

Repair of cyclobutane pyrimidine dimers or dimethylsulfate damage in DNA is identical in normal or telomerase-immortalized human skin fibroblasts

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ABSTRACT

The progression of a normal cell to senescence *in vivo* and *in vitro* is accompanied by a reduction in the length of the telomeres, the chromosome capping segments at the end of each linkage group. However, overexpression of the reverse transcriptase subunit (HTERT) of the ribonucleoprotein telomerase restores telomere length and delays cellular senescence. Although some data exist in the literature with respect to survival, no molecular data have shown that DNA repair in telomerase-immortalized cells is normal. Several telomerase-immortalized human skin fibroblast cell lines were constructed from a primary human fibroblast cell line. The primary line and the telomerase-immortalized cell lines were treated with either ultraviolet (UV) radiation or dimethylsulfate (DMS). UV radiation principally produces cyclobutane pyrimidine dimers that are repaired by nucleotide excision repair, whereas DMS introduces mainly *N*-methylpurines repaired by base excision repair. Here, we show that repair of both types of damage in the telomerase-immortalized human skin fibroblast cell lines is identical to repair observed in normal skin fibroblasts. Thus, telomerase expression and consequent immortalization of skin fibroblasts do not alter nucleotide or base excision repair in human cells.

INTRODUCTION

Common to all eukaryotes, telomeres consist of repeated hexamers of the sequence TTAGGG/CCCTAA, which terminate the DNA strands at the ends of chromosomes (1). Repeats of 15 kb are considered long and can shorten to 5 kb in senescent

and geriatric cells. Telomeres of chromosomes in normally proliferating cell populations, such as keratinocytes and lymphocytes, are maintained at 10–12 kb, but shorten with each population doubling in cell culture as a predicted consequence of replication at DNA ends and the lack of telomerase expression in normal cells (2–6). Expression of the human telomerase reverse transcriptase (HTERT) subunit of the telomerase holoenzyme is necessary to maintain telomere length (7). Transfection of normal cells by constructs expressing HTERT results in telomere lengthening and an increased *in vitro* life span (8–11). Telomerase also has a role in tumorigenesis (12). Cancers and cancer cell lines in many cases express HTERT at high levels compared with their non-malignant progenitors (11,13–18). In *Saccharomyces cerevisiae*, telomere maintenance is dependent on the activities associated with repair of double-strand breaks (19). Telomerase is thus associated with telomere maintenance, DNA repair functions and cellular transformation.

Ultraviolet (UV) radiation is often considered one of the most wide spread carcinogens. The repair of UV damage by the nucleotide excision repair (NER) system has been characterized and many aspects of this repair at the molecular level are understood, including the proteins involved in recognition, elimination and re-synthesis. Moreover, the use of other DNA repair techniques has helped elucidate details about the process of eliminating damage from DNA. One technique in particular, ligation-mediated PCR (LMPCR), has provided information at nucleotide resolution about the process of NER (20,21). LMPCR monitors ligatable 5' phosphate strand breaks. Strand breaks with ligatable phosphate ends can be induced by either chemical or enzymatic reactions, but much less background is introduced if enzymatic means are employed to produce the sites (22–27). The most widely used enzymes to induce abasic sites or strand breaks appropriate for LMPCR are DNA glycosylases (28–34). NER of cyclobutane pyrimidine dimers (CPDs) is sequence context dependent and the 5' limit of transcription coupling in human genes has been identified (21). Other details of NER

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and links to human disease have been elucidated using LMPCR (20,35,36). In these studies, it has proven a valuable technique in examining repair at nucleotide resolution in human cells.

Damage inflicted by DNA methylating agents is repaired by base excision repair (BER) and also, to some extent, by NER (37). The series of steps involved in the removal of 7-meG and 3-meA were once thought to occur exclusively by BER, but their repair is now known to involve NER (37–40). Only limited data exist on the repair of dimethylsulfate (DMS) damage by BER at nucleotide resolution in human cells using LMPCR (41). Although there is a sequence context-dependent repair of 7-meG and 3-meA, 3-meA is generally repaired faster than 7-meG. In contrast to NER, repair of alkylated bases in transcribed genes occurs without any strand bias (41–43). Despite the contribution of NER to repair of *N*-methylpurines, the major repair of at least 3-meA is via BER. Therefore, measuring the repair of *N*-methylpurines reflects the BER capacity.

