

## Characteristics of DNA repair induced by DNA polymerase $\beta$ in hepatoma cells after $\gamma$ -ray irradiation

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### Abstract

**AIM:** To investigate the effects of DNA repair induced by DNA polymerase  $\beta$  in hepatoma cells after  $\gamma$ -ray irradiation.

**METHODS:** Cell nuclei were prepared from mouse model (SMMC LTNM), in which human hepatoma cells are transplanted on nude mice. The nuclei were then irradiated with  $^{60}\text{Co}$ - $\gamma$  rays at different dose levels or dose rates. A selective inhibitor test was then used to detect the effects of the radiation on DNA repair using N-ethylmaleimide (NEM) and ddTTP as selective inhibitors to DNA polymerases  $\gamma$  and  $\beta$  respectively.

**RESULTS:**  $^3\text{H}$ -TTP incorporation into irradiated nuclei or calf thymus DNA was significantly higher than that the rate at which it is incorporated into non-irradiated nuclei when either DNA polymerase  $\alpha$  or  $\gamma$  was inhibited. When both NEM and ddTTP are present, the  $^3\text{H}$ -TTP incorporation in irradiated DNA was not significantly different from the non-irradiated nuclei. Furthermore,  $^3\text{H}$ -TTP incorporation into DNA of SMMC-LTNM hepatoma nuclei was higher than that of normal hepatocyte nuclei ( $P < 0.01$ ). This suggests that DNA repair induced by DNA polymerase  $\beta$  was more active in hepatoma cell nuclei than in normal hepatocyte nuclei.

**CONCLUSION:** DNA polymerase  $\beta$  may be more responsive to

DNA damage in some tumor cells than that in normal cells, which may facilitate the cells to repair DNA damages from radiation more efficiently.

**Key words:** DNA polymerases; DNA repair  $\gamma$ -rays; Liver neoplasms; Liver/radiation effects

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### INTRODUCTION

DNA polymerase  $\beta$  (pol  $\beta$ ), which is the smallest and simplest polymerase, exists in most vertebrate cells. The exact cellular roles of pol  $\beta$  are still obscure, especially in the repair of DNA damaged from  $\gamma$ -ray exposure<sup>[1-3]</sup>. However, recent studies showed that pol  $\beta$  could participate in DNA repair synthesis after  $^{60}\text{Co}$ - $\gamma$  ray irradiation. In the present study, the differential characteristics of pol  $\beta$  in radiation-induced DNA damage in DNA repair between hepatoma cells and normal liver cells was investigated.

### MATERIALS AND METHODS

#### Nuclei preparations

BALB/c or NC nude mice bearing SMMC-LTNM hepatomas were killed and the livers and SMMC-LTNM hepatomas were harvested. The tissues were washed with Hanks solution, cut into pieces, homogenized and filtered through a nylon net. The cells were collected by centrifuge and the nuclei were isolated as described previously<sup>[4]</sup>.

#### Sample irradiation

Cell nuclei suspensions or calf thymus DNA solutions (for the controls; Sigma Co.) were put in 1.5 mL sterile Eppendorf tubes in an ice bath before being transferred into a field of  $^{60}\text{Co}$ - $\gamma$  rays ( $1.85 \times 10^{15}$  Bq) and irradiated with 1-40 Gy at 1-10 Gy/min dose rates. The samples were maintained in an ice bath throughout the procedures. The DNA repair synthesis test was carried out immediately after irradiation.

#### Assay for DNA repair synthesis

DNA repair synthesis was measured in the following reaction

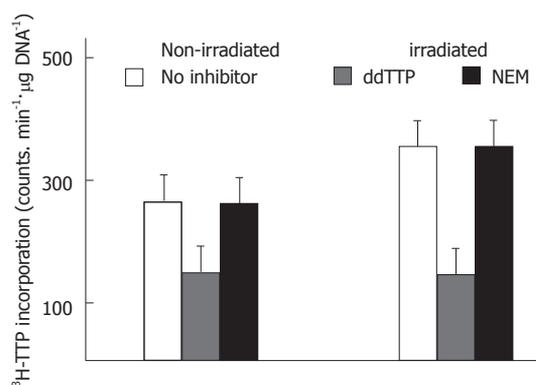


Figure 1 Effects of selective inhibitors on DNA repair synthesis regulated by DNA polymerase  $\beta$ .

