

# A method on theoretical simulation of chromosome breaks in cells exposed to heavy ions

Jianshe Yang<sup>1,2,3</sup>, Wenjian Li<sup>1</sup>, Xigang Jing<sup>1</sup>, Zhuanzi Wang<sup>1</sup>, Qingxiang Gao<sup>1</sup>

<sup>1</sup>Institute of Modern Physics, Chinese Academy of Sciences, LAN Zhou, <sup>2</sup>Life Science School of Northwest Normal University, LAN Zhou, <sup>3</sup>Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai, China

**Background.** The aim of this study is to assess an easy and quick method on simulating chromosome breaks in cells exposed to heavy charged particles.

**Methods.** The theoretical value of chromosome break was calculated, and the validated comparison with the experimental value by using a premature chromosome condensation technique was done.

**Results.** A good consistence was found to be appeared between the theoretical and experimental value.

**Conclusions.** This suggested that a higher relative biological effectiveness of heavy ions was closely correlated with its physical characteristics and besides, a safe approach on predicting chromosome breaks in cells exposed to heavy ions at off-line environment come to be considered. Furthermore, three key factors influencing the theoretical simulation was investigated and discussed.

*Key words:* theoretical simulation; chromosome breaks; cells; heavy ions

## Introduction

Radiotherapy is one of the most effective methods for the treatment of malignant tumors. GSI in Germany and HIMAC in Japan have successfully treated hundreds of patients with carcinomas by using accelerated heavy ions, such as carbon and silicon.<sup>1</sup> In comparisons with treatments involving X-rays and  $\gamma$ -rays, the high rate of cure with heavy ions is due to physical characteristics, such as high linear energy

transfer (LET) at the Bragg peak region, low side-scattering etc. As reported in previous studies, cells exposed to various radiations resulted in chromosome breaks including chromatid discontinuity, misalignment of the distal, chromatid ring and so on.<sup>2-7</sup> These potential changes possibly cause the death of cells.<sup>1,7</sup> Since Gotoh *et al.* have reported the chemically induced premature condensed chromosome technique in 1995, an easy and quick method on detecting chromosome breakage was widely applied in the radiobiological and oncology works.<sup>8</sup> Murakami *et al.* used atom force microscope (AFM) to assess the accuracy of chemically induced PCC breakages in comparison with the results acquired through the light microscope vision; there

Received 27 December 2007

Accepted 29 January 2008

Correspondence to: Prof. Jianshe Yang, PhD, No. 509, Nanchang Road, Lanzhou City 730000, PR China; Phone: +86 931 4969342; Fax: +86-931 4969201; E-mail: yangjs@impcas.ac.cn

was no significant difference between two methods, and thus it was validated that the PCC technique was suitable and reliable for radiation induced chromosome damage analysis.<sup>9</sup> Suzuki *et al.*<sup>4-7</sup>, Kawata *et al.*<sup>2,3</sup> have employed different heavy ions with various LET and X rays to investigate the radiation induced chromosome breaks both in human normal fibroblast cells and malignant cells. The number of chromatid breaks was found to be linearly correlated with the absorbed dose of radiation. In comparisons with experiments involving X-rays or  $\gamma$ -rays, more isochromatid breaks were produced by the exposure to heavy ions, while chromatid-type breaks were dominantly possessed when cells were exposed to X/gamma rays.

Before the clinical treatment can begin, the therapeutic regimen must be defined and during this stage information on the individual patient radiosensitivity would be of great medical value. Several methods have been developed to measure cell radiosensitivity, for example, the colony assay and the cytoplasm-blocked micronuclei assay.<sup>10-13</sup> Previous data have shown that these two methods are not ideal. Briefly, the colony assay is the classic method for detecting radiosensitivity. The assay is precise but the formation of a clone takes at least 7 days. Conflicting views have been held concerning the detection of cell radiosensitivity with the cytoplasm-blocked micronuclei method.<sup>14,15</sup> Some scientists consider that there is a good relationship between the radiation-induced micronuclei and cell radiosensitivity, but others do not agree. Our previous works further improved the PCC technique in the area of chromosome analysis. Therefore, we have found the radiation induced chromatid /isochromatid breaks were closely correlated with cell surviving when exposed to heavy charged carbon ions.<sup>16,17</sup> The results suggested chemically induced PCC breaks could be possibly re-

garded as a good signal to predict radiosensitivity when cells exposed to high LET radiations.

