NASOPHARYNGEAL CARCINOMA RADIOSENSITIVITY PREDICTION BY CYTOKINESIS-BLOCK MICRONUCLEUS ASSAY

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ABSTRACT

Cytokinesis-block micronucleus method is used to evaluate the radiosensitivity of a nasopharyngeal carcinoma cell line (CNE-1) and biopsies obtained from 31 patients with nasopharyngeal carcinoma. The number of micronuclei increases with the radiation dose. A good correlation was found between the radiosensitivity determined by the micro-nucleus assay and that measured by the colony-forming assay in CNE-1 cell line (r=-0.998). Moreover, the results of micronucleus assay for tumor cells from biopsies of patients with primary carcinoma look promising for the prediction of tumor radiosensitivity. These results are encouraging but need to be confirmed with a larger number of patients with a longer follow-up.

Keywords Micronucleus assay, Radiosensitivity, Nasopharyngeal carcinoma

1 INTRODUCTION

Cytokinesis-block micronucleus method, developed by Fenech and Morley in 1985[1], has been used to evaluate the radiosensitivity of tumor patients and predict the response of radiotherapy. The micronucleus numbers of peripheral blood lymphocytes have been shown increasing in a dose-dependent manner[2]. Studies on xenografted human and murine tumors have shown that the results of in vitro irradiation were not significantly different from those of in vivo irradiation for all tumors, and that a good correlation was found between the radiosensitivity determined by the micronucleus assay and that found with the colony-forming assay in six human tumors. Cells carrying a micronucleus have almost certainly lost their clonogenic ability[3]. Therefore, the micronucleus assay has the potential for studying tumor biopsies that can not be tested by the colony-forming assay[4]. Furthermore, because of its relative rapidity and high success rate, it is suitable for clinical application. In this investigation, the cytokinesis-block micronucleus assay is performed in a nasopharyngeal carcinoma cell line and the biopsies obtained from patients with this tumor as a potential method for predicting the radiosensitivity of nasopharyngeal carcinoma.

2 MATERIALS AND METHOD

2.1 Nasopharyngeal carcinoma cell line (CNE-1)

The CNE-1 cell line was obtained from Institute of Cell Biology, the Chinese Academy of Sciences. The tumor cells were grown in monolayer in RPMI-1640 medium

Manuscript received date: 1994-09-04

supplemented with 20% fetal calf serum incubated in a moist atmosphere of 5% CO_2 in air at 37 °C for all the micronucleus assays, colony-forming assays, and subsequent culture of the cells.

2.1.1 Micronucleus assay

At 24 h after plating the exponentially growing cell in tissue culture dishes (d=60 mm), irradiation was carried out by using a Gammacell-40 ^{137}Cs γ-ray machine. The dose rate was 1.12 Gy/min and the dose range was from 0 to 8 Gy. Fresh medium containing 3 μg/ml cytochalasin B(cyt-B, Sigma) was changed 2 h after irradiation. After the following 24 h culture, cells were fixed in situ and stained with Wright-Giemsa dye, the total number of micronuclei in the binucleate cells was determined by using a Nikon light microscope. Total 1000 binucleate cells were assessed per dish to measure the proportion of binucleate cells and the micronucleus frequency, respectively.

2.1.2 Colony-forming assay

The exponentially growing cells were irradiated 24 h after culture. The irradiation conditions and doses were the same as above (triplicate plates per point). Continually cultured 10 d after irradiation, cells were fixed in situ and stained, and then tumor colonies were totaled (cells≥50). The surviving fraction (SF) is given by

$$\mathrm{SF} = \frac{\text{Number of visible colonies}}{\text{Number of cells incubated} \times \text{Plating efficiency (PE/100)}}$$

Survival for each dose was calculated, and the survival curves were fitted to a linear equation.

2.2 Tumor cells from biopsies of primary nasopharyngeal carcinoma

Biopsy specimens were obtained from 31 patients with nasopharyngeal carcinoma, among which 20 specimens were successfully tested by micronucleus assay. There were 14 male patients and 6 female ones. The average age was 47.6, and the follow-up ranged from 1.5 to 2 a.

Tissue specimens were treated immediately, or they were stored in RPMI-1640 medium containing penicillin (150 u/ml), streptomycin (150 μg/ml) and 20% fetal calf serum at 4°C overnight. The following day tumor tissue was minced with scalpel blades and digested in a solution containing 0.5% Collagenase type Ⅳ and 0.005% DNase with constant slow stirring for 5 h. The cell suspension was centrifugalized and the pellet resuspended in Hank's balanced salt solution. A viable cell count was obtained by dye exclusion under phase contrast microscopy. The percentage of tumor cells was determined by using May-Grunwald-Giemsa staining and appropriate number (3×10^4 Cells/ml) of tumor cells were plated out in dishes using attachment medium containing fibronectin. Irradiation was carried out by using a ^{60}Co γ-ray machine, 72 h later; radiation doses are 0, 2 and 4 Gy. After irradiation, fresh medium was changed immediately, and Cyt-B (2 μg/ml) was added in. After the following 24 h culture, cells were fixed in situ and stained, and the micronuclei in the binucleate cells were totaled under microscope.

