Title

The Impact of Minor-Groove N2-Alkyl-2-deoxyguanosine Lesions on DNA Replication in Human Cells.

Journal

ACS Chemical Biology, 14(8)

Authors

Wu, Jun
Du, Hua
Li, Lin
et al.

Publication Date

2019-08-16

DOI

10.1021/acschembio.9b00129

Peer reviewed

HHS Public Access

Author manuscript

ACS Chem Biol. Author manuscript; available in PMC 2020 August 16.

Published in final edited form as:

ACS Chem Biol. 2019 August 16; 14(8): 1708–1716. doi:10.1021/acschembio.9b00129.

The Impact of Minor-Groove N^2–Alkyl-2′-deoxyguanosine Lesions on DNA Replication in Human Cells

Jun Wu, **Hua Du**, **Lin Li**, **Nathan E. Price**, **Xiaochuan Liu**, **Yinsheng Wang***

Department of Chemistry, University of California, Riverside, California 92521-0403, United States

Abstract

Endogenous metabolites and exogenous chemicals can induce covalent modifications on DNA, producing DNA lesions. The N^2 of guanine was shown to be a common alkylation site in DNA; however, not much is known about the influence of the size of the alkyl group in N^2-alkyldG lesions on cellular DNA replication or how translesion synthesis (TLS) polymerases modulate DNA replication past these lesions in human cells. To answer these questions, we employ a robust shuttle vector method to investigate the impact of four N^2-alkyldG lesions (i.e., with the alkyl group being a methyl, ethyl, *n*-propyl, or *n*-butyl group) on DNA replication in human cells. We find that replication through the N^2-alkyldG lesions was highly efficient and accurate in HEK293T cells or isogenic CRISPR-engineered cells with deficiency in polymerase (Pol) ζ or Pol η. Genetic ablation of Pol ι, Pol κ, or Rev1, however, results in decreased bypass efficiencies and elicits substantial frequencies of G → A transition and G → T transversion mutations for these lesions. Moreover, further depletion of Pol ζ in Pol κ- or Pol ι-deficient cells gives rise to elevated rates of G → A and G → T mutations and substantially decreased bypass efficiencies. Cumulatively, we demonstrate that the error-free replication past the N^2-alkyldG lesions is facilitated by a specific subset of TLS polymerases, and we find that longer alkyl chains in these lesions induce diminished bypass efficiency and fidelity in DNA replication.

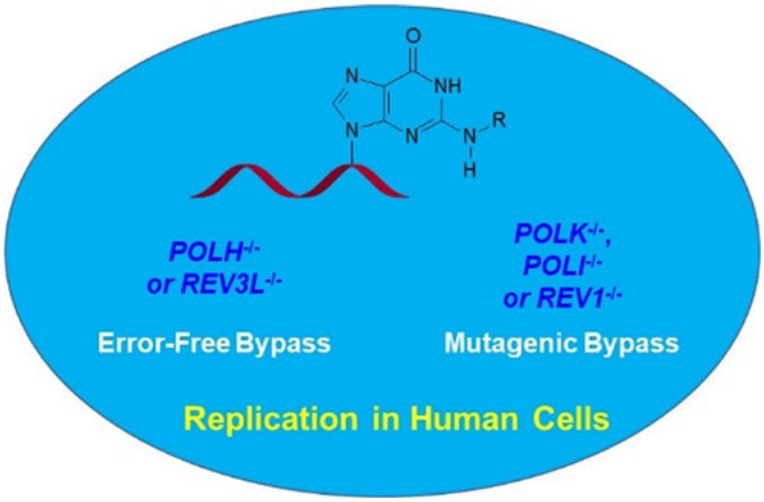
Graphical Abstract

***Corresponding Author:** Tel: (951) 827-2700. Fax: (951) 827-4713. Yinsheng.Wang@ucr.edu.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschem-bio.9b00129. Genotyping of CRISPR-Cas9 knockout cells; LC-MS and MS/MS results for the synthetic N^2-alkyldG-containing ODNs; LC-MS, MS/MS, and PAGE for analyzing the restriction fragments for PCR amplicons for replication products of lesion-containing genomes (PDF)

The authors declare no competing financial interest.



Alkylating agents are produced from endogenous metabolism and are ubiquitously found in the environment.[1,2] Exposure to these agents leads to the formation of DNA lesions, which may challenge genomic stability by impeding DNA replication and transcription and eliciting mutations.[1–3]

A large variety of DNA lesions are formed on 2′-deoxyguanosine (dG) in DNA. Many alkylating agents, apart from conjugating with the *N*7 and O^6 positions of guanine, can react with the N^2 of guanine.[1,2] In this vein, benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), formed from metabolic activation of benzo[*a*]pyrene, reacts predominantly with the N^2 of dG.[4] N^2-dG adducts are also produced by chemotherapeutic agents, including mitomycin C (MC) and its metabolite.[5,6] In addition, the N^2 of guanine is susceptible to reaction with formaldehyde, which could be induced endogenously, and acetaldehyde, a chemical that can be produced endogenously from ethanol metabolism or lipid peroxidation, and is also present in external sources, including diesel exhaust, cigarette smoke, etc.[7,8] Moreover, methylglyoxal, a glycolysis byproduct,[9] could modify DNA to yield the stable N^2-(1-carboxyethyl)-2′-deoxyguanosine (N^2-CEdG).[10–12]

To mitigate the adverse consequences of DNA adducts, cells have multiple DNA repair pathways to remove DNA lesions.[13] As part of the DNA damage tolerance mechanism, cells also employ translesion synthesis (TLS) DNA polymerases to assist the replication bypass of those lesions that normally block replicative DNA polymerases.[14] In this respect, polymerase κ and its orthologs in other organisms have been shown to promote the accurate bypass of various N^2-dG adducts, including those carrying simple alkyl groups,[15] carboxyalkyl groups,[11,16] a furfuryl functionality,[17] an N^2–N^2 guanine interstrand cross-link,[18] an acrolein-derived peptide cross-link,[19] or a bulky BPDE remnant.[20] Moreover, Pol ι, a unique TLS polymerase which can accommodate a non-Watson–Crick base pair into its active site,[21] is needed for the error-free replication across N^2-CEdG lesions in mammalian cells.[16]

In the present study, we, by utilizing a highly robust shuttle-vector-based method,[22] investigated systematically how the alkyl group size in the N^2-alkyldG lesions affects DNA

replication efficiency and accuracy in human cells. We also assessed how replication through these DNA lesions is influenced by genetic ablation of TLS DNA polymerases.

METHODS

Materials.

