Published in final edited form as:

DNA Repair (Amst). 2008 June 1; 7(6): 830–833. doi:10.1016/j.dnarep.2008.02.013.

Negligible impact of pol ι expression on the alkylation sensitivity of pol β -deficient mouse fibroblast cells

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Dear Editor

DNA repair plays a major role in maintaining genomic integrity such that deficiency, as well as an excess of DNA repair, has a significant impact. For example, the absence of DNA polymerase β (pol β) expression in mice results in embryonic lethality, while over-expression of pol β causes cataracts [1,2]. Alkylated DNA bases in higher eukaryotes are repaired predominantly by the base excision repair (BER) pathway. During “single-nucleotide” BER, an alkylated base is removed by the damage specific glycosylase, 3-methyladenine DNA glycosylase, and the resulting abasic site is cleaved by apurinic/apyrimidinic endonuclease. This results in a nick with a 5' dRP group that can be removed by the dRP lyase activity of pol β ; the same polymerase then fills the single-nucleotide gap. Finally, a DNA ligase seals the nick completing repair (reviewed in [3]).

Mouse embryonic fibroblasts (MEFs) deficient in pol β demonstrate increased sensitivity to alkylating agents such as methyl methanesulfonate (MMS) [4]. Hypersensitivity is moderate, and cellular extracts from pol β null cells have some residual BER activity, suggesting that other polymerases could participate in BER. Reversal of the MMS hypersensitivity requires the dRP lyase activity of pol β [5]. DNA polymerase λ (pol λ) also has dRP lyase activity and can function as a back up BER enzyme in pol β null cells [6,7].

Another DNA polymerase carrying dRP lyase activity is DNA polymerase ι (pol ι) [7,8]. This polymerase is a member of the Y-family of DNA polymerases and was identified due to its homology with DNA polymerase η (pol η) [9]. Based on *in vitro* biochemical data and on the structure of the enzyme, it has been proposed that pol ι participates in DNA translesion synthesis during bypass of a damaged template [10,11]. Gene expression at the mRNA level is modest in most tissues, with strongest expression in testis and ovarian cells [9]. The data on the biological roles of this enzyme are contradictory. Pol ι is expressed at a high level in some human cancer cells and also is associated with increased frequency of mutagenesis in certain cell types; lowering the expression of this enzyme decreased mutation frequency [12]. Deletion of the pol ι gene in human Burkitt's lymphoma cells reduced inducible somatic hypermutation (SHM), and this process could be restored by expression of pol ι [13,14]. At the same time, a

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mouse strain deficient in expression of pol ι demonstrated the same ability for SHM as mice carrying the wild-type pol ι gene [15,16]. Down-regulation of human pol ι in HEK 293 cells showed no effect on resistance to UV-induced damage as well as to UVC-induced mutagenesis [17].

Recent studies have revealed that MEFs derived from the 129 mouse strain carry a genomic mutation causing premature termination of the pol ι transcript and preventing expression of active enzyme [16]. Therefore, we analyzed MEF lines developed in this laboratory, and that are deficient and proficient in pol β , for expression of pol ι . Utilizing RT-PCR, sequencing and immunoblotting, we confirmed that pol β -deficient M β 19tsA MEFs are also deficient in expression of pol ι (Fig. 1 and data not shown). This is essentially in agreement with previously published data from another laboratory [18]. Since MEF cells deficient in BER are hypersensitive to alkylating agent-induced DNA damage, we next determined the resistance of MEFs to the monofunctional alkylating agent MMS. This agent methylates predominantly adenine and guanine bases producing mono-adducts that block DNA replication [19] and are removed from DNA primarily by glycosylase-initiated pol β -dependent BER [5].

To identify a role for pol ι in MEF cellular resistance to MMS in a pol β null background, we used M β 19tsA cells that lack expression of both pol ι and pol β (Fig. 1). As a control, we used M β 16tsA and 92TAg cells that are pol β and pol ι proficient, and 88TAg cells deficient in pol β and proficient in pol ι expression (Fig. 1). Treatment with MMS resulted in growth inhibition of cells in a dose dependent manner (Fig. 2A) as was shown previously [4,20]. MMS had relatively less effect on wild-type M β 16tsA and 92TAg cell lines, especially at lower concentrations, whereas M β 19tsA and 88TAg pol β null cell lines showed clear hypersensitivity to MMS (Fig. 2A). While the LD₁₀ doses for wild-type M β 16tsA and 92TAg were indistinguishable (~2.2 mM MMS), the LD₁₀ for M β 19tsA and 88TAg were dissimilar (0.75 and 1.25 mM, respectively). Therefore, pol β deficient cells expressing pol ι (88TAg) showed increased resistance to MMS compared with cells that were additionally deficient in pol ι (M β 19tsA) (Fig. 2A).

The pol β -deficient MEFs used in this study had different origins. To minimize the effect of genetic background variability, we expressed mouse pol ι in M β 19tsA pol ι - and pol β -deficient cells at a level equal to, and greater than, the wild-type level. Analysis of growth inhibition revealed that expression of pol ι did not affect the resistance of these cells to the alkylating agents MMS and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (Fig. 2B and C). Thus, we conclude that the difference in MMS sensitivity of the pol β -deficient M β 19tsA and 88TAg cell lines is not likely to be a result of the difference in pol ι expression. Finally, since it has been proposed that pol ι participates in error-prone bypass across thymine dimmers, we tested the sensitivity of cells expressing pol ι to UV radiation. While the individual clones have slight variations, we could not find any statistically significant differences between cells expressing pol ι and those expressing control vector (Fig. 2D).

There are at least 15 DNA polymerases currently described in mammalian cells [21]. Three of these polymerases, pol α , pol γ , and pol ϵ are indispensable, while others showed various effects on MEF cell viability. The functional interactions or overlap between these enzymes are of special interest. Thus, the polymerases in higher organisms appear to either substitute for each other or participate in similar processes. Our results demonstrate that expression of pol ι has negligible impact on the alkylation damage sensitivity of cells lacking pol β .

Acknowledgements

This research was supported by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences, and the National Institute of Child Health and Human Development.

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Fig. 1.

Expression of pol ι in MEFs. Western blotting of whole cell extract (20 μg) isolated from Mβ16tsA (lane 1), Mβ19tsA (lane 2), 92TAg (lane 3), and 88TAg (lane 4) cell lines. Cell lines were grown in DMEM medium supplemented with 10% FBS (HyClone, Logan, UT). Extract preparation and immunoblotting were as described previously [20,22].

Fig. 2. Growth inhibition assay after exposure to DNA damaging agents. (A) 92TA γ (closed squares), 88TA γ (open squares), M β 16tsA (closed circles) and M β 19tsA (open circles) were treated with MMS for 1 h. (B) M β 19tsA (open circles) and M β 19tsA expressing pol τ (closed diamonds) were exposed to (B) MMS (1 h), (C) MNNG (1 h), and (D) UV. Growth inhibition assays were performed as described previously [22]. Briefly, MEFs were plated at a density of 40,000 cells/well in six-well dishes and the following day, were treated with a range of concentrations of DNA damaging agent in growth medium without antibiotics. After exposure, cells were washed, fresh medium was added, and cells were incubated in a 10% CO₂ incubator for 4–5

days. Cells (triplicate wells for each concentration) were counted, and results were expressed as the number of cells in agent-treated wells relative to untreated wells (i.e., % Control Growth).