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Polk mutant mice have a spontaneous mutator phenotype

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ABSTRACT

Mice defective for the *Polk* gene, which encodes DNA polymerase kappa, are viable and do not manifest obvious phenotypes. The present studies document a spontaneous mutator phenotype in *Polk*$^{-/-}$ mice. The initial indication of enhanced spontaneous mutations in these mice came from the serendipitous observation of a postulated founder mutation that manifested in multiple disease states among a cohort of mice comprising all three possible *Polk* genotypes. *Polk*$^{-/-}$ and isogenic wild-type controls carrying a reporter transgene (the λ-phage *cII* gene) were used for subsequent quantitative and qualitative studies on mutagenesis in various tissues. We observed significantly increased mutation frequencies in the kidney, liver, and lung of *Polk*$^{-/-}$ mice, but not in the spleen or testis. G:C base pairs dominated the mutation spectra of the kidney, liver, and lung. These results are consistent with the notion that Polκ is required for accurate translesion DNA synthesis past naturally occurring polycyclic guanine adducts, possibly generated by cholesterol and/or its metabolites.

1. Introduction

Vertebrate cells are endowed with at least nine specialized DNA polymerases, all of which copy native DNA with markedly reduced fidelity and are devoid of 3′ → 5′ proofreading exonuclease activity [1]. *In vitro*, these enzymes have been shown to support replicative bypass [translesion DNA synthesis (TLS)] past numerous types of base damage that can arrest high fidelity DNA replication in living cells. One of these DNA polymerases, Polη, supports replication past thymine–thymine (and presumably other) cyclobutane pyrimidine dimers (CPD) [2]. Regardless of the fact that Polη displays extremely low fidelity when copying native DNA, TLS past thymine-containing CPD is remarkably accurate *in vitro* [2]. This is apparently also the case in living cells, since humans and mice defective in Polη activity display typical clinical features of xeroderma pigmentosum (XP), including enhanced UV radiation-dependent mutagenesis and increased predisposition to skin cancer [3,4].

These observations suggest that some, if not all, specialized DNA polymerases evolved to relieve arrested DNA replication associated with specific types of naturally occurring base damage of either environmental (such as that caused by UV radiation from the sun in the case of Polη) or spontaneous origin [1]. This notion embraces the nuance that while TLS by an appropriate specialized polymerase is largely error-free, its absence invokes one or more other such enzymes to subserve this function. Cells are thereby rescued from the lethal consequences of arrested DNA replication, but in a manner that generates an increased mutational burden [1].

Cognate substrates for specialized DNA polymerases other than Polη have not yet been identified. However, a number of reported observations suggest that DNA polymerase kappa (Polκ) may have evolved to support error-free bypass of polycyclic N^2-guanine adducts in DNA. It is of significant interest that the promoter region of the mouse (and human) *Polk* gene (but not the promoter regions of other specialized polymerases) contains two canonical arylhydrocarbon receptor-binding sites [5]. Such sites bind polycyclic aromatic ligands with high affinity and the ensuing receptor–ligand complex ultimately promotes the transcriptional activation of genes required for the catabolism of polycyclic aromatic hydrocarbons [5]. Consistent with this observation, cells derived from a *Polk*$^{-/-}$ mouse strain are hypersensitive to killing in the presence of BPDE [6], and Polκ (but not Polη) is specifically required for recovery from checkpoint arrest associated with exposure of mouse cells to this polycyclic aromatic compound [7]. Additionally, chicken DT40 *Polk*$^{-/-}$ cells manifest increased sensitivity to killing and increased chromosomal abnormalities associated with exposure to polycyclic estrogen analogs such as tamoxifen and 4-hydroxyestradiol [8]. Finally, *in vitro* [6,9–12] and *in vivo* [13] studies indicate that Polκ efficiently bypasses various polycyclic N^2-guanine adducts in a largely error-free manner, supporting the preferential incorporation of dC [10].

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Volcanoes and natural fires are well known spontaneous sources of benzo[*a*]pyrene and other polycyclic compounds [14], and may have conceivably promoted selection of Polκ during evolution. However, plants and microorganisms are endowed with numerous polycyclic aromatic phenolic compounds, glycosides, and alkaloids [15]. Additionally, though not planar in its native configuration, cholesterol and a plethora of metabolic derivatives of this compound, including sex hormones and steroids, can be aromatized to polycyclic derivatives that can covalently interact with DNA [16]. In this regard it is intriguing that *Polk* mRNA (but not *Poli* or *Poll* mRNA) is particularly highly expressed in the adrenal cortex of embryonic and adult mice, the site of steroid biosynthesis [17].

These considerations, coupled with the knowledge that many polycyclic aromatic compounds bind in the minor groove of DNA and covalently attach to the N^2-position of guanine, anticipate that *Polk*$^{-/-}$ mice may carry an increased spontaneous mutational burden with a spectrum consistent with error-prone bypass (TLS) of N^2-guanine adducts.

Here we document that cells from an independently derived *Polk* mutant mouse [18] are also distinctly sensitive to exposure to benzo[*a*]pyrene diol epoxide (BPDE). We also document increased levels of spontaneous mutations in various (but not all) tissues from these *Polk*$^{-/-}$ mice. Furthermore, we show that the spectra of enhanced mutagenesis were indeed consistent with mutations primarily involving G:C base pairs, potentially implicating guanine as a source of spontaneous DNA damage.

