Expression of Fas (CD95/APO-1) Antigen Induced by Radiation Therapy for Diffuse B-Cell Lymphoma: Immunohistochemical Study

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ABSTRACT

Most malignant lymphomas show relatively high degrees of radiosensitivity, in which apoptosis has been shown to play an important role. Recently, the Fas (CD95/APO-1)/Fas ligand system has been identified as a key regulator of apoptosis in some types of lymphoma cell lines. In this study, we aimed to determine whether Fas antigen expression is induced by radiotherapy for malignant lymphoma and to clarify its possible correlation with the therapeutic effect of radiation therapy. Fifty-six patients with tumors of the tongue, oropharynx, and maxillary sinus were examined; four were confirmed as malignant lymphoma, and the rest were identified as squamous cell carcinoma. After obtaining the patients’ informed consent, biopsies were performed before treatment and at doses of 4, 10, and 20 Gy of radiotherapy, and specimens were preserved in liquid nitrogen until further examination. Serial sectioning of 6 μm was performed using a cryostat, and samples were immunohistochemically stained using the streptavidin-biotin peroxidase method and a monoclonal antibody against Fas. Two of the four patients with malignant lymphoma showed Fas antigen expression on their tumor tissue at 4 and 10 Gy of radiotherapy. These tumors showed high radiosensitivity and disappeared at a dose of 20 Gy of radiotherapy. In samples from these two patients, DNA ladder formation was identified at 10 Gy.

In 52 squamous cell carcinomas, staining for the Fas antigen showed negative or only slightly positive results. However, in one of the cases of squamous cell carcinoma, lymphocytes infiltrating into cancer tissue showed Fas antigen expression at 4 Gy of irradiation, and these lymphocytes disappeared on the tumor tissue at 10 Gy.

Therefore, the high radiosensitivity of malignant lymphoma among our samples could be explained by the over-expression of Fas antigen induced by small doses of radiation therapy, and Fas ligand could be produced by infiltrating lymphocytes or may be expressed simultaneously on the lymphoma cells.

INTRODUCTION

Radiation-induced apoptosis is commonly seen in lymphomas (1). For example, in a mouse lymphoma, 50–60% of the cells have been reported to show signs of apoptotic death within 3 h after irradiation (2). Therefore, apoptosis is considered to be involved if a tumor shows high radiosensitivity to a relatively low dose of radiation (3).

Clinically, most malignant lymphomas show high radiosensitivity, but some (especially recurrent malignant lymphoma) show radioresistance. The difference in radiosensitivity among malignant lymphomas has not yet been fully clarified. Recently, the involvement of the Fas/Fas ligand system was reported in chemotherapy-induced apoptosis for some types of lymphoma cell lines (4). Fas (CD95/APO-1) is a member of the tumor necrosis factor receptor/nerve growth factor receptor superfamily, which mediates apoptosis upon oligomerization (5–7). Therefore, the Fas/Fas ligand system is considered to be a key regulator of apoptosis (8–10).

In regard to radiation therapy, it is possible that the Fas antigen is induced by small doses of radiotherapy for some types of tumors that show high radiosensitivity. Thus, our study aimed to evaluate Fas antigen expression induced by low-dose irradiation for malignant lymphoma and the antigen’s possible correlation with the therapeutic effect of radiation.

MATERIALS AND METHODS

The subjects of this study were a total of 56 patients with malignancies of the head and neck region seen at the Kochi Medical School Hospital from January 1993 to December 1995. After obtaining patients’ informed consent, pieces of approximately 0.3 × 0.3 × 0.3 cm were cut from obviously viable portions of the tumors before and after the delivery of 4, 10, and 20 Gy (cumulative dose) of irradiation. Fifty-two of the 56 patients’ tumors were histopathologically diagnosed as squamous cell carcinomas, and another 4 patients’ tumors were confirmed as malignant lymphomas. All patients with malignant lymphoma did not receive any previous chemotherapy and were treated at the time of initial diagnosis.

Samples from these tissue specimens were immediately stored in liquid nitrogen and subsequently cut into 6-μm-thick serial sections in the cryostat. These sections were immunohis-
Expression of Fas Antigen Induced by Radiotherapy

RESULTS

Two of four patients with malignant lymphoma (Tables 1 and 2) showed Fas antigen expression in their tumor cells at cumulative doses of 4 and 10 Gy of radiotherapy (Figs. 1 and 2); because these lymphoma cells showed characteristic findings of apoptosis at cumulative doses of 10 (Fig. 3, case 1) or 20 Gy (Fig. 2, case 2) and these tumors disappeared clinically at the cumulative dose of 20 Gy, they are considered highly radiosensitive. Another patient with malignant lymphoma (case 4) showed no Fas antigen expression during radiotherapy, and viable tumor cells were still observed on H&E-stained sections at a dose of 20 Gy. For one patient (case 3), the immunohistochemical staining yielded unreadable results; therefore, the results were not included in the analysis.

Among the 52 squamous cell carcinomas, all showed no expression or only faint expression of Fas antigen. However, in one of the cases with squamous cell carcinoma, lymphocytes infiltrating into cancer tissue showed Fas antigen expression at a dose of 4 Gy of irradiation (Fig. 4), and these lymphocytes disappeared in the tumor tissue at 10 Gy. As for the DNA ladder formation assays, a DNA ladder was confirmed at 10 Gy of irradiation on the samples of the malignant lymphomas from cases 1 and 2 and was not observed on the malignant lymphomas of cases 3 and 4, nor in another six patients with squamous cell carcinomas (Fig. 5). DNA ladder formation was identified only on malignant lymphomas that showed Fas antigen expression induced by irradiation.