As a part of our characterization of telomerase-transformed human skin fibroblasts, we examined UVC repair patterns at global, strand and nucleotide levels to determine whether the expression of HTERT alters NER or BER in such cells. In previous work, HTERT overexpression has been shown to immortalize human skin fibroblasts and skin fibroblasts from individuals suffering from the heritable human disease xeroderma pigmentosum (8,10). Persons with xeroderma pigmentosum are hypersensitive to UV radiation. HTERT-immortalized cell lines from both normal individuals and xeroderma pigmentosum individuals maintain the same UV sensitivity and unscheduled DNA synthesis as the non-immortalized counterpart lines (10), but unscheduled DNA synthesis and survival are indirect measures of the repair of DNA adducts. A recent report has examined telomerase-transformed oral fibroblasts (44). A number of assays for repair were also performed to indicate that repair is faster in oral fibroblasts expressing telomerase. A single assay based on strand-specific repair indicated that there was at least a slight preference for repair of UV damage. It is possible that the repair at various levels is different in telomerase-immortalized versus primary cell lines. In this paper, we studied the repair of CPDs and methylpurines in three different clones overexpressing telomerase in diploid human skin fibroblasts. In contrast to the telomerase-immortalized oral fibroblasts (44), we found that telomerase immortalization of skin fibroblasts did not alter DNA repair patterns at any level after UV or DMS exposure at global or nucleotide resolution. Thus, the use of telomerase-immortalized cells is a practical model for the study of NER and BER of DMS damage in human cells.

MATERIALS AND METHODS

Enzymes and chemicals

Enzymes including bacteriophage T4 UV endonuclease V, *Escherichia coli* DNA photolyase, AlkA and *Pfu* DNA polymerase were from laboratory stocks. DNA ligase was obtained from Promega (Madison, WI) and used according to the manufacturer's instructions. DMS was from Sigma (St Louis, MO).

Cell culture

Human foreskin fibroblasts isolated in our laboratory, HF57, or telomerase-immortalized equivalents, HTERTG, were grown in DMEM + 10% fetal calf serum and maintained in standard cell culture conditions. Cultures were split at ratios of 1:4, fed every 2 days and passaged with trypsin/EDTA. Five million log-phase HF57 p8 cells were used for each electroporation (BioRad), in Schultz buffer (45) with 5–50 μ g linearized pBABEHYGRO-HTERT (a gift from Dr Robert Weinberg), 450 V DC, 960 μ F and 400 μ l volume. Following electroporation, cells were returned to a 10 cm culture dish. After 48 h, cultures were treated with 20 μ g/ml Hygromycin B for 3 days, followed by 2 weeks recovery. Three bouts of Hygromycin B selection were conducted throughout 5 weeks. Many positive Hygromycin B-resistant clones formed, but only five were positive by RT-PCR for telomerase expression. Individual clones were grown and used in these experiments. The lines HTERTB, HTERTC and HTERTG have been grown to passage 21 in the absence of selection and all three lines overexpressed the HTERT cDNA.

Cytogenetics

Late log-phase cultures were used for standard chromosome preparation (46). Metaphase chromosomes were counted and evaluated by GTG banding (46,47). One hundred metaphases of HF57 and its telomerase-transformed derivative, HTERTG, were counted.

Survival curves

HF57 or HTERTG cells were treated with either a germicidal lamp with different fluxes of UV radiation measured at 254 nm or different concentrations of DMS with $2\text{--}3 \times 10^5$ cells on a 150 mm plate (treatments are described below). Following treatment, the cells were incubated for 48 h, harvested and counted for trypan blue exclusion using a hemacytometer. Determinations were conducted in quadruplicate.

Repair of UV damage

For UV treatment, 15 cm plates of mitotically active fibroblasts at 90–95% confluence were washed with isotonic saline and covered with a sterile steel pan. The germicidal lamp was warmed up for 2 min and the covering pan was removed to expose the cells. Desired exposure time was calculated to deliver 20 J/m^2 at 254 nm using a UVX monitor (UVP, Upland, CA). Cells were fed culture medium and incubated for 0–48 h to allow DNA repair. At various time points, the cells were harvested.

Repair of DMS damage

For DMS treatment, 15 cm plates of HF57 or telomerase-immortalized human skin fibroblasts were washed with isotonic saline and the cells were treated with 1 mM DMS in DMEM medium without serum for 15 min at room temperature. Cells were fed culture medium and allowed to repair DNA damage for 0–72 h. At various time points, the cells were harvested.

Isolation of DNA

At different time points following UVC irradiation or DMS treatment, the cells were harvested by mild trypsinization.

Nuclei were prepared using a standard protocol (41). Briefly, the cytoplasm and intact nuclei were centrifuged for 15 min at 4°C at 3000 r.p.m. in an SS38 rotor. Nuclei were re-suspended in a standard buffer and lysed by treatment with Proteinase K and RNase A for 3–5 h at 37°C. The digested nuclei were extracted twice with each of the following solvents: buffer-equilibrated phenol, phenol:chloroform (1:1) and chloroform. Following the last extraction, the DNA was precipitated with ethanol, washed with 80% ethanol and re-suspended in 1 mM Tris-HCl, pH 7.5, 0.1 mM EDTA. DNA was quantified using the absorbance at 260 nm.