2. Materials and methods

2.1. *Polk*$^{-/-}$ mice

Mice carrying the *Polk*$^{tm1.1Esp}$ allele have been previously described [18]. Mice were screened for a naturally occurring mutation in the *Poli* gene of 129sv mice and were found to be wild-type for the gene. Mice were in a mixed 129 × C57BL/6 background and were housed in either a conventional mouse facility that was not specific-pathogen-free (SPF) or in an SPF facility. Food, water, and housing were the same between the facilities. Food (6% fat mouse chow) and water were provided ad libitum.

2.2. Big Blue *Polk*$^{-/-}$ and Big Blue *Polh*$^{-/-}$ mice

Big Blue mice (named for the *lacI* color-based plaque screening) were obtained from Stratagene (C57BL/6 strain background). These mice carry 80 copies of the chromosomally integrated λ-LIZ shuttle vector, which harbors the lambda *cII* reporter gene. Our *Polk*$^{-/+}$ mice (129/Ola backcrossed twice into C57BL/6; ∼75% C57BL/6) were mated with Big Blue mice to obtain *BB-Polk*$^{+/+}$ or *BB-Polk*$^{-/-}$ animals carrying 40 or 80 copies of the λ-LIZ shuttle vector. Similarly, *Polh*$^{-/+}$ (129/Ola/C57BL/6) mice from the laboratory of Dr. Raju Kucherlapati [4] were mated with Big Blue mice from Stratagene to obtain *BB-Polh*$^{-/-}$ animals carrying 40 or 80 copies of the λ-LIZ shuttle vector. Male and female mice for each genotype were housed in a specific-pathogen-free facility.

2.3. Genotyping

DNA was isolated from tails with a tissue DNA kit (formerly Gentra Systems now Qiagen, Valencia, CA). Genotyping for Polk was performed as previously reported [18]. For *Polh* genotyping, primers XPV-F7 (5′-AAGGGACAAGCGAACAGAGA-3′), XPV-R14 (5′-AGCAATATCACAGGCCCAAC-3′), and XPV-R1 (5′-TCACTTCAACACTAGCTTCCC-3′) were used in combination at a 1:1:1 concentration at a 58 °C annealing temperature to amplify either a 500 bp fragment (mutant), a 370 bp fragment (WT), or both (heterozygous). For detection of the λ-LIZ shuttle vector, primers CII-F (5′-CCACACCTATGGTGTATG-3′) and CII-R (5′-CCTCTGCCGAAGTTGAGTAT-3′) were used to PCR-amplify a 432-bp band containing the *cII* gene using a 52 °C annealing temperature with 5% DMSO. Sequencing reactions were carried out according to the manufacturer's protocol using the ABI 3100 Genetic Analyzer (ABI, Foster City, CA). To further determine whether mice were hemizygous (40 copies) or homozygous (80 copies) for the λ-LIZ shuttle vector, Q-PCR was used to quantify relative *cII* copy numbers using primers CII-F1 (5′-CTGCTTGCTGTTCTTGAATGGG-3′) and CII-R1 (5′-CGCTCGGTTGCCGCC-3′) with Stratagene's Brilliant Q-PCR Mastermix. Primers were used at an optimized concentration of 0.5 mM.

2.4. Isolation of DNA and packaging into λ-phage

Tissues harvested at the time of sacrifice (at 3,9, or 12 months of age) from *BB-Polk*$^{-/-}$ or *BB-Polk*$^{+/+}$ mice (relatively half male, half female) were flash frozen and stored at −80 °C. DNA was isolated from kidney, liver, lung, testis, or spleen using the RecoverEase DNA isolation kit (Stratagene) as directed. 12 μL of DNA was used for packaging into λ-phage using Transpack packaging extract (Stratagene).

2.5. Transformation into E. coli

Packaging extracts were diluted in 966 μL of SM buffer. Triplicates of 100× dilutions were generated and 100 μL or 20 μL of each triplicate was transformed into G1250 *E. coli* culture (in $MgSO_4$, OD = 0.5) for phage titering. The remaining packaged DNA was used to transform G1250 *E. coli* cells for the selection of *cII*$^{-}$ mutants. Cells were plated onto TB1 plates using heated TB1 top agar cooled to 55 °C. Titer plates were grown at 37 °C overnight and screening plates were grown at room temperature (24 °C) for 48 h.

2.6. Verification of putative λ-*cII*$^{-}$ mutants

Putative mutant plaques were cored, transferred to a 96-well plate containing 250 μL of SM buffer per well, and stored at 4 °C. Putative plaques were individually transformed and plated (1 μL per transformation) at low density on TB1 media, and grown at the selective temperature (24 °C) for 48 h. Plaques visible by 48 h were cored and transferred to a new 96-well plate containing 250 μL of SM buffer per well and stored at 4 °C indefinitely.

2.7. PCR amplification and sequence analysis of *cII*$^{-}$ mutants

Verified mutant plaques immersed in SM buffer were directly used as PCR templates. CII-F and CII-R (Stratagene) primers were used to amplify the promotor region immediately upstream of the *cII* gene and the *cII* open reading frame. A total of 5 μL of each PCR reaction was treated with 2 μL ExoSap-It enzyme (GE Healthcare) and incubated at 37 °C for 30 min, followed by a heat-shock at 80 °C for 15 min. Each sample was sequenced with the CII-R primer using the ABI Big Dye Terminator Cycle Sequencing Kit on an automated ABI Prism 3100 Genetic Analyzer.

2.8. Mutation frequencies

Raw mutation frequencies were corrected for "jackpot" mutations and wild-type sequences as previously described [19]. The corrected mutation frequency was determined as the total number of independent mutations per sample divided by the total number of plaque forming units screened (PFUs) per sample. For each experimental group, mutation frequencies from 4 to 7 experiments were averaged to represent the median mutation frequency. Mutation spectrum data was combined for each experimental group (in