All reagents, if not specifically described, were purchased from Thermo Fisher Scientific or Sigma-Aldrich. Solid-phase DNA synthesis reagents, unmodified oligodeoxyribonucleotides (ODNs), enzymes, and [γ–^{32}P]ATP were obtained from Glen Research Co. (Sterling, VA), Integrated DNA Technologies (Coralville, IA), New England Biolabs (Ipswich, MA), and PerkinElmer (Piscataway, NJ), respectively. HEK293T cells with *POLI*, *POLH*, *POLK*, and *REV3L* genes being individually ablated by CRISPR were described previously.[23] The isogenic cells with single depletion of *REV1*, or dual depletion of *POLK* and *POLI*, *POLK* and *REV3L*, or *POLI* and *REV3L*, were produced with the same method. The successful depletion of these genes was confirmed by Sanger sequencing analysis and, for *REV1*, by Western blot (Figure S1). The following guide sequences were employed in the current study, where the underlined sequences indicate the PAM motif:

Human *REV1* (Rev1): ATCAGATGCTGCTATGCAGAAGG.

Human *POLK* (Pol κ): ATCCATGTCAATGTGCACTATGG.

Human *REV3L* (Pol ζ): AATGAGCCAACCTGAGTCACAAG.

Western Blot.

The Western blot experiments were conducted using approximately 30 μg of protein lysate. Antibodies for human REV1 and GAPDH and the goat secondary antimouse antibody conjugated with horseradish peroxidase were procured from Santa Cruz Biotechnology, and the dilution factors for the three antibodies were 1:1000, 1:50000, and 1:2000, respectively.

ODN Synthesis.

The 12-mer ODNs carrying an N^2-MedG, N^2-EtdG, N^2-*n*PrdG, or N^2-*n*BudG at a specific site were synthesized following a previously reported convertible nucleotide approach,[24,25] where the fluorine atom in 2-fluoro-2′-deoxyinosine in ODNs was substituted with the corresponding alkylamines (Figure 1b). The identities of the 12-mer ODNs containing the four N^2-alkyldG lesions were confirmed by liquid chromatography-mass spectrometry (LC-MS) and tandem MS (MS/MS) experiments (Figures S2–S5), following published procedures.[26]

Plasmid Construction, Replication, Progeny Isolation, and Replication Product Analysis.

The methods for the construction of the lesion-containing plasmids were described in detail elsewhere,[27] except that the aforementioned 12-mer N^2-alkyldG-containing ODNs were employed as the lesion-containing insert. Cellular replication and progeny isolation, PCR amplification, and polyacrylamide gel electrophoressis (PAGE) as well as LC-MS/MS analysis were carried out as previously described,[27] with the exception that the primer

employed for strand-specific PCR was the same as what was used previously for the replication studies of 8,5′-cyclo-2′-deoxyguanosine-containing DNA.[22]

RESULTS AND DISCUSSION

We set out to investigate the degrees to which the efficiency and fidelity of DNA replication in human cells are perturbed by N^2-alkyldG lesions carrying different sizes of alkyl groups, where we considered four lesions, with the alkyl groups being a methyl (N^2-MedG), ethyl (N^2-EtdG), *n*-propyl (N^2-*n*PrdG), and *n*-butyl (N^2-*n*BudG) (Figure 1). These lesions were chosen on the basis of the fact that exposure to various environmental carcinogens and some anticancer agents can give rise to covalent attachment of different alkyl groups on the N^2 position of dG, as described above.

We first prepared several ODNs housing an N^2-alkyldG adduct at a specific site (Figure 1), validated the identities of these ODNs by ESI-MS and MS/MS (Figures S2–S5), and ligated them into double-stranded shuttle vectors (Figure 2). A damage-free competitor genome, which contained three additional nucleotides, was used as an internal reference to correct for the variations in transfection efficiencies among different experiments. After cellular replication, all plasmid genomes were extracted from cells, and the residual parental plasmids were eliminated by DpnI and exonuclease III digestion. The sequence segment of interest in the extracted progeny plasmids was subsequently amplified by strand-specific PCR (Figure 2b).[22] The resulting PCR products were subjected to NcoI and SfaNI cleavage (Figure 3a), and the digestion products were analyzed by LC-MS/MS and PAGE (Figures 3 and 4 and Figures S6–S8).

The Effects of N^2-alkyldG Lesions on the Perturbation of DNA Replication Rate in HEK293T Cells.

We found that increasing size of the N^2-alkyl group elicits subtle differences in the efficiency of replicative bypass in parental HEK293T cells, where all four lesions were bypassed at high efficiencies (60–80%) (Figure 5a). We also investigated how individual or combined ablation of TLS DNA polymerases influences the bypass of these lesions in HEK293T cells. We observed that single ablation of Pol η or Pol ζ did not alter the bypass efficiencies of the N^2-alkyldG lesions (Figure 5a). This result suggests that, in the presence of other TLS polymerases, Pol η and Pol ζ do not assume appreciable roles in bypassing the N^2-alkyldG lesions. Depletion of Pol ι, on the other hand, caused significant drops (to ~38–50%) in the bypass rates for all of the N^2-alkyldG lesions (Figure 5a), and depletion of Pol κ also gave rise to significant diminutions in bypass efficiencies for N^2-MedG and N^2-EtdG (Figure 5a). To investigate whether Pol ι and Pol κ act cooperatively or independently, we explored the impact of their concurrent ablation on the bypass efficiencies of the N^2-alkyldG lesions. Our results showed that their codepletion led to bypass efficiencies that are similar to what were observed for cells depleted of Pol ι alone, suggesting that Pol ι and Pol κ act cooperatively when bypassing these N^2-alkyldG lesions.

We next examined how combined depletion of Pol ζ, which was previously found to function during the extension step of TLS,[28,29] modulates the efficiencies of replication across the N^2-alkyldG lesions in the HEK293T cells depleted of Pol κ or Pol ι. We observed

that simultaneous removal of Pol ζ and Pol ι (Figure S1), or codepletion of Pol κ and Pol ζ, conferred significant reductions in bypass rates (to ~13–29 and 16–33%, respectively). Together, these data show that Pol ζ supports the TLS of the N^2-alkyldG lesions in cells depleted of Pol κ or Pol ι.

Previous studies showed that DNA polymerase Rev1 played an indispensable scaffolding role for the recruitment of TLS polymerases in the Y-family, including Pol η, Pol ι, and Pol κ.[30,31] Hence, we generated Rev1-deficient HEK293T cells by CRISPR-Cas9, confirmed the successful knockout of *REV1* gene by Sanger sequencing and Western blot analysis (Figure S1), and explored the function of this polymerase in the replication across the N^2-alkyldG lesions. As displayed in Figure 5a, we observed a substantial decline in the bypass rates for the N^2-alkyldG lesions upon loss of Rev1 (~13–36%), underscoring the important function of Rev1 in supporting the replication through these lesions in human cells.

The Effects of N^2-alkyldG Lesions on the Perturbation of DNA Replication Accuracy in HEK293T Cells.

