

• BRIEF REPORTS •

Comparative study on radiosensitivity of various tumor cells and human normal liver cells

Jian-She Yang, Wen-Jian Li, Guang-Ming Zhou, Xiao-Dong Jin, Jing-Guang Xia, Ju-Fang Wang, Zhuan-Zi Wang, Chuan-Ling Guo, Qing-Xiang Gao

Jian-She Yang, Wen-Jian Li, Guang-Ming Zhou, Xiao-Dong Jin, Jing-Guang Xia, Ju-Fang Wang, Zhuan-Zi Wang, Chuan-Ling Guo, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, Gansu Province, China
Qing-Xiang Gao, Life Science School of Lanzhou University, Lanzhou 730000, Gansu Province, China

Supported by the National Natural Science Foundation of China, No. 10335050; Ministry of Science and Technology of the People's Republic of China, No. 2003CCB00200

Correspondence to: Dr. Jian-She Yang, Radiobiology Laboratory, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, Gansu Province, China. tuyangjs@impcas.ac.cn
Telephone: +86-931-4969338 Fax: +86-931-4969201
Received: 2004-11-03 Accepted: 2004-11-19

Abstract

AIM: To investigate the radiation response of various human tumor cells and normal liver cells.

METHODS: Cell lines of human hepatoma cells (SMMC-7721), liver cells (L02), melanoma cells (A375) and cervical tumor (HeLa) were irradiated with ^{60}Co γ -rays. Cell survive was documented by a colony assay. Chromatid breaks were measured by counting the number of chromatid breaks and isochromatid breaks immediately after prematurely chromosome condensed by Calyculin-A.

RESULTS: Linear quadratic survival curve was observed in all of four cell lines, and dose-dependent increase in radiation-induced chromatid and isochromatid breaks were observed in GB2B phase. Among these four cell lines, A375 was most sensitive to radiation, while, L02 had the lowest radiosensitivity. For normal liver cells, chromatid breaks were easy to be repaired, isochromatid breaks were difficult to be repaired.

CONCLUSION: The results suggest that the γ -rays induced chromatid breaks can be possibly used as a good predictor of radiosensitivity, also, unrejoined isochromatid breaks probably tightly related with cell cancerization.

© 2005 The WJG Press and Elsevier Inc. All rights reserved.

Key words: Radiosensitivity; Tumor cells; Normal; Liver cells

Yang JS, Li WJ, Zhou GM, Jin XD, Xia JG, Wang JF, Wang ZZ, Guo CL, Gao QX. Comparative study on radiosensitivity of various tumor cells and human normal liver cells. *World J Gastroenterol* 2005; 11(26): 4098-4101
<http://www.wjgnet.com/1007-9327/11/4098.asp>

INTRODUCTION

Liver cancer is one kind of malignancy tumor with the highest incidence and clinical risk, especially popular in China. As till now, radiotherapy is the most acceptable and effective method. Low LET (Linear energy transfer) rays, such as X-ray, γ -ray, were often used. Radiobiologists aim to develop an assay or a combinative 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^[1-5]. But as a routine predictive assay, it will take at least 2 or 3 weeks to form the clone, is unlikely to be used for clinical diagnosis and treatment.

The PCC (premature chromosome condensation) technique is very useful for measuring the radiation-induced chromatin breaks in all of the cell cycles^[6-9], especially in G_2 phase. In the present study, we investigated the G_2 phase radiosensitivity of human hepatoma cells and normal liver cells, compared with other two type of human malignancy tumors. The results showed that the radiosensitivity of normal liver cells is lowest among these four cell lines, suggest that in the course of liver cancer radiotherapy, liver cells will less injured than hepatoma cells and other two tumor cell lines when absorbed same dose of radiation.

MATERIALS AND METHODS

Cell culture and irradiation

Four cell lines (From CCTCC) were grown in RPMI-1640 medium supplemented with 10% fetal calf serum at 37 °C in 50 mL/L CO_2 , additional 0.25 U/mL insulin was added into the L02 culture medium.

