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SHORT REPORT

Leukemic cell line, KG-1 has a functional loss of hOGG1 enzyme due to a point mutation and 8-hydroxydeoxyguanosine can kill KG-1

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We tested the cytotoxic action of 8-hydroxyguanine (8ohG) by observing the viability of several leukemic cell lines (KG-1, U937, Jurkat and K 562) in the presence of 8-hydroxydeoxyguanosine (8ohdG), a nucleoside of 8ohG. It was found that 8ohdG showed cytotoxic action only to KG-1 and that only KG-1 showed a homozygous arginine 209 to glutamine mutation in the hOGG1 gene with an almost negligible hOGG1 enzyme activity. Possibly, the selective cytotoxicity in 8ohdG to KG-1 may be due to its low capacity to cope with an increase in the 8ohG level in DNA resulting from the incorporation of 8ohdG present in the culture media. The mutational impairment of hOGG1 in KG-1 is the first report in leukemic cell lines. Using KG-1 with impaired hOGG1, we demonstrated cytotoxicity of 8ohdG probably due to its incorporation into cellular DNA. This new property of KG-1 may allow it to serve as an useful tool for studies of OGG1, oxidative DNA damage and the cytotoxic action of 8ohG. *Oncogene* (2000) **19,** 4476–4479.

Keywords: KG-1; 8-hydroxyguanine; hOGG1; mutation; cytotoxicity

We tested the cytotoxicity of 8-hydroxyguanine (8ohG) by observing whether growths of four leukemia cell lines (KG-1, U937, Jurkat and K562) are affected when they are cultured in the presence of 8-hydroxydeoxyguanosine (8ohdG, a nucleoside of 8ohG). The cytotoxicity of 8ohG was assumed by the fact that 8ohG is a base analog, many of which have proven to be cytotoxic and are used in anti-cancer therapy (Calabresi and Chabner, 1991).

In this investigation, we found that 8ohdG inhibited the growth of only KG-1 and no other cell line tested (Figure 1a). Why does this base analog exhibit differential or selective cytotoxicity? We assume that the impaired repair activity of 8ohG is one of the likeliest reasons. It is possible that the exogeneous 8ohdG added to the culture media might be incorporated in cellular DNA. If cells have a normal repair activity, they can cope with the increase of 8ohG in DNA resulting from such an incorporation by removing the modified guanine residues from DNA. However, cells without such repair ability cannot survive the resulting accumulation of 8ohG in DNA.

We found an enzyme which repairs 8ohG in DNA, by removing 8ohG as a free base (glycosylase activity) and then cleaving the phosphodiester bonds 3′ and 5′ to the resulting AP (AP lyase activity) site in *E. coli* (Chung *et al*., 1991a; Tchou *et al*., 1991) and mammals (Chung *et al*., 1991b; Yamamoto *et al*., 1992; Lee *et al*., 1993). Recently, genes have been found which encode this enzyme in yeast (OGG1) (Auffret Van Der Kemp *et al*., 1996; Nash *et al*., 1996) and human (hOGG1; human analog of yeast OGG1) (Radicella *et al*., 1997; Roldan-Arjona *et al*., 1997; Arai *et al*., 1997; Aburatani *et al*., 1997; Lu *et al*., 1997). In order to elucidate a possible mechanism for the observed differential cytotoxicity of the exogenous 8ohdG, we compared the status of the hOGG1 gene in four leukemic cell lines in terms of enzyme activity and gene sequence, and also tested the incorporation of the exogenous 8ohdG into DNA of cells impaired in hOGG1 function.

As mentioned above, Figure 1a shows selective or differential cytotoxicity of 8ohdG as indicated by the observation that 8ohdG added to media inhibited the growth of only KG-1 of the four cell lines tested. In order to determine a possible reason for this selective cytotoxicity, we compared the activity of the hOGG1 enzyme of KG-1 with that of U937, which is one of the cell lines resistant to the cytotoxic action of 8ohdG. The hOGG1 enzyme was assayed in terms of glycosylase activity (Figure 2a) and AP lyase activity (Figure 2b). As is shown in Figure 2, both glycosylase and AP lyase activities were almost negligible in KG-1 compared to U937. The other two resistant cell lines showed activities almost identical to those of U937 (data not shown). KG-1 and U937 showed no difference in the band densities in Western blot using a polyclonal rabbit antibody to hOGG1 protein (Figure 3). However, we found a point mutation in the hOGG1 gene of KG-1, which was CGA→CAA (arginine→glutamine) at codon 229 of exon 4 (Table 1). Thus, the activity loss of the hOGG1 enzyme in KG-1 possibly may be due to mutational impairment and not to the low level of the enzyme protein. In Figure 1b, we demonstrated that the exogeneous 8ohdG was incorporated into DNA of KG-1 but not into that of U937. It is of importance to note that basal level of 8ohdG in DNA of KG-1 was higher than that in DNA of U937. This 229 mutation was homozygous since all the exon 4s obtained from more than 10 subcloned colonies showed the CAA sequence.

