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Research Article

Suppression of γ-irradiation-induced deletion mutations under Parp-1 deficiency in mice

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Abstract: Poly (ADP-ribose) polymerase-1 (Parp-1) functions in DNA repair, acting as a sensor for DNA strand breaks and recruiting DNA repair proteins to the site of DNA damage. To investigate the impact of Parp-1 in processing of DNA damages and genomic stability after γ-irradiation, we performed in vivo mutation analysis utilizing gpt delta transgenic mice in the liver and brain under Parp-1 deficiency. The mutant frequencies increased 3-fold in Parp-1+/+ mice in the liver 3 days after γ-irradiation at 8 Gy, whereas those of Parp-1 knockout (Parp-1-/-) mice did not increase. When the mutation spectra in the livers were analyzed, the frequencies of simple-type deletion mutations were lower in the Parp-1-/- mice. Notably, one base deletion mutations at hot-spots of 4-6 mononucleotide repeats were 4-fold lower in the Parp-1-/- liver ($P<0.05$). In the brain, the mutant frequencies did not increase in both genotypes after γ-irradiation. These results suggest that PARP-1 is required in the induction of γ-irradiation-induced deletion mutations in the liver possibly through supporting inaccurate DNA repair processes.
Keywords: γ-irradiation, PARP-1, deletion mutation, liver, brain.

INTRODUCTION

Elucidation of DNA damage responses after ionizing radiation (IR) is important to understand the mechanisms of both ionizing radiation (IR)-induced carcinogenesis and radiation therapy of cancer. Major DNA lesions caused by IR are single and double strand DNA breaks (SSB and DSB). These lesions efficiently activate poly (ADP-ribose) polymerase-1 (Parp-1) [1-4]. Parp-1 catalyzes polyADP-ribosylation using NAD as a substrate and functions in DNA repair, acting as a sensor for DNA strand breaks and recruiting DNA repair proteins to the site of DNA damage. Parp-1 physically interacts with various proteins involved in base excision repair (BER), including XRCC1 [5, 6], DNA ligase III [6], and also proteins involved in non-homologous end-joining (NHEJ), such as Ku70/80 [7] and DNA-PK [7, 8]. Parp-1-/- mice show increased sensitivity to alkylating agents, γ-irradiation [9-12]. We and others have previously shown that Parp-1-/- mice show increased frequencies of tumor incidence after treatment with alkylating agents, including N-nitrosobis(2-hydroxypropyl)amine (BHP) and azoxymethane [9, 13, 14]. Deletion mutations are augmented after treatment with BHP [9]. This could contribute to the augmented susceptibility to carcinogenicity induced by alkylating agents. Parp-1-/- mice also show increased incidences of spontaneous development of tumors after aging [15, 16]. In advanced ages, Parp-1-/- mice showed increased frequencies of deletion mutations in the liver [17].

Although Parp-1-/- mice show augmented sensitivity to whole-body γ-irradiation [10, 18], the detailed role of Parp-1 in repair of γ-irradiation-induced DNA lesions has not been fully understood. By analyzing the mutation profiles of the knockout mice, it is possible to evaluate the impact on DNA repair in vivo. Therefore we investigated whether mutation

incidence and its spectra after γ-irradiation were altered by Parp-1 deficiency in mice. For this purpose, here we analyzed the mutation profiles after γ-irradiation, using gpt delta transgenic mice intercrossed with Parp-1-/- mice. These mice harbor two novel mutation marker genes, red/gam and gpt genes, which are not functional in mice. Because IR mainly causes SSB and DSBs, we used the mutation marker, red/gam gene, which can detect deletion mutations of various sizes. Different from the effect on alkylating agent-induced DNA damage, we demonstrated that Parp-1 deficiency led to suppression of γ-irradiation-induced deletion mutations both in the liver and brain. Therefore, the presence of Parp-1 is suggested to promote induction of γ-irradiation-induced deletion mutations, probably through enhancing inaccurate DSB repair.

MATERIALS AND METHODS

Animal experiments

Parp-1-/-/gpt delta and Parp-1+/+/gpt delta mice were previously established and maintained [19]. The mice possess mixed genetic background of C57BL/6, ICR and 129Sv. Male mice were fed a basal diet (CE-2, Clea Japan). Whole body irradiation were carried out using a ^{60}Co γ-irradiator at 0.2 Gy/sec. Mice were irradiated with different doses and survival was observed for 30 days to determine the irradiation doses. For mutation analysis, three days after irradiation, mice were anesthetized and sacrificed at the ages of 4 months (n=4-5 for each genotype) and genomic DNA was isolated from immediately frozen tissues as previously described [9]. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of the National Cancer Center Research Institute.

