学 位 論 文 の 要 旨

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学 位 論 文 名　Cyclopamine Induces Eosinophilic Differentiation and Upregulates CD44 Expression in Myeloid Leukemia Cells

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論 文 内 容 の 要 旨

INTRODUCTION

Hedgehog (Hh) signaling plays an important role in the regulation of stem/progenitor cell expansion. The Hh pathway is activated in some leukemia cell lines and primary leukemia cells. The Hh pathway may be important as a novel therapeutic target. Cyclopamine, a plant-derived teratogenic steroidal alkaloid, inhibits the Hh pathway. Recent reports have indicated that cyclopamine inhibits proliferation and induces apoptosis in several malignancies *in vitro* and *in vivo*. The effect of cyclopamine in leukemia is still unknown. We have examined the differentiating effects of cyclopamine in leukemia cells.

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. Human myeloid leukemia cell lines were maintained in RPMI-1640 medium supplemented with fetal bovine serum and gentamicin. Suspensions of these cells were incubated with or without the test compounds in multidishes. Superoxide-generating oxidase was determined by the ability of the cells to reduce nitroblue tetrazolium (NBT). Surface expression of myelomonocytic antigens was determined by monoclonal antibody labeling and flow cytometry.

RESULTS AND DISCUSSION

Cyclopamine concentration-dependently inhibited the proliferation of human myeloid leukemia HL-60 cells, and induced NBT reduction, which is a typical marker of myelomonocytic differentiation. Cyclopamine also induced morphological changes in HL-60 cells toward granulocytes with large reddish granules. Untreated HL-60 cells showed weak and diffuse staining by the Luxol-fast-blue method (a marker of eosinophils) and differentiated cells contained granules that were strongly positive for Luxol-fast-blue staining. Cyclopamine significantly enhanced the expression of the surface antigen CD11b in a concentration-dependent manner. The drug also enhanced the expression of CD44 antigen. The expression of CD44 was not affected by other differentiation inducers such as all-*trans* retinoic acid (ATRA) and 1α,25-dihydroxyvitamin D_3 (VD3). Cyclopamine significantly enhanced the expression of CD44 mRNA in a concentration-dependent manner. Ligation of CD44 with some monoclonal antibodies against CD44 can induce the differentiation of leukemia cells. When the cells were treated with anti-CD44 antibody and cyclopamine, anti-CD44 antibody-induced NBT reduction was enhanced by cyclopamine in a concentration-dependent manner. VD3- and ATRA-induced NBT reduction was also enhanced by cyclopamine. We examined the effects of other Hh inhibitors on HL-60 cells and two of four Hh inhibitors had no differentiation-enhancing effects. On the other hand, tomatidine, which is cyclopamine analogue and is not Hh inhibitor, showed similar differentiation-enhancing effects. These findings suggest that the differentiating effects of cyclopamine are not associated with the inhibitory activities toward Hh signaling. Cyclopamine significantly enhanced CD44 expression in leukemia cells

that were isolated from some AML patients. The upregulation of CD11b, CD14 or CD15 was observed in all cases with upregulation of CD44 expression by cyclopamine. The combined treatment with cyclopamine and other differentiation-inducers more than additively enhanced myelomonocytic differentiation in leukemia cells that were isolated from some AML patients.

CD44 expression is a function of eosinophil maturation of hematopoietic progenitor cells and an activation marker of human eosinophils. Cyclopamine induced HL-60 cells to differentiate into granulocytes with eosinophilic granules and the upregulation of CD44 expression. The eosinophilic differentiation of leukemia cells by chemicals has not been reported, although several chemicals have been reported to induce neutrophilic differentiation of leukemia cells. Cyclopamine may also induce ligand(s) of CD44 during the differentiation of HL-60 cells.

CONCLUSION

Cyclopamine can induce the differentiation of myeloid leukemia cell lines and AML cells in primary culture. The treated cells showed intracytoplasmic eosinophilic granules, which were stained with Luxol-fast-blue. Combined treatment with cyclopamine and a monoclonal antibody to ligate CD44 more than additively induced the differentiation of leukemic cells. These results may provide useful information for the development of a CD-44-targeted therapy in AML.