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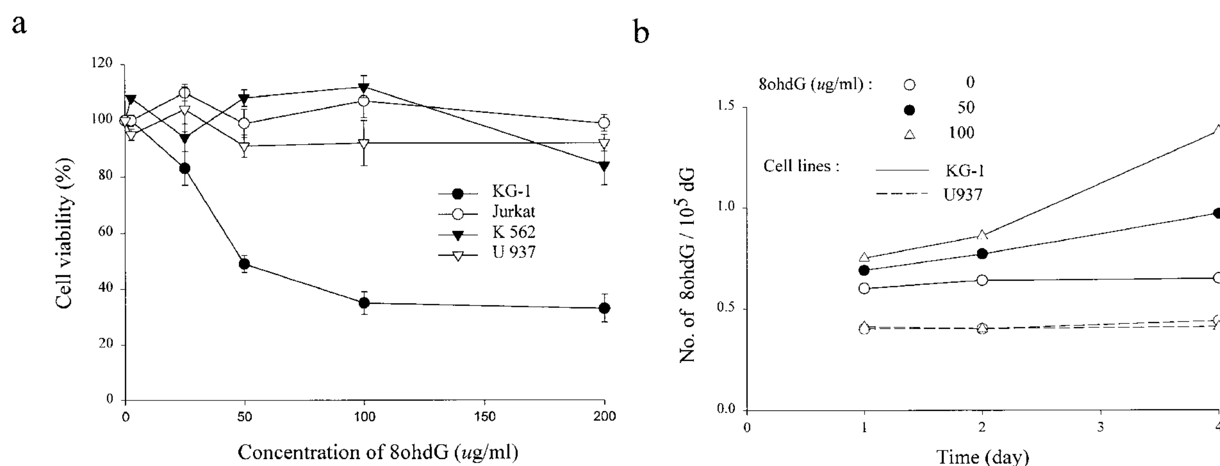

Figure 1 Cytotoxic effect of exogenous 8ohdG on various leukemic cell lines and its incorporation into cellular DNA. (**a**) Jurkat (human T-acute lymphoid leukemic cells), U937 (human monocytic leukemic cells), K562 (human myelomonocytic leukemic cells) and KG-1 (human acute myelocytic leukemic cells) were grown in RPMI 1640 containing 2 mM glutamine, 10% heat-inactivated fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml) at 37°C in 5% CO_2. Cells at the exponential phase were collected and transferred into each well (about 10^4-10^5 cells in 180 μl/well). The cells were incubated for 4 days in the presence of various amounts of 8ohdG (0–200 μg/ml) at 37°C in 5% CO_2 in a total reaction volume of 200 μl. The cytotoxic effect of 8ohdG was assayed using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Sigma Chemical Co., St. Louis, MO, USA) as described previously (Carmichael *et al*., 1987). Briefly, MTT (0.1 mg/well; 50 μl of 2 mg/ml MTT solution) was added to each well. After 4 h incubation, plates were centrifuged at 800 *g* for 5 min and supernatants were aspirated. The formazan crystals in each well were dissolved in 150 μl DMSO and the A_{540} was read on a scanning multiwell spectrophotometer (Molecular Device Co., Sunnyvale, CA, USA). The results presented are the mean±s.e. of three independent experiments. (**b**) During the culture in the presence of various concentrations of 8ohdG, cells were harvested at times indicated. DNA was isolated from the harvested cells with genomic DNA extraction kit (Wako junyaku, Tokyo, Japan) and assayed for 8ohdG as previously described (Kasai *et al*., 1986). Data are means of the two experiments