We determined the identities of the mutagenic replication products based on PAGE and LC-MS/MS analyses of the restriction digestion products of PCR amplicons from the isolated progeny plasmids (Figures 3 and 4 and Figures S6–S8). No mutagenic products were found for the N^2-alkyldG lesions in parental HEK293T cells (Figure 3). Depletion of Pol η or Pol ζ did not compromise the fidelity of replication past the N^2-alkyldG lesions (Figure S8), which is consistent with the corresponding replication bypass data described above.

Appreciable rates of G → T and G → A mutations were observed for all N^2-alkyldG lesions, in all other polymerase-deficient backgrounds examined (Figure 5b,c, Figure 6). In this vein, G → A transition was detected at frequencies of ~10–25% in Pol ι- or Pol κ-deficient cells, accompanied by low frequencies (~4–15%) of G → T substitution. Thus, dTMP and, to a lesser extent, dAMP are misincorporated opposite the N^2-alkyldG lesions. This result also reveals that, when in the absence of Pol κ or Pol ι, other TLS polymerases may assume a back-up role in bypassing the N^2-alkyldG lesions, albeit at the expense of poorer fidelity. This finding is in line with the prior observations that purified human Pol κ and Pol ι could catalyze accurate and efficient nucleotide incorporation opposite N^2-dG adducts.[15,16] Further ablation of Pol ζ in Pol ι- or Pol κ-depleted cells led to significant increases in G → A mutations for all four lesions relative to single ablation of Pol ι or Pol κ (Figure 5b,c, Figure 6b,c). Significant elevations in G → T mutations were also found for N^2-*n*PrdG upon deletion of Pol ζ in Pol κ-depleted cells and for N^2-MedG and N^2-*n*BudG upon removal of Pol ζ in Pol ι-depleted cells (Figure 5b,c and Figure 6b,c). Together, Pol κ and Pol ι are likely the major polymerases involved in the accurate nucleotide insertion opposite the N^2-alkyldG lesions during TLS in HEK293T cells. Loss of Rev1 also led to substantial rates of G → A transition (to ~11–20%) and G → T transversion (~4–11%) mutations (Figure 5b,c), which perhaps can be attributed to the scaffolding role of Rev1 in assembling Y-family polymerases(i.e., Pol ι and Pol κ) during TLS of the N^2-alkyldG lesions.[30,31]

In all genetic backgrounds where we observed mutagenic bypass of the N^2-alkyldG lesions, the G → A and G → T mutation rates increased with the alkyl group size. For example, the

frequencies for the G → A and G → T mutations in Pol ι-depleted cells range from ~10% for N^2-MedG to ~17% for N^2-*n*BudG and from ~7% for N^2-MedG to ~13% for N^2-*n*BudG, respectively (Figure 5b,c, Figure 6b,c). The elevated mutation frequencies are associated with diminished bypass efficiencies of these lesions. Thus, longer alkyl chains elicit lower bypass rate and decreased fidelity during replication past the N^2-alkyldG lesions in human cells.

Many different types of DNA lesions are induced in cells upon alkylating agent exposure,[2,32] and N^2-dG adducts are of particular interest owing to the relatively poor efficiency in repair of these minor-groove lesions.[33] However, the mutagenicity and cytotoxicity of the alkylated N^2-dG lesions or the involvement of TLS DNA polymerases in supporting the replication across the N^2-alkyldG lesions in human cells has not been systematically investigated.

Here, we systematically investigated the cytotoxicity and mutagenicity of N^2-alkyldG lesions in HEK293T cells and CRISPR/Cas9-engineered cells with individual or combined depletion of TLS polymerases. We observed that alkyl groups located at the N^2 of guanine did not significantly impede DNA replication and no mutagenic products were found in parental HEK293T cells that are proficient in TLS, or the isogenic cells with depletion of Pol ζ or Pol η. We also found that the efficiencies and fidelities of DNA replication across the N^2-alkyldG lesions are significantly reduced in cells with Pol ι and Pol κ being ablated individually or in combination (Figures 5 and 6). Our results are consistent with the data from a number of previous *in vitro* biochemical studies showing the capability of DNA Pol κ in preferentially incorporating the correct dCMP opposite N^2-dG adducts.[11,15–20] For instance, Choi et al.[15] found that the ratios for misincorporating dGMP and dTMP (relative to the insertion of the correct dCMP) opposite N^2-MedG and N^2-EtdG were less than 0.01. This can be attributed to the unique active site structure of Pol κ, where an opening toward the DNA minor groove could accommodate readily the alkyl groups conjugated with the N^2 of the template guanine in the active site.[34,35] Hence, our results provide important *in vivo* evidence to substantiate the conclusion that N^2-dG adducts constitute cognate lesions for DinB DNA polymerases.[11,17,36]

Pol ι-catalyzed nucleotide incorporation across the N^2-CEdG lesions was previously shown to be both accurate and efficient,[16] and here we revealed that Pol ι promotes the accurate replicative bypass of N^2-alkyldG lesions in human cells. Depletion of Pol ζ in Pol ι- or Pol κ-deficient background induced a further diminution in bypass efficiencies (Figure 5a). Pol ζ is known to extend proficiently from the DNA strand after nucleotide incorporation opposite DNA lesions.[28,29] Therefore, the accurate bypass of the N^2-alkyldG lesions may proceed through incorporation of the correct dCMP opposite the lesion by Pol ι or Pol κ, followed by extension of the primer from the resulting N^2-dG:dC base pair by Pol κ or Pol ζ, thereby acting in a cooperative fashion.

In cells without Pol ι or Pol κ, the TLS of the N^2-alkyldG lesions occurred with lower fidelity and efficiency (Figure 5b,c, Figure 6). TLS mediated by Pol η might lead to mutations at the site of N^2-alkyldG lesions, which parallels what was observed recently for the bypass of N^2-CMdG and N^2-CEdG lesions in MEF cells.[16] Along this line, Pol η was

found to insert not only the correct dCMP but also the incorrect dAMP and dTMP opposite a number of N^2-alkylated dG lesions.[16,37] Removal of Rev1 led to appreciable rates of G → A and G → T substitutions (Figure 5b,c), which is in line with the previous notion that Rev1 serves as a scaffold for the recruitment of Y-family polymerases in mammalian cells.[30,31]

We also compared the findings made from the current study with what were previously observed for replication past other minor-groove DNA lesions. It was found that O^2-alkyldT lesions primarily direct T → A and T → G mutations, even in parental HEK293T cells.[27] We found here that the bypass of N^2-alkyldG lesions does not evoke mutations in parental HEK293T cells, or the isogenic cells lacking Pol ζ or Pol η. By sharp contrast, both Pol ζ and Pol η are required for the efficient bypass of the minor-groove O^2-alkyldT lesions. In addition, Pol ι and Pol κ are primarily involved in the accurate bypass of N^2-alkyldG lesions (Figure 5b,c, Figure 6b,c). Replication across 3-deaza-3-methyladenine (3-dMeA), which is a model minor-groove DNA lesion, exhibited similarities and differences from that across the N^2-alkyldG lesions.[38] In this vein, bypass of the 3-dMeA lesion was mediated by the combined action of Pol κ and Pol ι, where it was proposed that Pol ι incorporated a nucleotide across 3-dMeA, followed by Pol κ-mediated extension from the inserted nucleotide.[38] Pol ζ contributed to the extension step of TLS across the 3-dMeA lesion after nucleotide incorporation opposite the lesion catalyzed by other polymerases.[38] Different from what we observed for the N^2-alkyldG lesions, no mutagenic product was found in TLS opposite 3-dMeA in parental as well as Pol ι-, Rev1-, Pol θ-, or Pol ζ-knockdown cells, indicating highly accurate replication past this lesion in human cells.[38]