Even though, we do not think it is perfectly ideal to predict radiosensitivity by using an experimental PCC technique, heavy ions are of great capability in killing cells, the online detection would bring a vast irradiation risk to operators. Thus, the main idea of this study is to simulate the chromosome breaks and validate the simulation combined with the experimental PCC technique.

## Materials and methods

### *Simulation of chromosome breaks*

In radiobiology and therapy the absorbed dose is defined as the energy deposited per mass unit. By definition 1 Gray to 1 Joule per kilogram. If a thin volume-thin compared to changes in the energy loss of a particle- is irradiated by a parallel beam of particles, the dose in Gray in this volume is given as

$$D = 1.602 \times 10^{-9} \times F \times LET \times \frac{1}{\rho} \quad (\text{equ.1})$$

where D is the absorbed dose of cells, LET the energy loss rate,  $\rho$  the density of the stopping material and F the particle fluence i.e. the number of primary ions traversing the unit area. Commonly the density of cells was regarded as  $1\text{g}/\text{cm}^3$  in that the main content of cell is  $\text{H}_2\text{O}$ , thus the real-time particle fluence can be described as

$$F = \frac{D \times 1\text{g}/\text{cm}^3}{1.602 \times 10^{-9} \times LET} \quad (\text{equ. 2})$$

Supposed that each heavy ion could interact with chromosome effectively and

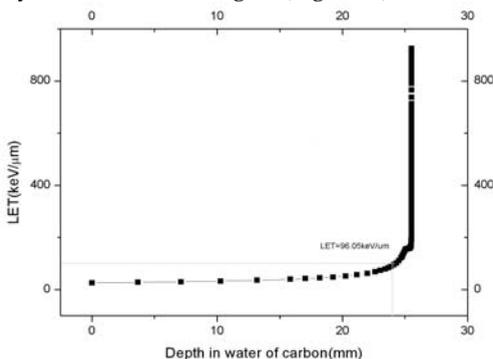
result in one chromatid break, the number (N) of radiation induced chromosome breaks could be calculated by the following equation

$$N = \frac{F \times \frac{\pi \phi^2}{4}}{\text{Number}_{\text{cells}}} \quad (\text{equ. 3})$$

### Cell culture and irradiation

Human normal liver cell line L02 (purchased from the Chinese Center for Type Culture Collection (CCTCC)) was grown in RPMI-1640 medium supplemented with 10% fetal calf serum at 37°C in 5% CO<sub>2</sub>, insulin 0.25 U/ml (Sigma production) was added to the culture medium. In the present study cell numbers need to be accurately counted, 2 ml cell suspension with density of 5×10<sup>6</sup> cells/ml were planted into φ 35 mm plastic dish to be irradiated.

L02 cells were irradiated with <sup>12</sup>C<sup>6+</sup> ion beams generated by the HIRFL with a dose range from 0 to 8Gy. The initial energy of <sup>12</sup>C<sup>6+</sup> ions was 80.55MeV/u, which was decreased by 13.58 mm Lucite (ρ = 1.2g/cm<sup>3</sup>) to 20MeV/u before it reached the cells. The LET was 96.05keV/μm when carbon ions interacted with the cells located in the region of the Bragg peak. LET was calculated by the Trim Program 92 which was written by Bierstadt and Zeigler (Figure 1).<sup>18</sup>



**Figure 1.** The relationship between the range and LET of carbon ions generated from HIRFL.

Dosimetry was performed with an air ionizing chamber where the uniformity of the carbon ion beams was 85%, as measured by CR39 technique.

