Article ID: 1007 - 4627(2005)03 - 0280 - 04

Kinetic Repair of Chromosome Breaks of Normal Human Liver Cells Induced by Low LET Rays*

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Abstract: We employed the prematurely chromosome condensation(PCC) technique to investigate the 48 h kinetic repair of normal human liver cell line L02 exposed to γ-rays. The results showed that chromatid-type and isochromatid-type breaks increased with the dose at 0 h measured by PCC, the number of chromatid-type breaks was several times more than that of isochromatid-type breaks. Further 24 h incubation after exposed to irradiation, both of these two type breaks decreased in different extent, 50% for chromatid-type one, 15% for isochromatid-type one at most, respectively. At 48th h, there was a slightly change of the chromosome breaks compared with that of 24th h($p > 0.05$). These results revealed that the main type of the chromosome breaks was chromatid-type after exposed to low LET rays, also, it was easy to repair. Though the isochromatid-type breaks was obviously less than that of the chromatid-type one, it was difficult to repair. It implied that the isochromatid-type breaks was the important factor causing cell death and canceration when cells were exposed to irradiations.

Key words: kinetic repair; chromosome break; human liver cell

CLC number: TL99 **Document Code**: A

1 Introduction

As we all know, we humankind was received many kinds of irradiations at any time, data[1—6] shows that these irradiation could induce chromosome damage, the damage will probably result in cancerization. Correct and effective repair of injured chromosome is very important to stop cancerization course. Kawata et al[6] investigated the early 700 min chromatid kinetic repair process of the normal human fibroblasts exposed to three kinds of heavy ions and γ-rays, showed that the G2-phase fraction decrease quickly with an incubation time after exposure to 440 keV/μm iron particles and γ-rays. Nasonova et al[7] also reported after 10 h of post-irradiation incubation, 60% of Ar ions induced excess fragments remained unrejoined, only 14% of X-rays-induced lesions were not rejoined.

Chemically induced prematurely chromosome condensation (PCC) technique using Calyculin A was introduced by Gotoh et al[8] and Durante et al[9]. By using this technique, it is easy to get the PCC in any cell-cycle phase, especially in G2-phase[10]. We used this technique to study the chromosome damage and kinetic repair process of normal liver cell line L02 within 48 h further incubation after exposed to γ-rays, while,

Received date: 6 Dec. 2004; **Corrected date**: 12 May. 2005

* **Foundation item**: National Natural Science Foundation of China(10335050); Dedicated Project of National Key Basic Research of Science and Technology Ministry of China(2003CCB00200)

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very little information is available concerning about such a long post-irradiation time.

2 Materials and methods

Normal human liver cell line L02 provided by the China center for type culture collection (CCTCC) were grown in RPMI 1640 supplemented with 10% fetal calf serum and 25 U/ml insulin at 37 ℃ in 5% CO_2. Exponentially growing L02 cells were irradiated with ^{60}Co γ-rays of 0.2 Gy dose rate.

After irradiation, cells were incubated for different repair time before chromosome were prematurely condensed using Calyculin A (BIOMOL® America) as described elsewhere[8,9,11]. Briefly, Calyculin A was dissolved in 100% ethanol as 1mmol stock solution; 50 nmol 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 ℃ in 5% CO_2. Chromosome spreads were then harvested by swelling cells in 75 mmol KCl for 20 min at 37 ℃ and fixing with Carnoy's (1: 3 of glacial acetic acid and methanol) 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 G2-phase cells were scored for each dose point according to the standard criteria[12]. 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 was scored as two breaks. The total chromatid breaks were calculated by summing the production of chromatid-type and isochromatid-type breaks.

3 Results and Discussion

Figure 1 shows the correlation between chromatid-type/isochromatid breaks and dose at 0, 24 and 48 h after radiation. At each time point, chromatid-type breaks and isochromatid breaks are increased linearly with the dose, and the absolute number of chromatid-type breaks is much more than that of isochromatid ones, this phenomenon was described by Kawata et al[13]. It suggested that during the emitting range, the low LET rays could not deposit enough energy that penetrate the sister chromatids synchronously, which would result in isochromatid-type breaks. So, most of breaks of chromosome are chromatid-type.

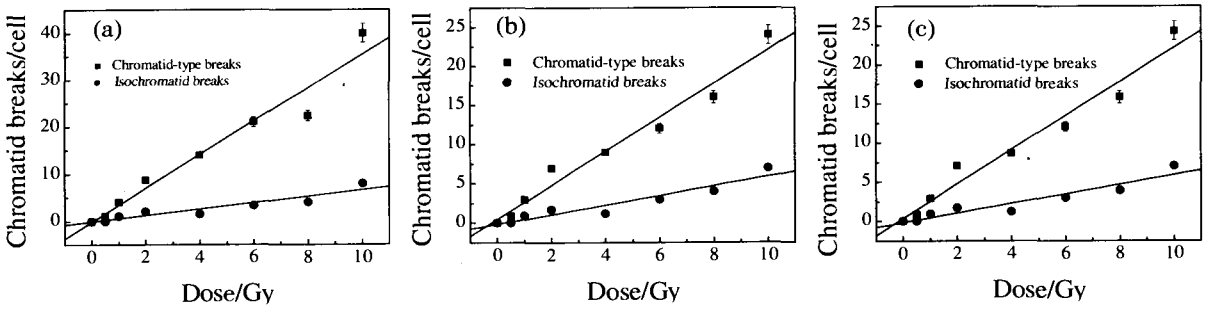

Fig. 1 Correlation between chromatid-type/isochromatid breaks and dose at 0, 24 and 48 h after exposed to ^{60}Co γ-rays. (a) 0 h after irradiation; (b) 24 h after irradiation; (c) 48 h after irradiation.