Table 1 Effect of DNA polymerase  $\beta$  on the repair of  $\gamma$ -rays irradiated nuclei DNA at different dose rates ( $\bar{x} \pm s$ )

Dose rate (Gy·min <sup>-1</sup> )	<sup>3</sup> H-TTP incorporation (counts · min <sup>-1</sup> · μg DNA <sup>-1</sup> )	
	Hepatoma nuclei	Hepatocyte nuclei
1	281 ± 50	207 ± 24
5	282 ± 27	204 ± 18
10	284 ± 37	208 ± 22
Control	236 ± 35 <sup>a</sup>	179 ± 17 <sup>a</sup>

$n = 7$ , <sup>a</sup> $P < 0.05$  compared with irradiated group.

mixture. Each reaction mixture contained 0.1 mL sample solution (nuclei or calf thymus DNA) and 0.1 mL DNA repair synthesis reaction solution. The reaction solution contained, at a final concentration, 18 mmol/L Tris-HCl (pH8.5), 2 mmol/L dithiothreitol, 40 mmol/L MgCl<sub>2</sub>, 4 mmol/L ATP, 2 mmol/L dCTP, dGTP, dATP, and 3.7 × 10.4 Bq <sup>3</sup>H-TTP. Selective inhibitors (NEN or ddTTP) were used as required. For the calf thymus DNA samples, rat DNA polymerase  $\beta$  (a gift from Dr. Akio Matsukage) was also put in the reaction mixtures. After incubation at 37 °C for 120 min with shaking, 1 mL reaction stopping solution containing 1 mol/L perchloric acid and 20 mmol/L sodium pyrophosphate was added to each reaction mixture. The radioactive DNA product was collected on a piece of cellulose paper, and the incorporation of <sup>3</sup>H-TTP was measured using a scintillation counter.

## RESULTS

### Effect of pol $\beta$ in DNA repair synthesis

In order to evaluate the role of pol  $\beta$  in DNA radiation damage repair, we first examined the effects of radiation on DNA repair by comparing the amount of <sup>3</sup>H-TTP incorporation in irradiated and non-irradiated calf thymus DNA (10 Gy, 5 Gy/min). The <sup>3</sup>H-TTP incorporation was much higher ( $P < 0.01$ ) in irradiated DNA than in non irradiated DNA (Figure 1). This increase was also present in DNA treated with NEM. However, when ddTTP was added to the reaction mixture to inhibit pol  $\beta$ , the amount of <sup>3</sup>H-TTP incorporation was similar in the irradiated and non-irradiated DNA (Figure 1).

Next, we examined DNA repair in hepatoma and normal liver nuclei. As seen with the calf-thymus DNA, the incorporation of <sup>3</sup>H-TTP into both irradiated SMMC-LTNM hepatoma nuclei and normal liver nuclei was higher ( $P < 0.05$ ) than that in non irradiated nuclei (Table 1). To investigate whether pol  $\beta$  is responsible for the DNA repair synthesis in irradiated nuclei, a subset of irradiated hepatoma and normal liver nuclei were treated with the pol  $\beta$  selective inhibitor ddTTP. As illustrated in Figure 2, the incorporation of <sup>3</sup>H-TTP did not significantly increase in nuclei treated with ddTTP; however, nuclei from both hepatoma and normal liver that were not treated with ddTTP had a significant increase in the incorporation of <sup>3</sup>H-TTP ( $P < 0.01$ ).

### Characteristics of DNA repair synthesis induced by pol $\beta$ in hepatoma

The characteristics of DNA repair synthesis induced by pol  $\beta$  in SMMC-LTNM hepatoma nuclei were compared with that in normal hepatocyte nuclei after different doses of  $\gamma$ -ray exposure. We found that the increment of <sup>3</sup>H-TTP incorporation (<sup>3</sup>H-TTP incorporation of

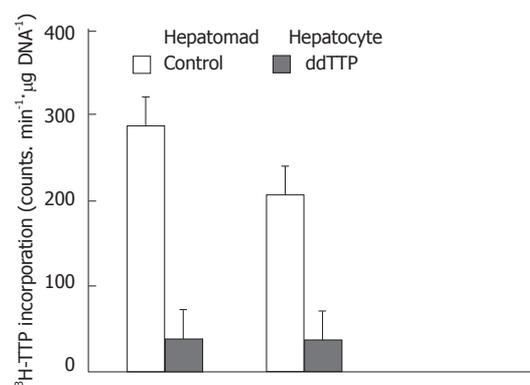


Figure 2 Inhibition effect of ddTTP on the DNA repair synthesis regulated by polymerases in nuclei of hepatomas and hepatocytes.