We would like to note some limitations of the shuttle-vector-based method when investigating how DNA damage modulates cellular DNA replication. First, the replication across DNA lesions situated in episomal plasmids may not fully recapitulate cellular replication where DNA is packaged into chromatin. Second, some translesion synthesis DNA polymerases were previously shown to function in DNA repair.[39–41] Hence, we cannot formally exclude the possibility that the augmented ability of the lesions to block DNA replication and induce mutations in some polymerase-deficient cells may also arise in part from the functions of these polymerases in repairing the N^2-alkyldG lesions.

In summary, our comprehensive replication study on a structurally defined group of N^2-alkyldG lesions reveals previously unrecognized effects that these minor-groove lesions exert on the efficiency and fidelity of cellular DNA replication. We demonstrate that the degrees of cytotoxicity and mutagenicity imparted by the N^2-alkyldG lesions depend on the bulkiness of the alkyl group, and Pol ι, Pol κ, and Rev1 are indispensable for the error-free bypass of these lesions. Additionally, we find that individual depletion of Pol η or Pol ζ does not appreciably affect the rate or fidelity of DNA replication across these lesions. Moreover, we reveal the cooperative roles of TLS polymerases, and the possible redundant role of Pol ζ as an extender, while bypassing these lesions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (R01 ES025121). N.E.P. was supported in part by an Institutional T32 training grant (T32 ES018827).

REFERENCES

(1). Shrivastav N, Li D, and Essigmann JM (2010) Chemical biology of mutagenesis and DNA repair: cellular responses to DNA alkylation. Carcinogenesis 31, 59–70. [PubMed: 19875697]

(2). Liu S, and Wang Y (2015) Mass spectrometry for the assessment of the occurrence and biological consequences of DNA adducts. Chem. Soc. Rev 44, 7829–7854. [PubMed: 26204249]

(3). You C, and Wang Y (2016) Mass spectrometry-based quantitative strategies for assessing the biological consequences and repair of DNA adducts. Acc. Chem. Res 49, 205–213. [PubMed: 26758048]

(4). Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, and Hainaut P (2002) Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. Oncogene 21, 7435–7451. [PubMed: 12379884]

(5). Tomasz M, Lipman R, Chowdary D, Pawlak J, Verdine GL, and Nakanishi K (1987) Isolation and structure of a covalent cross-link adduct between mitomycin C and DNA. Science 235, 1204–1208. [PubMed: 3103215]

(6). Tomasz M (1995) Mitomycin C: small, fast and deadly (but very selective). Chem. Biol 2, 575–579. [PubMed: 9383461]

(7). Cheng G, Shi Y, Sturla SJ, Jalas JR, McIntee EJ, Villalta PW, Wang M, and Hecht SS (2003) Reactions of formaldehyde plus acetaldehyde with deoxyguanosine and DNA: formation of cyclic deoxyguanosine adducts and formaldehyde cross-links. Chem. Res. Toxicol 16, 145–152. [PubMed: 12588185]

(8). Brooks PJ, and Theruvathu JA (2005) DNA adducts from acetaldehyde: implications for alcohol-related carcinogenesis. Alcohol 35, 187–193. [PubMed: 16054980]

(9). Thornalley PJ (1996) Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification–a role in pathogenesis and antiproliferative chemotherapy. Gen. Pharmacol 27, 565–573. [PubMed: 8853285]

(10). Frischmann M, Bidmon C, Angerer J, and Pischetsrieder M (2005) Identification of DNA adducts of methylglyoxal. Chem. Res. Toxicol 18, 1586–1592. [PubMed: 16533023]

(11). Yuan B, Cao H, Jiang Y, Hong H, and Wang Y (2008) Efficient and accurate bypass of N^2-(1-carboxyethyl)-2′-deoxyguanosine by DinB DNA polymerase *in vitro* and *in vivo*. Proc. Natl. Acad. Sci. U. S. A 105, 8679–8684. [PubMed: 18562283]

(12). Synold T, Xi B, Wuenschell GE, Tamae D, Figarola JL, Rahbar S, and Termini J (2008) Advanced glycation end products of DNA: Quantification of N^2-(1-carboxyethyl)-2′-deoxyguanosine in biological samples by liquid chromatography electrospray ionization tandem mass spectrometry. Chem. Res. Toxicol 21, 2148–2155. [PubMed: 18808156]

(13). Friedberg EC, Walker GC, Siede W, Wood RD, Schultz RA, and Ellenberger T (2006) DNA Repair and Mutagenesis; ASM Press, Washington, D.C.

(14). Ohmori H, Friedberg EC, Fuchs RPP, Goodman MF, Hanaoka F, Hinkle D, Kunkel TA, Lawrence CW, Livneh Z, Nohmi T, Prakash L, Prakash S, Todo T, Walker GC, Wang Z, and Woodgate R (2001) The Y-family of DNA polymerases. Mol. Cell 8, 7–8. [PubMed: 11515498]

(15). Choi JY, Angel KC, and Guengerich FP (2006) Translesion synthesis across bulky N^2-alkyl guanine DNA adducts by human DNA polymerase κ. J. Biol. Chem 281, 21062–21072. [PubMed: 16751196]

(16). Yuan B, You C, Andersen N, Jiang Y, Moriya M, O'Connor TR, and Wang Y (2011) The roles of DNA polymerases κ and ι in the error-free bypass of N^2-carboxyalkyl-2′-deoxyguanosine lesions in mammalian cells. J. Biol. Chem 286, 17503–17511. [PubMed: 21454642]

(17). Jarosz DF, Godoy VG, Delaney JC, Essigmann JM, and Walker GC (2006) A single amino acid governs enhanced activity of DinB DNA polymerases on damaged templates. Nature 439, 225–228. [PubMed: 16407906]

(18). Minko IG, Harbut MB, Kozekov ID, Kozekova A, Jakobs PM, Olson SB, Moses RE, Harris TM, Rizzo CJ, and Lloyd RS (2008) Role for DNA polymerase κ in the processing of N^2-N^2-guanine interstrand cross-links. J. Biol. Chem 283, 17075–17082. [PubMed: 18434313]

(19). Minko IG, Yamanaka K, Kozekov ID, Kozekova A, Indiani C, O'Donnell ME, Jiang Q, Goodman MF, Rizzo CJ, and Lloyd RS (2008) Replication bypass of the acrolein-mediated deoxyguanine DNA-peptide cross-links by DNA polymerases of the DinB family. Chem. Res. Toxicol 21, 1983–1990. [PubMed: 18788757]