Therefore, the high radiosensitivity of many malignant lymphomas could be explained by the overexpression of Fas induced by small doses of radiation therapy. Although Fas ligand was not identified in this study, the ligand could perhaps be produced by infiltrating lymphocytes or may be induced simultaneously in lymphoma cells with Fas antigen.

DISCUSSION

Each organ and cell shows various degrees of radiosensitivity. But the mechanisms that determine radiosensitivity have not yet been clarified. Among various types of malignant tu-
Malignant lymphoma Fas

Fig. 1 At pretreatment, Fas expression was negative, but at 4- and 10-Gy cumulative doses of radiation, Fas expression was obvious in lymphoma cells (case 1; original magnification, ×200).

Fig. 2 At pretreatment, Fas expression was negative, but at a 10-Gy cumulative dose of radiation, Fas expression was strongly positive in lymphoma cells, and at 20 Gy, most lymphoma cells showed pyknosis on their H&E-stained sections, which is considered characteristic of apoptosis (case 2; original magnification, ×200).

Malignant lymphoma

Most malignant lymphomas show remarkably high radiosensitivity in vivo. Actually, apoptosis has been demonstrated as a type of cell death induced by the irradiation of some types of lymphoma cell lines (16).

Therefore, the existence of some unknown mechanism governing radiation-induced apoptosis has been speculated for a long time. Recently, it has been determined that the Fas/Fas ligand system plays a major role in the death system of various types of cells (8–10), and that some anticancer agents exert their effect via this system through the physiological concentrations of the drugs (4).

For many years, we have been studying changes in cancer tissue during radiotherapy using immunohistochemical methods and various types of monoclonal antibodies against lymphocyte...
Fig. 3 Photomicrographs of H&E-stained sections of pretreatment and 4- and 10-Gy cumulative doses of radiation are shown. Most lymphoma cells at 10 Gy of radiation show shrinkage and chromatin condensation, which are characteristic findings of apoptosis (case 1; original magnification, ×200).

Fig. 4 At a cumulative dose of 4 Gy of radiotherapy, tumor-infiltrating lymphocytes (arrows) showed positive staining for anti-Fas, in contrast to the negative staining of tumor cells for the antibody (original magnification, ×200).
Fig. 5  Of the 56 patients, DNA ladder formation assay using 2% agarose gel electrophoresis was performed on 10 samples at 10 Gy of irradiation, and DNA ladders were confirmed on samples from cases 1 and 2 (Lanes 6 and 9, respectively). DNA fragment size markers are shown in Lanes M1 and M2 (500 and 100 bp, respectively). In Lane P, a typically fragmented genome DNA derived from normal tissue was assayed as a control.

subsets (13, 14), fibronectin (17), Ki-67 (18), and oncogene products (19).

Actually, literature reports on the incidence of Fas antigen in diffuse B-cell lymphomas involving immunohistochemistry with large numbers of lymphomas indicate that 75% of follicular lymphomas and up to 56% (26–56%) of diffuse lymphomas express Fas antigen (20–22). In contrast to these reports, there are many experimental results that Fas/APO-1 is absent on the majority of B-cell lymphomas (23–25). Therefore, this theme is still controversial. Moreover, there has been no report as of yet concerning Fas antigen expression induced by irradiation.

More than 50 patients with head and neck tumors have been examined, 4 of whom suffered from malignant lymphoma. In one of these four patients, we did not obtain a useful immunostaining, possibly due to endogenous peroxidase activity, but two of the four patients showed obvious positive staining for Fas antigen on their tumor tissues at 4- and 10-Gy cumulative doses of radiation therapy, and their tumors responded very well to radiotherapy and disappeared at a 20-Gy cumulative dose of radiotherapy.

Moreover, in these two patients, DNA ladder formation was identified on tumor samples obtained at 10 Gy; therefore, an occurrence of apoptosis was confirmed. Another patient showed negative staining for Fas on the tumor tissue before and during radiotherapy, and viable lymphoma cells were still observed in the tissue at a cumulative dose of 20 Gy of radiotherapy.

As mentioned above, our findings demonstrate that the Fas/Fas ligand system is involved in the mechanism of high radiosensitivity in malignant lymphomas. However, obtaining specimens serially before and during radiation therapy is considerably difficult, especially for malignant lymphoma. Our study was coincidentally achieved by examining tumors of the head and neck region. Actually, some head and neck tumors, especially those of the oropharynx, were identified as malignant lymphomas several weeks after the patient’s first visit to the Otorhinolaryngology Clinic. Therefore, it must be a really rare experience to have been able to get specimens of malignant lymphomas serially before and during radiation therapy.

For the reason mentioned above, the number of patients in this study is very limited, but our results are still worthwhile in showing radiation-induced Fas antigen expression in malignant lymphoma involving apoptosis and its possible correlation with therapeutic effect of radiotherapy. To augment these findings, we need to further explore the mechanism of the irradiation-induced augmentation of Fas antigen using some lymphoma cell lines and flow cytometry, and these experiments are now being carried out in our laboratory.

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