Tumor Res. **32**, 57−63 (1997)

Irradiation Enhances Proto-Oncogene c-erbB-2 Expression in Human Adenocarcinoma Cells

Masato Hareyama[1)*], Kohzoh Imai[2)], Koh-ichi Sakata[1)], Atsushi Oouchi[1)], Masaaki Adachi[2)], Takao Endo[2)], Yuji Hinoda[2)], Takaharu Shonai[1)], and Kazuo Morita[1)]

[1)] *Department of Radiology,* [2)] *Department of First Internal Medicine, Sapporo Medical University, School of Medicine, South 1, West 16, Chuo-ku, Sapporo 060-0061, Japan.*

ABSTRACT

We investigated the effect of irradiation on the surface antigen expression of proto-oncogene c-erbB-2 on human adenocarcinoma cell lines. Cultured human colonic adenocarcinoma cell BM314 and gastric adenocarcinoma cells MKN45 were irradiated to investigate the expression of the Erb B-2 protein. Interferon-gamma (IFN-γ) was also used to treat these cancer cells. The expression of ErbB-2 showed remarkable increases on the surface of the membrane. Such upregulation was shown to be dose dependent, namely, higher radiation doses were associated with increased antigen expression. However, IFN-γ administration did not show an increased expression of proto-oncogene c-erbB-2. These findings may explain partially the increased immunogenity of tumor cells following irradiation. The effect of irradiation is distinct from that of IFN-γ administration, suggesting that a different mechanism of action is present.

Key words : c-erbB-2, Radiation, Flow cytometry, Human adenocarcinoma cell

INTRODUCTION

In addition to the direct cytocidal effects, the indirect effects of radiation have recently been studied (1, 2). However, there have been only a few investigations describing the immunological effects of radiation on the surface of tumor cells (3-8). We reported that the expression of tumor-associated antigens such as YH206 and CEA, major histocompatibility complex (MHC) Class I antigen and intercellular adhesion molecule (ICAM) -1 in cultured human adenocarcinoma cells, is enhanced after low-dose irradiation (9-11).

*To whom requests for reprints should be addressed

Proto-oncogene c-erb B-2, discovered by Akiyama et al. (12), has been classified as a gene resembling an EGF (epidermal growth factor) receptor gene (13). Its product is believed to be associated with tumor cell proliferation and prognosis (14, 15). In the present study, we investigated the effects of radiation on proto-oncogene c-erb B-2 product.

MATERIALS AND METHODS

Tumor cell lines

Human colonic adenocarcinoma cells BM314 and human gastric adenocarcinoma cells MKN45 were used. Both lines were maintained at 37℃, 5% CO_2 in RPMI-1640 medium containing 10% fetal calf serum.

Monoclonal antibodies

Monoclonal antibody (MoAb) E-907 (IgG 1) to proto-oncogene c-erb B-2 product (16) was used in this study.

X-ray irradiation

Tumor cell lines on a rotating plate were irradiated with a X-ray generator (SOFTEX M-150W, Softex Co.,Tokyo, Japan) operating at 150 kVp and 9 mA with a 0.5-mm aluminum filter at a dose rate of 1.16 Gy/minute.

Treatment of cultured cells

The untreated and recombinant human interferon-γ (IFN, a generous gift from TORAY, Kamakura, Japan) treated cells were designated as the controls, while the x-ray irradiated cells were subdivided into 3 dosage groups (5, 10 and 12Gy). 5 X 10^5 BM314 cells or 1 X 10^6 MKN cells with 10ml of medium in plastic containers were incubated for 24 hours and either exposed to radiation or treated with 200u/ml of IFN at room temperature. One hundred twenty hours after culturing began, without further changes of the medium, the cells were harvested and the supernatants were collected. The cells were counted using a coulter counter (Coulter Electronics, Hialeak, FL). Both cells at the time of collection were in the stationary phase.

Flow cytometry

For an indirect fluorescent antibody procedure, MoAb E-907 was used to detect and proto-oncogen c-erb B-2 product on the collected cells. After one hour of incubation on ice, the cells were exposed to a 1:10 dilution of fluorescein isothiocyanate-labeled goat anti-mouse Ig (Tago, Burlingame, CA) for another 30 minutes on ice, after which they were fixed by 70% ethanol and preserved at 4℃.

These cells were then analyzed for green fluorescence on flow cytometry (Epics-C, Coulter Electronics). The 488-nm line of a 500mW argon ion laser was used to excite fluorescence using a 530-nm short-pass filter. For each sample, 3 X 10^4 cells were counted. The experiment was performed three more times and similar data were obtained each time.

