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THE RADIOBIOLOGIC CHARACTERISTICS OF DNA POLYMERASE β IN HEPATOMAS

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To investigate the effects of γ rays on DNA polymerase β properties and its DNA repair functions before or after γ rays exposure, DNA polymerase β activity, gene expression and mRNA levels in SMMC-LTNM hepatomas born on nude mice or the samples of the liver cancer tissues from 15 patients were measured with ^{3}H-TTP incorporation test, immunocytochemistry and cytoplasmic dot hybridization analysis, respectively. Irradiation was carried out with ^{60}Co-γ rays at ice bath. It was found that DNA polymerase β activity, gene expression and the amount of mRNA were much higher in hepatoma cells than those in normal hepatocytes (P<0.01). *In vitro* studies, the enzyme activity both in hepatoma and normal liver cells appeared unchanged within 40 Gy γ -ray exposure. Following whole-body exposure of the nude mice bearing SMMC-LTNM with 2 Gy or 4 Gy of γ rays, DNA polymerase β activity in hepatoma increased temporarily at 48 hours post-irradiation, and its gene expression seemed more active. The enzyme mRNA increased to 1.76-fold of the control group. 72 hours after exposure, all of these changes returned to normal levels. DNA polymerase β participated in DNA repair synthesis and this effect was different between hepatoma and hepatocytes because there were some biologic differences of the enzyme between hepatoma cells and normal liver cells. These data suggested that DNA polymerase β activity, its gene expression and mRNA level in hepatomas could increased temporarily after γ rays exposure, which may facilitate the cells to repair DNA damages from radiation.

Key words: Liver neoplasm, DNA repair, Radiobiology, DNA polymerases, Gene expressions

DNA polymerase β (pol β) is one of five cellular DNA polymerases in vertebrates and the exact cellular roles of pol β is still obscure, especially in repair of DNA damaged from γ -ray exposure. However, recent studies showed that pol β could participate in DNA repair synthesis after ^{60}Co-γ rays irradiation.[1] In the present study, pol β activity, its gene expression and mRNA levels in hepatoma and normal liver cells were detected, and the effects of γ-ray exposure on these molecular characteristics were observed. The relations between pol β biologic characteristics and the DNA radiation damage repair functions was also discussed.

MATERIALS AND METHODS

Tissues and Nuclei Suspension Preparation

SMMC-LTNM hepatoma, a transplanted human liver cancer borne on BALB/c nude mice, was purchased from the nude mice feeding room of the Department of Pathology in our university. The mice were sacrificed by cervical dislocation. Their livers and SMMC-LTNM hepatomas were taken out. The tissues were washed by Hank's solution, then cut in

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pieces, homogenized and filtered through a nylon net. The cells were collected by centrifuge and both kinds of nuclei were prepared as described previously. Cancer tissues samples of human liver were collected from operation patients in Dong Fang Hospital. Normal liver tissues were taken from persons died of accidents.

Assay for DNA pol β Activity

Pol β activity was detected in the following reaction mixture. Each reaction mixture contained, in a final reaction volume of 200 μl, 0.1 ml nuclei, 9 mmol/L Tris-HCl (pH8.5), 1 mmol/L dithiothretol, 20 mmol/L $MgCl_2$, 2 mmol/L ATP, 1 mmol/L dCTP, dGTP, dATP, 3.7 × 10^4Bq ^{3}H-TTP, 10 mmol/L N-ethylmaleimide, and some amount of activated DNA. After incubation at 37 ℃ for 120 min with shaking, 1 ml stopping reaction solution containing 1 mol/L perchloric acid and 20mmol/L sodium pyrophosphate was added to each reaction mixture. Radioactive DNA product was collected on a piece of cellulose paper. The incorporation of ^{3}H-TTP was measured by scintillation counting.

Cytoplasmic Dot Hybridization

To detect pol β mRNA, cytoplasmic dot hybridization described by White[2] was used. In brief, cells (1-5 × 10^6) were washed with phosphate-buffered salts, and repelleted by centrifugation in a sterile, 1.5-ml Eppendorf tube. After resuspension in 45 μl aliquots of 5% Nonidet P-40 with 5 min of mixing on ice in between. Following pelleting of nuclei, 50 μl of the supernatant were transferred to a sterile 1.5-ml tube containing 30 μl of 20 × NaCl/Cit (0.15 mol/L NaCl, 0.015 mol/L trisodium citrate) plus 20 μl of 37% (w/w) formaldehyde. The mixture was then incubated at 60 ℃ for 15 min, and stored at -70 ℃. For analysis, 5 μl of each sample were serially diluted with 15 × NaCl/Cit in a 96-well microtiter plate to yield a final volume of 200 μl, and 100μl of each dilution were applied with suction to 3-mm diameter spot on a nitrocellulose sheet. The nitrocellulose sheet was then baked (80 ℃,90 min). Prehybridization of the nitrocellulose, preparation of pol β ^{32}P-labeled cDNA probes (PUC 9-10 F and PUC 9-10 S, the generous gifts from Professor Akio Matsukage) by random primers DNA labeling system, hybridization, autoradiography, and quantification by scanning were performed as described previously.