### Chromosome preparation

Calyculin A (BIOMOL America), used as the PCC inducer, was dissolved in 100% ethanol as a 1 mmol/L stock solution. In order to induce chromatid breaks, 50 nmol/L of calyculin-A was added to cell cultures 5 min before the irradiation. Cells were incubated for a further 30 min at 37°C in 5% CO<sub>2</sub>. The chromosome spread was harvested by swelling cells in 75 mmol/L of KCl for 20min at 37°C and fixed with Carnoy's fixative. A final wash and fixation were completed before placing the cells on a glass slide and drying at 37°C and 85% relative humidity.

The cells were stained with 5% Giemsa (5ml original Giemsa solution was diluted with 47.5ml 1/15M Na<sub>2</sub>HPO<sub>4</sub> and 47.5ml 1/15M KH<sub>2</sub>PO<sub>4</sub>) for 20 min. According to the standard criteria, more than 40 G<sub>2</sub>-phase cells were scored for each dose level.<sup>19</sup> Briefly, the chromatid discontinuity, misalignment of the region distal to the lesion, or a non-stained region longer than the chromatid width was considered as a chromatid break. An isochromatid break was considered as two breaks that occurred at the same position on each of two sister chromatids, *i.e.* a lesion through the two q arms or p arms of the chromosome was regarded as an isochromatid break. One isochromatid break was therefore scored as two breaks. The total chromatid breaks were calculated by summing the numbers of chromatid and isochromatid breaks. A total of 20 non-irradiated cells were examined; there were very few spontaneous chromatid breaks. The mean number of chromatid breaks in non-irradiated cells was subtracted from the mean number observed in ir-

radiated cells to provide the experimental data given in the result section.

*Statistical analysis*

All data were analyzed statistically with SPSS 8.0; note the data at each point are Mean ± Standard Error.

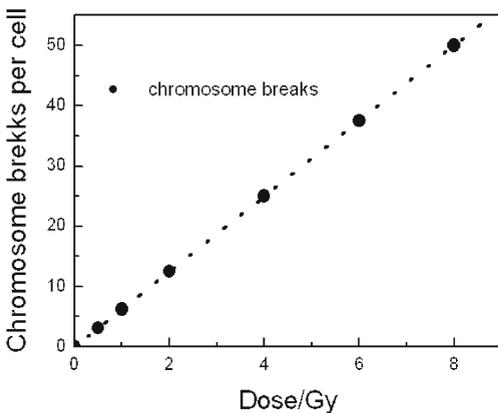
**Results**

*Simulation of the chromatid breaks if L02 cells exposed to  $^{12}\text{C}^{6+}$  ions*

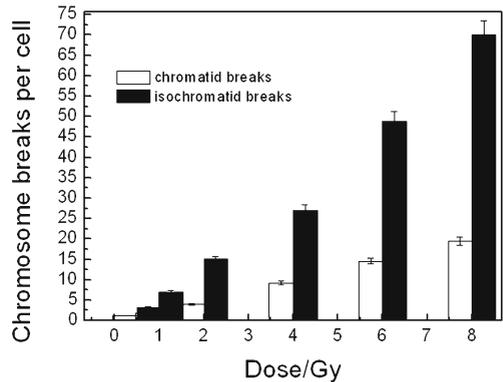
If each ion interacted with cell resulted in just one chromatid break, the number of chromatid breaks could be simulated according to the equation 3 described above. Figure 2 shows the relationship between the absorbed dose and radiation induced chromatid breaks, fitted curve suggested an increasing linear tendency.

*Experimental chromatid type and number in L02 cells exposed to  $^{12}\text{C}^{6+}$  ions*

By using the premature chromosome condensation technique, two types of chro-



**Figure 2.** Correlation between chromosome breaks and ion flux of  $^{12}\text{C}^{6+}$ . Assumed that one  $^{12}\text{C}^{6+}$  ion could produce one chromosome break ( $n=1$ )

