学 位 論 文 の 要 旨

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学　位　論　文　名　Gene Expression Profiles in Differentiating Leukemia Cells Induced by Methyl Jasmonate Are Similar to Those of Cytokinins and Methyl Jasmonate Analogs Induce the Differentiation of Human Leukemia Cells in Primary Culture

発　表　雑　誌　名　Leukemia
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論 文 内 容 の 要 旨

Introduction

Jasmonic acid and its methyl ester, methyl jasmonate (MJ), are fatty acid-derived cyclopentanones in plants that play major roles in defense against insects and disease. We previously reported that jasmonates can induce myeloid leukemia cell lines to undergo differentiation into granulocytes with some monocytic properties, and one novel derivative (methyl 4,5-didehydrojasmonate, DDHJ) is a particularly promising therapeutic agent for differentiation therapy of leukemia.

Myeloid leukemia cells are induced to differentiate into mature granulocytes and macrophages by various inducers. During the differentiation of leukemia cells, the expression of leukemia-associated genes is down-regulated and many maturation-associated genes are normally expressed, as in the maturation of normal neutrophils and monocytes. Our previous results indicate that the gene expression profiles associated with exposure to isopentenyladenine (IPA) are quite different from those with all-*trans* retinoic acid (ATRA), although both inducers effectively induce the granulocytic differentiation of myeloid leukemia HL-60 cells, suggesting that IPA-induced differentiation is regulated by transcription factors different from those in the differentiation induced by ATRA. In the present investigation, we examined and compared the gene expression

profiles in leukemia cells treated with MJ and other differentiation inducers.

Although differentiation-inducing agents potently affect the differentiation of established cell lines, they may have only modest differentiation-inducing activity in freshly isolated leukemic cells. Therefore, we sought to determine whether DDHJ could affect the differentiation of leukemic cells from patients with acute myeloid leukemia (AML) and other hematological malignancies to determine whether DDHJ could be useful for differentiation therapy against myeloid leukemia.

Materials and methods

The diagnosis and classification of AML were according to the FAB criteria. Leukemic bone marrow specimens were collected at diagnosis, after all of the patients gave their written informed consent for sample collection in accordance with institutional policy. Only specimens that contained at least 70% leukemia cells were studied. Assay of cell growth and properties of differentiated cells was performed by cell numbers count, the reduction of nitroblue tetrazolium chloride (NBT), morphological changes of malignant cells, and expression of myelomonocytic antigens. cDNA microarray analysis was performed using with a cDNA microarray representing about 1,000 different human genes specified for human cancer. Gene expression analysis of S100P was performed by RT-PCR and quantitative RT-PCR reaction method.

Results and discussion

Changes in gene expression of HL-60 cells treated by MJ, IPA, cotylenin A (CN-A), ATRA or 1α,25-dihydroxyvitamin D_3 (VD3) were examined. All the inducers significantly up-regulated the expression of two genes, such as tumor necrosis factor-α-induced protein 1 and amyloid beta (A4) precursor protein. The gene for the calcium-binding protein S100P was the most up-regulated gene in cells treated with MJ, IPA and CN-A, whereas ATRA and VD3 did not significantly affected the gene expression. Similar changes were also observed in expression of activating transcription factor 3 and Clk-associating RS-cyclophilin mRNAs. Expression of five genes was significantly up-regulated by both MJ and IPA but not by the others and only three genes are differentially regulated by MJ and IPA. The time course of the expression of S100P mRNA revealed that MJ rapidly induced the expression of this gene in HL-60 cells, suggesting that the rapid induction of the expression of this gene is involved in the induction of differentiation induced by MJ, just like by IPA and

CN-A. The same result as the accumulation of S100P transcript was greatly induced was obtained by quantitative RT-PCR analysis. The present results indicate that MJ also effectively induces S100P mRNA and the general gene profile is similar to that with IPA, suggesting that the mode of action is largely shared in MJ- and IPA-induced differentiation. Interestingly, these inducers are known as plant growth regulators.

Since there are stereoisomers in DDHJ, we examined the difference in activity of these isomers on the growth and differentiation of leukemia cells. (+)Methyl 4,5-didehydrojasmonate (natural DDHJ) is more potent than (-)methyl 4,5-didehydrojasmonate (unnatural DDHJ) in inhibiting the cell growth, inducing NBT reduction, morophologic changes and expression of cell surface antigens of HL60 cell . The growth of leukemia cells from patients with hematological malignancies treated with DDHJ for 7 days showed most of the malignant cells were viable, suggesting that the effect of the jasmonate is cytostatic. We analyzed NBT reduction, non-specific esterase activity, differentiation-associated antigens such as CD11b and CD14, and morphological changes, and the results indicate that natural DDHJ effectively enhanced the functional and morphological differentiation of leukemia cells in some patients. Jasmonate and its analogs are used as flavors in foods and cosmetics, suggesting that these compounds are quite safe and clinical useful. MJ at 3 mM concentration induced perturbation of mitochonria in hepatoma and leukemia cells, whereas mitochondria from quiescent and mitogenically stimulated normal human peripheral blood lymphocytes were not affected by MJ, suggesting that jasmonates are preferentially cytotoxic toward malignant cells. Flescher and his collaborators indicated that MJ and its analogs exhibit anti-cancer activity in vivo, as well as in vitro. MJ was therapeutically effective in an animal model of lymphoma, and its analog significantly suppressed metastasis of murine melanoma, suggesting that these compounds are clinical useful.

Conclusion

Methyl 4,5-didehydrojasmonate significantly stimulated both the functional and morphological differentiation of leukemia cells that had been freshly isolated from patients with hematological malignancies. Jasmonate derivatives may be promising therapeutic agents for differentiation therapy of leukemia.