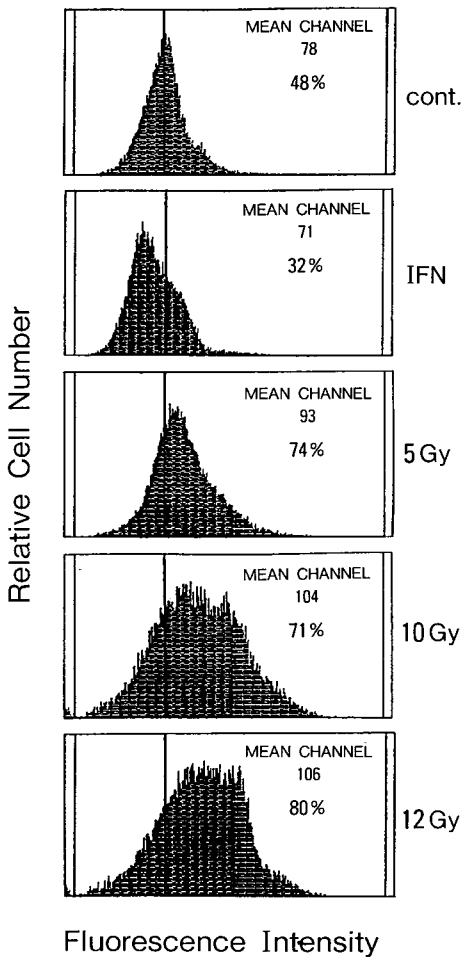

Fig. 1 Analysis of c-erbB2 expression on the membrane of BM314 cells by flow cytometry. Control cells, cells exposed to 2000 U of interferon-γ, and cells exposed to 5, 10, and 12 Gy. Cells were treated 24 hours after cultivation and incubated for another 96 hours before collection.

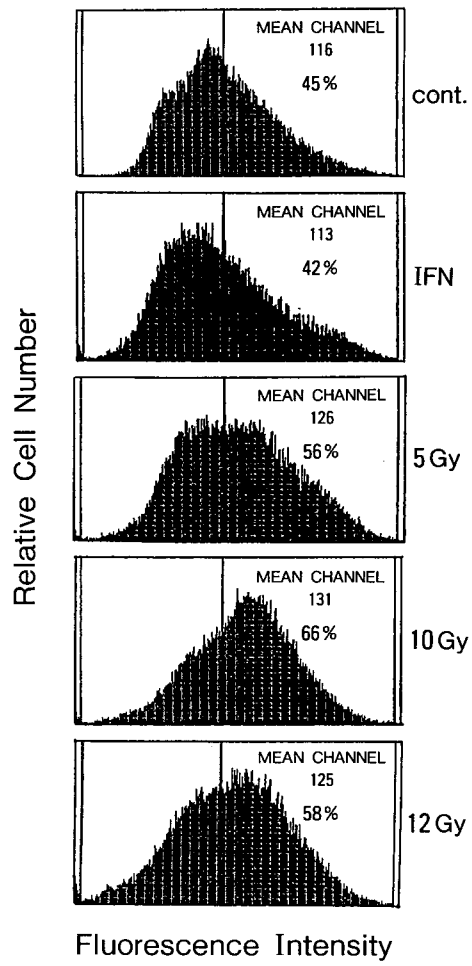

Fig. 2 Analysis of c-erbB2 expression on the membrane of MKN45 cells by flow cytometry. Control cells, cells exposed to 2000 U of interferon-γ, and cells exposed to 5, 10, and 12 Gy. Cells were treated 24 hours after cultivation and incubated for another 96 hours before collection.

Table 1 Changes of the expression on the tumor cells with X-ray irradiation and interferon-γ

Treatment	Tumor-associated Ag (CEA mRNA)	MHC Class I	ICAM-1 (mRNA)	c-erb B2
X-ray	↑ ↑ (↑ ↑)	↑	↑ (↑)	↑ ↑
IFN	→ (→)	↑ ↑	↑ ↑ (↑ ↑)	→

RESULTS

To evaluate the effect of irradiation on the expression of c-erbB-2, human adenocarcinoma cells were irradiated with X-ray. Fig 1 shows the expression of c-erb B-2 product on the surface of BM314 cells by flow cytometry. The mean channel of fluorescent intensity was 78 in the untreated population and 71 in the INF-treated cell population ; but with an increase in the radiation dosage, the number of cells with greater intensities increased. The mean channel was 106 (1.6-fold) in the 12-Gy irradiated cell population. Figure 2 shows the results of the analysis of MKN45 cells. The mean channel was 116 in the untreated poplulation, while the 10-Gy irradiated cell population showed an increase to 131. The figure for the IFN-treated cell population was 113 (second panel of Fig. 2), showing no increase over the control.

