Physics Journal
Vol. 1, No. 3, 2015, pp. 290-294
http://www.aiscience.org/journal/pj

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

Yang Jianshe[1, *], Zhao Hua[2]

[1]Life Science School, Longyan University, Longyan, China
[2]Agricultural School, Gansu Agriculture University, Lanzhou, China

Abstract

To investigate the radiation response of hepatoma, SMMC-7721 cells were irradiated with ^{60}Co γ-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

Received: July 20, 2015 / Accepted: August 30, 2015 / Published online: November 11, 2015

1. Introduction

Cancers is one of the most seriously lethal diseases. Radiobiologists aimed 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 [1-6]. 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 [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, γ-rays and heavy ions [5, 9, 10, 12-18]. With the introduction of the premature chromosome condensation technique [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}Co γ-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_2.

2.2. Irradiation

Exponentially growing SMMC-7721 cells were irradiated with γ-rays generated by using ^{60}Co source with a dose rate

* Corresponding author
E-mail address: yangjs@impcas.ac.cn (Yang Jianshe)

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% CO_2. 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 G_2-phase cells were scored for each dose point and meanwhile counted the G_1-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: $S=exp(-0.03D-0.06D^2)$, $R^2=1$. α and β value was 0.03 and 0.06, respectively.

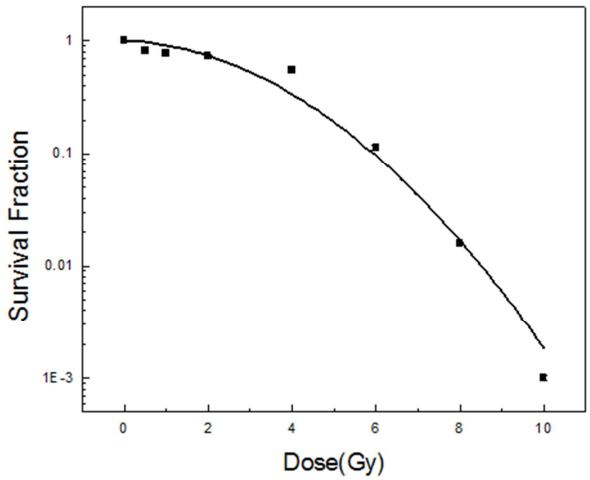


Fig. 1. Survival fraction of SMMC-7721 irradiated with γ-rays.

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 G_2 phase condensed chromosome are especially easy to get[25]. At each dose point, when 40 G_2 chromosome condensed cells were counted, there were 5 to 8 G_1 chromosome condensed cells came into the sight, see Tab. 1. So, in this study, PCC inducing efficiency was about 12.5~20% compared G_1 with G_2 phase of cell cycle.

Tab. 1. Ratio of chromosome prematurely condensed G_1 cells to G_2 cells.

Dose(Gy)	0	0.5	1	2	4	6	8	10
G_1 cells	7	6	5	5	6	8	7	8
G_2cells	40	40	40	40	40	40	40	40
G_1/ G_2 (%)	17.5	15	12.5	12.5	15	20	17.5	20

3.3. Initial Chromatid-Type Breaks After Irradiated with γ-rays in G_1 and G_2 Phase

Figure 2 shows the number of G_1 and G_2 chromatid-type breaks per cell as a function of dose, respectively. The number of chromatid breaks was linearly with the dose either in G_1 phase or G_2 phase. At the same dose point, the number of chromatid breaks per cell of G_1 phase was smaller than that of G_2 phase. The production of G_2 chromatid breaks per cell was nearly four times more than G_1 phase.

