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Ionizing Radiation Sensitivity of DNA Polymerase Lambda-Deficient Cells

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INTRODUCTION

Strand breaks and oxidized bases induced in the DNA by ionizing radiation are critical lesions that, if unrepaird, can lead to cell killing and mutation. Two complementary double-strand break (DSB) repair pathways exist in mammalian cells, non-homologous end joining (NHEJ) and homologous recombination (HR). In addition, ionizing radiation-induced lesions are targets of base excision repair (BER). Deficiencies in all of these pathways lead to increased sensitivity to ionizing radiation, indicating that all are important for genomic repair. Within these pathways, DNA polymerases are thought to play a critical role in repair of radiation-induced damage. At least 19 different DNA polymerases are expressed in mammalian cells. These polymerases differ from each other according to the specialized roles they have during replication, DNA repair, bypass of DNA damage, and somatic hypermutation. In vitro assays show a potential involvement of DNA polymerase lambda in non-homologous end joining and base excision repair. In this study, we investigated whether DNA polymerase lambda played a significant role in determining ionizing radiation sensitivity. Despite increased sensitivity to hydrogen peroxide, lambda-deficient mouse embryonic fibroblasts displayed equal survival after exposure to ionizing radiation compared to their wild-type counterparts. In addition, we found increased sensitivity to the topoisomerase inhibitors camptothecin and etoposide in the absence of polymerase lambda. These results do not reveal a major role for DNA polymerase lambda in determining radiosensitivity in vivo.

Ionizing radiation induces a diverse spectrum of DNA lesions, including strand breaks and oxidized bases. In mammalian cells, ionizing radiation-induced lesions are targets of non-homologous end joining, homologous recombination, and base excision repair. In vitro assays show a potential involvement of DNA polymerase lambda in non-homologous end joining and base excision repair. In this study, we investigated whether DNA polymerase lambda played a significant role in determining ionizing radiation sensitivity. Despite increased sensitivity to hydrogen peroxide, lambda-deficient mouse embryonic fibroblasts displayed equal survival after exposure to ionizing radiation compared to their wild-type counterparts. In addition, we found increased sensitivity to the topoisomerase inhibitors camptothecin and etoposide in the absence of polymerase lambda. These results do not reveal a major role for DNA polymerase lambda in determining radiosensitivity in vivo.

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studies have indicated a potential role of DNA polymerase lambda in DSB repair and/or BER, although the in vivo role is unknown.

DNA polymerase lambda is a member of the X family of DNA polymerases, comprising other enzymes involved in DNA repair processes such as DNA polymerase beta, polymerase mu, and terminal deoxy-nucleotidyl-transferase (TdT). Due to its bypass polymerase activity on templates with base lesions such as abasic sites, it has been implicated in translesion synthesis (TLS), a process allowing lesion bypass during replication. Other data demonstrating repair deficiency when lacking in a classical in vitro BER assay suggested a possible contribution in BER, as shown by Braithwaite et al. (1). However, in earlier studies, DNA polymerase lambda was shown to be capable of filling gaps and promoting end joining in an in vitro NHEJ assay (2, 3). Recent data indicate this involvement to be selective for DNA with complementary overhangs (4, 5). The observed interaction with ligase IV (LigIV) underlines its proposed role in NHEJ (6). Only a few studies have addressed DNA polymerase lambda’s role in vivo or in cellular systems. In vivo, polymerase lambda-deficient mice were viable and fertile. These mice showed normal immunoglobulin hypermutation (7). Furthermore, recent data indicated a participation of polymerase lambda in heavy chain rearrangement (8). Polymerase lambda-deficient embryonic stem cells did not show hypersensitivity to H2O2 or methyl methane sulfonate (MMS), ultraviolet (UV) light, or ionizing radiation (9). However, lambda-deficient mouse embryonic fibroblast cells have been found to be hypersensitive to oxidative damage produced by hydrogen peroxide (H2O2) and 5-hydroxymethyl-2’-deoxyuridine (HmdUrd), indicating a potential role in response to oxidative damage and BER (10). Our own studies have shown a significant role of BER in determining radiation response (11–13). We showed that BER deficiency, by DNA polymerase beta deficiency, resulted in sensitization to ionizing radiation. Consistent with lambda’s proposed role in NHEJ or BER, Capp et al. showed increased radiosensitivity in cells expressing a dominant negative to DNA polymerase lambda (14). In contrast, primary lambda-deficient mouse embryonic fibroblasts were not found to have increased sensitivity to ionizing radiation or bleomycin in earlier studies (8). However, strongly reduced proliferation and a concomitantly reduced percentage of cells in S phase in these...
primary lambda-deficient cells could have influenced survival after exposure to ionizing radiation. This alteration in proliferation has been attributed to oxidative damage response (8). Since ionizing radiation induces a DNA damage spectrum that partly overlaps with the spectrum induced by oxidative damage, we questioned whether the oxidative damage-sensitive population might have been under-represented in the radiation response analysis.

