Genes and Environment, Vol. 35, No. 4 pp. 105–109 (2013)

Short communication

DNA Polymerase λ Promotes Mutagenesis Induced by 8-Oxo-7,8-dihydroguanine (8-hydroxyguanine) Paired with Adenine

Hiroyuki Kamiya[1] and Masahiro Kurokawa

Graduate School of Science and Engineering, Ehime University, Ehime, Japan

Received March 27, 2013; Revised April 19, 2013; Accepted April 26 2013
J-STAGE Advance published date: July 1, 2013

DNA polymerase λ preferentially inserts dCTP opposite 8-oxo-7,8-dihydroguanine (G^O, 8-hydroxyguanine) *in vitro*, and this function is considered to be important after A base removal from G^O:A pairs by the MUTYH DNA glycosylase. However, dCTP incorporation would promote A:T→C:G transversion mutations induced by 8-oxo-7,8-dihydro-dGTP (dG^OTP), the mutation triggered by the incorporation of dG^OTP opposite A in the template DNA. In this study, double-stranded plasmid DNA containing the G^O:A pair, an intermediate in the dG^OTP mutagenesis pathway, was transfected into human cells in which DNA polymerase λ was knocked down. The knockdown of DNA polymerase λ significantly reduced the frequency of A:T→C:G transversion mutations induced by the G^O:A pair although the knockdown effect was small. These results suggested that DNA polymerase λ is involved in the mutagenesis processes of G^O, generated by dG^OTP, in the nucleotide pool.

Key words: 8-oxo-7,8-dihydroguanine, 8-hydroxyguanine, DNA polymerase λ, siRNA, knockdown

Introduction

Reactive oxygen species (ROS) are formed endogenously by normal oxygen metabolism, as well as by many environmental mutagens and carcinogens. ROS oxidize bases in both DNA and the nucleotide pool, thus producing mutagenic base damage (1). Other modifications, such as the loss of a base moiety and strand breaks, are also induced by ROS. The damage thus produced causes mutagenesis, carcinogenesis, neurodegeneration, and aging (2,3). 8-Oxo-7,8-dihydroguanine (G^O, also known as 8-hydroxyguanine) is an important modified base produced by ROS, and more than 100 G^O residues are generated per cell per day. (4–6). The G^O base can pair with both the C and A bases and is highly mutagenic in *Escherichia coli* and mammalian cells, in which it induces G:C→T:A transversion mutations (7–16). The oxidized base is also formed when dGTP is oxidized by ROS (17,18). The damaged deoxyribonucleotide, 8-oxo-7,8-dihydro-dGTP (dG^OTP), is mutagenic in living cells and specifically induces A:T→C:G transversions (19–21). This type of mutation is likely triggered by the incorporation of dG^OTP opposite A in the template DNA by DNA polymerases (pols), resulting in the formation of G^O:A base pairs (22). The incorporation of dCTP opposite G^O by DNA pols would be the next step in the mutagenesis pathway of dG^OTP. The ratio of dCTP to dATP incorporated opposite G^O is considered to be one of the determinants of dG^OTP mutagenicity.

The G^O:A base pair is recognized by the mammalian base excision repair enzyme, MUTYH DNA glycosylase, which removes the unmodified A base from the G^O:A pair (23–26). The removal of A results in gap formation, and DNA pols incorporate dCTP and dATP opposite G^O in the gap. In addition to DNA pol β, DNA pol λ, another X-family DNA pol, reportedly performs gap-filling (27,28). The nucleotide insertion opposite G^O by DNA pol λ is accurate, and dCTP is preferentially incorporated relative to dATP. However, this ''error-free'' dCTP incorporation would promote the A:T→C:G transversion mutation induced by dG^OTP. Thus, the mutagenicity of dG^OTP would be affected by both the amount and recruitment efficiency of DNA pol λ. Therefore, it would be interesting to assess the mutagenicity of G^O:A, an intermediate in the dG^OTP mutagenesis pathway, in living cells with reduced amounts of DNA pol λ.