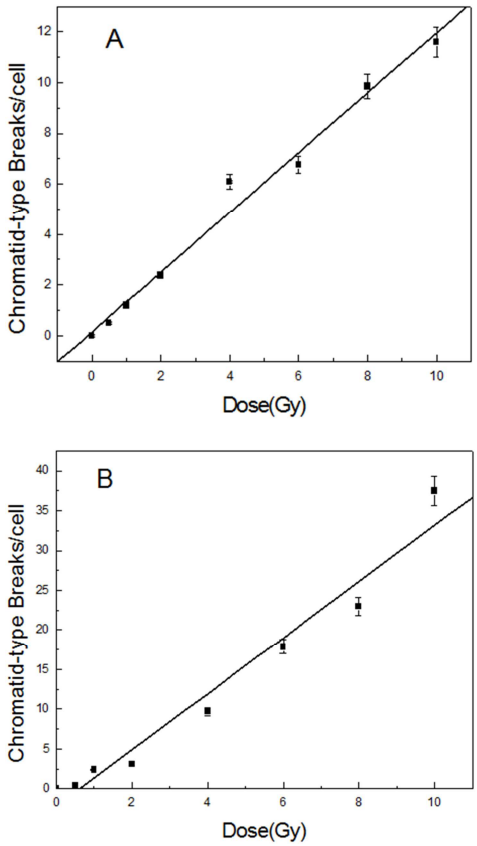


Fig. 2. Chromatid-type breaks of G_1 and G_2 PCC SMMC-7721 cells. A: G_1 PCC; B: G_2 PCC.

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 $y=0.01+0.2x$, $R^2=0.99$.

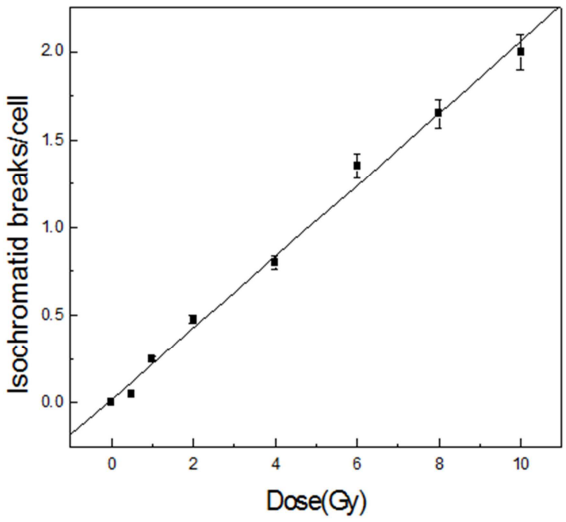
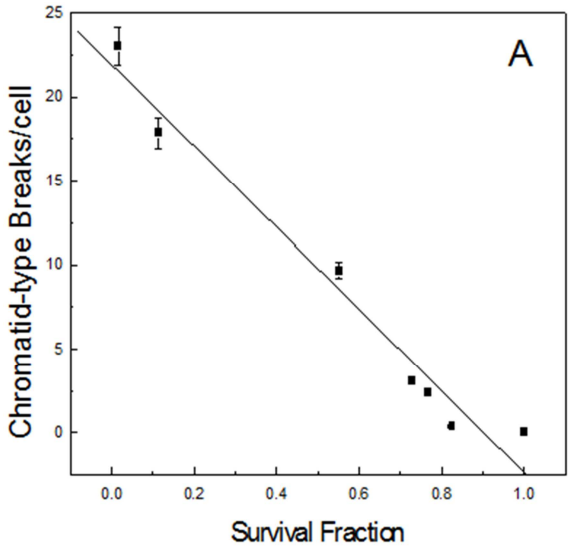


Fig. 3. Isohromatid-type breaks of G_2 PCC SMMC-7721 cells.

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.



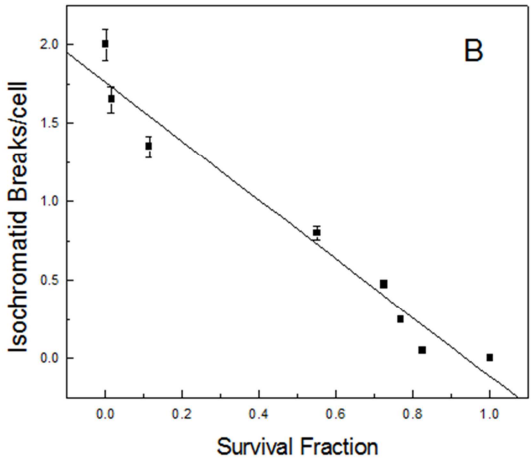


Fig. 4. G_2 chromatid-type breaks and isochromatid-type breaks as a function of cell survival fraction of SMMC-7721 cells. A: chromatid-type breaks; B: isochromatid-type breaks.