The Spi$^-$ assay

The Spi assay was carried out with a modification as described previously [9]. The frequencies of background mutants were less than 10^{-8} in the Spi$^-$ assay and were negligible as described elsewhere [20]. The frequencies of Spi$^-$ mutants were presented without subtracting the background mutant frequencies.

Analysis of mutation spectra

To determine the sequences of the deleted region and their surrounding sequences, PCR and Southern blot hybridization method was used as previously described [9] and as shown in the scheme of Fig. 1. In gpt delta mice the detectable deletion mutations in the red/game gene were generated exclusively in the gam gene, therefore, the sequence analysis was carried out in the gam gene as previously described [9]. DNA sequencing of the mutated region was performed with a CEQ^{TM} DTCS Quick Start Kit (Beckman Coulter).

Statistical analysis

The statistical significance of differences in mutant or mutation frequencies between the two groups were analyzed by using the Mann-Whitney U test. When P value is less than 0.05, the difference was considered significant.

RESULTS AND DISCUSSION

Lower frequencies of IR-induced deletion mutation in the liver of Parp-1-/- mice

It is known that DNA replication should occur to fix the mutation, therefore three days after γ-irradiation was chosen as the time point to analyze mutations as previously described [21]. Irradiation dose of 8 Gy was chosen because the survival for at least 30 days was observed for both genotypes (data not shown). Because we focused on the deletion mutations that could be generated after DNA strand breaks, the mutation was analyzed with the red/gam gene, in which deletion mutations of various sizes could be detected. As shown Fig. 2A, the mutant frequencies of the red/gam gene was increased in Parp-1+/+ mice after 8 Gy irradiation ($P<0.05$) but not in Parp-1-/- mice.

We further analyzed the mutation spectrum of the red/gam gene (Fig. 2B). Deletion mutations are the major type of mutations as reported previously [19]. When mutations were categorized into simple-type and complex-type, accompanying insertions or rearrangements [9], Parp-1-/- mice showed 2.5-fold lower deletion mutation frequencies of simple-type mutation compared to Parp-1+/+ mice, whereas there was no difference in the mutation frequencies of complex-type mutations, that harbor insertion or rearrangements, between the genotypes (Fig. 2B). When deletion mutations were classified by the deletion sizes of single base, 2-1,000 bases, and more than 1,000 bases, all sizes of deletion mutations were lower in Parp-1-/- mice (Fig. 2C).

As the hot spots of single base mutations in the red/gam gene are known to be located at 4-6 base mononucleotide repeat sequences [19], we analyzed the distribution of mutations in the gam gene (Fig. 3A). The distribution of single base deletion mutations were rather concentrated in the middle positions but widely distributed in the Parp-1+/+ mice. Of note, when the effect on the repeat sequence length around the mutated bases was analyzed (Fig. 3B), the frequencies of single base deletion mutation on 4-6 mononucleotide repeat sequences were 4-fold lower in Parp-1-/- mice ($P<0.05$).