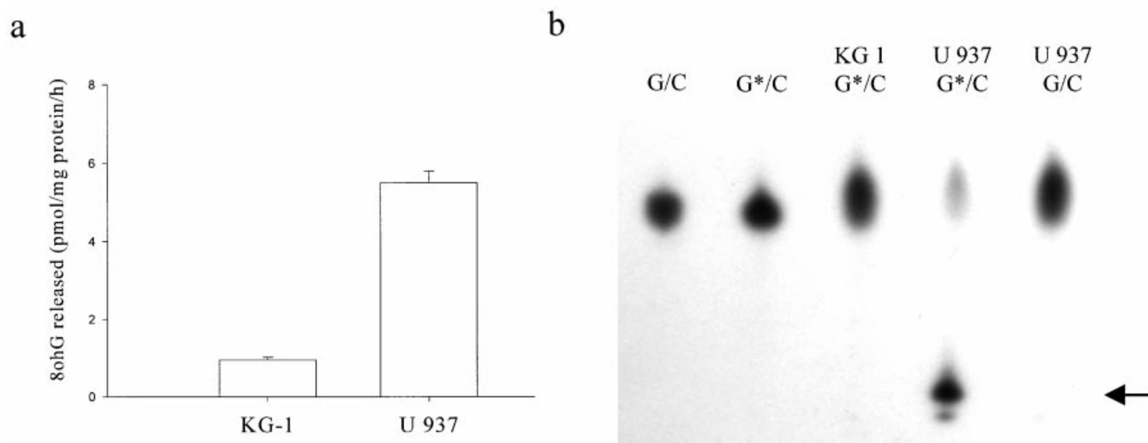

Figure 2 Activity of hOGG1 enzyme in KG-1 and U937. KG-1 and U937 were cultured as described in Figure 1. The cells at the exponential phase were centrifuged (800 *g* for 5 min). Cell pellets were then suspended in 2 volumes of homogenation buffer (50 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 5% glycerol and 0.05% 2-mercaptoethanol, pH 7.5) and homogenized. The homogenates were mixed with streptomycin (final concentration 1.5%) to remove nucleic acids. Supernatants obtained by centrifugation were dialyzed extensively against the homogenation buffer and used as cell extracts for the hOGG1 activity assay. All the procedures were performed below 4°C and the protein amount was determined using the bicinchoninic acid method (Smith *et al*., 1985) using bovine serum albumin as a standard. The enzyme was assayed for glycosylase activity to remove 8ohG from DNA strand, and for AP lyase activity to cleave the DNA strand at the site of 8ohG. (**a**) glycosylase activity: A 21-mer oligonucleotide containing 8ohG (5′-CAGCCAATCAGTG*CACCATTC-3′; G*; 8ohG) was chemically synthesized (The Midland Certified Reagent Co., Medland, TX, USA). The oligonucleotide was annealed with its complementary oligonucleotide and the resulting duplex DNA was used as the assay substrate. The duplex substrate DNA (20 pmol) was incubated with the cell extracts (2 mg protein) at 30°C for 2 h in 1 ml of reaction mixture (50 mM Tris-HCl, 50 mM KCl and 1 mM EDTA, pH 7.5). The reaction was terminated by heating at 90°C for 3 min. The amount of 8ohG released into the reaction media was assayed according to the procedure described by Lee *et al*. (1993). Results quoted are the mean±s.e. of two independent experiments. (**b**) AP lyase activity: The above 21 mer oligonucleotide containing 8ohG and the same 21 mer but containing G at the site of 8ohG were labeled with ^{32}P at the 3′ terminus according to the method of Tu and Cohen (1980) but with slight modification, as described previously (Kim *et al*., 1996) and annealed with the unlabeled complementary oligonucleotide. Each of the duplex DNAs (0.2 pmol) was incubated with the cell extract (50 μg protein) in 20 μl of 50 mM Tris-HCl, 50 mM KCl, and 1 mM EDTA (pH 7.5) at 37°C for 15 min. Cleaved substrate DNA fragments at the 8ohG position were detected autoradiographically as described previously (Chung *et al*., 1991a; Kim *et al*., 1996). G*/C: duplex DNA containing 8ohG and G/C: duplex DNA containing G at the position of 8ohG. The arrow indicates the fragment cleaved at the 8ohG position. Only the cell extract of U937 but not KG-1 was able to cleave the G*/C. As expected, G/C was not cleaved by either of the cell extracts

Table 1 Mutation of hOGG1 gene in KG-1

Exon	*Codon*	*Nucleotide position*	*Sequence change*	*Amino acid change*
4	229	954	CGA→CAA	Arg→Gln

Genomic DNA was isolated from cells at the exponential phase, which were cultured as described in Figure 1. Mutation in the hOGG1 gene were screened by the polymerase chain reaction based on single strand conformation polymorphism (PCR–SSCP) analysis as described previously (Kohno *et al*., 1998). Samples showing abnormal bands in PCR–SSCP were subjected to PCR and the PCR products obtained were sequenced using a Taq dideoxy terminator cycle sequencing kit on an ABI 377 DNA sequencer (Perkin-Elmer, Branchburg, NJ, USA)