(20). Suzuki N, Ohashi E, Kolbanovskiy A, Geacintov NE, Grollman AP, Ohmori H, and Shibutani S (2002) Translesion synthesis by human DNA polymerase κ on a DNA template containing a single stereoisomer of dG-(+)- or dG-(−)-anti-N^2-BPDE (7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]-pyrene). Biochemistry 41, 6100–6106. [PubMed: 11994005]

(21). Nair DT, Johnson RE, Prakash S, Prakash L, and Aggarwal AK (2004) Replication by human DNA polymerase-ι occurs by Hoogsteen base-pairing. Nature 430, 377–380. [PubMed: 15254543]

(22). You C, Swanson AL, Dai X, Yuan B, Wang J, and Wang Y (2013) Translesion synthesis of 8,5′-cyclopurine-2′-deoxynucleo-sides by DNA polymerases η, ι, and ζ. J. Biol. Chem 288, 28548–28556. [PubMed: 23965998]

(23). Wu J, Li L, Wang P, You C, Williams NL, and Wang Y (2016) Translesion synthesis of O^4-alkylthymidine lesions in human cells. Nucleic Acids Res. 44, 9256–9265. [PubMed: 27466394]

(24). Harris CM, Zhou L, Strand EA, and Harris TM (1991) New strategy for the synthesis of oligodeoxynucleotides bearing adducts at exocyclic amino sites of purine nucleosides. J. Am. Chem. Soc 113, 4328–4329.

(25). Cao H, Jiang Y, and Wang Y (2007) Stereospecific synthesis and characterization of oligodeoxyribonucleotides containing an N^2-(1-carboxyethyl)-2′-deoxyguanosine. J. Am. Chem. Soc 129, 12123–12130. [PubMed: 17877341]

(26). Gu C, and Wang Y (2007) In vitro replication and thermodynamic studies of methylation and oxidation modifications of 6-thioguanine. Nucleic Acids Res. 35, 3693–3704. [PubMed: 17517786]

(27). Wu J, Leng J, Wang P, Li L, and Wang Y (2018) Cytotoxic and mutagenic properties of minor-groove O^2-alkylthymi-dine lesions in human cells. J. Biol. Chem 293, 8638–8644. [PubMed: 29685891]

(28). Shachar S, Ziv O, Avkin S, Adar S, Wittschieben J, Reissner T, Chaney S, Friedberg EC, Wang Z, Carell T, Geacintov N, and Livneh Z (2009) Two-polymerase mechanisms dictate error-free and error-prone translesion DNA synthesis in mammals. EMBO J. 28, 383–393. [PubMed: 19153606]

(29). Johnson RE, Washington MT, Haracska L, Prakash S, and Prakash L (2000) Eukaryotic polymerases iota and zeta act sequentially to bypass DNA lesions. Nature 406, 1015–1019. [PubMed: 10984059]

(30). Guo C, Fischhaber PL, Luk-Paszyc MJ, Masuda Y, Zhou J, Kamiya K, Kisker C, and Friedberg EC (2003) Mouse Rev1 protein interacts with multiple DNA polymerases involved in translesion DNA synthesis. EMBO J. 22, 6621–6630. [PubMed: 14657033]

(31). Yoon JH, Park J, Conde J, Wakamiya M, Prakash L, and Prakash S (2015) Rev1 promotes replication through UV lesions in conjunction with DNA polymerases η, ι, and κ but not DNA polymerase ζ. Genes Dev. 29, 2588–2602. [PubMed: 26680302]

(32). Altshuler KB, Hodes CS, and Essigmann JM (1996) Intrachromosomal probes for mutagenesis by alkylated DNA bases replicated in mammalian cells: a comparison of the mutagenicities of O^4-methylthymine and O^6-methylguanine in cells with different DNA repair backgrounds. Chem. Res. Toxicol 9, 980–987. [PubMed: 8870985]

(33). Zewail-Foote M, Li VS, Kohn H, Bearss D, Guzman M, and Hurley LH (2001) The inefficiency of incisions of ecteinascidin 743-DNA adducts by the UvrABC nuclease and the unique structural feature of the DNA adducts can be used to explain the repair-dependent toxicities of this antitumor agent. Chem. Biol 8, 1033–1049. [PubMed: 11731295]

(34). Lone S, Townson SA, Uljon SN, Johnson RE, Brahma A, Nair DT, Prakash S, Prakash L, and Aggarwal AK (2007) Human DNA polymerase κ encircles DNA: implications for mismatch extension and lesion bypass. Mol. Cell 25, 601–614. [PubMed: 17317631]

(35). Jha V, Bian C, Xing G, and Ling H (2016) Structure and mechanism of error-free replication past the major benzo[a]pyrene adduct by human DNA polymerase kappa. Nucleic Acids Res. 44, 4957–4967. [PubMed: 27034468]

(36). Friedberg EC, Wagner R, and Radman M (2002) Specialized DNA polymerases, cellular survival, and the genesis of mutations. Science 296, 1627–1630. [PubMed: 12040171]

(37). Choi JY, and Guengerich FP (2005) Adduct size limits efficient and error-free bypass across bulky N^2-guanine DNA lesions by human DNA polymerase η. J. Mol. Biol 352, 72–90. [PubMed: 16061253]

(38). Yoon JH, Roy Choudhury J, Park J, Prakash S, and Prakash L (2017) Translesion synthesis DNA polymerases promote error-free replication through the minor-groove DNA adduct 3-deaza-3-methyladenine. J. Biol. Chem 292, 18682–18688. [PubMed: 28939775]

(39). Ogi T, and Lehmann AR (2006) The Y-family DNA polymerase κ (pol κ) functions in mammalian nucleotide-excision repair. Nat. Cell Biol. 8, 640–642. [PubMed: 16738703]

(40). McIlwraith MJ, Vaisman A, Liu Y, Fanning E, Woodgate R, and West SC (2005) Human DNA polymerase η promotes DNA synthesis from strand invasion intermediates of homologous recombination. Mol. Cell 20, 783–792. [PubMed: 16337601]

(41). Kawamoto T, Araki K, Sonoda E, Yamashita YM, Harada K, Kikuchi K, Masutani C, Hanaoka F, Nozaki K, Hashimoto N, and Takeda S (2005) Dual roles for DNA polymerase η in homologous DNA recombination and translesion DNA synthesis. Mol. Cell 20, 793–799. [PubMed: 16337602]

