

Unlike Catalyzing Error-Free Bypass of 8-OxodGuo, DNA Polymerase λ Is Responsible for a Significant Part of Fapy·dG-Induced G \rightarrow T Mutations in Human Cells

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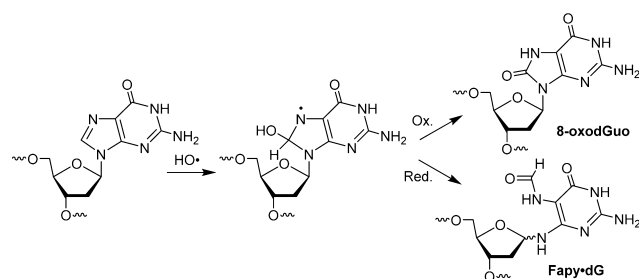
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S Supporting Information

ABSTRACT: 8-OxodGuo and Fapy·dG induced 10–22% mutations, predominantly G \rightarrow T transversions, in human embryonic kidney 293T cells in four TG*N sequence contexts, where N = C, G, A, or T. siRNA knockdown of pol λ resulted in 34 and 55% increases in the level of mutations in the progeny from the 8-oxodGuo construct in the TG*T and TG*G sequences, respectively, suggesting that pol λ is involved in error-free bypass of 8-oxodGuo. For Fapy·dG, in contrast, the level of G \rightarrow T mutations was reduced by 27 and 46% in the TG*T and TG*G sequences, respectively, suggesting that pol λ is responsible for a significant fraction of Fapy·dG-induced G \rightarrow T mutations.

Oxidative stress produces many different lesions in DNA, of which 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) has received a great deal of attention.^{1,2} Approximately 20% of the hydroxyl radicals that react with dG add to C8 to produce a radical at N7.³ The N7 radical generates 8-oxodGuo via one-electron oxidation, whereas fragmentation followed by reduction gives rise to the ring-opened formamidopyrimidine derivative, *N*-(2-deoxy-D-pentofuranosyl)-*N*-(2,6-diamino-4-hydroxy-5-formamidopyrimidine) (Fapy·dG) (Scheme 1).⁴ The

Scheme 1. Formation of 8-OxodGuo and Fapy·dG by Hydroxyl Radical through a Common Intermediate



relative amounts of these two lesions depend on the oxidation conditions, but frequently they are formed in comparable amounts.⁴ 8-OxodGuo and Fapy·dG are mutagenic in bacteria and mammalian cells.^{5–8} Many studies in prokaryotes and eukaryotes have demonstrated that 8-oxodGuo induces G \rightarrow T transversion as the predominant mutation.^{5,6}

Structural studies suggest that 8-oxodGuo leads to misincorporation of adenine in its *syn* conformation, leading to G:C \rightarrow T:A mutations.⁹ Fapy·dG also induces G \rightarrow T mutations.^{7,8} However, a recent study using a carbocyclic analogue of Fapy·dG indicates that the Fapy lesions remain in the *anti* conformation during replication, but that base-shifting and tautomerization result in mispairing with dA, ultimately leading to G \rightarrow T transversions.¹⁰ There are only a limited number of studies in which replication of 8-oxodGuo and Fapy·dG has been explored in the same sequence context using the same approach. In one comparative study in *Escherichia coli*, the bypass efficiency of Fapy·dG was found to be lower than that of 8-oxodGuo in four sequence contexts.⁷ Induction of the SOS functions resulted in the increased bypass efficiency of 8-oxodGuo, whereas it has no effect on Fapy·dG, suggesting that the SOS enzymes are not involved in the translesion synthesis (TLS) of Fapy·dG. In addition, Fapy·dG is less mutagenic than 8-oxodGuo in *E. coli* in all four sequences.⁷ In contrast to this result, in two sequence contexts examined in the simian kidney (COS-7) cells, Fapy·dG was found to be ~25% more mutagenic than 8-oxodGuo. The Fapy·dG-induced G \rightarrow T mutation frequency (MF) was as high as 30% in the TG*T sequence, which was nearly 4-fold relative to that in the TG*A sequence.⁸