Exponentially growing cells were irradiated with γ -rays generated by ^{60}Co source with a dose rate 0.2 Gy/min at Lanzhou medicine college, dose range was from 0 to 8 Gy.

Chromosome preparation

Calyculin-A (BIOMOL America) was used as the PCC inducer, which was dissolved in 100% ethanol as 1 mmol/L stock solution; 50 nmol/L 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 min at 37 °C in 50 mL/L CO_2 . Chromosome spreads were then harvested by swelling cells in 75 mmol/L KCl for 20 min at 37 °C and fixed 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.

Chromosome was stained with 5% Giemsa for 20 min. More than 40 G_2 phase cells were scored for each dose point according to the standard criteria^[10]. 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 breaks were scored two breaks. The total chromatid breaks were calculated by summing the production of chromatid and isochromatid breaks. The process of kinetic repair of damaged chromosome by PCC technique was same as initial ones.

RESULTS

G_2 PCC chromosome spreads

Calyculin A is a phosphatase inhibitor, can effectively induce the cells into G_2 phase by very little time. It is easy to detect the chromosome damage using PCC technique. Figure 1 shows the G_2 PCC chromosome spreads of L02 cell lines exposed to 8 Gy of γ -rays and SMMC-7721 cell lines of 0.5 Gy.

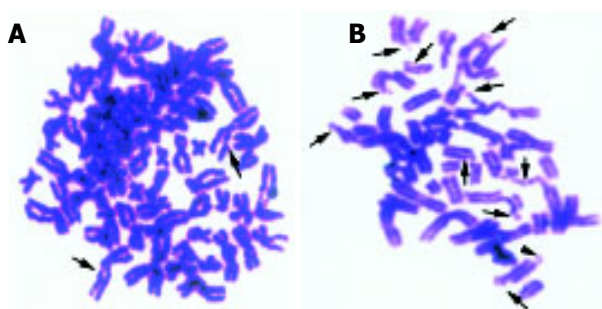


Figure 1 G_2 phase prematurely condensed chromosome spreads. **A:** SMMC-7721 cells after exposed to the γ -rays of 0.5 Gy; **B:** L02 cells after exposed to the γ -rays of 8 Gy. Arrows show the chromatid breaks, arrowheads show the isochromatid breaks.

Survival curves

Colony assay is a routine method for analyzing the cell survival fraction. Classical model of cell survival curve after exposed to low LET rays, such as X-ray, γ -ray, was linear quadratic. In present study, four cell lines' fitted survival curve were well agreement with the linear quadratic model. Figure 2 shows the detail of it.

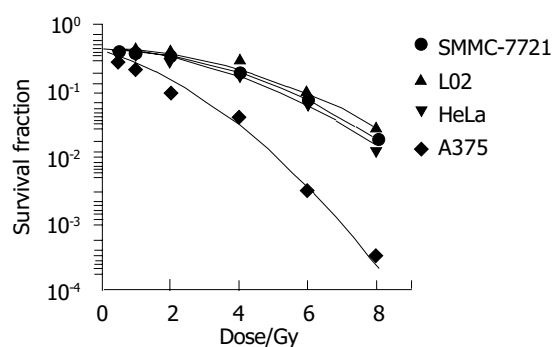


Figure 2 Cell survival curve after exposed to γ -rays. All of four cell lines have a linear quadratic survival curve, which were good agreement with classical model.

Initial chromatid and isochromatid breaks after irradiated with γ -rays in G_2 phase

Figures 3A and B express the chromatid and isochromatid breaks of four cell lines after irradiated with various absorbed dose. Though some inter-exchanges or inner-exchanges exist in chromosome spreads, its number was rare. We only observed the main chromosome break type, i.e., chromatid and isochromatid breaks. Chromatid and isochromatid breaks linearly increased with the dose increase of these four cell lines, the number of chromatid breaks was much more than that of isochromatid breaks at each dose point.

