primed by incubation for 24 h with 100 n*M* RA responded to cAMP-inducing agents and DIF, which alone were inactive on this cell line. A decrease in synthesis of some protein(s) seems to favor RA-induced differentiation because priming with RA occurs even better

at a concentration of cycloheximide that inhibits growth completely. However, the continuous presence of the latter agent inhibited maturation. Another finding was that HL-60, but not U-937, primed with RA responded with maturation to ATP and other nucleoside triphosphates, suggesting a role of phosphorylation reactions at the plasma membrane.

Thus our results indicated that cAMP-inducing agents are potent modulators of RA-induced differentiation of both HL-60 and U-937. Therefore a cAMP-dependent phosphorylation reaction(s) might modulate the differentiation response to RA. RA could induce a cAMP-dependent protein kinase (PK) or a substrate which is phosphorylated in the process of differentiation. Treatment of HL-60 for 8–16 h with RA gave a dose-dependent increase in cytosol cAMP-dependent PK I while PK II was decreased. Typical functional changes occur in the plasma membrane as a result of terminal maturation, leading to a capacity for motility, phagocytosis, and secretion. An increase was found in cAMP-independent PK activity of a fraction enriched in plasma membranes from HL-60 treated for less than 1 day with RA. Concomitantly a change in the phosphoprotein pattern of the plasma membrane was observed judging from SDS electrophoresis after incubation of membranes with γ-^{32}P ATP. These changes were seen before morphological maturation. Therefore they may be related to the mechanisms for induction of differentiation.

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