In this study, double-stranded plasmid DNA containing the G^O:A pair was transfected into human cells, in which DNA pol λ was knocked down. We found that the knockdown of DNA pol λ reduced the frequency of A:T→C:G transversion mutations induced by the G^O:A

[1]Correspondence to: Hiroyuki Kamiya, Graduate School of Science and Engineering, Ehime University, 2-5 Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan. Tel: +81-89-927-9609, E-mail: hirokam@ehime-u.ac.jp
doi: org/10.3123/jemsge.2013.002

pair. These results suggested that DNA pol λ is involved in the mutagenesis processes of G^O, generated by the oxidation of dGTP in the nucleotide pool.

Materials and Methods

Materials: Oligodeoxyribonucleotides containing G^O and T (control) (29) were purchased from Nihon BioService (Asaka, Japan). They were synthesized and chemically phosphorylated at their 5′-ends on the support during synthesis, and were purified by HPLC, as described previously (30). Their sequences are 5′-dGCAGACTCG^{O}AAATCTGCCGTCAT-3′ (G^O-96) and 5′-dGCAGACTCTAAATCTGCCGTCAT-3′ (T-96). Other oligodeoxyribonucleotides were obtained from Sigma Genosys Japan (Ishikari, Japan) in purified forms. The Stealth Select 3 RNAi was obtained from Life Technologies (Carlsbad, CA, USA). The following RNAs were used: λ sense, 5′-AAUAGAAGCAUCCU GCUCUGCCUUG-3′; λ antisense, 5′-CAAGGCAG AGCAGGAUGCUUCUAUU-3′. Stealth RNAi Negative Control Med duplex (Life Technologies) was used as the negative control, according to the recommended GC content.

Plasmid DNAs containing G^O: The single-stranded form of pZ189-107K/402E was obtained by superinfection of *E. coli* JM109, containing pZ189-107K/402E, with the helper phage VCS-M13 (Agilent Technologies, Santa Clara, CA, USA), followed by polyethylene glycol precipitation and proteinase K treatment, as described (31–33). Double-stranded plasmid DNA containing the G^O:A pair at position 96 of the *supF* gene was constructed, essentially as described (34). Namely, the single-stranded forms of pZ189-107K/402E (5 μg, 2.9 pmol) and the G^O-96 oligodeoxyribonucleotide (16 pmol) were annealed and treated with T4 DNA pol (2 units, F. Hoffmann-La Roche, Basel, Switzerland) and T4 DNA ligase (350 units, Takara, Otsu, Japan). The DNA was treated with Dam methylase (16 units, New England Biolabs, Ipswich, MA, USA), which methylates the N^6-position of adenine in 5′-GATC-3′ sequences, to restore the bacterial methylation pattern. The control T:A plasmid was prepared by the same procedures.

Mutagenesis experiments: U2OS cells (3.0×10^4 cells) were cultured in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum, at 37°C under a 5% CO_2 atmosphere for 24 h. The siRNA against DNA pol λ (10 pmol) was introduced into U2OS cells by Lipofectamine 2000 (Life Technologies), according to the supplier's recommendations. After 24 h, the plasmid DNA containing G^O:A (100 ng, 29 fmol) was transfected with Lipofectamine (Life Technologies). After 48 h of culture, the plasmid DNA replicated in the cells was recovered by the method of Stary and Sarasin (35). The recovered plasmid DNA was treated with *Dpn*I, which recognizes 5′-GA*TC-3′ sequences (A* represents N^6-methyladenine), to digest the unreplicated plasmids maintaining the bacterial methylation pattern.

Determination of *supF* mutant frequency: The *Dpn*I-treated DNA was electroporated into *E. coli* KS40/pOF105 (36), using a Gene Pulser II transfection apparatus with a Pulse Controller II (Bio-Rad, Hercules, CA, USA). The *supF* mutant frequency was determined as the ratio of white and faint blue colonies to total colonies, on agar plates containing ampicillin, chloramphenicol, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, and isopropyl-β-D-thiogalactopyranoside (37).

Reverse transcription-quantitative PCR analysis of mRNA: Total RNA was extracted from U2OS cells with the TRIzol reagent (Life Technologies), and was treated with RNase-free DNase I (Takara) to degrade the genomic DNA in these samples. First-strand cDNA synthesis was performed on 1 μg of total RNA with an Applied Biosystems High Capacity RNA-to-cDNA Kit (Life Technologies), according to the manufacturer's instructions. Each of the mRNA transcripts was measured by quantitative PCR (qPCR) with a Bio-Rad CFX96 Real Time PCR System and EvaGreen chemistry (Bio-Rad), using the following primers: Pol λ upper, 5′-dGCCTGTGCCCTGCTCT ACTT-3′; Pol λ lower, 5′-dACTCATGCCCTTGG TTTTGG-3′. Data are expressed as the ratio to the GAPDH mRNA, which was determined using the following primers: GAPDH upper, 5′-dAACTTTGGTAT CGTGGAAGG-3′; GAPDH lower, 5′-dGTCTTCT GGGTGGCAGTGAT-3′.

