Supplementary material

SUPPLEMENTARY MATERIALS AND METHODS

Genotype determination: OGG1 codon 326 genotype had been determined in the LCLs used in this work by TAQMAN chemistry (Applied biomarker assay C3095552-1) (data not presented). The status of this SNP and the genotype of the two other *OGG1* SNPs reported to show variations in the SNP500 cancer population (rs1801127 and rs4986999) (http://snp500cancer.nci.nih.gov/snplist.cfm) were determined by direct sequencing of DNA extracted from the 10 LCLs using NucleoSpin ® Tissue columns (MACHERY-NAGEL). The region surrounding the Ser326Cys SNP was amplified using 10uM of forward (5'-CCCCAGTGGATTCTCATTG) and reverse (5'-ATCCCCCACCCCATCTTG) primers and for the SNPs rs1801127 and rs4986999 the primer pairs given on the SNP500 website were used. The amplifications were carried out in a total volume of 20µl containing the appropriate primer pairs, 10 ng of genomic DNA, 1 U of TaqPlatinium (INVITROGEN), 0.2mM final dNTP mix, 1.5 mM final $MgCl_2$.. The PCR conditions were 95°C for 3 mins, then 45 cycles of (95°C-40s, 58°C-40s, 72°C-40s) for rs 1052133 and rs1801127 amplification or 45 cycles of (95°C-40s, 60°C-40s, 72°C-40s) for rs4986999 amplification; followed by a 10 min hold at 72°. PCR products were checked on an agarose gel and then 5µl of the PCR product was purified using ExoSap (USB) following the manufacturer's recommendations and sequenced using the BigDye V1.1 on a Applied Biosystems Genetic Analyser 3130. The sequencing confirmed the expected codon 326 genotypes and wildtype alleles were detected at SNPs rs1801127 and rs4986999 in all the ten cell lines.

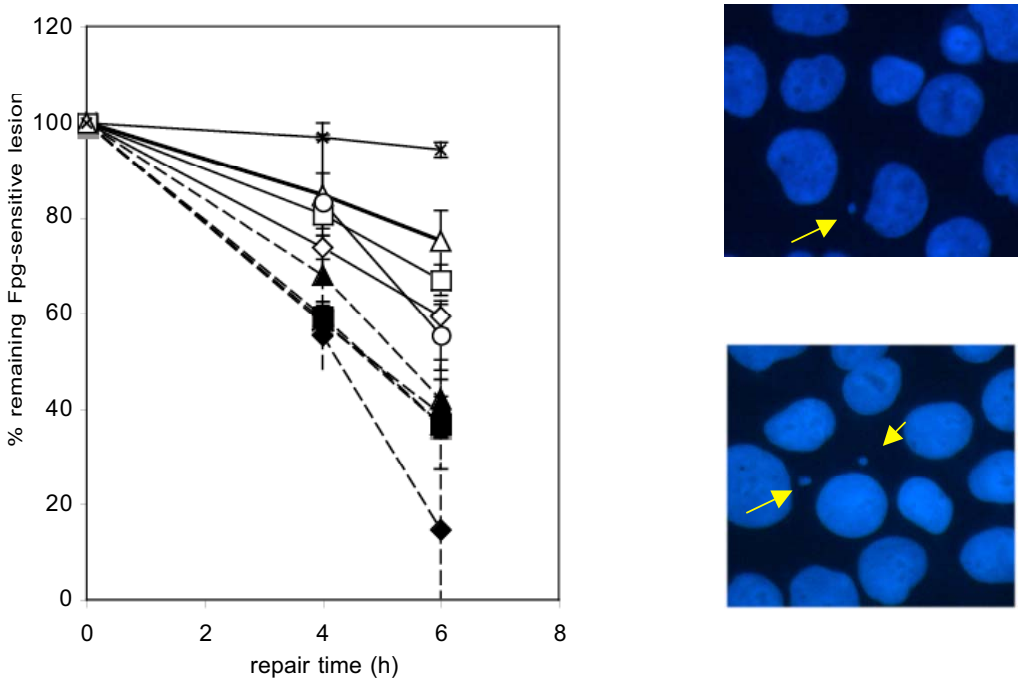
Cellular repair kinetics. For the analysis of cellular repair rates, additional oxidative guanine modifications were generated in exponentially growing cells ($1x10^6$ /ml) by irradiation in the presence of the photosensitizer Ro19-8022 (70 nM) for 10 min on ice with visible light (166 kJ/m^2 between 400 and 600 nm) from a 1000 W halogen lamp (Philips PF811) at 38 cm distance in 10 ml phosphate buffer (137 mM NaCl, 2.7 mM KCl, 8.3 mM NaH_2PO_4, 1.5 mM KH_2PO_4, pH 7.4). The removal of the induced modifications was then followed by means of an alkaline elution technique in combination with the repair glycosylase Fpg protein as described previously. The assay makes use of the fact that DNA containing single-strand breaks (SSB) or having been incised at substrate modifications by Fpg elutes more rapidly from a membrane filter than intact DNA. The numbers of (unrepaired) modifications therefore can be calculated from the slopes of the elution curves. In brief, cells after damage