Using transformed mouse embryonic fibroblasts with equal growth and cell cycle phase distribution patterns here, we show that, despite exhibiting $H_2O_2$ hypersensitivity, lambda-deficient cells were not more sensitive to ionizing radiation. In addition, we show increased sensitivity to other non-oxidative DSB-inducing agents such as etoposide and camptothecin.

**MATERIALS AND METHODS**

**Cell Lines**

Polymerase lambda-deficient mice were generated by Bertocci et al. as described previously (7). RT-PCR analysis confirmed DNA polymerase lambda expression status. Transformed polymerase lambda-deficient (pol $\lambda^{-/-}$) mouse embryonic fibroblasts and the isogenic wild-type (pol $\lambda^{+/+}$) counterparts were generated by the 3T3 protocol from these mice, as described previously by Bertocci et al. (8). Pol $\lambda^{+/-}$ and pol $\lambda^{-/-}$ cells were grown at 37°C in a 95% air/5% $CO_2$ incubator in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum. Growth curves were obtained after seeding 0.5 $\times$ 10$^4$ and 10$^4$ cells/well in six-well dishes in triplicate and counting at various times.

**Colony-Forming Survival Assay**

Colony-forming survival assays were performed as described previously (13). Briefly, cells were seeded and, after 6 h, irradiated using a 137Cs irradiation unit at a dose rate of 0.9 Gy/min. For $H_2O_2$, camptothecin and etoposide sensitivity, cells were seeded and treated after 6 h with $H_2O_2$ (Sigma) for 1 h or with camptothecin or etoposide for 2 h in medium without fetal calf serum and antibiotics. Exposure to serum-free medium for 2 h did not alter S-phase content or replication as measured by BrdU labeling. Data analysis showed an equal growth and cell cycle phase distribution patterns here, partly overlaps with the spectrum induced by oxidative damage. Since ionizing radiation induces a DNA damage spectrum that overlaps with the spectrum induced by oxidative damage, we questioned whether the oxidative damage-sensitive population might have been under-represented in the radiation response analysis.

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significantly more sensitive to ionizing radiation than the wild-type cells (Fig. 2a), confirming previous growth inhibition analyses (8). In contrast, lambda-deficient cells appeared to be more radioresistant. However, this was significant only at 6 Gy. We then questioned whether the lack of sensitization was caused by the assay used, with growth assays emphasizing growth inhibition rather than killing. We therefore repeated the H2O2 sensitivity study with a colony-forming survival assay. The data confirmed the previous growth inhibition study, shown in Fig. 1b, with DNA polymerase lambda-deficient cells being significantly more sensitive to H2O2 than wild-type cells, with SF50 values of 89.1 ± 12.2 μM and 44.9 ± 4.4 μM (mean values ± SD; P = 0.004) for wild-type and pol λ−/− cells, respectively, giving a dose ratio of 2.0 ± 0.4 (Fig. 2b). In summary, our data demonstrate no major involvement of DNA polymerase lambda in the response to ionizing radiation.

Sensitivity of pol λ−/− Cells to Camptothecin and Etoposide

Both damaging agents, H2O2 and ionizing radiation, induce similar oxidative lesions. Nevertheless, lambda-deficient cells exhibited hypersensitivity to H2O2 but surprisingly not to ionizing radiation. This discrepancy prompted us to analyze two other DNA strand break-inducing agents without inducing oxidized base damage. For this purpose, we incubated polymerase lambda-deficient and wild-type cells with topoisomerase inhibitors. Type I topoisomerase inhibitors such as camptothecin bind irreversibly to the DNA-topoisomerase I complex, thereby generating enzyme-trapped cleaved DNA. Etoposide, a type II topoisomerase inhibitor, enhances double-strand cleavage by complexation with topoisomerase II. We found that pol λ−/− cells showed an increased sensitivity to camptothecin compared to wild-type cells, as determined by growth inhibition assays (Fig. 3a). The IC10 values were 1.4 ± 0.4 and 0.5 ± 0.2 μM (mean values ± SD; P = 0.02) for wild-type and pol λ−/− cells, respectively, with a dose ratio of 3.2 ± 0.9. Furthermore, pol λ−/− cells were significantly more sensitive to etoposide than wild-type cells (Fig. 3b), with IC50 values of 1.0 ± 0.1 and 0.6 ± 0.1 μM (P = 0.001) for wild-type and pol λ−/− cells, respectively, with a dose ratio of 1.9 ± 0.4. We repeated the growth inhibition studies for both drugs with a colony-forming survival assay and again found that DNA polymerase lambda-deficient cells were significantly more sensitive than wild-type cells (Fig 3c). As with the growth assays, the surviving fractions of the cell lines diverged at greater killing after etoposide treatment. Taken together, these results suggest the involvement of polymerase lambda in the repair of DNA double-strand breaks of non-oxidative origin.

