An Improved Predictive Assay for Radiotherapy to Human Hepatoma Measured by Prematurely Chromosome Condensation Technique

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Abstract

To investigate the radiation response of hepatoma, SMMC-7721 cells were irradiated with $^{60}\text{Co}$ $\gamma$-rays. Initial chromatid breaks were measured by counting the number of chromatid breaks and isochromatid breaks. A dose-dependent increase in radiation-induced chromatid/isochromatid breaks was observed in $G_1$ and $G_2$ phase respectively. A good relationship was found between cell survival and chromatin breaks. This study implied that low LET radiation-induced chromatid/isochromatid breaks can be possibly used as a good predictor of radio sensitivity of SMMC-7721 hepatoma cells.

Keywords

Predictive Assay, Radiotherapy, Hepatoma, Prematurely Chromosome Condensation Technique

1. Introduction

Cancers is one of the most seriously lethal diseases. Radiobiologists aimed to develop an assay or a combative assay to predict the radiation response of human cancers. Precise prediction of response to radiation could provide the basis for selecting and designing clinical treatment project. Colony assay or growth assay has the good correlation with the radiation response\cite{1-6}. But as a routine predictive assay, it will take at least two or three weeks to form the clone, is unlikely to be used for clinical diagnosis and treatment.

The premature chromosome condensation (PCC) technique is very useful for measuring the radiation-induced chromatin breaks in all of the cell cycles\cite{7-11}, especially in $G_2$ phase. Many researchers have reported a linear dose response in various cell lines irradiated by sorts of radiations, such as X-rays, $\gamma$-rays and heavy ions\cite{5, 9, 10, 12-18}. With the introduction of the premature chromosome condensation technique\cite{19-21}, it is easy to study early radiation-induced chromosome damage.

Previous studies about PCC mostly emphasized on the $G_2$ phase of cell cycle, very little information concerning about the $G_1$ phase even the relationship between $G_1$ and $G_2$ phase. In this study, we employed a chemically induced technique with Calyculin-A to investigate the initial chromatid breaks in SMMC-7721 cells (human hepatoma cells) condensed in $G_1$ and $G_2$ phase after exposed to $^{60}\text{Co}$ $\gamma$-rays.

2. Material and Method

2.1. Cell Culture

Human hepatoma cells SMMC-7721(From CCTCC) were grown in RPMI-1640 medium supplemented with 10% fetal calf serum at 37°C in 5% CO\textsubscript{2}.

2.2. Irradiation

Exponentially growing SMMC-7721 cells were irradiated with $\gamma$-rays generated by using $^{60}\text{Co}$ source with a dose rate...
0.2Gy/min at Lanzhou medicine college, Lanzhou, China.

2.3. Premature Chromosome Condensation and Chromosome Preparation

Calyculin-A (BIOMOL America) was used as the PCC inducer, which was described else where [9, 10, 22] was dissolved in 100% ethanol as 1mM stock solution; 50nM of Calyculin-A was added to the cell cultures before irradiation to score the initial chromatid breaks. Then, cells were incubated for a further 30 minutes at 37°C in 5% CO₂. Chromosome spreads were then harvested by swelling cells in 75 mM KCl for 20 minutes at 37°C and fixing with Carnoy’s fixation. A final wash and fixation in the same fixative was completed before dropping cells onto a glass slide and hot humidity drying.

2.4. Observation and Scoring After Giemsa Staining

Chromosome was stained with 5% Giemsa for 20 minutes. More than 40 G₂-phase cells were scored for each dose point and meanwhile counted the G₁-phase cells using the standard criteria [23]. Briefly, chromatid discontinuing, misalignment of the distal to the lesion or a non-stained region longer than the chromatid width was classified as a break. Isochromatid-type breaks were scored two breaks. The total chromatid breaks were calculated by summing the production of chromatid-type and isochromatid-type breaks.

3. Results

3.1. Colony Assay

Figure 1 shows the survival fraction as a function of dose of SMMC-7721 cells after irradiated with γ-rays, the survival curve was linear quadratic, equation was: \( S=exp(-0.03D-0.06D^2) \). \( R^2=1 \). \( \alpha \) and \( \beta \) value was 0.03 and 0.06, respectively.

3.2. PCC Inducing Efficiency

Calyculin-A, an inhibitor of protein serine/threonine phosphatase, can induce PCC in different phase of cell cycle[7,24] and G₂ phase condensed chromosome are especially easy to get[25]. At each dose point, when 40 G₂ chromosome condensed cells were counted, there were 5 to 8 G₁ chromosome condensed cells came into the sight, see Tab. 1. So, in this study, PCC inducing efficiency was about 12.5~20% compared G₁ with G₂ phase of cell cycle.