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 isochomatid-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 G_2 phase, because of chromosome reduplication and assemblage for mitosis, chromosome were tightly condensed, so the probability of electron hit target in G_2 phase was higher than that in G_1 phase. In this study, the production of chromatid breaks in G_1 phase was smaller than that of G_2 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 G_2 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

[1] Girinsky, T, Bernheim, A., Lubin, R., Tavakolirazavi, T., Baker, F., Janot, F., Wibault, P., Cosset, J.M., Duvillard, P., Duverger, A. and 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. 30 (1994) 789-794.

[2] West, C.M., Davidson, S.E., Burt, P.A., Hunter, R.D. The intrinsic radiosensitivity of cervical carcinoma: correlations with clinical data. Int. J. Radiat. Oncol. Biol. Phys. 31 (1995) 841-846.

[3] West, C.M., Davidson, S.E., Roberts, S.A., Hunter, R.D. The independence of intrinsic radiosensitivity as a response factor for prognostic patient to radiotherapy of carcinoma of cervix. Br. J. Cancer 76 (1997) 1184-1190.

[4] Coco Martin, J.M., Mooren, E., Ottenheim, C., Burrill, W., Nunez, M.I., Sprong, D., Bartelink, H. and Begg, A.C. Potential of radiation-induced chromosome aberrations to predict radiosensitivity in human tumor cells. Int. J. Radiat. Biol. 75 (1999) 1161-1168.

[5] Kawata, T., Ito, H., George, K., Wu, H., Uno, T., Isobe, K. and Cucinotta, F.A. Radiation-induced chromosome aberrations in Ataxia Telangiectasia cells: high frequency of deletions and misrejoining detected by fluorescence in situ hybridization. Radiat. Res. 159 (2003) 597-603.

[6] Buch, K., Peters, T., Nawroth, T., Sänger, M., Schmidberger, H., Langguth, P. Determination of cell survival after irradiation via clonogenic assay versus multiple MTT Assay-a comparative study. Radiat. Oncol. 7 (2012) 1.

[7] Gotoh, E., Asakawa, Y. and Kosaka, H. Inhibition of protein serine/threonine phosphatases directly induces premature chromosome condensation in mammalian somatic cells. Biomed. Res. 16 (1995) 63-68.

[8] Durante, M., Frusawa, Y., George, K., Gialanella, G., Greco, O., Grossi, G., Matsufuji, N., Pugliese, M., Yang, T.C. Rejoining and misrejoining of radiation-induced chromatin breaks. IV. Charged particle. Radiat. Res. 149 (1998a) 446-454.

[9] Kawata, T., Gotoh, E., Durante, M., Wu, H., George, K., Furusawa, Y. and Cucinotta, F.A. High-LET radiation-induced aberrations in prematurely condensed G_2 chromosome of human fibroblasts. Int. J. Radiat. Biol. 76 (2000) 929-938.

[10] Kawata, T., Durante, M., Frusawa, Y., George, K., Takai, N., Wu, H. and Cucinotta, F.A. Dose-response of initial G_2-chromatid breaks induced in normal human fibroblasts by heavy ions. Int. J. Radiat. Biol. 77 (2001) 165-174.

[11] Torsten, G., Hang, C., Gerald, F., James, C., Sylvain, V.C., Mary, H., Bahram, P., Bjorn, R. Persistence of γ-H2AX and 53BP1 foci in proliferating and non-proliferating human mammary epithelial cells after exposure to γ-rays or iron ions. Int. J. Radiat. Biol. 87 (2011) 696-710.

[12] Pantelias, G.E., Maillie, H.D. 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. 149 (1985) 67-72.

[13] Cornforth, M.N. and Goodwin, E.H. The dose-dependent fragmentation of chromatin in human fibroblast by 3.5-MeV α particles from 238Pu: Experimental and theoretical consideration pertaining to single-track effects. Radiat. Res. 127 (1991) 61-71.

[14] Suzuki, M., Watanabe, M., Suzuki, K., Nakano, K. and Matsni, K. Heavy-ion induced chromosome breakage studied by premature chromosome condensation (PCC) in Syrian hamster embryo cells. Int. J. Radiat. Biol. 62 (1992) 581-586.

[15] Suzuki, M., Watanabe, M., Kanai, T., Kase, Y., Yatagai, F., Kato, T. and Matsubara, S. LET dependence of cell death, mutation induction and chromatin damage in human cells irradiated with accelerated carbon ions. Adv. Space Res. 18 (1996) 127-136.

