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Evaluation of radiosensitivity of human tumor cells after irradiation of γ-rays based on G2-chromosome aberrations

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Abstract The aim of the present investigation is to determine initial G2-chromosome aberrations and to validate whether the G2-chromosome aberrations can predict the cellular clonogenic survival in human tumor cell lines. Cell lines of human ovary carcinoma cells (HO8910) and human hepatoma cells (HepG2) were irradiated with a range of doses and assessed both for initial G2-chromosome aberrations and for cell survival after γ-irradiation. The initial G2-chromosome aberrations were measured by counting the number of G2-chromatid breaks after irradiation, detected by the premature chromosome condensation technique, and the G2-assay method. Cell survival was documented by a colony formation assay. A linear-quadratic survival curve was observed in both cell lines. The dose–response results show that the numbers of G2-chromatid breaks increase with the increase in dose in the two cell lines. At higher doses (higher than 4 Gy) of irradiation, the number of G2-chromatid breaks for the G2-assay method cannot be determined because too few cells reach mitosis, and hence their detection is difficult. A good correlation is found between the clonogenic survival and the radiation-induced initial G2-chromatid breaks per cell ($r = 0.9616$). The present results suggest that the premature chromosome condensation technique may be useful for determining chromatid breaks in G2 cells, and the number of initial G2-chromatid breaks holds promise for predicting the radiosensitivity of tumor cells.

Keywords Colony formation assay, G2-chromatid breaks, Premature chromosome condensation technique, G2-assay
CLC numbers Q345+.2, R73-33

1 Introduction

It is well established that the radiosensitivity is dependent on cell-cycle progression, and among stages of interphase, G2 phase is the most radiosensitive followed by G1 phase, and S phase, which is the least radiosensitive, whereas G0 phase is radioresistant under conditions of oxygen deprivation and sufficient repair time [1-3]. Previous studies have concluded that the G2 phase is a very important stage. Some researchers have studied the chromosome aberrations in G2 phase [4-7].

There are two main techniques to study the chromosome aberrations in G2 cells, namely, G2-assay and G2-PCC (premature chromosome condensation) techniques. The G2-assay method is used to evaluate G2-chromosome aberrations by analyzing the subsequent metaphases after irradiation, and the number of G2-chromosome aberrations studied by the G2-assay method can reflect cell radiosensitivity[6,7]. The G2-PCC technique is used to evaluate the induction of G2 chromosome aberrations directly in G2 phase instead of metaphase cells [7, 8].

To investigate whether the G2-PCC technique can substitute for the G2-assay method to study cell radi-

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osensitivity, the chromatid breaks in G2 phase induced by γ-rays were measured using the G2-assay and the G2-PCC techniques in this study. The correlation between the radiation-induced G2-chromatid breaks and the cellular clonogenic radiosensitivity was analyzed.

2 Experimental

2.1 Cell culture

Human ovary carcinoma cells (HO8910) and human hepatoma cells (HepG2) were grown in 1640 medium (Gibco) supplemented with 10% foetal calf serum, 100 $U \cdot mL^{-1}$ penicillin, and 100 $mg \cdot mL^{-1}$ streptomycin at 37°C in 5% CO_2 with 95% humidity.

2.2 Gamma irradiation

Gamma rays were generated from a ^{60}Co source (Radiology Department, Affiliated No. 1 Hospital, Lanzhou University). HO8910 cells and HepG2 cells were irradiated at doses of 0, 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 Gy, respectively, with a dose rate of 0.2 $Gy \cdot min^{-1}$.

2.3 Survival of cells

After γ-irradiation, cells were plated at a density of about 100 surviving cells per 6 cm culture dish and incubated for approximately14 days, and the cells were then fixed and stained with a solution of Giemsa. Colonies of more than 50 cells were counted as survivors. The number of colonies per dish was counted, and the fractions containing surviving cells were calculated as the ratio of plating efficiencies for the irradiated to unirradiated cells. Plating efficiency is defined as the quotient obtained by dividing the number of plated cells by the number of colonies. The survival data were fitted to the linear-quadratic model: $\ln S = -\alpha D - \beta D^2$, where S is the survival fraction, and D is the radiation dose.