Statistical analysis: Statistical significance was examined by the Student's *t*-test. Levels of $P < 0.05$ were considered to be significant.

Results

The formation of G^O:A base pairs and the incorporation of dCTP opposite G^O would occur within the mutagenesis pathway of dG^OTP (22). In this study, we constructed plasmid DNAs containing the G^O:A base pair at position 96 of the *supF* gene (38). This position was one of the A:T→C:G hot spots in the *supF* gene when dG^OTP was introduced (20,21). Plasmid DNA containing T:A at position 96 was also constructed.

Human U2OS cells expressing wild-type p53 and Rb were treated with the siRNA against DNA pol λ, and the knockdown of DNA pol λ was measured by reverse transcription-qPCR (Table 1). The siRNA treatment of U2OS cells reduced the amount of DNA pol λ mRNA. The knockdown efficiency was 62% at 24 h after siRNA introduction, which was the time point when the plasmid DNAs were transfected.

The siRNA treatment did not affect the *supF* mutant

Table 1. Amounts of DNA pol λ mRNA in knocked-down U2OS cells*

24 h	48 h	72 h
0.38 ± 0.03	0.48 ± 0.02	0.52 ± 0.01

*The amount of mRNA was measured by reverse transcription-qPCR at 24, 48, and 72 h after siRNA introduction. The amount of mRNA was normalized relative to the amount of human GAPDH mRNA present in each sample. Values relative to those in U2OS cells treated with the control siRNA are shown. Experiments were performed three times. Data are expressed as means ± SEM.

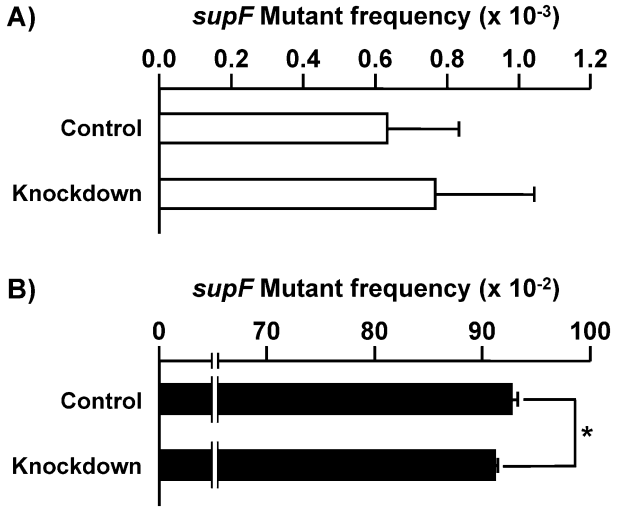

Fig. 1. Effects of the knockdown of DNA pol λ in U2OS cells on the mutant frequencies induced by G^O:A. (A) Mutant frequencies upon transfection of the control plasmid containing T:A at position 96. (B) Mutant frequencies upon transfection of the plasmid containing G^O:A at position 96. Experiments were performed three (T:A) and four (G^O:A) times. Data are expressed as means + SEM. $^*P < 0.05$ vs. control siRNA.

frequencies in the experiments using the T:A plasmid ($\sim 7 \times 10^{-4}$, Fig. 1A). When the plasmid DNA with the G^O:A pair at position 96 was introduced into U2OS cells treated with the control (Med) siRNA, the *supF* mutant frequency was 93×10^{-2} (93%), which is quite high (Fig. 1B). This high mutant frequency was due to the MUTYH protein, which removes A opposite G^O (29). The *supF* mutant frequency was decreased to 91×10^{-2} in the cells in which DNA pol λ was knocked down. Although the knockdown effect was small, it was statistically significant, suggesting that this DNA pol enhances the G^O:A-induced mutations.

We analyzed the sequences of the *supF* genes in the colonies that grew on the selection agar plates (Table 2). As expected, the A:T→C:G transversion was found in almost all of the progeny plasmid DNAs of the G^O:A plasmid. Only one colony contained the A:T→C:G mutation, at position 96 for the transfection of the control T:A plasmid (Table 2).