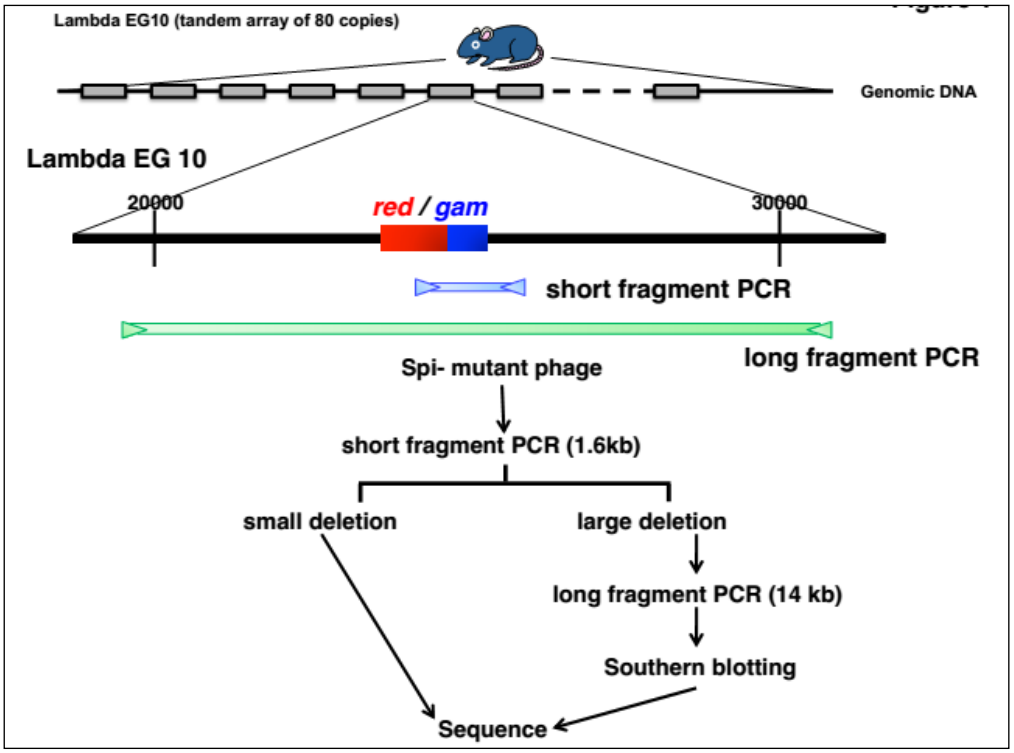

Fig-1: Scheme of the mutation analysis of the red/gam genes using gpt delta mice

Methodology of analysis of red/gam gene mutations using the gpt delta mice. The strategy for determination of mutated sequences using short and large range PCR and sequencing is shown.

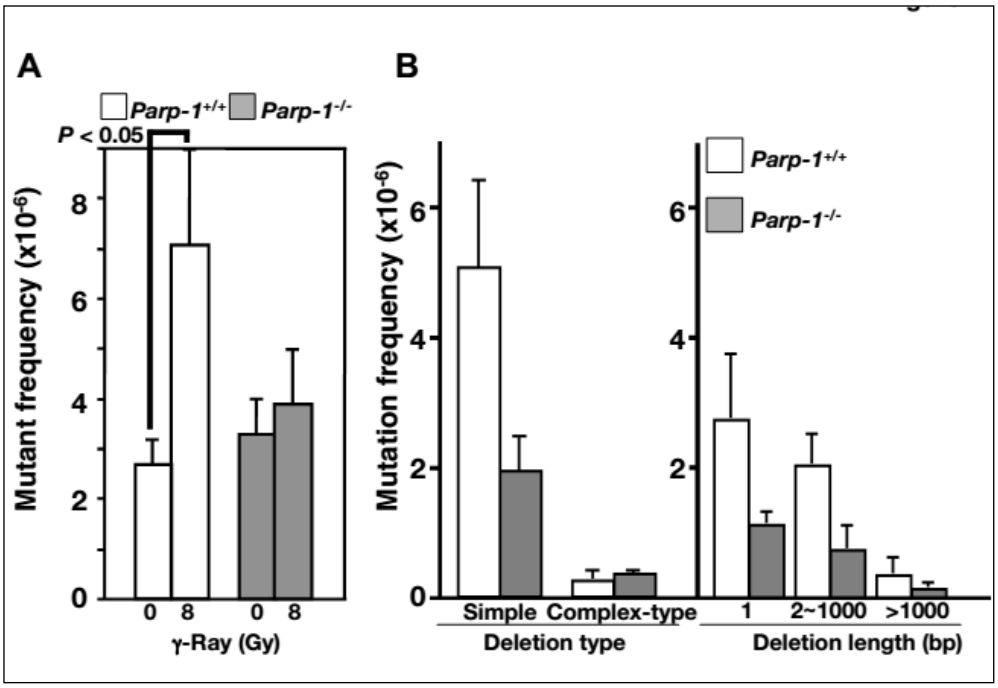

Fig- 2: Mutation analysis of the red/gam genes 3 days after 8 Gy irradiation in the livers of Parp-1+/+ and Parp-1-/- mice

A: Mutant frequencies of the red/gam gene.

B. Mutation spectra of the red/gam gene. Deletion mutations were classified into simple- and complex-type mutations (left panel) and into the size of deletions (right panel).