<table>
<thead>
<tr>
<th>Dose(Gy)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁ cells</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>G₂ cells</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>G₁/G₂ (%)</td>
<td>17.5</td>
<td>15</td>
<td>12.5</td>
<td>12.5</td>
<td>15</td>
<td>20</td>
<td>17.5</td>
<td>20</td>
</tr>
</tbody>
</table>

3.3. Initial Chromatid-Type Breaks After Irradiated with γ-rays in G₁ and G₂ Phase

Figure 2 shows the number of G₁ and G₂ chromatid-type breaks per cell as a function of dose, respectively. The number of chromatid breaks was linearly with the dose either in G₁ phase or G₂ phase. At the same dose point, the number of chromatid breaks per cell of G₁ phase was smaller than that of G₂ phase. The production of G₂ chromatid breaks per cell was nearly four times more than G₁ phase.
3.4. Initial Isochromatid-Type Breaks After Irradiated with γ-rays in G$_2$ Phase

Figure 3 shows the number G$_2$ isochromatid-type breaks per cell as a function of dose. The number of isochromatid breaks has the linear relationship with the dose. Linear regression analysis result was $y=0.01+0.2x$, $R^2=0.99$.

3.5. Correlation Between Cell Survival Fraction and Chromatid Breaks

Figure 4 shows the number of G$_2$ chromatid-type breaks and isochromatid-type breaks per cell as a function of the cell survival fraction. Using the linear regression analysis, in the G$_2$ phase, both chromatid-type breaks and isochromatid-type breaks have the relatively good correlation with the cell survival fraction after exposed to low LET γ-rays, though some data points deviated from fitted lines, the increasing trend of chromatid breaks with the survival fraction decrease was obvious.

4. Discussion

Calyculin-A is a potential PCC inducer in all cell cycles, especially in G$_2$ phase. In this study, PCC inducing efficiency on G$_2$ phase was about 5~8 times much than that of G$_1$ phase, which was good agreement with other studies [8, 21, 26-29]. Linear dose-response relationship was found between cell survival fraction and the chromatid breaks after the SMMC-7721 cells were exposed to $^{60}$Co γ-rays in this study. This relationship has proved by many previous studies [5, 10, 15, 30, 31]. Either in G$_1$ phase or G$_2$ phase, the linearly increase of chromatid breaks was well correlated with the cell survival fraction decline.

An increased production of chromatid breaks induced by X-ray irradiation has been reported by Durante et al. [32], an increased production of isochromatid breaks produced by exposure to X-ray was also reported by Kawata et al. [9, 10] and Yang et al. [33]. In this study, with the increase of irradiation dose the production of chromatid-type and isochromatid-type breaks linearly increased. It suggests that with the higher dose, the electrons which hit the target-chromosome increased, so, the production increased.

Yet, the absolutely production increase of isochromatid-type breaks was smaller than that of chromatid-type one in G$_2$ phase. Kawata et al. [10] has reported that after low LET irradiation, the chromatid-type breaks dominated, while for high LET rays, such as heavy ions, isochromatid-type breaks dominated, suggesting that most isochromatid breaks resulted from two separate breaks on sister chromatids induced by independent electron tracks. For low LET rays, it can not deposit enough energy during unit range to penetrate sister chromatids meantime, so, most breaks were chromatid-type ones.

In G$_1$ phase, chromatids was relatively loose conglomerated,
but in G₂ phase, because of chromosome reduplication and assemblage for mitosis, chromosome were tightly condensed, so the probability of electron hit target in G₂ phase was higher than that in G₁ phase. In this study, the production of chromatid breaks in G₁ phase was smaller than that of G₂ phase.

5. Conclusion

Radiotherapy for cancers is a very useful method. Earlier diagnosis and the design of treatment project are very important for clinicians and patients. In this study, chemically induced premature chromosome condensation technique was used for quick and exquisite detection of correlation between chromosome breaks with irradiation dose. Linearly increased chromatid-type breaks implied a good correlation between initial chromosome aberration and radiosensitivity of SMMC-7721 cells especially in G₂ phase after low LET irradiation. These results strongly support the possibility of chemically induced PCC technique for predicting the radiosensitivity of hepatoma cells irradiated with low LET radiations.

Acknowledgements

We would express our thanks to all of the workers in the HIRFL. This work was supported by the Talent Project form Longyan University (Grant No. LB20040015).

References


