学 位 論 文 の 要 旨

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学　位　論　文　名	Tamoxifen Enhances the Differentiation-Inducing and Growth-Inhibitory Effects of All-*trans* Retinoic Acid in Acute Promyelocytic Leukemia Cells
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論 文 内 容 の 要 旨

INTRODUCTION

All-*trans* retinoic acid (ATRA) induces a dramatic response in the treatment of patients with acute promyelocytic leukaemia (APL), leading to complete remission, but most patients will eventually experience a relapse. The combination of ATRA and anthracyclines results in an overall remission rate of up to 95% and a cure rate that now exceeds 80%. However, hematologic toxicity was observed in the ATRA/anthracycline group and liver toxicity and long Q-T syndrome were observed in patients in the ATRA/arsenic trioxide group. Since anthracyclines and arsenic compounds are genotoxic and carcinogenic, long-term treatment with these drugs should be avoided as much as possible. Although recent therapy against APL has a high cure rate, further improvements are needed. To identify a compound that would be useful for APL therapy when combined with ATRA, we have screened various compounds that are less toxic and clinically available, such as inhibitors of signal transduction pathways and physiologically active molecules. As a result, the most effective agent we identified was tamoxifen. We found that tamoxifen effectively enhances the differentiation-inducing and growth-inhibitory effects of ATRA in APL cells. Tamoxifen is a selective estrogen receptor modulator that is used as the first-line treatment for estrogen receptor-positive breast cancer. However, multiple non-estrogen receptor-mediated mechanisms have been implicated in the antitumor effects induced by tamoxifen in estrogen receptor-negative tumors. Therefore, in the present study, we sought to clarify the combined effects of ATRA and tamoxifen on the growth

and differentiation of human myeloid leukaemia cells including APL cells.

MATRERIALS AND METHODS

The HL-60 cell line, derived from an AML patient, and NB4 and HT93 promyelocytic leukaemia cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 80 μg/ml gentamicin at 37°C in a humidified atmosphere of 5% CO_2 in air. Suspensions of cells (5×10^4 cells/ml) in 2 ml of culture medium were incubated with or without the test compounds in multidishes. Cell numbers were counted in a Model Z1 Coulter Counter. The viable cells were examined by the MTT assay. Differentiation of APL cell lines was evaluated by NBT reduction and morphological changes in cell smears stained with May-Grunwald-Giemsa solution and surface expression of CD11b by monoclonal antibody labeling and flow cytometry.

NB4 cells (3×10^3 per dish) were plated into 3 ml of a semisolid medium with 0.8% methylcellulose and 20% fetal bovine serum in triplicate multiwell plates for 8 days. Colonies in enlarged photographs were measured and those greater than 0.4 mm in diameter were counted. Nucleated cells (1,000 cells/ml/dish) were placed in 1 ml of a semi-solid medium containing haematopoietic growth factors in a 24-well plate and incubated for 8 days.

Cells were packed after being washed with cold PBS, and then lysed at 1.5×10^7 cells/ml in sample buffer. The resultant lysates were resolved on 10% SDS-polyacrylamide gels. The proteins were transferred electrically from the gel to an Immobilon-P membrane and immunoblotted with antibody. Horseradish peroxidase (HRP)-conjugated antibody was used as a secondary antibody. The bands were developed by treatment with the Immun-Star HRP Chemiluminescent Kit for 5 min at room temperature, and detected using a Fuji Lumino Image Analyzer LAS-4000 system.

The study protocol was approved by the Ethics Committee of Shimane University and written informed consent was obtained from all subjects. All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University and they were handled according to our institutional guidelines.

RESULTS AND DISCUSSION

Tamoxifen concentration dependently inhibited the proliferation of APL cells and induced

the expression of CD11b, a surface marker of myelomonocytic differentiation. Under treatment with ATRA, although malignant cells did not grow, most of the cells were still viable even at a high concentration. In the presence of tamoxifen, ATRA concentration dependently reduced the numbers of viable cells.

The combination of ATRA and tamoxifen significantly suppressed colony formation by APL cells but hardly affected colony formation by normal mouse bone marrow cells. Both the number of colonies and the colony size in the treated cultures were similar to those in untreated cultures. These results suggest that APL cells were more sensitive to combined treatment with ATRA and tamoxifen than normal haematopoietic cells. The growth-inhibitory effect of tamoxifen on APL cells was prevented by treatment with α-tocopherol, a membrane stabilizer and lipiophilic antioxidant. This suggest that tamoxifen induces oxidative stress and membrane damage. Tamoxifen enhanced ATRA-induced NBT reduction in the APL cell lines. Tamoxifen also enhanced the morphologic differentiation of APL cells induced by APL cells.

We analyzed morphological changes and viable cell number of APL cells in primary culture. Combined treatment with ATRA and tamoxifen significantly induced the granulocytic differentiation of primary APL cells, suggesting that tamoxifen enhances the granulocytic differentiation of primary APL cells induced by ATRA. Under treatment with ATRA, although primary APL cells did not grow, most of the cells were still viable even at a high concentration. In the presence of tamoxifen, ATRA concentration-dependently reduced the numbers of viable cells.

CONCLUSION

This study suggested that plasma concentrations of tamoxifen that are high enough to enhance the differentiation and growth arrest of APL cells can be approached with an acceptable toxicity profile. The combination of ATRA and tamoxifen should be considered for the treatment of APL patients in whom it is difficult to apply arsenic trioxide or anthracyclines.