Table 2. Mutations at position 96, found in progeny plasmids replicated in U2OS cells with knocked-down DNA pol λ*

Mutation	T:A		G^O:A	
	Control	Knockdown	Control	Knockdown
Single base substitutions				
Transition				
A:T→G:C	0 (0)	0 (0)	0 (0)	0 (0)
Transversion				
A:T→T:A	0 (0)	0 (0)	0 (0)	1 (5)
A:T→C:G	0 (0)	1 (4)	20 (100)	19 (95)
Others	0 (0)	0 (0)	0 (0)	0 (0)
No mutation	13 (100)	22 (96)	0 (0)	0 (0)
Total colonies analyzed	13 (100)	23 (100)	20 (100)	20 (100)

*All data are represented as cases found (%).

Discussion

When DNA is directly oxidized, G^O:A base pairs are formed after dATP insertion opposite G^O during DNA replication. In this situation, the removal of the A base by MUTYH and the subsequent incorporation of dCTP by DNA pol λ provide a useful defense system against the oxidized base. However, this DNA repair pathway leads to the formation of a G^O:C base pair, at the site where dG^OTP is incorporated opposite A. Thus, DNA pol λ is expected to promote A:T→C:G mutations. Indeed, the knockdown of DNA pol λ decreased the frequency of A:T→C:G mutation caused by the G^O:A base pair at position 96, which was used as a dG^OTP mutagenesis pathway intermediate in this study (Fig. 1B). Therefore, this DNA pol would enhance the frequency of dG^OTP-induced A:T→C:G mutations. The decreased A:T→C:G mutation by the DNA pol λ knockdown indicated that at least one other DNA pol incorporates dATP opposite G^O. DNA pol β reportedly performs gap-filling, and inserts dATP more frequently than DNA pol λ (28). Thus, if DNA pol β conducted the gap-filling, then the A:T→C:G mutation rate would be suppressed. Meanwhile, the knockdown of DNA pol η slightly decreased the A:T→C:G transversion frequency in human cells (38). The results obtained in the previous and present studies indicated that two translesion synthesis DNA pols, λ and η, in addition to DNA pol β, are involved in the pathway generating the A:T→C:G transversion mutations induced by G^O:A pairs.

As shown in Table 1, the knockdown efficiency was 62% at 24 h after siRNA introduction, which was the time point when the plasmid DNAs were transfected. The *supF* mutant frequency was 93×10^{-2} and 91×10^{-2} in the cells treated with the control and anti DNA pol λ siRNAs, respectively, when the plasmid DNA with the G^O:A pair at position 96 was introduced (Fig. 1). The value (91×10^{-2}) would reflect contribution of DNA pol λ that was present in the knockdown cells. Thus, more evident knockdown effect would be expected when more

effective knockdown conditions would be found.

A possible defense system against the G^O:A pair, formed by dG^OTP incorporation, is the removal of the G^O base paired with A. In *E. coli* cells, endonuclease III (Nth) performs this function, and the mutagenicity of dG^OTP is higher in *nth* cells than wild type cells (39). However, the knockdown of the human counterpart, the NTH1 protein, did not affect either dG^OTP- or G^O:A-caused mutations (29). Further studies will be needed to examine whether human/mammalian cells contain this type of enzyme(s).

In conclusion, the knockdown of DNA pol λ decreased the mutagenicity of G^O paired with A in human U2OS cells. This result suggested that DNA pol λ is involved in the mutagenesis processes of G^O, generated by dGTP oxidation in the nucleotide pool. In addition to the DNA pols, other cellular enzymes play crucial roles in the mutagenesis processes of damaged DNA and DNA precursors (40). Experiments to reveal their roles are currently in progress.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgments: We thank Professor Satoru Suzuki and Ms. Mitsuko Ogo for assistance with the reverse transcription-qPCR experiments. This work was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Takeda Science Foundation.