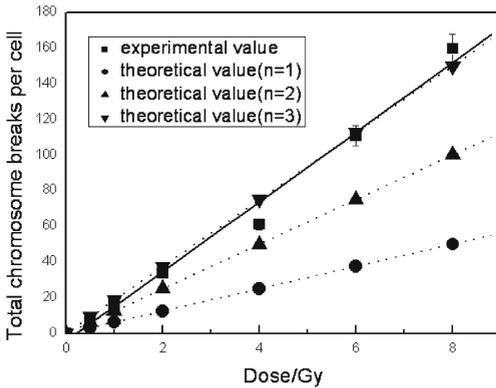


**Figure 3.** Experimental type and number of chromosome breaks of L02 cell irradiated with  $^{12}\text{C}^{6+}$  ions chromatid breaks, isochromatid breaks, ( $p<0.001$ )

matid break were observed under light microscope, *i.e.*, chromatid-type and isochromatid break were induced by the accelerated carbon ion irradiation. With the increasing absorbed dose, both of two types of the chromatid number increased, while the number of isochromatid breaks were significantly higher than that of chromatid-type ones at each dose point (Figure 3).

*Comparison experimental number of chromatid breaks with simulated ones*

An isochromatid break was considered as two breaks that occurred at the same position on each of two sister chromatids, *i.e.* a lesion through the two q arms or p arms of the chromosome was regarded as an isochromatid break. One isochromatid break was, therefore, scored as two breaks. The total chromatid breaks were calculated by summing the numbers of chromatid and isochromatid breaks. According to Figure 4, the same increasing linear tendency was apparent both in simulation and experiment regarded the relationship between the absorbed dose and the number of chromatid breaks. Given one ion produced just one break (named  $n=1$ ), vast discrepancy appeared between simulation and experiment. When simulated curve with  $n=3$ , a



**Figure 4.** Comparison of simulated number of chromosome break exposed to  $^{12}\text{C}^{6+}$  ions with experimental one simulation, experiment

good consistency was found.

## Discussion

In comparisons with treatments involving X-rays and  $\gamma$ -rays, the high rate of cure with heavy ions is due to biophysical characteristics, such as high LET at the Bragg peak region, high relative biological effectiveness (RBE), low oxygen enhancement ratio (OER), low side-scattering etc.<sup>1</sup> Among these characteristics, high RBE is of most importance in the fact that the equivalent dosimetry of heavy ions would result in much more, even several times, cure ratio than that of low LET rays. Our previous studies confirmed that accelerated carbon ions were much more effective in inducing chromatid breaks than those of gamma rays.<sup>16</sup>

What makes heavy ions hold such priority in inducing chromatid break? Whether it is due to pure physical reaction or combination of the biological and physical reaction? The previous study suggested that bio-system would not be activated immediately by the radiation excitation, and the repair of the injured chromosome occurs in 2-12 h after the exposure.<sup>20</sup> It is obvious that the initial chromatid breaks result from the pure physical

interaction. Kawata *et al.*<sup>2,3</sup> regarded a large amount of isochromatid breaks as a sign of cells exposed to high LET radiation, and mechanism of this phenomenon was explicated as a tensely energy deposition at target volume. The result of this study was in agreement with theirs. Recent works by Yang *et al.*<sup>21</sup> supposed that chromatid breaks were linearly negatively related with cell surviving; they suggested chemically induced chromatid breaks measured by PCC technique and can be acted as a quick and precise predictor of radiosensitivity when several normal and tumor cell lines exposed of heavy charged carbon particles.

In this work, carbon ions were used to induce L02 cells to produce chromatid breaks, the experimental result was in good agreement with the theoretical simulation when supposed that each ion leads to three breaks. This suggested some probability of theoretical simulation in place of experiment works to predict radiosensitivity.

Though ion influence (F) and absorbed dose (D) could be accurately detected by professional apparatus, the simulation results approached in this study just to express an ideal status which could be described as:

1. Distribution of carbon beams was uniform, i.e., the uniformity of radiation equate to 1;
2. Cells fully and uniformly covered the culture dish bottle and with single layer, no interspaces exist among cells;
3. Chromosomes occupied all the inner space of a whole cell.