Table 2 Comparison of the increment of <sup>3</sup>H-TTP incorporation of irradiated hepatoma nuclei with that of hepatocyte nuclei (counts · min<sup>-1</sup> · μg DNA<sup>-1</sup>,  $\bar{x} \pm s$ )

Dose rate (Gy·min <sup>-1</sup> )	Increment of <sup>3</sup> H-TTP incorporation	
	Hepatoma nuclei	Hepatocyte nuclei
1	45 ± 8.0 <sup>b</sup>	28 ± 2.0
5	46 ± 4.4 <sup>b</sup>	29 ± 4.1
10	48 ± 6.3 <sup>b</sup>	32 ± 5.1

$n = 7$ , <sup>b</sup> $P < 0.01$  compared with hepatocyte nuclei group.

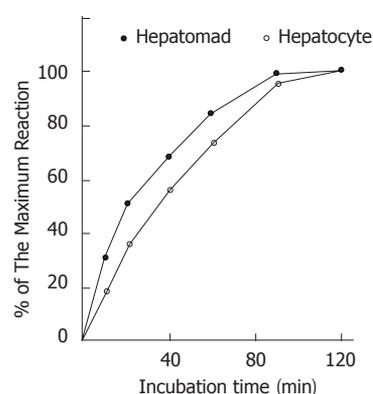


Figure 3 Extent of <sup>3</sup>H-TTP incorporating into irradiated nuclei of hepatoma and hepatocyte at different incubation time induced by DNA polymerase  $\beta$ .

irradiated nuclei-<sup>3</sup>H-TTP incorporation of non irradiated nuclei) of hepatoma nuclei were much higher than that in normal hepatocyte nuclei ( $P < 0.01$ ) at all dose rate groups under the same reaction conditions and same absorbed dose (10 Gy) (Table 2).

Further investigation revealed that there was a significant difference in the reaction speed of DNA repair synthesis induced by pol  $\beta$  between the two kinds of nuclei. The reaction induced by pol  $\beta$  in hepatoma nuclei was faster than that in hepatocyte nuclei. The incubation time, which the <sup>3</sup>H-TTP incorporation increased to 50% of the maximum, was about 18 min in hepatoma nuclei and about 32 min in normal hepatocyte nuclei (Figure 3).

## DISCUSSION

DNA damage caused by ionization can be partly repaired under proper conditions<sup>[5]</sup>. However, the mechanism of DNA repair is not clearly understood<sup>[6,7]</sup>. The role of pol  $\beta$  in DNA repair after ionization radiation exposure is one of the problems remaining to be solved. For example, which polymerase is responsible for DNA repair synthesis after  $\gamma$ -ray exposure has been a controversy in radiobiology. The evidence obtained in this study further demonstrates that pol  $\beta$  does participate in DNA repair synthesis and it is an important enzyme in the process of DNA repair after  $\gamma$ -ray exposure.

This study also found some intriguing differences in the DNA repair induced by pol  $\beta$  between SMMC-LTNM hepatoma cells and normal hepatocytes. We found that SMMC-LTNM hepatoma cells had a faster DNA repair synthesis reaction, a higher incorporation of <sup>3</sup>H-TTP after radiation damage, and a lower dose of radiation

required to reach the maximum  $^3\text{H-TTP}$  incorporation. All these characteristics suggest that the DNA repair synthesis induced by pol  $\beta$  in hepatoma cells was strong. In other experiments we found that the gene of pol  $\beta$  was overexpressed and the enzyme activity was high in hepatomas. These findings are meaningful in the radiotherapy of tumors. Cell death in radiotherapy is closely related to the extent of DNA damage and DNA repair. Cells in which their pol  $\beta$  gene is overexpressed and which have high enzymatic activity have a stronger ability for DNA repair synthesis, and therefore, more cells could theoretically survive the killing effects of radiation. The resistance to ionization radiation of some tumor cells may be related with the effects of pol  $\beta$ . It is highly necessary to investigate the radiobiologic characteristics of pol  $\beta$  in more tumors, especially in those tumors which are resistant to radiation, and to study the radiosensitizing effects by the way of selectively inhibiting the pol  $\beta$  in tumor cells.

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