[16] Suzuki, M., Kase, Y., Kanai, T., Yatagai, F. and Watanabe, M. LET dependence of cell death and chromatin-break induction in normal human cells irradiated by neon-ion beams. Int. J. Radiat. Biol. 72 (1997) 497-503.

[17] Peter, E.B., Hossein, M. A comparison of G_2 phase radiation-induced chromatid break kinetics using calyculin-PCC with those obtained using colcemid block. Mutagenesis 22 (2007) 359-362.

[18] Peter, E.B., Hossein, M., Christie, M. G2-phase chromatid break kinetics in irradiated DNA repair mutant hamster cell lines using calyculin-induced PCC and colcemid-block. Mutat. Res. 657 (2008) 8-12.

[19] Johnson, R.T., Rao, P.N. Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei. Nature 226 (1970) 717-722.

[20] Hittelman, W.N., Rao, P.N. Premature chromosome condensation. I. Visualization of x-ray-induced chromosome damage in interphase cells. Mutat. Res. 23 (1974) 251-258.

[21] Cornforth, M.N., Bedford, J.S. X-ray-induced breakage and rejoining of human interphase chromosomes. Science 222 (1983) 1141-1143.

[22] Wang, Q., Zhou, G., Jing, X.G., Li, W.J., Yang, J.S. Avoid online radiation risk: Theoretical simulation of chromosome breaks in cells exposed to heavy ions. Adv. Space Res. 47 (2011) 2039-2043.

[23] Savage, J.R.K. Classification and relationships of induced chromosomal structural changes. J. Med. Genetics 13 (1975) 103-122.

[24] Bergs, J.W., Ten, C.R., Rodermond, H.M., Jaarsma, P.A., Medema, J.P., Darroudi, F., Buist, M.R., Stalpers, L.J., Haveman, J., Van Bree, C., Franken, N.A. Premature chromosome condensation by hyperthermia. Int. J. Hyperthermia 25 (2009) 220-228.

[25] Gotoh, E., Kawata, T. and Durante, M. Chromatid breaks rejoining and exchange aberration formation following γ-ray exposure: analysis in G_2 human fibroblasts by chemical-induced premature chromosome condensation. Int. J. Radiat. Biol. 75 (1999) 1129-1135.

[26] Waldern, C.A., Johnson, R.T. Analysis of interphase chromosome damage by means of premature chromosome condensation after X- and ultraviolet-irradiation. P.N.A.S. 71 (1974) 1137-1141.

[27] Pantelias, G.E., Maillie, H.D. The use of peripheral blood mononuclear cell prematurely condensed chromosomes for biological dosimetry. Radiat. Res. 99 (1984) 140-150.

[28] Goodwin, E.H., Blakely, S.M., Chen, D.J. and Cornforth, M.N. The effect of track structure on cell inactivation and chromosome damage at a constant LET of 120keV/μm. Adv. Space Res. 18 (1996) 93-98.

[29] Francesca, B., Andrea, O. A model of chromosome aberration induction: applications to space research. Radiat. Res. 164 (2005) 567–570.

[30] Pandita, T.K., Hittleman, W.N. The contributions of DNA and chromosome repair deficiencies to the radiosensitivity to Ataxia-Telangiectasia. Radiat. Res. 131 (1992) 214-223.

[31] Sasai, K., Evans, J.W., Kovacs, M.S., Brown, J.M. Prediction of human cell radiosensitivity: Comparison of colon genetic assay with chromosome aberrations scored using premature chromosome condensation with fluorescence in situ hybridization. Int. J. Radiat. Oncol. Biol. Phys. 30 (1994) 1127-1132.

[32] Durante, M., Gialanella, G., Grossi, G.F., Nappo, M., Pugliese, M., Bettega, D., Calzolari, P., Chiorda, G.N., Ottolenghi, A. and Tallone-Lombardi, L. Radiation-induced chromosomal aberrations in mouse 10T1/2 cells: dependence on the cell-cycke stage at the time of irradiation. Int. J. Radiat. Biol. 65 (1994) 437-447.

[33] Yang, J.S., Li, W. J., Zhou, G. M., Jin, X.D., Jing, X.G., Wang, J.F., Wang, Z.Z., Guo, C.L., Gao, Q.X. Comparative study on radiosensitivity of various tumor cells and human normal liver cells. World J. Gastroenterol. 26 (2005) 4098-4101.