It was concluded that irradiation caused increases in the expression of c-erb B-2 product on the cell surface, but that IFN had no effect on it.

DISCUSSION

Radiotherapy is the most effective modality for treatment of locally advanced carcinoma. Moreover, total body or half body irradiation by fractionated low doses irradiation not including the primary lesion in the irradiated field has been reported to be an effective therapeutic modality (17-18). The efficacy of these low dose irradiation can not be explained by the cytocidal effect of the radiation alone from a radiobiologic point of view. In addition to the cytotoxic activity of irradiation, there may be some process of cellular immunological

damage involved in modulating the immune response.

Proto-oncogene c-erbB-2 has been classified as a gene resembling an EGF receptor gene. Its product is abundantly and relatively frequently expressed in epithelial adenocarcinoma such as breast and stomach cancers and is therefore currently noted as a marker molecule for immunotherapy (20) or radiolabeled tumor detection (21).

The present study showed that X-ray irradiation, but not IFN administration, caused increases in the expression of c-erbB-2 on the tumor cell surface. We have recently reported that X-ray irradiation causes a marked increase in the expression of adenocarcinoma-associated antigens such as YH206 and CEA and that the irradiated cells showed slight, but clear increases in the expression of ICAM-1. However, IFN-γ administration caused marked increases in MHC Class I antigen. Table 1 shows the changes of various antigens treated by irradiation and IFN-γ administration which we found. Thus it is interesting that immune responses can be modified by altering the quantities of various antigens on the tumor cell surface. We believe that it is extremely significant to observe the fluctuations of the antigens on the surface of tumor cells caused by irradiation. The effect of X-ray irradiation on the tumor cell surface antigens including the mRNA level, is distinct from that of IFN administration suggesting that a different mechanism is operating in these two modalities.

The mechanism involved in the augmentation of these various antigens by low dosage irradiation has not yet been elucidated. As for IFN actions, the presence of the IFN consensus sequence is known to be responsible at the gene level for antigens such as CEA (22). It is known that T cells produce endogeneous IFN-γ, which when produced in tumor cells up-regulates the level of expression of MHC Class I and ICAM-1 in the cells, thus becoming instrumental in immunological surveillance. It has been thought that radiotherapy caused an immunosuppressive effect in the host. It has been observed, however, that X-ray irradiation increases the level of various membrane antigens of tumor cells, offering a possibility of facilitating their recognition by CTL (23) or other related cells (24). In such situations, it would be necessary to select an appropriate dosage so as not to cause damage to cells of the immune system. It is necessary to perform in vivo research on this subject. The present study indicated for the first time that certain dosages of radiation have an effect not only on the expression of the carcinoembryonic antigen and ICAM-1 but also on the expression of c-erbB-2 in tumor cells.

ACKNOWLEDGEMENT

This work was supported by grants for Cancer Research from the Japanese Ministry of Education, Science, and Culture (M. H., K. I.).