For the past two decades, the role of oxidative stress and 8-oxodGuo in human diseases has been a very active area of research.^{11,12} As such, the mechanism of TLS of 8-oxodGuo was studied extensively in vitro using purified human DNA polymerases (pols).^{13–16} It was also investigated in human cells.^{17–19} 8-OxodGuo does not severely block the human replicative pols, but pol α , pol δ , and pol ϵ extend an 8-oxodGuo:dA mispair much more efficiently than the correct 8-oxodGuo:dC pair.^{2,13,19} Despite this, TLS of 8-oxodGuo in human cells is largely error-free.^{19,20} In comparison to the B-family enzymes pol α , pol δ , and pol ϵ , the X-family enzyme pol λ bypasses 8-oxodGuo more faithfully.²¹ It incorporates the correct nucleotide (dC) opposite 8-oxodGuo 1200-fold more efficiently than dA in the presence of RP-A and PCNA.²² A key role of MUTYH and pol λ in the repair of 8-oxodGuo:dA mispair was recognized.²³ A subsequent study showed the existence of a pathway in which error-free TLS of 8-oxodGuo is

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accomplished by a switch of pol δ with pol λ .²⁴ The importance of this switch is enhanced in this work in which it was established that pol β and pol η are not involved in the error-free pathway.

To improve our understanding of the mutagenic mechanism of 8-oxodGuo and Fapy-dG in human cells, we replicated four sets of vectors containing 8-oxodGuo or Fapy-dG located in the TG*N sequence context (where N = C, G, A, or T and G* = 8-oxodGuo or Fapy-dG) in human embryonic kidney (HEK) 293T cells. In each case, the progeny were analyzed for mutations using oligonucleotide hybridization, followed by DNA sequencing.^{25,26}

8-OxodGuo and Fapy-dG were significantly mutagenic in HEK293T cells (Figure 1). The total MF ranged from 10 to

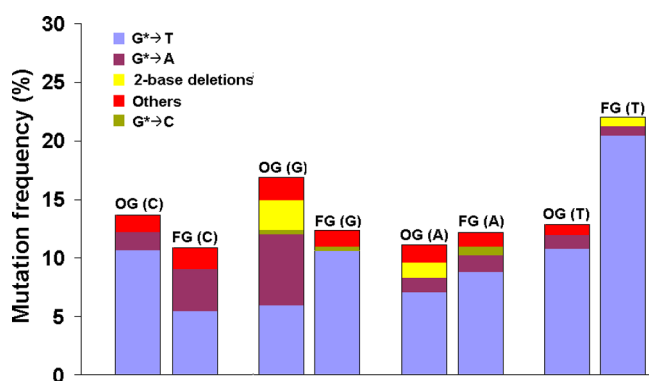


Figure 1. Comparative mutagenesis of 8-oxodGuo (OG) and Fapy-dG (FG) in HEK293T cells in TG*N sequence, where N represents C, G, A, or T shown in parentheses. The data represent the average of two independent experiments (shown in Table S1 of the Supporting Information). Others include semitargeted mutations near the lesion as indicated in Table S1 of the Supporting Information.

22%, and in each sequence context, they exhibited a distinct pattern of mutations. In the TG*T sequence, the MF of Fapy-dG was ~75% higher than that of 8-oxodGuo, whereas in the TG*C and TG*G sequences, the MF of 8-oxodGuo was 20–30% higher than that of Fapy-dG (Figure 1 and Table S1 of the Supporting Information). In the TG*A sequence, the MFs of the lesions were comparable. Although the most prevalent mutations induced by both Fapy-dG and 8-oxodGuo were G → T transversions, in the TG^{8-oxo}G and TG^{Fapy}C sequences (G^{8-oxo} and G^{Fapy} are bases of 8-oxodGuo and Fapy-dG, respectively), significant targeted G → A transitions also occurred (Figure 1 and Table S1 of the Supporting Information). In contrast to the results in simian kidney (COS-7) cells,⁸ in which the MF in the TG^{Fapy}T sequence was 4-fold higher compared to that in the TG^{Fapy}A sequence, the MF in HEK293T cells was only ~75% higher in the TG^{Fapy}T sequence than in the TG^{Fapy}A sequence. It should be noted, however, that the DNA sequence contexts beyond the immediate neighbors of the lesions in the work in COS-7 cells were different from those used in this study.