References

1 Kamiya H. Mutagenic potentials of damaged nucleic acids produced by reactive oxygen/nitrogen species: approaches using synthetic oligonucleotides and nucleotides: survey and summary. Nucleic Acids Res. 2003; 31: 517–31.
2 Halliwell B, Aruoma OI. DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. FEBS Lett. 1991; 281: 9–19.
3 Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA. 1993; 90: 7915–22.
4 Lindahl T. Instability and decay of the primary structure of DNA. Nature. 1993; 362: 709–15.
5 Kamiya H. Mutagenicities of 8-hydroxyguanine and 2-hydroxyadenine produced by reactive oxygen species. Biol Pharm Bull. 2004; 27: 475–9.
6 Kasai H, Kawai K, Li Y. Analysis of 8-OH-dG and 8-OH-Gua as biomarkers of oxidative stress. Genes Environ. 2008; 30: 33–40.
7 Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. Nature. 1991; 349: 431–4.
8 Kamiya H, Sakaguchi T, Murata N, Fujimuro M, Miura H, Ishikawa H, et al. *In vitro* replication study of modified bases in *ras* sequences. Chem Pharm Bull. 1992; 40: 2792–5.
9 Wood ML, Dizdaroglu M, Gajewski E, Essigmann JM. Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. Biochemistry. 1990; 29: 7024–32.
10 Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA. 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G→T and A→C substitutions. J Biol Chem. 1992; 267: 166–72.
11 Moriya M, Grollman AP. Mutations in the *mutY* gene of *Escherichia coli* enhance the frequency of targeted G:C→T:A transversions induced by a single 8-oxoguanine residue in single-stranded DNA. Mol Gen Genet. 1993; 239, 72–6.
12 Wagner J, Kamiya H, Fuchs RPP. Leading versus lagging strand mutagenesis induced by 7,8-dihydro-8-oxo-2′-deoxyguanosine in *E. coli*. J Mol Biol. 1997; 265: 302–9.
13 Kamiya H, Miura K, Ishikawa H, Inoue H, Nishimura S, Ohtsuka E. c-Ha-*ras* containing 8-hydroxyguanine at codon 12 induces point mutations at the modified and adjacent positions. Cancer Res. 1992; 52: 3483–5.
14 Moriya M. Single-stranded shuttle phagemid for mutagenesis studies in mammalian cells: 8-oxoguanine in DNA induces targeted G·C→T·A transversions in simian kidney cells. Proc Natl Acad Sci USA. 1993; 90: 1122–6.
15 Kamiya H, Murata-Kamiya N, Koizume S, Inoue H, Nishimura S, Ohtsuka E. 8-Hydroxyguanine (7,8-dihydro-8-oxoguanine) in hot spots of the c-Ha-*ras* gene: effects of sequence contexts on mutation spectra. Carcinogenesis. 1995; 16: 883–9.
16 Le Page F, Margot A, Grollman AP, Sarasin A, Gentil A. Mutagenicity of a unique 8-oxoguanine in a human Ha-*ras* sequence in mammalian cells. Carcinogenesis. 1995; 16: 2779–84.
17 Murata-Kamiya N, Kamiya H, Muraoka M. Kasai H. Comparison of oxidation products from DNA components by γ-irradiation and Fenton-type reactions. J Radiat Res. 1997; 38: 121–31.
18 Pursell ZF, McDonald JT, Mathews CK, Kunkel TA. Trace amounts of 8-oxo-dGTP in mitochondrial dNTP pools reduce DNA polymerase γ replication fidelity. Nucleic Acids Res. 2008; 36: 2174–81.
19 Inoue M, Kamiya H, Fujikawa K, Ootsuyama Y, Murata-Kamiya N, Osaki T, et al. Induction of chromosomal gene mutations in *Escherichia coli* by direct incorporation of oxidatively damaged nucleotides. J Biol Chem. 1998; 273: 11069–74.
20 Satou K, Kawai K, Kasai H, Harashima H, Kamiya H. Mutagenic effects of 8-hydroxy-dGTP in live mammalian cells. Free Radical Biol Med. 2007; 42: 1552–60.
21 Satou K, Hori M, Kawai K, Kasai H, Harashima H, Kamiya H. Involvement of specialized DNA polymerases in mutagenesis by 8-hydroxy-dGTP in human cells. DNA Repair (Amst). 2009; 8: 637–42.
22 Kamiya H. Mutations induced by oxidized DNA precursors and their prevention by nucleotide pool sanitization enzymes. Genes Environ. 2007; 29: 133–40.