But in fact, no evidence has been applied to support this ideal status. When these three factors neglected, the reliability of radiosensitivity would be discounted when radiotherapy regimen was established. Thus, the better simulation will inevitably be revised by three above factors; we named them approximation of radiation uniformity ( $K_1$ ), detection of cell coverage rate ( $K_2$ )

and approximation of chromosome density ( $K_3$ ). Based on these revisions, the equation (3) can be rewritten to

$$N' = \frac{F \times \frac{\pi\phi^2}{4}}{\text{Number}_{\text{cells}} \times K_2} K_1 K_3 \quad (\text{equ. 4})$$

As to different rays, their LET was not stable and fluctuates;  $K_1$  can be defined by the equation 5

$$K_1 = \frac{\sum_1^n de / dx}{de' / dx'} \quad (\text{equ. 5})$$

Where  $\sum_1^n de / dx$  is the sum of real-time LET value measured at the different time point,  $n$  the measure times,  $de' / dx'$  the one real-time LET value.

The equation 6 defined the cell coverage rate at culture dish bottle

$$K_2 = \frac{\text{Number}_{\text{cells}} \times S_{\text{cell}}}{\pi\phi^2 / 4} \quad (\text{equ. 6})$$

Where  $\text{Number}_{\text{cells}}$  is the number of cells grow in culture dish,  $S_{\text{cell}}$  the area of single cell vertically faced to ion beams,  $\pi\phi^2 / 4$  the inner area of culture dish.

At various phases of cell cycle, the chromosome agglomeration status and the content are different. Though it is not so well impacting the physical density of cells, the cross-section of the interaction between cells and ions closely linked with it. Chromosome in  $G_2$  phase, is of better configuration, which is selected to analyze chromatid breaks,  $K_3$  was denoted as

$$K_3 = \frac{G_2}{G_0 + G_1 + G_2 + S + M} = G_2$$

Where  $G_0$ ,  $G_1$ ,  $G_2$ ,  $S$ ,  $M$  were respectively the content percent of each phase cell number in all cells which were detected. This percent could be measured by flow cytometer (FCM).

In a word, considered these external factors, the simulation of chromatid breaks to predict the radiosensitivity in heavy ion radiotherapy project is of great possibility and feasibility.

### Conclusions

Chemically induced PCC technique can be used to analyze chromatid breaks induced by heavy ion, the radiation induced by initial chromatid/isochromatid breaks can be regarded as a possible good sign of intrinsic radiosensitivity of cells exposed to heavy charged ions, the theoretical simulation of radiation induced by chromatid breaks was a simple and convenient and safe approach to measure the radiosensitivity.

### Acknowledgements

We would express our thanks to all of the workers in the HIRFL. This work was jointly supported by the National Natural Science Foundation Committee (Grant No. 10335050), Sciences and Technology Ministry of People's Republic of China (Grant No. 2003CCB00200), K. C. Wong Education Foundation, Hong Kong. (Grant No. 20060037) and China Postdoctoral Science Foundation (Grant No. 20060400686).