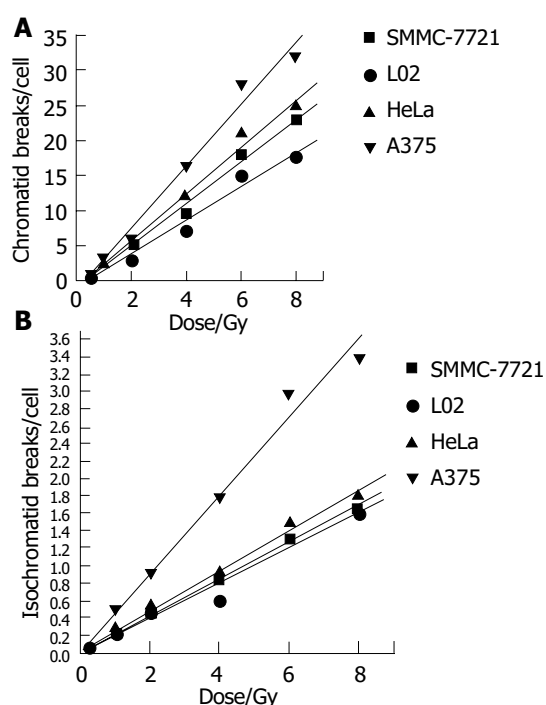


Figure 3 G_2 chromosome breaks at various absorbed dose. **A:** chromatid breaks; **B:** isochromatid breaks.

Kinetic repair of chromosome breaks of L02 cells in 48 h of post-irradiation time

Figures 4 and 5 show the unrejoined chromatid breaks/cell/Gy at 0, 24, and 48 h. In this kinetic process, almost

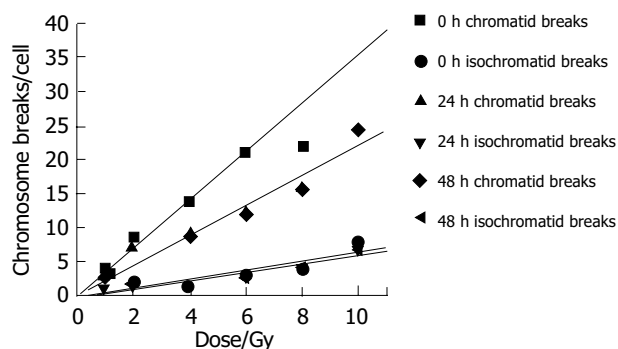


Figure 4 Correlation between chromatid breaks and absorbed dose at 0, 24, and 48 h after exposed to radiation.

50% chromatid breaks got repaired, for isochromatid ones, just 15% got repaired at most. Also we can see, during 24-48 h, both of these two type breaks were nearly the same level, i.e., from 2.16 to 1.93 breaks/cell/Gy for chromatid breaks, from 0.476 to 0.48 breaks/cell/Gy for isochromatid ones. However, from 0 to 24 h these values were 2.95-2.16 breaks/cell/Gy, 0.502-0.476 breaks/cell/Gy, respectively. Compared with chromatid breaks, isochromatid breaks nearly keep the original state within 48 h after exposed to radiation. This is to say, at the early 24 h after exposure, chromosome repair process finished.

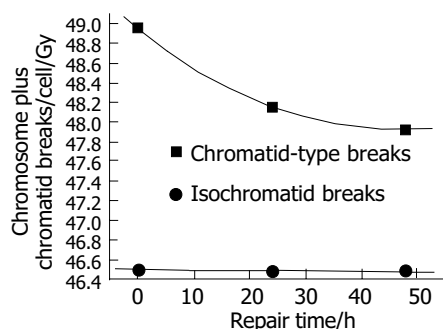


Figure 5 Kinetic repair of chromosome breakages within 48 h after L02 cell line was exposed to γ -rays. The chromatid breaks plus 46 (number of normal human chromosomes) make this change remarkably.

DISCUSSION

Liver cancer is of high risk and mortality. Besides the surgical excision, radiotherapy is the main method after the discovery of X-rays. Before radiotherapy, prompt and exact detection of radiosensitivity of target cells is of great importance.