Kawata et al[10] have reported that in G2-phase of cell cycle, chromosomes were easy to be prematurely condensed, in this phase, we can clearly distinguish two type of chromosome aberrations, named chromatid breaks and isochromatid breaks. In the study of Kawata et al[10], they found that high LET radiations were more effective at inducing isochromatid breaks, while the low LET radiations were more effective at inducing chromatid-type breaks. It suggests that with a higher dose, the number of electrons which hit the target (chromosome) increased, the production increased.

Figure 2 shows the unrejoined chromatid breaks/

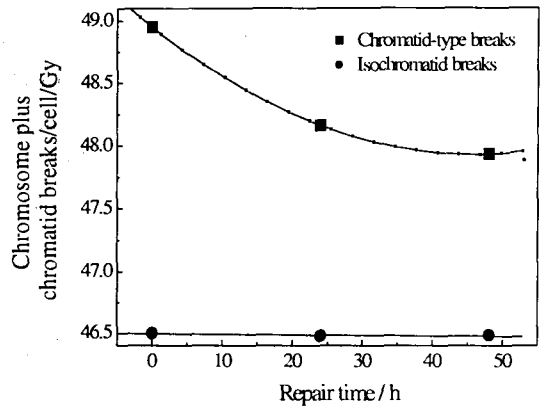

Fig. 2 Kinetic repair of chromosome breakages within 48 h after L02 cell line was exposed to γ-rays. The chromatid breaks plus 46 (number of normal human chromosomes) make this change remarkably.

cell · Gy^{-1} at 0, 24 and 48 h. In this kinetic process, almost 50% chromatid-type breaks got repaired, for isochromatid ones, just 15% got repaired at most. Also we can see, during 24 h to 48 h, both of these two type breaks are nearly in the same level, i. e., from 2.16 to 1.93 breaks/cell · Gy^{-1} for chromatid-type breaks, from 0.476 to 0.48 breaks/cell · Gy^{-1} for isochromatid ones. However, from 0 to 24 h these values are 2.95—2.16 breaks/cell · Gy^{-1}, 0.502—0.476 breaks/cell · Gy^{-1}, respectively. Compared with chromatid-type 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. Also, Suzuki et al[2] 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.

4 Conclusion

In this study, we have found the linearly increasing breaks for chromatid and isochromatid, very good repair for chromatid-type breaks and bad repair for isochromatid breaks. We concluded that the unrejoined isochromatid breaks is probably the main cause of cancerization for normal human liver cells after exposed to the low LET radiation.

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γ射线辐照人类正常肝细胞染色体损伤的动态修复*

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摘　要：应用早熟染色体凝集技术对人类正常肝脏细胞经γ射线照射导致的染色体损伤后48 h内的动态修复过程进行了研究。结果显示：照射后原初染色单体断裂和等点染色单体断裂数随着照射剂量的增加而增多，染色单体断裂显著多于等点染色单体断裂；经过24 h的继续培养，这两种类型的损伤都有不同程度的修复，约50%染色单体断裂得到修复，而等点染色单体断裂的修复率最多为15%；经过48 h的照射后培养，染色体损伤的水平与24 h相比没有显著差异。说明肝细胞经γ射线照射后染色体损伤的主要形式是染色单体断裂，易于修复；虽然等点染色单体断裂数量较少，但修复困难。由此表明，等点染色体断裂是细胞经γ射线照射后死亡和癌变的一个重要因素。

关 键 词：染色体断裂；动态修复；人肝细胞

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Indirect Measurement of $^{9}Be(p,\alpha)^{6}Li$ Reaction by Means of Trojan Horse Method**

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Abstract: The beryllium abundance acts as a key role for understanding the inhomogeneous Big Bang nucleosynthesis. In order to measure the $^{9}Be(p,\alpha)^{6}Li$ bare nucleus cross section and $S(E)$ factor at astrophysical energies, the Trojan Horse Method (THM) can be applied. The main feature of the method is that it allows to extract the energy dependence for the astrophysical $S(E)$ factor of bare nuclei at very low energies without any extrapolation, by measuring the cross section of an appropriate three body process. Thus the $^{9}Be(p,\alpha)^{6}Li$ has been studied by means of the THM applied to the $^{2}H(^{9}Be,\alpha^{6}Li)n$ at INFN-LNS, Catania, Italy. The two body reaction cross section has been studied in the energy range of $E_{cm}=0$—1 000 keV. Preliminary results are discussed and a comparison with direct data is made.

Key words: Trojan Horse Method; bare nucleus; Coulomb barrier; electron screening

* **基金项目**：国家自然科学基金重点课题(10335050)；国家重大基础研究前期研究专项基金资助项目(2003CCB00200)

** **Foundation item**: C. S. F. N. S. M., Catania, Italy