induction were washed twice and resuspended in culture medium supplemented with catalase (316 U/ml) and, if indicated, 0.5 mM DTT. The numbers of DNA modifications in these cells and in untreated control cells were quantified directly and after repair incubations under culture conditions for various times (2-6 h). For that purpose, 10^6 cells were lysed on a polycarbonate filter (2 mm pore size) and incubated for 50 min at 37°C with Fpg protein (1 μg/ml). Subsequently, the DNA was eluted with an alkaline buffer and the elution rate was determined. From the slopes of the elution curves obtained, the numbers of modifications incised by Fpg protein were obtained after subtraction of the number of SSB observed in parallel experiments without glycosylase treatment. To calculate the numbers of modifications induced by photosensitization, the numbers of SSB and Fpg-sensitive modifications determined in the undamaged control cells, which were incubated in parallel, were subtracted. The slopes obtained with γ-irradiated cells were used for calibration, assuming that 6 Gy induce one SSB per 10^6 bp (23).

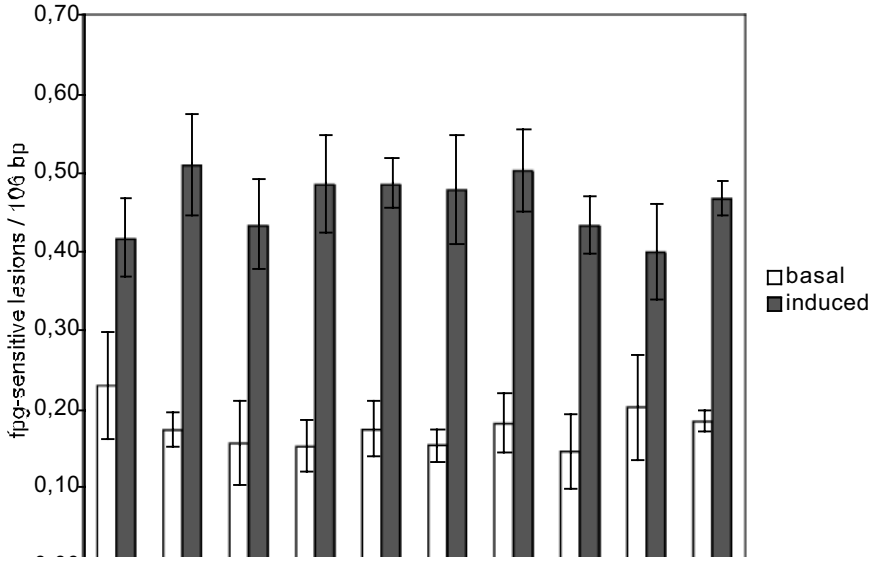
qPCR measurement of OGG1 transcript levels. Total RNA was extracted from 10^7 cells plated at $0.5.10^6$ cells/ml the day before extraction using Trizol (Invitrogen). Reverse transcription was carried out on 1 μg of RNA in a 20 μl reaction volume containing 1x first strand buffer, random primers (50 ng total) (Invitrogen), 0.5mM dNTP mix, 10mM DTT and 200 units of Superscript II (Invitrogen). The mRNA levels of the *OGG1* and *GAPDH* genes were measured following generation of the corresponding cDNA by real time PCR based on TAQMAN chemistry using a StepOnePlus machine from Applied Biosystems and the Taqman probes for *OGG1* (Applied Biosystems, Hs00249899_m1) and *GAPDH* (Applied Biosystems, Hs99999905_m1). The reaction mix contained 1μl of the cDNA, 1μl TAQMAN probe and primers, 1x gene expression master mix (Applied Biosystems) in a total volume of 10μl. The amplification protocol consisted of an initial denaturing and enzyme activation step at 95° for 10 min followed by 40 cycles at 95°C-15s, 60°C-1min. Two cDNAs were prepared from two independent RNA extractions from each cell line, with each cDNA analyzed in triplicate on one plate. The relative expression of *OGG1* gene in each cell line was then estimated using a semi-quantitative method by calculating the Dct value, defined as the difference in the threshold cycle (Ct) value for the target gene (*OGG1*) and the reference gene (*GAPDH*).

SUPPLEMENTARY RESULTS

Suppl. Fig. 1. Decreased 8-oxoG DNA repair activity and increased genetic instability in hOGG1 Cys326 cell lines. A. Lymphoblastoid cells homozygous for one of the two position 326 alleles were exposed to Ro 19-8022 plus light to induce oxidative DNA lesions and analysed by alkaline elution for the repair of Fpg-sensitive sites after 4 and 6 hours. Points represent the mean value ± SD for tripicate experiments for each indicated cell line. B. Representative micronuclei from Ser4 (above) and Cys4 cell lines (below).