3. RESULTS

3.1 Radiation response of CNE-1 cell line

3.1.1 CNE-1 dose-micronucleus frequency curves

The number of micronuclei in the binucleate cells increased significantly with radiation dose. Correlation between mean number of micronuclei per single binucleate cell (Y_1) and radiation dose (D in Gy) was well fitted to a linear quadratic equation $Y_1 = 0.048 + 0.204D$, $r = 0.996$, $P < 0.001$.

3.1.2 CNE-1 cell survival curve

The surviving fractions of CNE-1 cell line (Y_2) decreased with the increase in dose (D in Gy), $\lg Y_2 = 0.77 - 0.081\,D$.

3.1.3 Correlation between micronucleus frequency and surviving fraction of CNE-1 cell line

There was a good negative correlation between surviving fraction (Y_2) and the number of micronuclei in the binucleate cells (Y_1) in the dose range from 0 to 8 Gy, $Y_2 = 0.792 - 0.397Y_1$, $r = -0.998$, $P < 0.001$.

3.2 Radiation responses of nasopharyngeal carcinoma biopsies

In the total 31 biopsies, 4 specimens were contaminated. 7 specimens were unattached, and the other 20 specimens were successfully set up and then tested by the CB micronucleus assay. The success rate was 65%.

Fig.1 shows the results of micronucleus-forming. In the total 20 specimens set up, 8 specimens showed relatively high response, 5 specimens relatively low response, and the rest 7 median response. Nineteen cases had a good local control, and one patient (No.13), who was among the low response group, showed a local recurrence within 2 a.

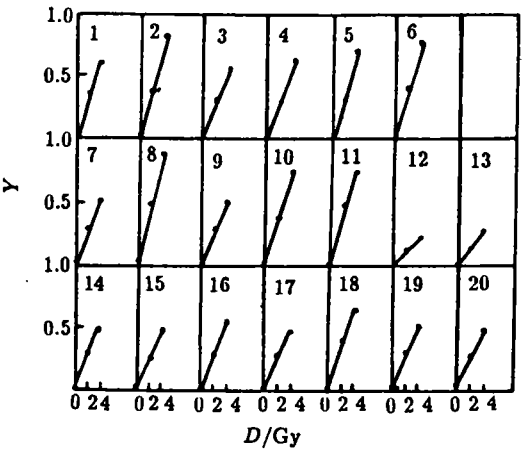

Fig.1 Micronucleus frequency per single binucleate cell (Y) as a function of the radiation dose from tumor biopsies

4 DISCUSSION

Studies on human tumors have demonstrated that tremendous heterogeneity of biological phenomena exists between individual tumors and even within the same tumor (inter- and intraindividual heterogeneity). From such studies it has become evident that an understanding of these heterogeneities and of the corresponding biological processes is very important for the therapeutic treatment of individual tumors.

Micronucleus assay plays an important role in evaluating radiation damage[5]. With the use of Cyt-B, which at appropriate concentrations prevents cytoplasmic but not nuclear division; cells that have undergone one mitosis can be recognized easily by their typical binucleate apperance[1]. Thus CB micronucleus assay overcomes the major drawback of the conventional micronucleus assay of including nondividing cells in the estimate, which makes the results unreliable. Therefore, this assay is considered to be more efficient than the conventional one [3,6]. A Cyt-B concentration of 2 μg/ml appears to be optimal in most cases for tumor cells[3]. This technique has been applied recently to estimate the correlation between peripheral blood lymphocytes and accumulated doses of tumor patients, and has gotten some encouraging results[7]. However, there have been few reports on applying this assay to human tumor biopsies. It was reported that there was a good correlation between the CB micronucleus frequency and dose by testing 19 squamous epithelium originated from renal cell carcinoma biopsies. The success rate was 50%, which was similar to previous results[8,9] and ours.

Since primary cultures of specimens from tumor biopsies is laborious, low plated, time consuming and easily contaminated[3], the correlation between the colony-forming assay and the CB micronucleus assay was analysed only on CNE-1 cell line. When it was shown that there was a good correlation between those two assays, we tested 20 human nasopharyngeal carcinoma biopsy specimens only by CB micronucleus assay, and then had a follow up ranged from 1.5 to 2 a. With head and neck cancers it was observed that the number of micronuclei increased after a fractionated radiation dose of 10 Gy (5×2 Gy) of photons in the majority of those tumor (10 of 12 tumors) that showed no local recurrence, while no increase was found in most of those cancers (12 of 14 tumors) that showed a local recurrence within 2 a[9]. Our results showed 19 of 20 cases had good local control. However, one case (No.13) of low micronucleus frequency group tended to recur recently. Because nasopharyngeal carcinoma belongs to median radiation sensitivity tumors, for those patients who had a relatively low micronucleus frequency assay, it needs further study and accumulation of cases whether it will become a valuable method in predicting the radiation sensitivity tumors.

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