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In vivo evidence that DNA polymerase kappa is responsible for error-free bypass across DNA cross-links induced by mitomycin C

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ABSTRACT

Translesion DNA synthesis (TLS) is an important pathway that avoids genotoxicity induced by endogenous and exogenous agents. DNA polymerase kappa (Polk) is a specialized DNA polymerase involved in TLS but its protective roles against DNA damage *in vivo* are still unclear. To better understand these roles, we have established knock-in mice that express catalytically-inactive Polk and crossbred them with *gpt* delta mice, which possess reporter genes for mutations. The resulting mice (inactivated Polk KI mice) were exposed to mitomycin C (MMC), and the frequency of point mutations, micronucleus formation in peripheral erythrocytes, and γH2AX induction in the bone marrow was determined. The inactivated Polk KI mice exhibited significantly higher frequency of mutations at CpG and GpG sites, micronucleated cells, and γH2AX foci-positive cells than did the Polk wild-type (Polk$^+$) mice. Recovery from MMC-induced DNA damage, which was evaluated by γH2AX induction, was retarded in embryonic fibroblasts from the knock-in mice when compared to those from the Polk$^+$ mice. These results suggest that Polk mediates TLS, which suppresses point mutations and DNA double-strand breaks caused by intra- and interstrand cross-links induced by MMC treatment. The established knock-in mice are extremely useful to elucidate the *in vivo* roles of the catalytic activity of Polk in suppressing DNA damage that was induced by a variety of genotoxic stresses.

1. Introduction

The human genome is continuously exposed to a variety of endogenous and exogenous genotoxic insults, e.g. by reactive oxygen species and alkylating agents from endogenous sources, and by cigarette smoke, aflatoxins, and chemotherapeutic agents from exogenous sources [1]. These agents induce DNA damage in the form of DNA adducts, abasic sites, DNA strand cross-links, and single- and double-stranded breaks in DNA [2,3]. Such lesions are repaired by a number of mechanisms within the cell but, because chromosomal replication occurs before all lesions are removed, DNA polymerases (Pols) involved in replication will inevitably encounter the lesions. One strategy that cells develop to deal with the lesions is translesion DNA synthesis (TLS), in which the replication fork directly passes over DNA damage by means of specialized Pols [4–6]. Unlike Pols responsible for chromosomal replication, which stall at or before the lesions [7,8], the specialized Pols can bypass DNA lesions and continue primer extension beyond them [9]. It is assumed that the replicative Pols would take over the primer from the specialized Pols after successful TLS, thereby accomplishing the whole chromosomal replication [10].

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Pol kappa (Polk) is a specialized Pol that belongs to the Y family, the most abundant class of Pols involved in TLS [11–14]. Polk is unique in that its orthologs are present in Eukarya, bacteria and Archaea [15–17]. Several lines of *in vitro* evidence suggest that Polk may be involved in TLS across a variety of DNA damage, such as N^2-guanyl adducts induced by polycyclic aromatic hydrocarbons and alkylating agents [18–23], a C8-guanyl adduct by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) [24], thymine glycol [25,26], 8-oxo-guanine [27,28], and interstrand DNA cross-links [29]. In addition to TLS, Polk is reported to be involved in nucleotide excision repair [30], replication checkpoint [31], repair of single-strand breaks in DNA [32], and microsatellite stability [33]. Knockout (KO) mice of Polk are viable and exhibit more frequent spontaneous mutations in liver, lung, and kidney in aged mice [34,35]. However, it is still unclear which of the DNA lesions Polk protects cells from *in vivo* (the whole body system) and what roles it plays in preventing cancer induced by environmental stresses.

To understand better the *in vivo* protective roles, we have established knock-in (KI) mice where inactive Polk is expressed from its cognate promoter. Two essential amino acids for Pol activity, Asp197 and Glu198, were changed to Ala and Ala in the KI mice (*Polk*$^{D197A-E198A}$ mice). We generated KI mice instead of KO mice because Polk interacts with other proteins, such as REV1 and PCNA [23,36–38]. REV1 interacts with Pol eta, Pol iota, and REV3L (a catalytic subunit of Pol zeta) [39], and PCNA interacts with a number of proteins involved in DNA transactions [40]. Therefore, a simple KO or knockdown of Polk might possibly modulate the functions of other proteins, thereby obscuring the intrinsic roles of Polk, which depend on a catalytic activity as a DNA polymerase and on protein–protein cross-talks with other DNA replication components to maintain the genome stability. We crossed the *Polk*$^{D197A-E198A}$ mice with *gpt* delta mice [41], which possess reporter genes for mutations *in vivo*. The *gpt* delta mice have been extensively employed in chemical mutagenesis, carcinogenesis, and radiation biology [42–44]. We exposed the resulting mice, namely, inactivated Polk mutant and *gpt* delta double transgenic mice (hereafter, referred to as inactivated Polk KI mice, and their counterparts, the Polk wild-type *gpt* delta mice, as Polk$^+$ mice) to mitomycin C (MMC). Then gene mutations and DNA damage induced by MMC in these mice were evaluated in bone marrow, which is widely known as a target tissue of MMC. We chose MMC as the first genotoxic agent to be assayed on inactivated Polk KI mice because the chemotherapeutic agent induces interstrand and intrastrand cross-links in DNA [45]. Interstrand cross-links prevent strand separation of DNA, which inhibits DNA replication, transcription, and translation. Although several Pols such as Pol zeta, Rev1, and Polk are suggested to be involved in TLS across lesions that have been unhooked from the cross-linked DNA during repair [46], *in vivo* evidence of which Pols participate in the repair process is missing. In addition, the Pols responsible for TLS across intrastrand cross-links, which are much more abundant after MMC treatment than the interstrand cross-links, have been less thoroughly investigated [47]. The results from inactivated Polk KI mice suggest that Polk plays significant roles in protecting cells against genotoxicity of the inter- and intrastrand