23 Takao M, Zhang QM, Yonei S, Yasui A. Differential subcellular localization of human MutY homolog (hMYH) and the functional activity of adenine:8-oxoguanine DNA glycosylase. Nucleic Acids Res. 1999; 27: 3638–44.
24 Slupska MM, Luther WM, Chiang JH, Yang H, Miller JH. Functional expression of hMYH, a human homolog of the *Escherichia coli* MutY protein. J Bacteriol. 1999; 181: 6210–3.
25 McGoldrick JP, Yeh YC, Solomon M, Essigmann JM, Lu AL. Characterization of a mammalian homolog of the *Escherichia coli* MutY mismatch repair protein. Mol Cell Biol. 1995; 15: 989–96.
26 Ohtsubo T, Nishioka K, Imaiso Y, Iwai S, Shimokawa H, Oda H, et al. Identification of human MutY homolog (hMYH) as a repair enzyme for 2-hydroxyadenine in DNA and detection of multiple forms of hMYH located in nuclei and mitochondria. Nucleic Acids Res. 2000; 28: 1355–64.
27 Maga G, Villani G, Crespan E, Wimmer U, Ferrari E, Bertocci B, et al. 8-Oxo-guanine bypass by human DNA polymerases in the presence of auxiliary proteins. Nature. 2007; 447: 606–8.
28 Maga G, Crespan E, Wimmer U, van Loon B, Amoroso A, Mondello C, et al. Replication protein A and proliferating cell nuclear antigen coordinate DNA polymerase selection in 8-oxo-guanine repair. Proc Natl Acad Sci USA. 2008; 105: 20689–94.
29 Suzuki T, Harashima H, Kamiya H. Effects of base excision repair proteins on mutagenesis by 8-oxo-7,8-dihydroguanine (8-hydroxyguanine) paired with cytosine and adenine. DNA Repair (Amst). 2010; 9: 542–50.
30 Kamiya H, Kasai H. Substitution and deletion mutations induced by 2-hydroxyadenine in *Escherichia coli*: Effects of sequence contexts in leading and lagging strands. Nucleic Acids Res. 1997; 25: 304–10.
31 Kamiya H, Kasai H. 2-Hydroxy-dATP is incorporated opposite G by *Escherichia coli* DNA polymerase III resulting in high mutagenicity. Nucleic Acids Res. 2000; 28: 1640–6.
32 Suzuki T, Harashima H, Kamiya H. Unexpectedly weak impacts of decreased p53 and retinoblastoma protein levels on mutagenesis by 8-oxo-7,8-dihydroguanine (8-hydroxyguanine). Genes Environ. 2011; 33: 103–8.
33 Sambrook J, Fritsch EF, Maniatis,T. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1989.
34 Tornaletti S, Maeda LS, Kolodner RD, Hanawalt PC. Effect of 8-oxoguanine on transcription elongation by T7 RNA polymerase and mammalian RNA polymerase II. DNA Repair (Amst). 2004; 3: 483–94.
35 Stary A, Sarasin A. Simian virus 40 (SV40) large T antigen-dependent amplification of an Epstein-Barr virus-SV40 hybrid shuttle vector integrated into the human HeLa cell genome. J Gen Virol. 1992; 73: 1679–85.
36 Obata F, Nunoshiba T, Hashimoto-Gotoh T, Yamamoto K. An improved system for selection of forward mutations in an *Escherichia coli supF* gene carried by plasmids. J Radiat Res. 1998; 39: 263–70.
37 Kamiya H, Kurokawa M. Mutagenic bypass of 8-oxo-7,8-dihydroguanine (8-hydroxyguanine) by DNA polymerase κ in human cells. Chem Res Toxicol. 2012; 25: 1771–6.
38 Kamiya H, Yamaguchi A, Suzuki T, Harashima H. Roles of specialized DNA polymerases in mutagenesis by 8-hydroxyguanine in human cells. Mutat Res. 2010; 686: 90–5.
39 Suzuki T, Yamamoto K, Harashima H, Kamiya H. Base excision repair enzyme endonuclease III suppresses mutagenesis caused by 8-hydroxy-dGTP. DNA Repair (Amst). 2008; 7: 88–94.
40 Kamiya H. Mutagenicity of oxidized DNA precursors in living cells: Roles of nucleotide pool sanitization and DNA repair enzymes, and translesion synthesis DNA polymerases. Mutat Res. 2010; 703: 32–6.