REFERENCES

1. Francois L, Troise GD, Chavaudra N, Malaise EP, Barski G. Comparative effect of local radiotherapy and surgery on cell mediated immunity against a mouse transplantable mammary tumor. Int J Cancer 1974, 13 : 629-639.
2. Ogawa Y, Maeda T, Seguchi H, Yoshida S, Inomata T, Hamada F. Changes in peripheral lymphocyte subsets during radiotherapy for lung cancer patients. Oncology 1986, 43 : 154-158.
3. Tilkin AF, Lafontaine NS, Acker AV, Boccadoro M, Urbain J. Reduced tumour growth after low-dose irradiation or immunization against blastic suppressor T cells. Proc. Natl. Acad. Sci. 1981, 78 : 1809-1812.
4. Maruo T, Shibata K, Kimura A, Hoshina M, Mochizuki M. Tumor-associated antigen, TA-4, in the monitoring of the effects of therapy for squamous cell carcinoma of the uterine cervix : Serial determinations and tissue localization. Cancer 1985, 56 : 302-308.
5. Ogawa Y, Maeda T, Seguchi, Hamada F, Inomata T, Nishioka M, Kamiike O, Morita M, Ohara S, Yoshida S. Evidence of existence of low dose radiation induced tumor immunity. Nippon Act Radiol 1987, 47 : 845-848.
6. Hallahan DE, Spriggs DR, Beckett MA, Kufe DW, Weichselbaum RR. Increased tumor necrosis factorα mRNA after cellular exposure to ionizing radiation. Proc. Natl. Acad. Sci. USA. 1989, 86 : 10104-10107.
7. Sherman ML, Datta R, Hallahan DE, Weichselbaum RR, Kufe DW. Ionizing radiation regulates expression of the c-jun protooncogene. Proc Natl Acad Sci USA 1990, 87 : 5663-5666.
8. Papathanasiou MA, Kerr NCK, Robbins JH, Mcbride W, Alamo JRI, Barrett SF, Hickson ID, Fornace AJ Jr. Induction by ionizing radiation of the gadd45 gene in cultured human cells : lack of medication by protein kinase C. Mol Cell Biol 1991, 11 : 1009-1016.
9. Hareyama M. Effect of radiation on the expression of tumor-associated antigens of human lung adenocarcinoma cells. Immunological study using monoclonal antibodies. Nippon Act Radiol 1988, 48 : 1553-1562.
10. Hareyama M, Imai K, Kubo K, Takahashi H, Koshiba H, Hinoda Y, Shidou M, Oouchi A, Yachi A, Morita K. Effect of radiation on the expression of carcinoembryonic antigen of human gastric adenocarcinoma cells. Cancer 1991, 67 : 2269-2274.
11. Hareyama M, Imai K, Oouchi A, Takahashi H, Hinoda Y, Tsujisaki M, Adachi M, Shonai T, Sakata K, Morita K. The effect of radiation on the expression of intercellular adhesion molecule-1 of human adenocarcinoma cells. Int. J. Rad. Oncol. Biol. Phys. 1998, 40 : 691-696.
12. Akiyama T, Sudo C, Ogawara H, Toyoshima K, Yamamoto T. The product of the human c-erb B-2 gene : a 185-kilodalton glycoprotein with tyrosine kinase activity. Science 1986, 232 : 644-1646.

13. Yamamoto T, Ikawa S, Akiyama T, Semba K, Nomura N, Miyajima N, Saito T, Toyashima K. Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor. Nature 1986, 319 : 234-234.
14. Yang JL, Ow KT, Russel PL, Ham JM, Crowe PJ. Higher expression of oncoprotein c-myc, c-erb B-2/neu, PCNA, and p53 in metastasizing colorectal cancer than in nonmetastasizing tumors. Ann Surg Oncol 1996, 3 : 574-579.
15. Nakano T, Oka K, Ishikawa A, Morita S. Correlation of cervical carcinoma c-erb B-2 oncogene with cell proliferation parameters in patients treated withradiation therapy for cervical carcinoma. Cancer 1997, 79 : 513-520.
16. Ishida T, Tsujisaki M, Motoya S, Masuya J, Hanzawa Y, Hirakawa T, Imai K, Yachi A. Preparation of monoclonal antibody reacting with transfectoma of c-erb B-2 gene and immunohistological investigation in gastric cancers. Scand J Immunol 1994, 39 : 459-466.
17. Johnson RE. Management of generalized malignant lymphoma with "systemic" radiotherapy. Br. J. Cancer (Suppl II) 1975, 31 : 450-455.
18. Shen R, Hornback NB, Lu LH, Chen LT, Brahmi Z, Broxmeyer HE. Low dose total body irradiation : A potent anti-retroviral agent in vivo. Int J Rad Oncol Biol Phys 1989, 16 : 165-170.
19. Takai Y, Ogawa Y, Nemoto K, Yamada S, Sakamoto K. Direct anti-tumor effect of low dose total (or half) body irradiation and changes of the functional subset of peripheral blood lymphocytes in non-Hodgkin's lymphoma patients after TBI (HBI). J Jpn Soc Ther Radiol Oncol 1991, 3 : 9-18.
20. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich A, Press MF. Studies of the HER-2/neu Proto-oncogene in human breast and ovarian cancer. Science 1989, 244 : 707-712.
21. Mangili F, Sassi I, Di-Rocco M, Leone BE, Garancini P. Santambrogio, G. Breast carcinoma detection with a combination of radiolabeled monoclonal antibodies. Promising results from immunohistochemistry studies. Cancer 1996, 78 : 1334-2339.
22. Friedman RL, Stark GR.α-interferon-induced transcription of HLA and metallothionein genes containing homologous upstream sequences. Nature 1985, 314 : 637-639.
23. Hallahan DE, Spriggs DR, Beckett MA, Kufe DW, Weichselbaum RR. Increased tumor necrosis factorα mRNA after cellular exposure to ionizing radiation. Proc. Natl. Acad. Sci. USA 1989, 86 : 10104-10107.
24. Tilkin, AF, Lafontaine NS, Acker AV, Boccadoro M, Urbain J. Reduced tumour growth after low-dose irradiation or immunization against blastic suppressor T cells. Proc Natl Acad Sci. 1981, 78 : 1809-1812.