2.4 PCC induction using calyculin-A

Calyculin-A (BIOMOL America) was used as the PCC inducer, which was dissolved in 100% ethanol as 1 $mmol \cdot L^{-1}$ stock solution; 50 $nmol \cdot L^{-1}$ of Calyculin-A was added to the cell cultures 5 min before irradiation to score the initial chromatid breaks. After irradiation, cells were incubated for another 30 min at 37 °C in 5% CO_2. Chromosome spreads were then harvested by allowing the cells to swell in 75 $mmol \cdot L^{-1}$ KCl for 20 min at 37 °C and fixed using Carnoy's fixation. A final wash and fixation in the same fixative was completed before the cells were dropped onto a glass slide and were subjected to hot humidity drying [5].

2.5 G2-assay method

After irradiation, the cells were incubated for 30 min at 37 °C in 5% CO_2 and Colcemid (0.2 $\mu g \cdot mL^{-1}$) was added. After 60 min of incubation, the cells were harvested as described above [6].

2.6 Observation

Chromosome was stained with 5% Giemsa for 20 min. Fifty well-spread metaphase or G2-phase cells were scored under oil immersion with a light microscope for each dose point according to the standard criteria [9]. In brief, chromatid discontinuation, misalignment of the distal region to the lesion or a non-stained region longer than the chromatid width was classified as a break. One isochromatid break was scored as two breaks. The total chromatid breaks were calculated by summing the production of chromatid and isochromatid breaks.

2.7 Statistical analysis

Each experiment was repeated thrice. Linear regression and Student's *t*-test were used to analyze the correlation between G2-chromosome radiosensitivity and cellular clonogenic radiosensitivity. $p < 0.05$ was considered statistically significant.

3 Results

3.1 G2-chromosome radiosensitivity after irradiation with γ-rays

Fig. 1 shows the G2-chromosome breaks in both cell lines after irradiation with various absorbed doses. For G2-PCC technique, the chromatid breaks show a linear increase with the increase in dose. The regression lines are as follows. HepG2: $Y=1.002+4.376X$, $r=0.998$ ($p<0.01$); HO8910: $Y=-0.882+3.749X$, $r=0.9914$ ($p<0.01$) (Fig. 1(a)). For G2-assay method, the number of G2-chromatid breaks per cell cannot be determined at doses higher than 4 Gy in both cell lines, because too few cells proceed to mitosis to be analyzed at metaphase. The regression lines are as follows. HepG2: $Y=0.125+0.539X$, $r=0.998$ ($p<0.01$);

HO8910: $Y=0.018+0.116X$, $r=0.952$ ($p=0.013$) (Fig. 1(b)).

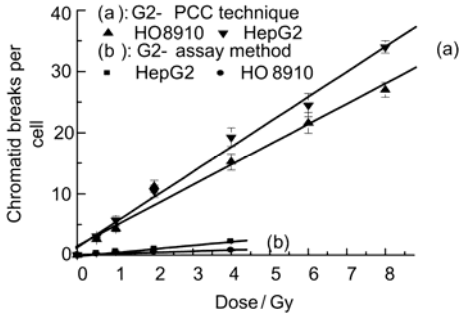

Fig. 1 The dose–response curves for the induction of G2-chromatid breaks studied by G2-PCC technique (a) and G2-assay method (b) for two cell lines.

During the experiment, some inter-exchanges or inner-exchanges were scored in chromosome spreads, but they were few. Only the main chromosome breaks aberration, i.e., chromatid and isochromatid breaks, was only observed by the authors of this study. The total chromatid breaks showed a linear increase with the increase in dose .

3.2 Survival curves

In this study, as shown in Fig. 2, the two cell lines' fitted survival curves agree well with the linear quadratic model. The α values of HepG2 and HO8910 were 0.2 and 0.08, respectively. SF2 of HepG2 and HO8910 were 0.455 and 0.652, respectively.

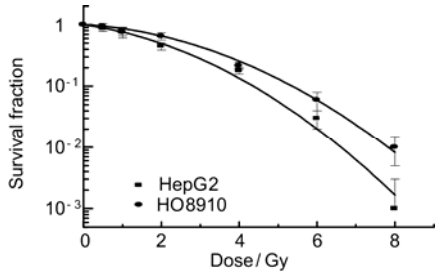

Fig. 2 Survival curves of two cell lines after γ-ray irradiation.

3.3 Correlation between cellular clonogenic survival and the G2-chromosome aberrations for the evaluation of radiosensitivity

To evaluate the radiosensitivity by the colony formation assay and the G2-chromosome methods, the number of G2-chromatid breaks after irradiation were plotted against the survival fractions in Fig. 3. The correlation coefficient of the two methods was 0.9616 ($p<0.01$) fixed by the G2-PCC technique and 0.9381 ($p<0.01$) fixed by the G2-assay method (data not shown) for the two cell lines.