Irradiation, DNA repair synthesis, Immuno-cytochemistry

All these were performed by using the techniques of our laboratory.

RESULTS

Changes of Pol β Activity in Hepatoma Cells after γ-ray Irradiation

Four groups of SMMC-LTNM hepatoma nuclei or hepatocyte nuclei suspension were irradiated with 0-40 Gy γ rays (5 Gy/min), and the pol β activity was detected. Before irradiation, pol β activity in hepatoma nuclei was much higher than that in normal hepatocyte nuclei (P <0.01). After irradiation *in vitro*, the enzyme activity in both kinds of nuclei did not decrease significantly (Table 1). However, when the SMMC-LTNM hepatomas borne on nude mice were irradiated with 2 Gy or 4 Gy of γ rays (1.478 Gy/min) *in vivo*, pol β activity in the tumor decreased slightly during the first 24 hours post-irradiation, then increased. It reached to 18.3% of control group at about 48 hours post-irradiation and gradually returned to normal level at about 72 hours (Figure 1).

Table 1. The activity of DNA polymerase β in hepatocyte and hepatoma from BALB/c nude mice after irradiation by different dose of γ-rays (cpm/μg DNA $\bar{\chi} \pm s$)

Cells	n	Absorbed Dose (Gy)			
		0	10	20	40
hepatocyte	15	610 ± 182	637 ± 56	626 ± 112	614 ± 138
hepatoma	21*	1639 ± 200	1694 ± 164	1620 ± 263	1616 ± 179

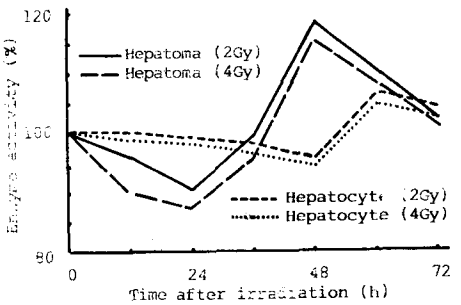


Fig. 1. Changes of DNA polymerase β activity in hepatoma and hepatocyte nuclei from nude mice after total body irradiation by γ-rays

The Molecular Basis of the Changes of pol β Activity in Hepatoma Cells

Immunocytochemistry test showed that pol β mainly located in the nuclei and scattered in cytoplasm. In the immuno-gold-silver staining assay with pol β antibodies, most of hepatoma cells (50-80%) were positive, in contrast with less positive hepatocytes (0-13%, Figure 2). Within first 24 hours post-irradiation, the content of the enzyme in nuclei seemed unchanged, but it slightly increased in cytoplasm. At 48 hours post-irradiation, pol β gene expression of hepatoma cells was more active than that of none irradiated ones ($P<0.05$). In liver cancer tissues from 15 operation patients, there were 60-85% pol β immuno-gold-silver staining positive cells. However, there were only 0-5% immuno-gold-silver staining positive cells in normal liver tissues. Cytoplasmic dot hybridization analysis demonstrated that pol β mRNA was much higher in hepatoma cells than in hepatocytes ($P<0.01$, Figure 3). After total-body irradiation with 2 Gy or 4 Gy of γ rays, pol β mRNA in SMMC-LTNM hepatoma cells increased. It increased to 1.76-fold of non-irradiated cells at about 48 hours post-irradiation, then gradually decreased to normal level 3 days later (Figure 4).

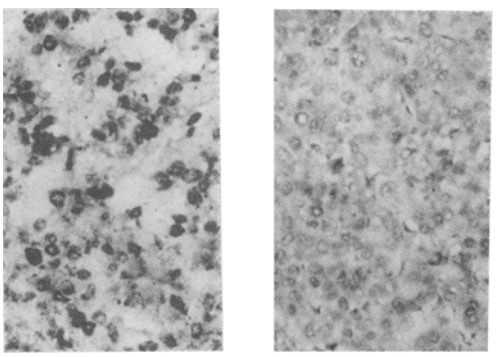
The Relation between pol β Radiobiologic Characteristic and DNA Repair Function

DNA repair synthesis analysis showed that ^{3}H-TTP incorporation induced by pol β was higher in 10 Gy γ -ray irradiated nuclei than that in non-irradiated ones (Table 2 $P<0.01$). Furthermore, there were some differences in DNA repair synthesis induced by pol β between hepatoma cells and normal hepatocytes. The incubation time that the ^{3}H-TTP incorporation of DNA repair synthesis induced pol β reached 50% of the maximum was less in hepatoma cells (about 18 min) than in normal liver cells (about 32 min), which indicated that DNA repair synthesis induced by pol β in hepatoma cells was stronger.