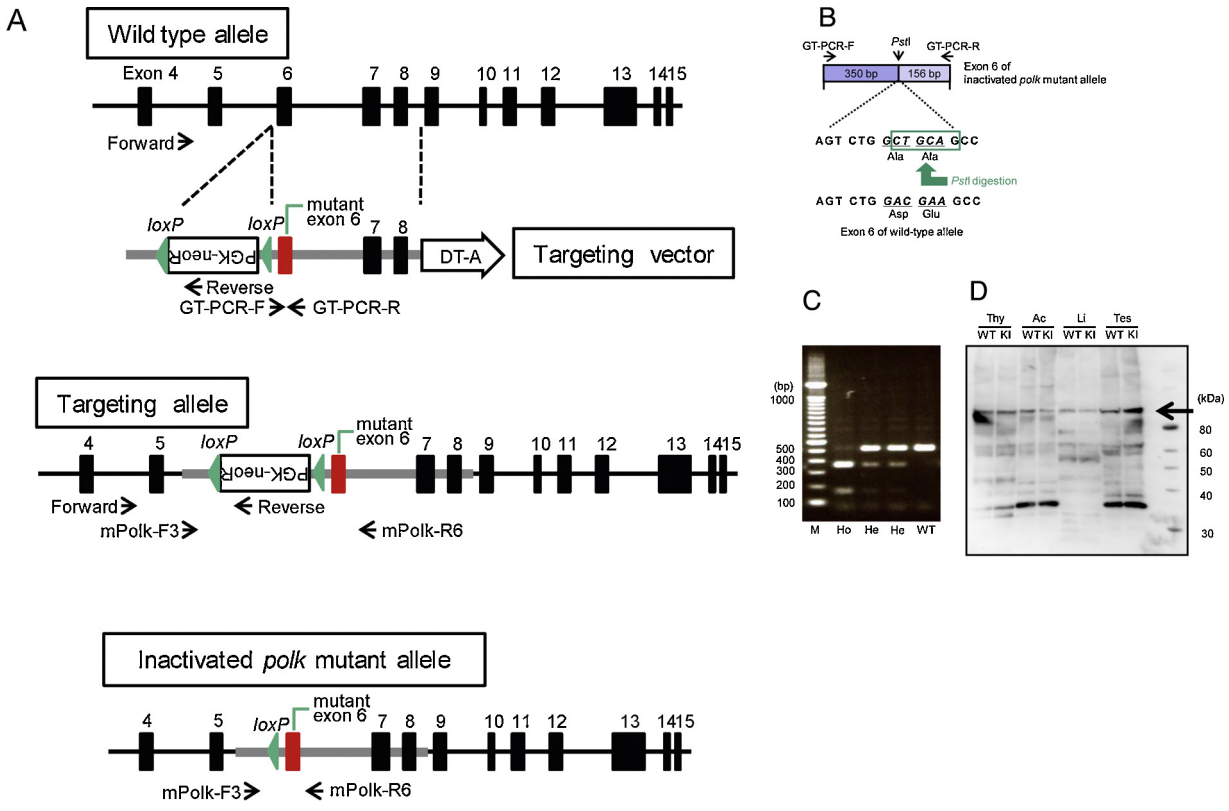

Fig. 1. Generation of inactivated Polk KI mice. (A) Structure of a targeting vector for inactivation of mouse *Polk*, and the predicted structures of the targeted and inactivated *Polk* mutant alleles. The latter allele was generated from the former by Cre-loxP mediated recombination. PCR primers for genotyping are shown by horizontal arrows. (B) The exon 6 of inactivated Polk KI mice. Wild type codons GAC GAA were substituted to GCT GCA in inactivated Polk KI mice to introduce the *Pst*I digestion site. (C) An agarose gel electrophoresis image for PCR products of mouse Polk exon 6 after *Pst*I digestion. Polk wild-type (WT) allele is insusceptible to *Pst*I digestion. A PCR product from homo genotype of *Polk* mutant (Ho) shows two fragments digested by *Pst*I. Hetero genotype of *Polk* mutant (He) shows both the wild-type allele band and the *Pst*I fragments. (D) Western blotting analyses of the wild-type Polk expressed in wild-type mice (WT) and the Polk derivative with D197A-E198A in *Polk*$^{D197A-E198A}$ mice (KI). Cell extracts (20 μg) of thymus (Thy), adrenal cortex (Ac), liver (Li), and testis (Tes) from the mice were applied to each lane. The arrow indicates the position of Polk and Polk derivative with D197A-E198A.

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