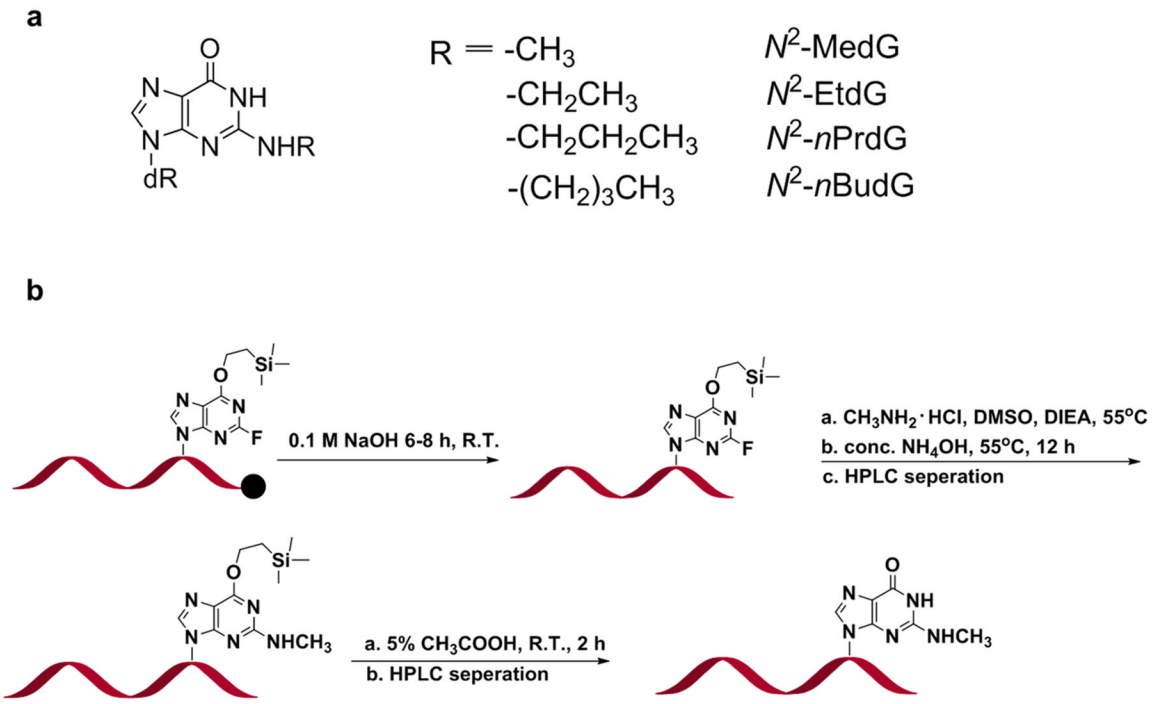


Figure 1.

N^2-alkyldG lesions examined in the present study. (a) The structures of the N^2-alkyldG lesions. (b) The post-oligomerization method for the incorporation of N^2-MedG into ODN.

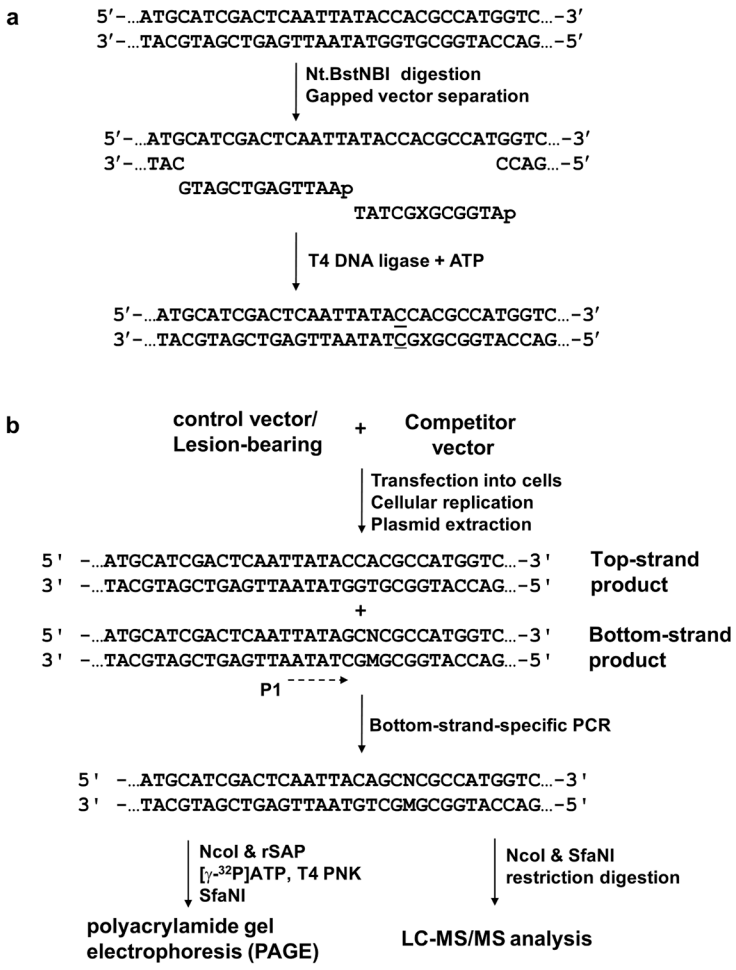


Figure 2.

Schematic diagrams outlining the procedures for the preparation of the lesion-bearing plasmids. (a) Experimental procedures for plasmid construction. The C/C mismatch site is underlined. (b) The SSPCR-CRAB assay. “P1” represents one of the PCR primers and contains a G at the 3′-terminus corresponding to the C/C mismatch site of the lesion-bearing genome. It also carries a C/A mismatch three nucleotides away from its 3′-terminus to improve the specificity of PCR. “M” and “N” designate the nucleotide incorporated at the lesion site during DNA replication and the paired nucleotide of “M” in the complementary strand, respectively.