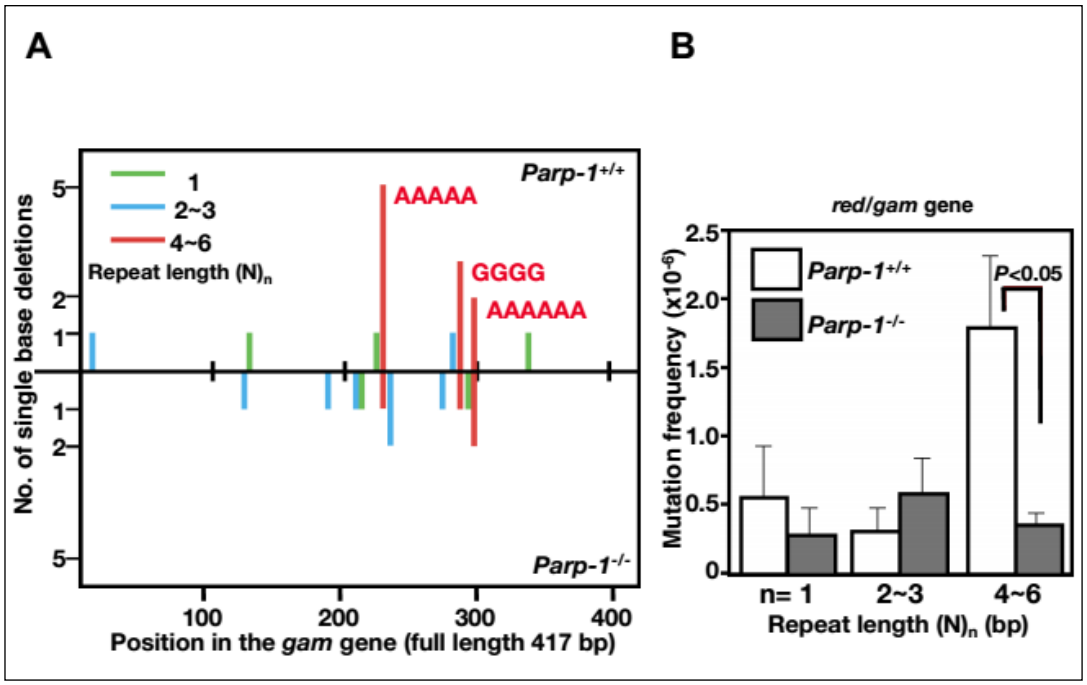

Fig- 3: Analysis of mutation spectra of the single base deletions in the gam gene of the livers.

A. Distribution of single-base deletions in the gam gene. Nucleotide 1 corresponds to the adenine residue in the 1st ATG of the gam gene. The green bars indicate the mutations not located in identical run sequences. Blue bars indicate those located in 2-3 base run sequences and red bars indicate those located in 4 or more base run sequences. B. Mutation frequencies of the single base deletion mutations on non-repeat, 2-3 base repeats, and 4-6 base repeats.