## References

- 1 Kraft G. Tumor therapy with heavy charged particles. *Prog Part Nucl Phys* 2000; **45**: 473-544.
- 2 Kawata T, Gotoh E, Durante M, Wu H, George K, Furusawa Y, et al. High-LET radiation-induced aberrations in prematurely condensed G<sub>2</sub> chromosome of human fibroblasts. *Int J Radiat Biol* 2000; **76**: 929-37.
- 3 Kawata T, Durante M, Frusawa Y, George K, Takai N, Wu H, et al. Dose-response of initial G<sub>2</sub>-chromatid breaks induced in normal human fibroblasts by heavy ions. *Int J Radiat Biol* 2001; **77**: 165-74.
- 4 Suzuki M, Watanabe M, Suzuki K, Nakano K, Matsui K. Heavy ion-induced chromosome breakage studied by premature chromosome condensation (PCC) in Syrian hamster embryo cells. *Int J Radiat Biol* 1992; **62**: 581-6.
- 5 Suzuki M, Watanabe M, Kanai T, Kase Y, Yatagai F, Kato T, et al. LET dependence of cell death, mutation induction and chromatin damage in human cells irradiated with accelerated carbon ions. *Adv Space Res* 1996; **18**: 127-36.
- 6 Suzuki M, Kase Y, Kanai T, Yatagai F, Watanabe M. LET dependence of cell death and chromatin-break induction in normal human cells irradiated by neon-ion beams. *Int J Radiat Biol* 1997; **72**: 497-503.
- 7 Suzuki M, Kase Y, Kanai T, Ando K. Correlation between cell death and induction of nonrejoining PCC breaks by carbon-ion beams. *Adv Space Res* 1998; **22**: 561-8.
- 8 Gotoh E, Asakawa Y, Kosaka H. Inhibition of protein serine/threonine phosphatases directly induces premature chromosome condensation in mammalian somatic cells. *Biomed Res* 1995; **16**: 63-8.
- 9 Murakami M, Minamihisamatsu M, Sato K, I Hayata. Structural analysis of heavy ion radiation-induced chromosome aberrations by atomic force microscopy. *J Biochem Biophys Methods* 2001; **48**: 293-301.
- 10 Girinsky T, Bernheim A, Lubin R, Tavakolirazavi T, Baker F, Janot F, et al. *In vitro* parameters and treatment outcome in head and neck cancers treated with surgery and/or radiation: cell characterization and correlation with local control and overall survival. *Int J Radia Oncol Biol Phys* 1994; **30**: 789-94.
- 11 West CM, Davidson SE, Burt PA, Hunter RD. The intrinsic radiosensitivity of cervical carcinoma: correlations with clinical data. *Int J Radiat Oncol Biol Phys* 1995; **31**: 841-6.
- 12 West CM, Davidson SE, Roberts SA, Hunter RD. The independence of intrinsic radiosensitivity as a prognostic factor for patient response to radiotherapy of carcinoma of cervix. *Br J Cancer* 1997; **76**: 1184-90.
- 13 Rosenblum ML, Knebel KD, Wheeler KT, Barker M, Wilson CB. Development of an *in vitro* colony formation assay for the evaluation of *in vivo* chemotherapy of a rat brain tumor. *In Vitro* 1975; **11**: 264-73.
- 14 Eastham AM, Atkinson J, West CM. Relationship between clonogenic cell survival, DNA damage and chromosomal radiosensitivity in nine human cervix carcinoma cell lines. *Int J Radiat Biol* 2001; **77**: 295-302.
- 15 Guo GZ, Sasai K, Oya N, Shibata T, Shibuya K, Hiraoka M. A significant correlation between clonogenic radiosensitivity and the simultaneous assessment of micronucleus and apoptotic cell frequencies. *Int J Radiat Biol* 1999; **75**: 857-64.
- 16 Yang JS, Li WJ, Zhou GM, Jin XD, Xia JG, Wang JF, et al. Comparative study on radiosensitivity of various tumor cells and human normal liver cells. *World J Gastroenterol* 2005; **11**: 4098-101.
- 17 Yang JS, Li WJ, Jin XD, Jing XG, Guo CL, Wei W, et al. Radiobiological response on human hepatoma and normal liver cells exposed to carbon ions generated by Heavy Ion Research Facility in Lanzhou. *Sci China Ser G* 2006; **49**: 72-6.
- 18 Ziegler JF, Biersack JP, Littmark U. The stopping and range of ions in solids. Oxford: Pergamon Press; 1985.
- 19 Savage JRK. Classification and relationships of induced chromosomal structural changes. *J Med Gene*, 1976; **13**: 103-22.
- 20 Nasonova E, Gudowska E, Ritter S, Kraft G. Analysis of Ar-ion and X-ray-induced chromatin breakage and repair in V79 plateau-phase cells by the premature chromosome condensation technique. *Int J Radiat Biol* 2001; **77**: 59-70.
- 21 Yang JS, Jing XG, Li WJ, Wang ZZ, Zhou GM, Wang JF, et al. Correlation between initial chromatid damage and survival of various cell lines exposed to heavy charged particles. *Radiat Environ Bioph* 2006; **45**: 261-6.