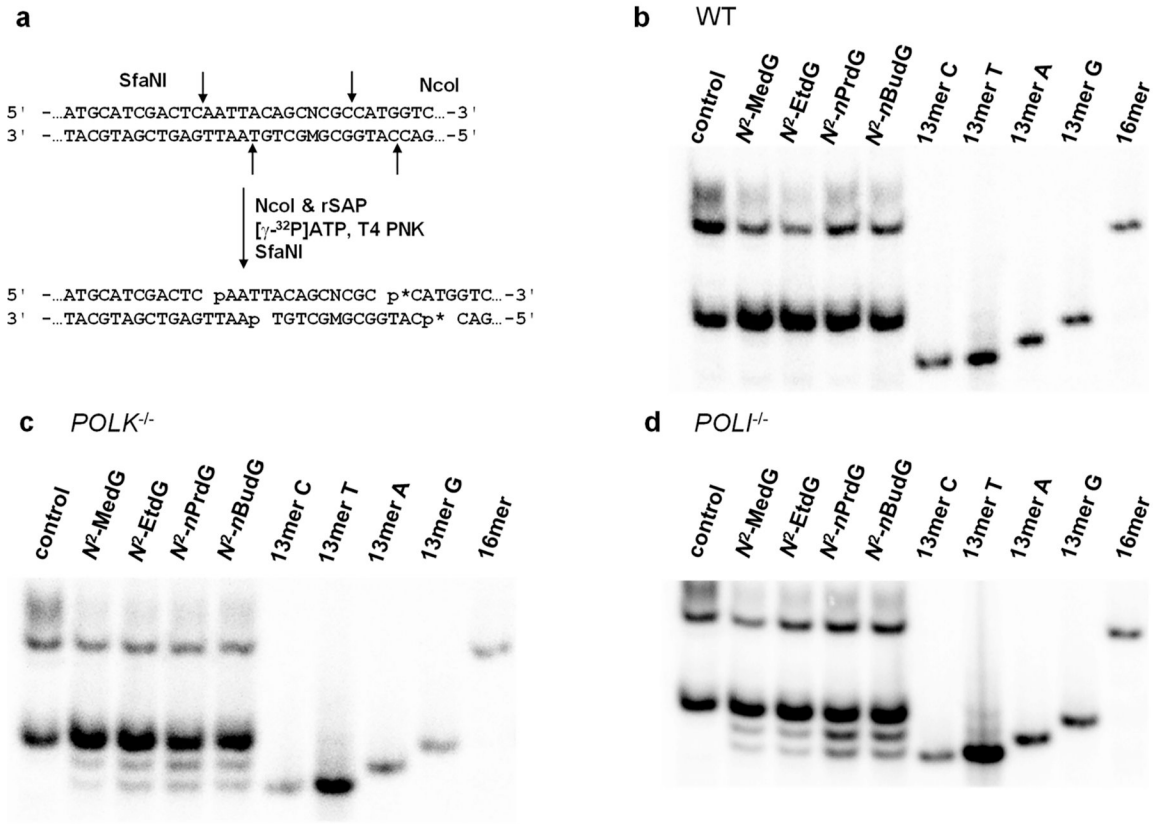


Figure 3.

Restriction digestion and post-labeling method for determining the bypass efficiencies and mutation frequencies of the N^2-alkyldG lesions in human cells. (a) Restriction digestion with NcoI and SfaNI and post-labeling assay (p* indicates a ^{32}P-labeled phosphate group). Restriction cleavage sites are designated with arrows. (b–d) Representative gel images showing the NcoI/SfaNI-produced restriction fragments of PCR amplicons from replication products isolated from HEK293T cells or the isogenic cells depleted of Pol κ or Pol ι. The restriction fragment arising from the competitor vector, i.e., d(CATGGCGATATGCTGT), is designated as “16mer Comp”; “13mer C”, “13mer A”, “13mer G”, and “13mer T” denote the standard synthetic ODNs d(CATGGCGNGCTGT), where “*N*” is C, A, G, and T, respectively.

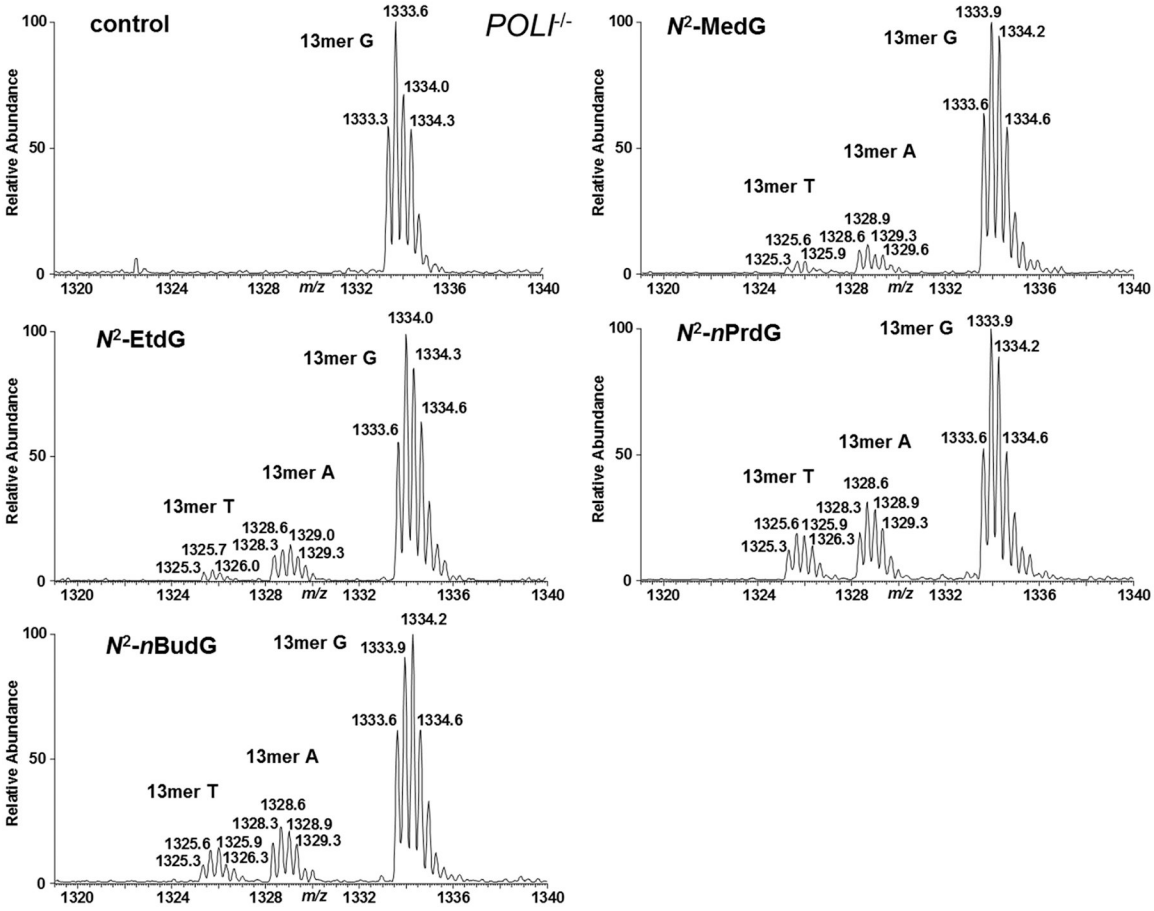


Figure 4.

Higher-resolution “zoom” scan ESI-MS for monitoring the $[M-3H]^{3-}$ ions of the lesion-containing strand of the restriction fragments of the PCR products from the replication of N^2-alkyldG lesions in HEK293T cells depleted of Pol ι. The G → A and G → T mutation products were further confirmed by MS/MS analyses, and representative MS/MS results for replication products of N^2-*n*PrdG-bearing plasmid are shown in Figure S6.