Table 2. The effect of DNA polymerase β on the repair of γ-ray irradiated nuclei DNA with different dose rat($\bar{x} \pm s$)

Dose rate	^{3}H-TTP Incorporation (cpm/μg DNA)	
(Gy/min)	Hepatoma nuclei	hepatocyte nuclei
1	281 ± 50	207 ± 24
5	282 ± 27	204 ± 18
10	284 ± 37	208 ± 22
Control	236 ± 35*	179 ± 17*

*$P<0.05$ vs irradiated group



Hepatoma Normal Liver

Fig 2. The immuno-gold-silver staining positive cells in hepatoma and normal liver tissues with DNA polymerase β antibodies.

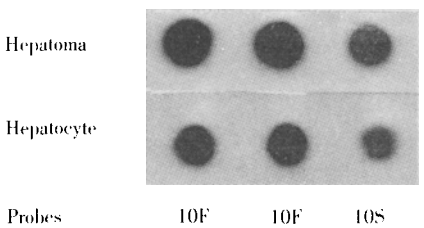


Fig 3. Cytoplasmic dot hybridization of DNA polymerase β mRNA from nude mice hepatoma with 10F and 10S probes

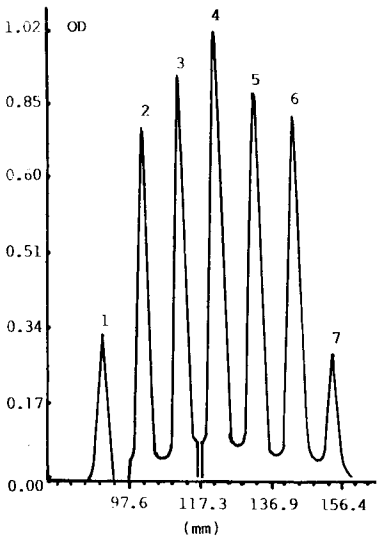


Fig 4. Changes of DNA polymerase β mRNA in hepatoma cells after 2Gy of γ-ray irradiatioin analysed by cytoplasmic dot hybridization . The post-irradiation time for image 1 to 7 was 0, 12, 24, 36, 48, 60 and 72 hours, respectively.

DISCUSSION

Which DNA polymerase is responsible for DNA repair synthesis has been the subject of recent controversy.[3] Our previous experiment showed that pol β was an important enzyme participating in DNA repair synthesis after γ-ray exposure. To study pol β radiobiologic characteristics may contribute tumor cells strong DNA repair ability. It was reported that the regulation of pol β gene is complicated and there are some gene regulate elements within cells. Detailed analysis of the 5'-flanking region of pol β gene has revealed that the silencer of this gene consists of long-dispersed multiple sub-elements.[4,5] The reason that there exist some differences of pol β between hepatoma cells and hepatocytes may be that the pol β gene regulate elements or protein regulators in hepatoma cells are different from normal cells. The promoter of this gene may be more active. We also found that the enzyme itself was relatively resistant to γ-ray exposure, and the enzyme activity in SMMC-LTNM hepatoma cells, expression and transcription enhanced temporarily after γ-ray irradiation *in vivo*. These changes may be a protective response of cells to DNA damage from radiation.

The radiobiologic molecular characteristics mentioned above are meaningful in DNA damage repair as well as the resistance to radiotherapy of tumors. Cell death occurring in radiotherapy is closely related with the extent of both DNA damage and DNA repair. It has been certificated that the process of DNA repair is a complicated enzymatic reaction. Pol β has an important role in DNA repair synthesis after γ rays exposure. It is suggested that the extent of its activity, gene expression and transcription may influence its DNA repair function. Cells in which the pol β gene over expresses and the enzyme activity is high have the strong ability of repair synthesis. The possible reason that some tumor cells have strong DNA repair ability found in other experiments may be in those cells the pol β activity was also higher and its gene expression activated further after irradiation. The resistance to ionization radiation of some tumors may be related with the effects of pol β. On the based of the findings of this paper, it can be indicated that tumor cells which are resistant to radiotherapy are possibly the cells in which the content and activity of DNA repair enzymes are higher, the genes of these enzymes can be easily activated by ionization radiation. So that the ability of tumor cells to repair DNA enhance and more tumor cells survive the killing effects of radiation.

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