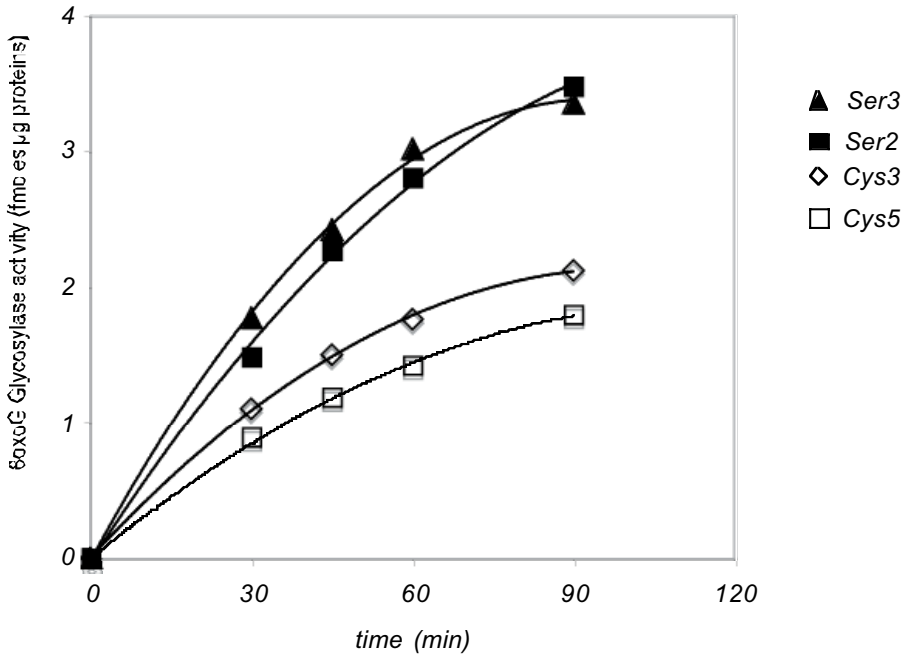


Suppl. Fig. 2. The number of basal and Ro 19-8022 plus light induced Fpg-sensitive lesions are similar in hOGG1-Ser326 and hOGG1-Cys326 cell lines. Lymphoblastoid cells homozygous for one of the two position 326 alleles were analysed by alkaline elution for the number of Fpg-sensitive sites under basal conditons and just after exposition to Ro 19-8022



plus light to induce oxidative DNA lesions. Bars represent the mean value ± SD for triplicate experiments for each indicated cell line.

Suppl. Fig. 3. Kinetic of 8-oxoG excision is decreased in hOGG1 Cys326 cells. Extracts (12µg) Ser2, Ser3, Cys3 and Cys5 cell lines were assayed for 8oxoG DNA excision rate as a function of time.

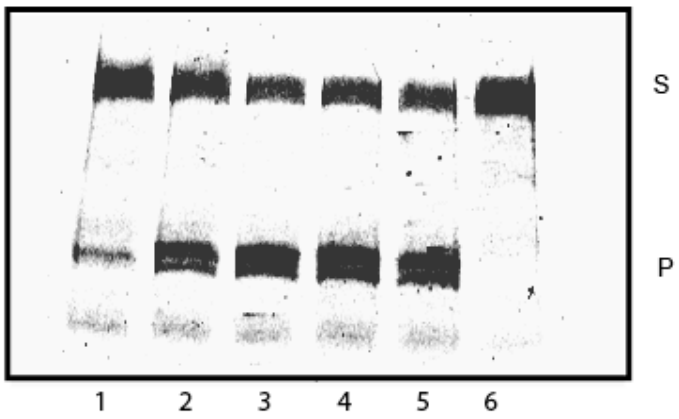


Suppl. Table 1. hOGG1 mRNA transcript levels are similar in hOGG1 Cys326 and hOGG1 Ser326 cell lines. cDNAs were prepared from two independent RNA extractions from each cell line, each cDNAs analyzed by qPCR in triplicate on one plate. The relative expression of OGG1 gene in each cell line was then estimated using a semi-quantitative method by calculating the Dct value, defined as the difference in the threshold cycle (Ct) value for the target gene (OGG1) and the reference gene (GAPDH).

Cell lines	Dct Mean/ ± S.D.	Dct Mean / genotype ± S.D.
Ser1	9,85 ± 0,0,3	
Ser2	9,60 ± 0,11	
Ser3	9,94 ±0,02	9,76 ± 0,14
Ser4	9,75 ±0,07	
Ser5	9,64 ± 0,15	
Cys1	10,12 ± 0,07	
Cys2	9,29 ± 0,03	
Cys3	9,71 ± 0,05	9,78 ± 0,32
Cys4	9,79 ± 0,05	
Cys5	9,98 ± 0,03	

Suppl. Fig. 4. 8-oxoG DNA glycosylase activity in HeLa cell lines. *A.* 8-oxoG DNA glycosylase activity of 10 µg extract of HeLa cells (lane 1) and 1 µg of HeLa cells expressing hOGG1-Ser-GFP (lane 2), hOGG1-Cys-GFP (lane 3), hOGG1-Ser-Flag (lane 4) and hOGG1-Cys-Flag (lane 5). S and P indicate substrate and product respectively. Lane 6 corresponds to the control without extract. *B.* Quantification of the activities.

A



B