DISCUSSION

In the present study, we investigated whether DNA polymerase lambda played a significant role in determining ionizing radiation sensitivity. Previous studies have shown contradictory results, with a lack of sensitization to ionizing radiation in lambda-deficient primary cells (8) and hypersensitivity in polymerase lambda dominant negative-expressing cells. However, studies on the deficient primary cells may have been hampered by the reduced growth rate
FIG. 2. Sensitivity of pol λ−/− cells to ionizing radiation and H2O2. Panel A: Clonogenic survival of pol λ−/− (○) and wild-type (●) cells after exposure to ionizing radiation. Panel B: Clonogenic survival of pol λ−/− (○) and wild-type (●) cells after exposure to H2O2 for 1 h. After irradiation or H2O2 exposure, the cells were returned to 37°C to allow colonies to develop. Data points are the means and standard deviations of five or three independent experiments with triplicate samples for panels A and B, respectively.

and considerably increased senescence, in particular when analyzed by a growth inhibition assay. A potential radiosensitive phenotype of the DNA polymerase lambda-deficient cells, in particular in S phase, could have been masked by a reduction in the S-phase cell population in the lambda-deficient cells to half of the control level, as observed previously in the primary cultures. Transformed cells, on the other hand, probably due to loss of cell cycle checkpoints, showed similar cell cycle phase distributions and growth rates for wild-type and lambda-deficient cells (Fig. 1b). Here we show that these transformed cells did not exhibit differences in radiation sensitivity (Fig. 2a) despite increased hypersensitivity to hydrogen peroxide. This indicates that the cells did not greatly adapt (due to selection) to oxidative damage during culture, thereby altering the oxidative damage response and/or repair. It is worthwhile noting that even though there were differences between the assays used, the growth inhibition assay (Fig. 1c) and the colony-forming survival assay (Fig. 2b), the results were generally in good concordance with each other. Differences between the two cell lines were similar to the results of by Braithwaite et al. (10). We observed a slight increase in radioresistance in DNA polymerase lambda-deficient cells. Although not significant for most doses, this could have resulted from the additional 10% of S-phase cells, which are generally more resistant to ionizing radiation. In conclusion, these results suggest that a deficiency in polymerase lambda is associated with an increased sensitivity to oxidative damage and strand breaks but not when these lesions are induced by ionizing radiation.

Two other DNA break-inducing agents, camptothecin and etoposide, both produced more killing in lambda-deficient cells (Fig. 3). In contrast to our results (Fig. 3c), Capp et al. found an increased resistance to camptothecin in hamster cells (CHO cells) expressing a catalytically inactive polymerase lambda (polλDN) (14). In addition, these cells were found to be more sensitive to ionizing radiation. Interestingly, sensitization reached levels that were comparable to that of NHEJ mutant cells. The inactive DNA polymerase lambda is expected to act in a dominant negative manner in the proposed repair pathways. It is likely that the mode of action is different from the lack of repair due to gene deficiency, in analogy with our polymerase beta dominant negative studies (11–13).

The dominant negative might have the capacity to block alternative backup polymerases and pathways, or it could interfere in processes unrelated to its wild-type lambda protein. In addition, the origin of the cell lines, mouse fibroblasts and hamster ovary cells, could result in different responses to damaging agents.

Based on the data presented here, we can only speculate on the possible role of lambda. Assuming that the observed sensitivities result from involvement in a common pathway, it is feasible that the nature of the lesion dictates DNA polymerase lambda’s interaction. Double-strand breaks produced by ionizing radiation are complex, often presenting as clustered lesions. In contrast, topoisomerase inhibition will cause breaks complexed to topoisomerases together with ligatable ends. Second, double-strand break induction by topoisomerase inhibitors and hydrogen peroxide are partly connected to replication. Replication could thereby present a mutual process in the lambda-dependent responses. Homologous recombination-deficient cells have been shown to be highly sensitive to camptothecin (15). Etoposide sensitivity showed a dependence on non-homologous end joining but also showed some dependence on homologous recombination (16, 17), indicating that the choice of pathway in response to the damaging agent might be related to DNA polymerase lambda dependence. Consistent with
such an interpretation, ionizing radiation response in most cell lines is less dependent on homologous recombination than on non-homologous end joining.

Repair of ionizing radiation damage is highly dependent on the non-homologous end-joining repair pathway, as shown by numerous studies, being dependent on the presence of non-homologous end-joining proteins such as Ku70/80, DNA-PK and LigIV. The lack of increased sensitivity to ionizing radiation of lambda-deficient cells found here therefore does not support a hypothesis of lambda’s sole involvement in non-homologous end joining after exposure to ionizing radiation. Our data rather suggest the involvement of another redundant polymerase (18). In summary, DNA polymerase lambda does not play an important role in determining sensitivity to ionizing radiation, despite the observed hydrogen peroxide sensitivity and proposed role in repair of DNA double-strand breaks.

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