Many researchers have reported a linear-quadratic cell survival curve in various cell lines irradiated by X-rays, γ -rays, also, linear relationship between chromosome breaks and absorbed dose was discovered^[5,8,9,11-15]. With the introduction of the premature chromosome condensation technique^[16-18], it is easy to study initial radiation-induced chromosome damage (Figure 1).

In the present study, fitted cell survival curve of four cell lines were linear quadratic (Figure 2), which were consistent with previous studies^[8-9]. Among these four cell lines, survival fraction of L02 was highest, A375 was lowest after absorbed same dose of radiation, suggested that human normal liver cells was of much higher resistance to γ -rays than human hepatoma cells, melanoma cells and cervical tumor cells. Radiation injury mainly resulted from oxidative free radicals, reductive glycogens synthesized in liver cells which interact with free radicals reduce the radiation injury.

Linear relationship was found between dose and the chromatid breaks after the cells were exposed to ^{60}Co γ -rays in this study (Figure 3). This relationship has proved by previous studies^[5,8,14,19,20]. An increased production of chromatid breaks induced by X-ray irradiation has been reported by Durante^[21-23], an increased production of isochromatid breaks produced by exposure to X-ray was also reported by Kawata^[8,9]. It suggested that with the higher

dose, the electrons which hit the target-chromosome increased, so, the production increased. While, the absolutely production increase of isochromatid breaks was smaller than that of chromatid breaks. Kawata *et al.*^[8], has reported that after low LET irradiation, the chromatid breaks dominated, while for high LET rays, such as heavy ions, isochromatid 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 ones.

Since normal liver cells were more radioresistant to γ -rays than other three kind of cancer cells, study the kinetic repair of chromosome damage of L02 cells has the essentiality. Data^[4,9,15,16] showed that these irradiation could induce the chromosome damage, the damage will probably result in cancerization. Correct and effective repair of injured chromosome is very important to stop cancerization course. Figures 4 and 5 show the correlation between chromatid breaks and dose at 0, 24 and 48 h after radiation. In the present study, at each time point, chromatid breaks and isochromatid breaks were increased linearly with the dose, and the absolute number of chromatid breaks was much more than that of isochromatid ones, this phenomenon was described by Kawata *et al.*^[8]; Suzuki *et al.*^[15], reported that this repairing process occurred during the early 10 h after irradiation. This means in the early 24 h, most of any kind of cells end their spontaneous repair process if they are injured. After 24 h, without any artificial factors, injured and normal cells will be stable and continue synthesizing, mitosing, and so on.

Though the radiotherapy by using common irradiation such as γ -ray is very effective, we could not neglect the side-effect which largely reduce the curative effect due to the injury of normal tissue. Heavy ions have the good biophysics characteristics such as Bragg peak of dose distribution which result in the lowest side scatter, higher RBE (relative biological effect) and lower OER (oxygen enhancement ratio). Present study will enrich the essential data for the Heavy-Ion-Radiotherapy project in the Institute of Modern Physics, Chinese academy of Sciences when the national great engineering HIRFL-CSR (heavy ion research facility of Lanzhou-cooling restore cycle) complete at 2005.

REFERENCES

- Girinsky T, Bernheim A, Lubin R, Tavakolirazavi T, Baker F, Janot F, Wibault P, Cosset JM, Duvillard P, Duverger A, Fertil B. *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 Radiat Oncol Biol Phys* 1994; **30**: 789-794
- 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**: 841-846
- 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**: 1184-1190
- Coco Martin JM, Mooren E, Ottenheim C, Burrill W, Nunez MI, Sprong D, Bartelink H, Begg AC. Potential of radiation-