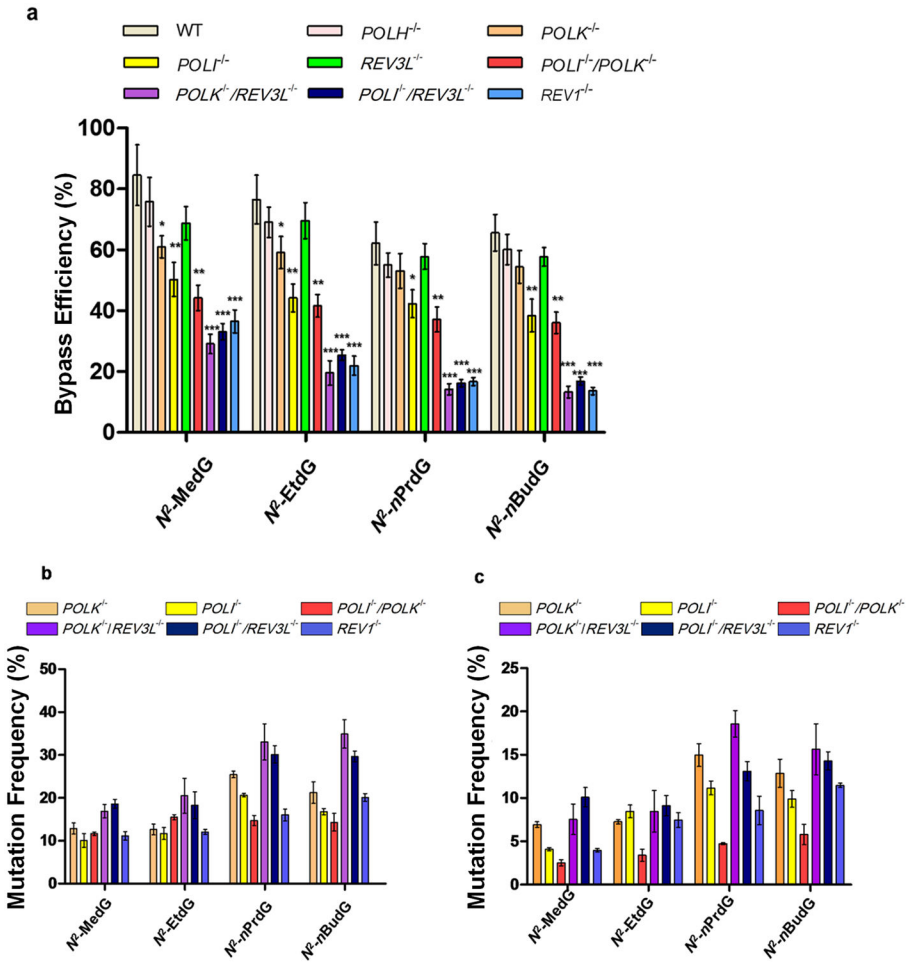


Figure 5.

Bypass efficiencies and mutation frequencies of the N^2-alkyldG lesions in HEK293T cells and the isogenic cells where the TLS polymerases were individually or simultaneously depleted by CRISPR/Cas9. Shown are the bypass efficiencies (a) and the frequencies for the G → A (b) and G → T (c) mutations observed for the N^2-alkyldG lesions. The data represent the mean and standard deviation of results acquired from three independent replication experiments. *, $0.01 \le p < 0.05$; **, $0.001 \le p < 0.01$; ***, $p < 0.001$. The p values were calculated using a two-tailed, unpaired t-test, and the values referred to the comparisons between HEK293T cells and the isogenic TLS polymerase-deficient cells.

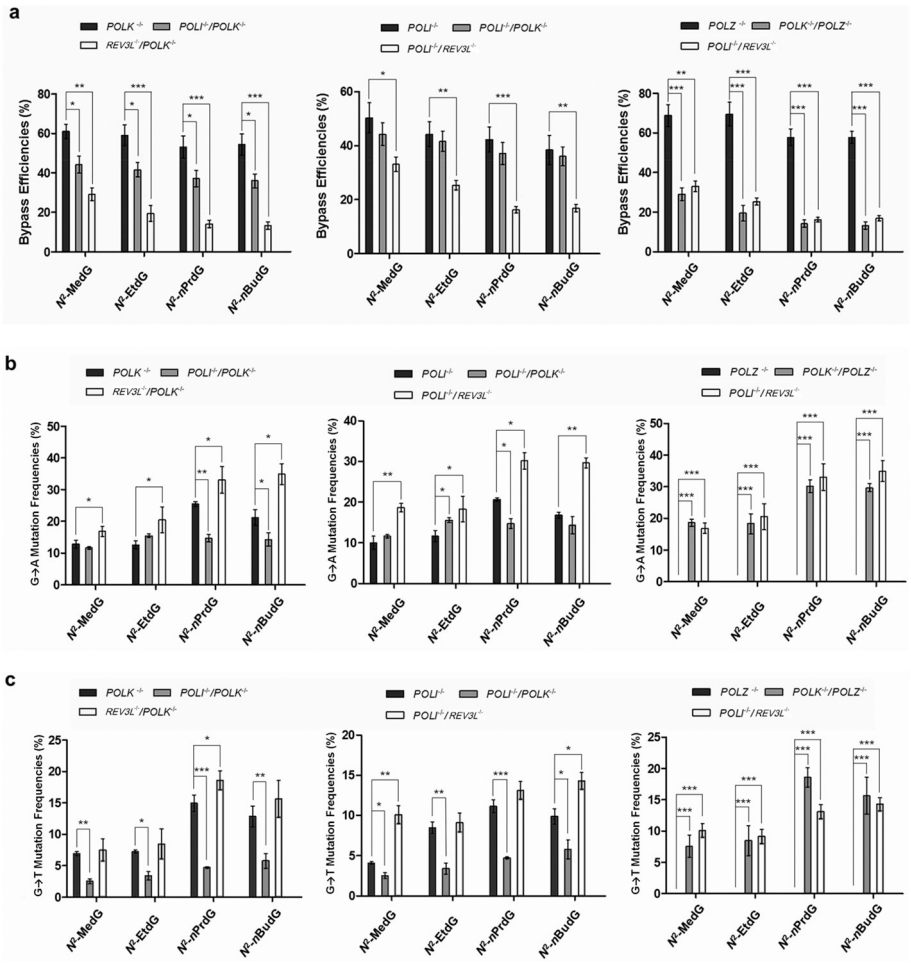


Figure 6.

Bypass efficiencies and mutation frequencies of the N^2-alkyldG lesions in HEK293T cells and the isogenic cells where the TLS polymerases were ablated individually or in combination by the CRISPR/Cas9 method. This figure is replotted from the data in Figure 5 so as to better compare the results obtained from single polymerase knockout cells with those from the corresponding double-knockout cells. Shown are the bypass efficiencies (a) and the frequencies for the G → A (b) and G → T (c) mutations observed for the N^2-alkyldG lesions. The data represent the mean and standard deviations of results from three independent replication experiments. *, $0.01 < p < 0.05$; **, $0.001 < p < 0.01$; ***, $p < 0.001$. The p values were calculated using a two-tailed, unpaired t test, and the values referred to the comparisons between wild-type and TLS polymerase deficient HEK293T cells.