Mutations in the hOGG1 gene have been reported in a small but significant number of cells, which are mostly of tumor origin, and the properties and consequences of these mutations have been studied. A serine 326 to cystein mutation (polymorphism) found in healthy individuals and gastric patients diminishes enzyme activity very slightly (Kohno *et al*., 1998; Dherin *et al*., 1999), and also has no association with gastric carcinogenesis (Shinmura *et al*., 1998). An arginine 154 to histidine mutation found in MKN1 (Shinmura *et al*., 1998), a stomach cancer cell line, does not affect the normal antimutagenic ability to remove 8ohG paired to C, but causes a substantial increase in its ability to remove 8ohG paired with bases other than C, particularly A (Bruner *et al*., 2000), which appears to make this enzyme promutagenic. But an arginine 131 to glutamine mutation discovered in a primary lung cancer makes the enzyme devoid of the activity to remove 8ohG paired to C (Chevillard *et al*., 1998). The guanidine group of arginine 131 appears to be involved extensively in hydrogen bonding interactions with neighboring residues. The smaller and less polar side chain of glutamine is not expected to participate in this interaction as effectively as that of arginine, which has an adverse effect on protein folding.

Recently, we (Choi *et al*., 1999) found an arginine 304 to tryptophan mutation in the two strains (SAMP1 and SAMP8) of senescence-accelerated mice (SAM) expressing a senescence-prone phenotype. This mutant enzyme of the SAMP strains has very low activity (about 10% of that of SAMR1, a SAM with the normal aging phenotype) and is thermolabile. Bruner *et al*. (2000) suggested that the hydrocarbon side chain of arginine 304 is packed into the protein core with the polar head group extruded into a water filled channel. The interaction of the tryptophan side chain probably disrupts the optimal packing of the protein core due to an increase in steric bulk. In this study, another mutation involving arginine→glutamine was also found in KG-1 but at position 229. The role of arginine 229 in hOGG1 has not been elucidated. However, this mutation is evidently expected to impair the enzyme activity since the polar and larger side chain of arginine is replaced by the less polar and smaller one of glutamine.

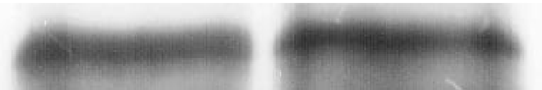

Figure 3 Western blot analysis for hOGG1 protein in KG-1 and U937. The cells (5×10^6) obtained from the cultures, as described in Figure 1, were lysed in a solution of 50 mM HEPES, 250 nM NaCl, 0.1% Nonidet P-40, and 1 mM phenylmethylsulfonyfluoride. Aliquots of the lysates (30 μg protein) were subjected to electrophoresis on a 12% SDS-polyacrylamide gel and the gel was transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA), and incubated with a primary rabbit anti-hOGG1 antibody (Alpha Diagnostic International, San Antonio, TX, USA). This was followed by further incubation with a goat anti-rabbit immunoglobulin G-horseradish peroxidase conjugate (Pierce, Rockford, IL, USA) and then exposed to X-ray film. Protein bands were detected using an enhanced chemiluminescence Western blotting detection kit (Amersham, Little Chalfont, Buckinghamshire, UK)

In this communication, we report two important findings. One is that KG-1 has functional loss of the hOGG1 enzyme due to the point mutation of CGA→CAA (arginine→glutamine) at codon 229. This mutation at a newly identified site is a first report of such in leukemia cell lines. Thus, we expect that KG-1 will serve as a useful model for studies on the roles of OGG1 and 8ohG in leukemia genesis. The second important finding is that exogeneous 8ohdG can be incorporated into cellular DNA and cytotoxic, which, however, were observed only in the cell having impaired activity of the OGG1 enzyme. The causal link of the deficiency of OGG1 activity to the cytotoxicity of exogeneous 8ohdG was further supported by the finding that on the culture of 18 cell lines (five cell lines with low activity of OGG1 and 13 cell lines with normal activity) in the presence of 8ohdG, growth inhibition was observed only in the five cell lines with a lower activity of hOGG1 enzyme and not in any of the 13 cell lines with normal activity (data not shown). However, the incorporation of 8ohdG into DNA of KG-1 observed in the study is in conflict with the report (Hayakawa *et al*., 1995) showing that guanylate kinase, which phosphorylates both GMP and dGMP to the corresponding nucleoside diphosphates, is totally inactive for 8-oxo-dGMP, which is formed from 8ohdG. Probably, guanylate kinase of KG-1 may also have a functional alteration which allows this enzyme to resume the activity to 8-oxo-dGMP. Now, we are studying the activity of guanylate kinase of KG-1 toward 8-oxo-dGMP.

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