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研究速報

Effect of Radiation on the Expression of Carcinoembryonic Antigen on the Membranes of Human Gastric Adenocarcinoma Cells
—Immunological Study Using Monoclonal Antibodies—

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ヒト胃癌細胞膜上の癌胎児性抗原に及ぼす放射線の影響
—モノクローナル抗体を用いた解析—

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Introduction

In recent years, a number of investigators[1)2)] have described an increase in the expression of the cell surface MHC antigens in the presence of either interferon-α or interferon-γ. This is of interest from the immunological point of view, since effector cells may accumulate to the tumor cells due to the enhanced expression of MHC antigens. However, the changes of antigenic expression of the tumor cell surface by irradiation has not yet been elucidated.

In this study, we investigated the effect of irradiation on the cell surface expression of CEA and MHC-class I antigen *in vitro* using monoclonal antibodies.

Materials and Methods

Cultured human gastric adenocarcinoma cells MKN45 were grown in RPMI 1640 medium containing 10% fetal calf serum, 20mM HEPES, streptomycin (50 μg/ml) and penicillin G (100 Units/ml). Cells were cultivated after trypsinization in plastic dishes (Falcon, 3003) at a concentration of 1×10^6 cells/dish. The cells were incubated for 24hr. at 37°C in a 5% CO_2 incubator and irradiated in the dishes with a dosage of 5 or 10 Gy. Irradiation was carried out with a Softex M-150W X-ray source operating at 150 kVp and 9 mA with 0.5 mm Al filter, at the dose rate of 1.16 Gy/min. Five days after treatment, the cells were harvested. Monoclonal antibodies CEA3 and HH1 were used to detect CEA and MHC-class I antigen, respectively. After 1 hr. of incubation on ice, the cells were exposed to a 1:10 dilution of FITC-labeled rabbit anti-goat IgG (Cappel) for another 30 min. on ice. After extensive washing with cultured medium, the cells were then

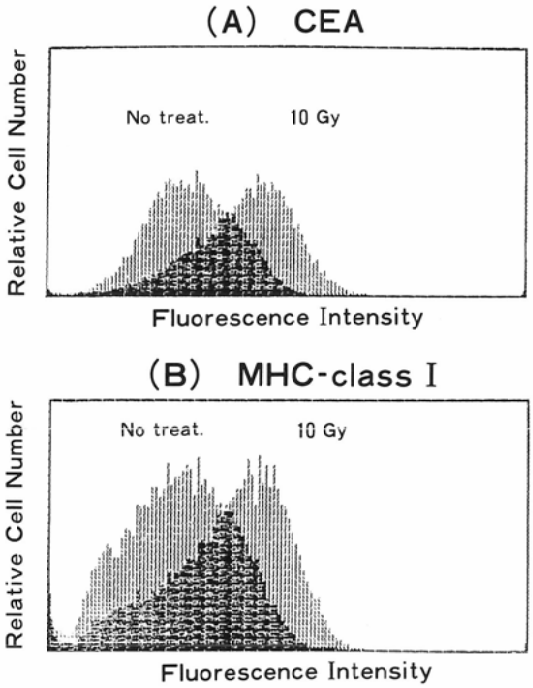

Fig. 1 Pictures of the effect of radiation upon the expression of CEA (A) and MHC-class I antigen (B). Each antigen was clearly enhanced with a dosage of 10 Gy.

fixed by 70% ethanol to store in a refrigerator. The cells were analyzed for green florescence on flow cytometry (EPICS-C, COULTER ELECTRONICS). Each experiment was repeated three times.

Results and Discussion

Fig. 1 shows the analysis of CEA expression (A) of the cell surface by flow cytometry. The peak of fluorescence intensity in untreated cells and cells treated with irradiation of 10 Gy was 80 and 112 channels, respectively. On the other hand, the peak of fluorescence intensity of MHC-class I antigen (B) in untreated cells and cells treated with irradiation of 10 Gy was 80 and 112 channels, respectively. These results revealed a 1,4-fold increase in the expression of cell surface CEA after irradiation of the cells. MHC-class I antigen on the cell surface showed a similar tendency after treatment. Also, there was significant enhancement in the cell surface antigens with a dosage of 5 Gy. Msirikale et al.[3] reported that the use of external radiation for hepatoma increased the accumulation of ^{131}I labeled polyclonal anti-ferritin antibody without increasing the uptake in normal liver. Leichner et al.[4] suggested that an increased macromolecular transport due to tumor vascular permeability after external-beam irradition may cause increased accumulation of radiolabeled scanning agents which are bound to polyclonal antibodies. To our knowledge, there have been few reports concerning the change of expression of antigens on the tumor cell surface after irradiaion.

Our results suggest that irradiation plays a key role in the change of the expression of CEA on the tumor cells which is one of the most useful tumor-associated antigens for adenocarcinomas[5]. Therefore, an appropriate dosage of irradiation to the cells might be beneficial to the host, since effector cells (macrophages, cytotoxic killer cells and/or NK cells) could respond to tumor associated-antigens and/or MHC-class I antigen on the membrane of tunor cells to destroy them. It is possible that enhancement of the expression of MHC-class I antigen would help cytotoxic killer cells to recognize the tumor cells *in vitro*. Analysis of the effector cells which may accumulate to the tumor cells *in vivo* after irradiation will be necessary to further investigate the role of irradiation as a biological response modifier.

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