- induced chromosome aberrations to predict radiosensitivity in human tumor cells. *Int J Radiat Biol* 1999; **75**: 1161-1168
- 5 **Kawata T**, Ito H, George K, Wu H, Uno T, Isobe K, Cucinotta FA. Radiation-induced chromosome aberrations in ataxia telangiectasia cells: high frequency of deletions and misrejoining detected by fluorescence *in situ* hybridization. *Radiat Res* 2003; **159**: 597-603
 - 6 **Gotoh E**, Kawata T, Durante M. Chromatid break rejoining and exchange aberration formation following γ -ray exposure: analysis in GB2B human fibroblasts by chemical-induced premature chromosome condensation. *Int J Radiat Biol* 1999; **75**: 1129-1135
 - 7 **Durante M**, Frusawa Y, George K, Gialanella G, Greco O, Grossi G, Matsufuji N, Pugliese M, Yang TC. Rejoining and misrejoining of radiation-induced chromatin breaks. IV. Charged particle. *Radiat Res* 1998; **149**: 446-454
 - 8 **Kawata T**, Gotoh E, Durante M, Wu H, George K, Frusawa Y, Cucinotta FA. High-LET radiation-induced aberrations in prematurely condensed G2 chromosome of human fibroblasts. *Int J Radiat Biol* 2000; **76**: 929-937
 - 9 **Kawata T**, Durante M, Frusawa Y, George K, Takai N, Wu H, Cucinotta FA. Dose-response of initial G2-chromatid breaks induced in normal human fibroblasts by heavy ions. *Int J Radiat Biol* 2001; **77**: 165-174
 - 10 **Savage JR**. Classification and relationships of induced chromosomal structural changes. *J Med Genet* 1976; **13**: 103-122
 - 11 **Pantelias GE**, Maillie HD. Direct analysis of radiation-induced chromosome fragments and rings in unstimulated human peripheral blood lymphocytes by means of the premature chromosome condensation technique. *Mutat Res* 1985; **149**: 67-72
 - 12 **Bedford JS**, Goodhead DT. Breakage of human interphase chromosomes by alpha particles and X-rays. *Int J Radiat Biol* 1989; **55**: 211-216
 - 13 **Goodwin EH**, Blakely EA, Tobias CA. Chromosomal damage and repair in G1-phase Chinese hamster ovary cells exposed to charged-particle beams. *Radiat Res* 1994; **138**: 343-351
 - 14 **Cornforth MN**, Goodwin EH. The dose-dependent fragmentation of chromatin in human fibroblast by 3.5-MeV α particles from ^{238}Pu : Experimental and theoretical consideration pertaining to single-track effects. *Radiat Res* 1991; **127**: 64-74
 - 15 **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**: 581-586
 - 16 **Suzuki M**, Watanabe M, Kanai T, Kase Y, Yatagai F, Kato T, Matsubara S. LET dependence of cell death, mutation induction and chromatin damage in human cells irradiated with accelerated carbon ions. *Adv Space Res* 1996; **18**: 127-136
 - 17 **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
 - 18 **Johnson RT**, Rao PN. Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei. *Nature* 1970; **226**: 717-722
 - 19 **Hittelman WN**, Rao PN. Premature chromosome condensation. I. Visualization of x-ray-induced chromosome damage in interphase cells. *Mutat Res* 1974; **23**: 251-258
 - 20 **Cornforth MN**, Bedford JS. X-ray-induced breakage and rejoining of human interphase chromosomes. *Science* 1983; **222**: 1141-1143
 - 21 **Pandita TK**, Hittelman WN. The contribution of DNA and chromosome repair deficiencies to the radiosensitivity to ataxia-telangiectasia. *Radiat Res* 1992; **131**: 214-223
 - 22 **Sasai K**, Evans JW, Kovacs MS, Brown JM. Prediction of human cell radiosensitivity: comparison of colongenic assay with chromosome aberrations scored using premature chromosome condensation with fluorescence *in situ* hybridization. *Int J Radiat Oncol Biol Phys* 1994; **30**: 1127-1132
 - 23 **Durante M**, Gialanella G, Grossi GF, Nappo M, Pugliese M, Bettega D, Calzolari P, Chiorda GN, Ottolenghi A, Tallone-Lombardi L. Radiation-induced chromosomal aberrations in mouse 10T1/2 cells: dependence on the cell-cycle stage at the time of irradiation. *Int J Radiat Biol* 1994; **65**: 437-447

Science Editor Li WZ Language Editor Elsevier HK