学 位 論 文 の 要 旨

氏名　　堀江　昭好

学 位 論 文 名　Induction of Differentiation of Myeloid Leukemia Cells in Primary Culture in Response to Lithocholic Acid Acetate, a Bile Acid Derivative, and Cooperative Effects With Another Differentiation Inducer, Cotylenin A

発 表 雑 誌 名　Leukemia Research

（巻，初頁～終頁，年）　32, 1112～1123, 2008

著 者 名　Akiyoshi Horie, Miho Akimoto, Hiroto Tsumura, Makoto Makishima, Takeshi Taketani, Seiji Yamaguchi, Yoshio Honma

論 文 内 容 の 要 旨

Introduction

The active form of vitamin D, 1α,25-dihydroxyvitamin D_3 (VD_3), inhibits proliferation and induces the differentiation of leukemia cells. However, the therapeutic effects of VD_3 for patients with myeloid malignancies have been very modest because it has hypercalcemic effects. Several analogues of VD_3 that show anti-cancer activity and only weak activity for inducing hypercalcemia have been developed, but they are not yet available for the clinical treatment of cancer or leukemia. Recently, vitamin D receptor (VDR) was found to function as a receptor for secondary bile acids such as lithocholic acid (LCA), and its derivative LCA acetate. Since it has been suggested that LCA acetate induces an alternative conformation in VDR, which results in differential cofactor recruitment and selective physiological functions, a structure-function analysis of vitamin D analogs suggests that VD_3 and its analogs induce non-genomic VDR actions and that adverse effects are at least partly attributable to nongenomic mechanisms. A recent report showed that LCA acted as a compensatory VDR ligand in vitamin D-deficient rats and that the administration of LCA

did not increase serum calcium levels over physiological ranges, suggesting that LCA acetate may relatively induce genomic actions in the intestine without hypercalcemia. LCA acetate may induce VDR target genes via genomic action. These findings suggest that LCA acetate may be a better analog for differentiation therapy against myeloid leukemia.

Materials and methods

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. Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2 in air. Cell numbers were counted in a Model ZM Coulter Counter. Superoxide-generating oxidase was determined by the ability of the cells to reduce NBT upon exposure to 12-*O*-tetradecanoyl phorbol-13-acetate. Morphological changes were examined in cell smears stained with May-Grünwald-Giemsa solution. Surface expression of myelomonocytic antigens was determined by monoclonal antibody labeling and flow cytometry using a FACSCAN.

Results and discussion

LCA acetate concentration-dependently inhibited the growth and induced the NBT reduction of leukemia cells. Monoblastic leukemia cell lines are more sensitive to LCA acetate than myeloblastic or promyelocytic leukemia cell lines. Leukemia cells from leukemia patients were also induce to undergo functional and morphological differentiation by LCA acetate, although most of the LCA acetate-treated cells were in early stages of monocytic differentiation.

LCA acetate alone may not be used in the clinical treatment of leukemia because a high concentration of LCA acetate is required to induce the growth inhibition and differentiation of leukemia cells. Therefore, we examined the effects of suboptimal concentrations of LCA acetate in combination with other differentiation inducers. Combined treatment with LCA acetate and cotylenin A was the most effective at inhibiting cell

proliferation among the combinations of LCA acetate and other differentiation inducers, and the differences were statistically significant ($p<0.05$). Synergism between LCA acetate and cotylenin A was also observed in the induction of NBT reduction, CD11b, CD14, lysozyme activity, α-naphthyl acetate esterase activity and morphological changes.

LCA acetate activated mitogen-activated protein kinase (MAPK) in leukemia cells before inducing differentiation, and this activation was needed to elicit differentiation and growth arrest, as with VD_3. Although cotylenin A alone did not essentially activate MAPK, it effectively enhanced LCA acetate -induced MAPK activity. The enhancing effect of cotylenin A may, at least in part, contribute to the synergistic effects of LCA acetate and cotylenin A on differentiation and growth arrest. While cotylenin A effectively induced the expression of S100P mRNA, LCA acetate hardly affected such expression. In combined treatment, LCA acetate did not affect the cotylenin A-induced expression of S100P mRNA. Cotylenin A immediately induced the expression of S100P mRNA and protein, and the differentiation of HL-60 cells was suppressed by treatment with antisense oligonucleotides against S100P, which suggests that S100P plays an important role in cell differentiation. Cotylenin A enhanced the LCA acetate-induced differentiation pathway that included MAPK and p21, whereas LCA acetate did not affect the cotylenin A-induced differentiation pathway that included S100P. These results suggest that the expression of differentiation-associated genes is regulated by several pathways.

Conclusion

LCA acetate induced the differentiation of human leukemia cells. Treatment with a combination of LCA acetate and cotylenin A, an inducer of the differentiation of leukemia cells, was more effective than that with LCA acetate or cotylenin A alone at inducing monocytic differentiation.