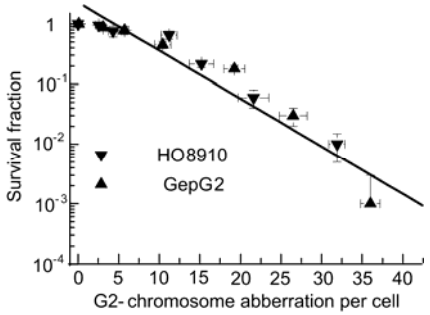

Fig. 3 Relation between survival fraction and the number of initial G2-chromatid breaks per cell for two cell lines studied by G2-PCC technique.

4 Discussion and conclusions

The present results confirm the linearity of G2-chromatid breaks in relation to the absorbed dose (Fig.1) [6, 10], and the data obtained by the G2-PCC technique may reflect more accurately the radiation damage at the chromatin level, which is not influenced by the effects of cell cycle and/or interphase cell death, as compared with the G2 assay. The results described in this study further show that it is easy to study the radiation-induced chromatid damage with the introduction of the premature chromosome condensation technique [11].

Consistent with previous studies [10, 12], the fitted cell survival curves of both cell lines are linear quadratic in this study. And different radiosensitivities are consistent with different aberration induction frequencies in both the human tumor cell lines (Figs. 1 and 2). These imply that the scoring of G2 chromosome breakage holds promise for predicting the radiosensitivity of individual tumor cells.

The colony formation assay is regarded as a good measure of radiosensitivity [13]. As the test sample often mixes with fibroblasts in the culture and the assay needs a long incubation time of 1–2 weeks, the applicability of this assay as a predictive test for radiosensitivity is limited. The micronucleus frequency after irradiation seems to correlate well with the cell lethality [14]. It takes several days to culture cells for this assay. The lack of correlation between micronucleus frequency and radiosensitivity has been reported for malignant melanomas and ovarian cancers [15]. Surrogate

assays for the cell lethality have also been reported as pulsed-field gel electrophoresis (PFGE) and single-cell gel electrophoresis (comet assay), both of which measure DNA double-strand breaks [16]. But some investigators reported the lack of correlation between radiosensitivity and DNA double-strand break induction or rejoining in human tumor cell lines [17]. Other assays should, therefore, be developed so that they can be routinely used in a clinical setting. In this study, an attempt has been made to validate the use of radiation-induced G2-chromatid breaks as a measure of cellular radiosensitivity. One of the prerequisites for the potential use of such a rapid assay is that the correlation with the clonogenic survival should be distinct. In this research, a good correlation was found between the cell survival and the G2-chromatid break aberrations in both cell lines studied by the G2-PCC technique($r=0.9616$, $p<0.01$) (Fig. 3) or by the G2-assay method ($r=0.9381$, $p<0.01$). Although the correlation between the cell survival and the number of G2-chromatid breaks per cell studied by the G2-assay method was also high and statistically significant, the correlation was lower than that studied by the G2-PCC technique. Furthermore, the G2-assay also requires cell division, and, the number of G2-chromatid breaks cannot be determined when cells receive a higher dose, say 4 Gy, due to the radiation-induced G2 delay. Whereas with the G2-PCC technique, the result can be more rapidly obtained and cannot be affected by the radiation-induced G2 delay and cell division, and it only takes 30 min to culture cells. In addition, an important ultimate objective of these studies is to detect radiation-induced chromosome aberration in primary tumor material. Because of the lack of appropriate material, it will be very difficult to obtain adequate metaphase cells, especially for the tumor cells that exhibit poor growth. Undoubtedly, the G2-PCC technique is preferable to predict the radiosensitivity of individual tumor cells compared with the G2-assay method.

To our knowledge, little research was made on the relationship between cell killing and G2-chromatid breaks using the G2-PCC techniques. Some studies have only involved G2-chromosome aberration and indicated that cells with more chromosome aberrations are more radiosensitive with the G2 assay method [7, 18]. Also, some studies dealt with the relationship between the induction of non-rejoining PCC breaks in interphase cells and cellular radiosensitivity, and a good correlation between them was found [12, 19].

In conclusion, it is suggested that the PCC technique is useful for determining chromosome radiosensitivity in G2 cells, and the number of initial G2-PCC breaks induced by radiation can be used for the evaluation of the cellular clonogenic radiosensitivity. To assess the general applicability of this approach, further studies are required to expand the number of human tumor cell lines.

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