Given the recognized role of pol λ in error-free bypass of 8-oxodGuo,^{21,24} we compared Fapy-dG and 8-oxodGuo mutagenesis in pol λ knockdown HEK293T cells. We chose the TG*T and TG*G sequences, because in the TG*T sequence Fapy-dG was more mutagenic than 8-oxodGuo, whereas in the TG*G sequence, the reverse was true (Figure 1). The location of 8-oxodGuo in the TG*G sequence is also of interest because of the diverse mutational spectrum observed in this sequence context, which included a high frequency of G → A mutations

and a moderate frequency of dinucleotide deletions (Figure 1). The control for this experiment was HEK293T cells treated with negative control (NC) siRNA (Figure 2), which had essentially no effect on mutagenesis and showed results very consistent with those induced by the lesions in untreated HEK293T cells (Figure 1).

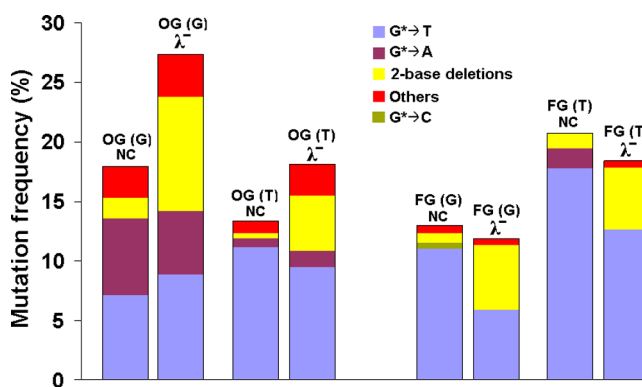


Figure 2. Mutational frequency of 8-oxodGuo (OG) and Fapy-dG (FG) in HEK293T cells also transfected with NC siRNA or siRNA for pol λ knockdown. HEK293T cells were plated in six-well plates at 25% confluence. After a 24 h incubation period, they were transfected with appropriate siRNA (NC or pol λ) and Lipofectamine. One day before the transfection of the plasmid, cells were seeded in 24-well plates at 50% confluence. Cells were then cotransfected with another aliquot of siRNA and either the control or the lesion-containing plasmid. After a 24 h incubation, progeny plasmids were isolated and analyzed as described previously.²⁵ The data represent the average of two independent experiments (shown in Table S2 of the Supporting Information). Others include semitargeted mutations near the lesion as indicated in Table S2 of the Supporting Information.

For 8-oxodGuo, the total MF increased in both sequence contexts upon knockdown of pol λ . In the TG^{8-oxo}T sequence, the MF increased from 13 to 18%, and in the TG^{8-oxo}G sequence, it increased from 18 to 27% (Figure 2 and Table S2 of the Supporting Information). This suggests that pol λ plays a role in the error-free bypass of 8-oxodGuo, as reported in murine and human cells.²⁴ It is noteworthy that 8-oxodGuo-induced G → T transversions were not significantly affected upon knockdown of pol λ . Instead, a large increase in the level of dinucleotide deletions occurred in pol λ knockdown cells. It suggests, therefore, that pol λ prevents dinucleotide deletions induced by 8-oxodGuo. In contrast, MF of Fapy-dG in the TG^{Fapy}T sequence decreased only marginally from 20.9 ± 0.9 to 17.5 ± 0.6% (Figure 2 and Table S2 of the Supporting Information). In the TG^{Fapy}G sequence, the MF of Fapy-dG was nearly unaltered (i.e., changed from 12.8 ± 1.3 to 11.4 ± 1.6%) (Table S2 of the Supporting Information). In striking divergence to the results from replication of the 8-oxodGuo construct, the G → T mutations induced by Fapy-dG decreased dramatically in the progeny from 11 to 6% in the TG^{Fapy}G construct and from 18 to 13% in the TG^{Fapy}T construct (Figure 2). This suggests a role for pol λ in part of Fapy-dG-induced G → T mutations. Even though structural studies are necessary to provide insight into the mechanism of these mutations, at least two different base-pairing modes of Fapy-dG with dA are possible (Figure 3). On the basis of the 8-oxodGuo mutagenic mechanism,^{9,27} *syn* Fapy-dG can generate Hoogsteen pairing with *anti* dA (Figure 3B). Alternatively, *anti* Fapy-dG can pair with *anti* dA (Figure 3D) as reported at the active site of Bst Pol