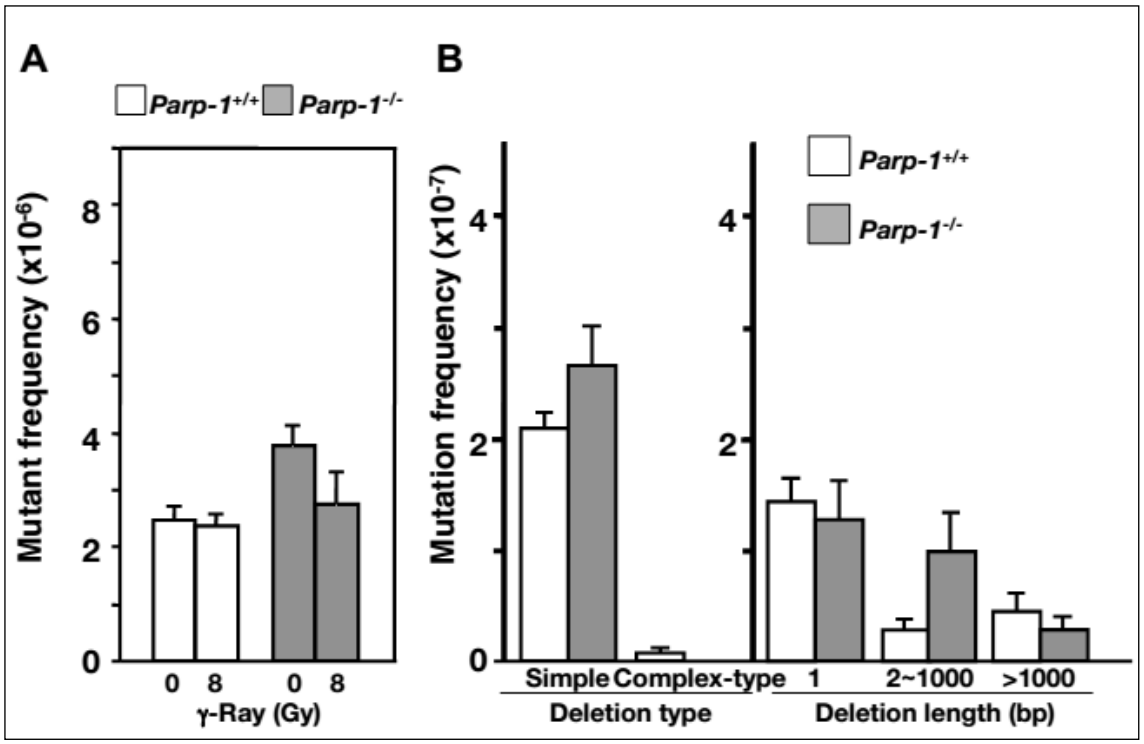

Fig-4: Mutation analysis of the red/gam genes 3 days after 8 Gy irradiation in the brains of Parp-1+/+ and Parp-1-/- mice

A: Mutant frequencies of the red/gam gene

B: Mutation spectra of the red/gam gene. Deletion mutations were classified into simple- and complex-type mutations (left panel) and into the size of deletions (right panel).

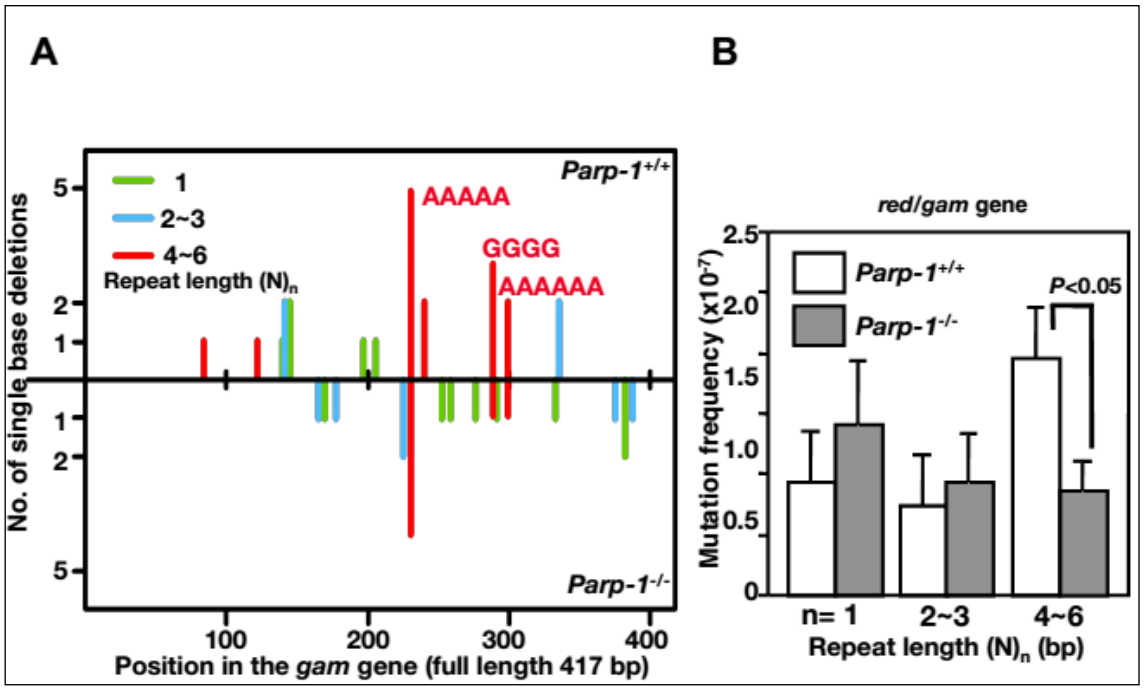

Fig-5: Analysis of mutation spectra of the single base deletions in the gam gene of the brains.

A. Distribution of single-base deletions in the gam gene in the brain. Nucleotide 1 corresponds to the adenine residue in the 1st ATG of the gam gene. The green bars indicate the mutations not located in identical run sequences. Blue bars indicate those located in 2-3 base run sequences and red bars indicate those located in 4 or more base run sequences. B. Mutation frequencies of the single base deletion mutations on non-repeat, 2-3 base repeats, and 4-6 base repeats.