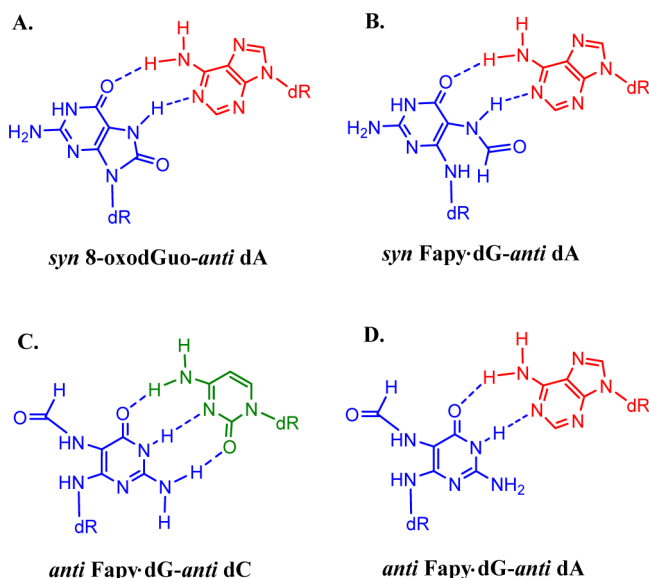


Figure 3. Similar to mispairing of *syn* 8-oxodGuo with *anti* dA (as shown in panel A),^{9,27} the base pairing of *syn* Fapy-dG may occur with *anti* dA (B). The postulated base pairing modes of *anti* Fapy-dG with *anti* dC (C) for error-free and with *anti* dA (D) for mutagenic bypass are also shown. The *anti* Fapy-dG base pairing with *anti* dA (as shown in panel D) was observed at the active site of *Bst* Pol I.¹⁰

I.¹⁰ However, currently there is no experimental evidence to specify how pol λ might be involved in G \rightarrow T mutations during TLS of Fapy-dG.

A common observation upon replication of vectors containing 8-oxodGuo and Fapy-dG in pol λ knockdown cells was the large increase in the level of dinucleotide deletions, which involved the lesion-containing G and its 3' or 5' neighboring base. For 8-oxodGuo, the level of dinucleotide deletions increased from 0.4 to 4.3% in the TG^{8-oxo}T sequence and from 1.9 to 8.9% in the TG^{8-oxo}G sequence in pol λ knockdown cells (Figure 2 and Table S2 of the Supporting Information). For Fapy-dG, likewise, the level of dinucleotide deletions increased from 1.3 to 5.8% in the TG^{Fapy}T sequence and from 0.9 to 5.2% in the TG^{Fapy}G sequence in pol λ knockdown cells (Figure 2 and Table S2 of the Supporting Information). The polymerase responsible for the dinucleotide deletions was not identified. However, a 4-fold increase in the level of small deletions by 8-oxodGuo was reported in HeLa cells in which pol λ was knocked down.²⁴

Though a similar study with Fapy-dG in human cells has not been performed, replication of the related MeFapy-dG (Figure 4), which contains a methyl group at position 5, in simian kidney cells resulted in a significant frequency of dinucleotide deletions in the TXT sequence, even in the presence of all functional pols.²⁸

In conclusion, the results of this study established a fundamental difference between the mechanism of TLS of 8-oxodGuo and that of Fapy-dG in human cells in that pol λ is

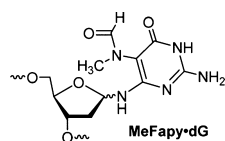


Figure 4. Structure of MeFapy-dG.

responsible for a fraction of G \rightarrow T mutations induced by Fapy-dG, whereas it conducts predominantly error-free bypass of 8-oxodGuo.

■ ASSOCIATED CONTENT

Supporting Information

Mutation data of 8-oxodGuo and Fapy-dG in TG*N sequences in HEK293T cells (Table S1) and in pol λ knockdown cells (Table S2) and reverse transcription polymerase chain reaction efficiency of pol λ knockdown (Figure S1). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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