Lower frequencies of IR-induced deletion mutation in the brain of Parp-1-/- mice

In the brain, the red/gam gene mutant frequencies showed no increase in both Parp-1+/+ and Parp-1-/- mice after 8 Gy irradiation (Fig. 4A). However, a further analysis of mutation spectra was carried out. The mutations were mostly the simple-type mutations in the both genotypes. The frequency of deletion mutation of 2-1,000 bases was higher in Parp-1-/- mice, although it is not statistically significant (Fig. 4B). The frequencies of single base deletion mutation on 4-6 base mononucleotide repeat were 2-fold lower in Parp-1-/- mice (Fig. 5B, $P<0.05$). The distribution of mutations in the gam gene was slightly different between the genotypes (Fig. 5A); the single base mutations were mostly observed between nucleotide number 150-400 in Parp-1-/- mice whereas they were widely distributed in Parp-1+/+ mice. The distribution pattern of single base mutations was different from that observed in the liver. Distribution of single base deletion mutations between nucleotide number 150-400 was also previously observed in the liver of aged Parp-1-/- mice [17]. After treatment with alkylating agent BHP, single base deletion mutations were previously observed in the liver between nucleotide number 150-400 in the both genotypes [9]. These results suggest after γ-irradiation, the mutation spectra is different between the Parp-1+/+ and Parp-1-/- brains, although the levels of overall mutant frequency is not apparently different.

The role of Parp-1 in IR-induced mutations

Here we showed that Parp-1 deficiency suppressed γ-irradiation-induced deletion mutations in the mice liver. Mutation spectrum analysis revealed that frequencies of simple-type deletions and deletion of different sizes were lower compared to Parp-1+/+ case. This decreased frequency of deletion mutations is similar to the cases in Ku70 [21] and Ku86 [22] knockout mice, which are essential factors of classical type NHEJ repair. It is thus suggested that PARP-1 could be involved in the generation of deletion mutations through imprecise NHEJ repair like Ku70 and Ku86. We further demonstrated that frequencies of single base deletions at 4-6 base mononucleotide repeats, which are the hot-spots, were significantly less in Parp-1-/- mice after γ-irradiation ($P<0.05$). Also in the brain, single base deletions at 4-6 base mononucleotide repeats were lower under Parp-1 deficiency after γ-irradiation ($P<0.05$). These hot-spot single base deletion mutations at 4-6 base mononucleotide repeats are considered to occur either through slippage reactions during DNA repair or replication, or through the inaccurate NHEJ [18]. The current study suggests the presence of PARP-1 somehow enhances single base deletion on the mononucleotide repeat sequences after γ-irradiation.

We also observed tissue differences in the effect of Parp-1 deficiency in deletion mutations after γ-irradiation. In the liver, Parp-1 deficiency caused suppression of deletion mutations, whereas in the brain

no increase of the red/gam mutations in each genotype was observed. The differences in active DNA repair pathways in these tissues may have affected to these differences. Both NHEJ and BER are considered to work as repair pathways in the livers, whereas BER could be a predominant DNA repair in the brain [23]. In the adult brain of mice, DNA replication that is required to fix mutations may not frequently occurred [24]. Because we analyzed mutations three days after irradiation, this time-point choice may also have limited the numbers of the detectable mutations. Longer time-course experiments and fractionation of cell types for mutation analysis will help to elucidate mechanism of the tissue difference.

There is also a distinct difference in the effect of Parp-1 deficiency on mutations induced by γ-irradiation and alkylating agents in the livers. In the case of γ-irradiation, we observed suppression of deletion mutations, whereas after alkylating agent treatment, we observed an increase of deletion mutations [9]. This difference is possibly due to the structure of DNA lesions caused directly or indirectly by γ-irradiation and alkylating agents. In the case of γ-irradiation, DNA strand break termini mainly possess modified dirty ends including 3'-phosphate and 3'-phosphoglycolate. There is a possibility that under Parp-1 deficiency, processing of these dirty ends after γ-irradiation might be less efficient, leading to the suppression of deletion mutations. On the other hand, DSBs produced after alkylating agent treatment could be processed even in the absence of Parp-1 through imprecise repair and generate deletion mutations. These mechanistic issues should be further addressed in reconstituted repair systems with detailed molecular analysis.

From the present results, it is considered to be important to evaluate the tumor incidences in Parp-1-/- mice in different tissues after γ-irradiation to understand the role of PARP-1 in genome stability and carcinogenesis after IR. The current results also indicate a possibility that combination of PARP inhibitors with radiation therapy may have a lower risk for introducing deleterious mutations compared to the combination with alkylating agents at least in the livers.

CONCLUSION

The current study suggests that the presence of PARP-1 increases introduction of deletion mutations possibly through supporting imprecise repair processes after γ-irradiation.

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