Isolation of RNA and RT-PCR assay for HTERT expression

The supernatant or cytoplasmic fraction with the total RNA was isolated from cells cultured in 100 mm Petri dishes using RNeasy as recommended by the manufacturer and stored at –80°C in deionized distilled water (TEL-TEST, Inc., Friendswood, TX). Following addition of chloroform, the solution was centrifuged and the supernatant was transferred to a new Eppendorf tube. The RNA was precipitated using isopropanol and re-suspended in 0.1× TE with 0.5 U of RNase inhibitor. RNA was quantified using the absorbance at 260 nm.

The primers used for RT-PCR are as follows: HTERT, TGACACCTCACCTCACCCAC and CACTGTCTTCCGC-AAGTTCAC; and for the GAPDH control, CGGAGTCAAC-GGATTTGGTCGTAT and AGCCTTCTCCATGGTGGTG-AAGAC. RT-PCR was performed using a kit obtained from Invitrogen using MMLV reverse transcriptase (San Diego, CA). Amplification was performed for 30 cycles with an initial denaturation of 4 min at 94°C, followed by cycles with subsequent denaturation for 30 s at 94°C, annealing of 40 s at 52°C and extension of 40 s at 72°C. Products were separated using 1% agarose gel electrophoresis.

Cleavage of DNA at CPDs

DNA (10 µg) from UV-treated cells was reacted with T4 UV endonuclease V followed by photoreactivation of the adducts using *E.coli* photolyase. T4 UV endonuclease V and photolyase-treated samples were electrophoresed on an alkaline agarose gel and analyzed for global DNA repair using a FluoroImager (Molecular Dynamics, Sunnyvale, CA) with ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Briefly, each lane of the denaturing agarose gel FluoroImager scan was divided into 100 boxes using ImageQuant software. A linear background function was used to subtract background. The molecular weight standards were plotted and fit using a polynomial function to convert each of the 100 boxes into a molecular weight. The volume function of ImageQuant was used to determine the contribution of each box to the weight average molecular weight, M_w . The fraction of the contribution of each of the boxes to the overall molecular weight was determined. The number of breaks found in genomic DNA can be calculated from the reciprocal of the M_w .

Cleavage of DNA at N-methylpurines

DNA (10 µg) from UV-treated cells was reacted with 1 µg of methylpurine DNA glycosylase (MPG) for 30 min at 37°C followed by cleavage at damage sites by treatment with alkali at 37°C. An aliquot of 100 µl of 2 M piperidine was added to

the MPG-treated DNA at the end of the 30 min enzymatic reaction, and the incubation continued at 37°C for 15 min to induce breaks at abasic sites via β-elimination, to leave a ligatable phosphate end for LMPCR analysis. The MPG/mild piperidine-treated DMS-modified DNA was supplemented to a final concentration of 0.3 M sodium acetate and 1 µl of 20 mg/ml glycogen was added. The DNA was ethanol precipitated, washed using 80% ethanol, and the excess piperidine removed by evaporation. DNA was re-suspended in 1 mM Tris-HCl, pH 7.6, 0.1 mM EDTA, separated using an alkaline agarose gel electrophoresis and analyzed for global DNA repair using a FluoroImager with ImageQuant software as for the UVC-treated DNA.

LMPCR

LMPCR was performed using a protocol employing *Pfu* Turbo DNA polymerase that efficiently amplifies 0.5 µg of DNA in a 10 µl volume (48,49). Labeling of the sample was accomplished using near infrared dyes as described previously. Quantification of bands was performed using ImageQuant software.

Calculated strand-specific repair analysis

The rate of repair to 50% of the initial CPD damage at individual base positions in exons 5–8 of the human P53 gene was determined using LMPCR. The repair rates were summed for each strand and divided by the number of bases with CPD damage analyzed on each strand. This average repair rate to 50% repair was used as the repair rate on each strand.

Telomere length assay

Average telomere length was determined using a commercial system from BD Pharmingen. Briefly, control genomic DNA is digested with restriction endonucleases, which degrade all DNA except the telomere repeat sequences (TTAGGG). DNA was electrophoresed, blotted to a charged nylon membrane and probed with biotinylated oligonucleotides complementary to the telomere repeat. Detection was accomplished with a streptavidin-horseradish peroxidase conjugate, which cleaves a substrate producing light at 410 nm. The membrane was exposed to X-ray film.

RESULTS

Telomerase-transformed HTERTG human skin fibroblasts have the characteristics of early passage normal human skin fibroblasts

Normal human skin fibroblasts (designated HF57) at passage 8 prepared in this laboratory were transformed using electroporation with pBABE-Hygro containing the complete HTERT cDNA. The HTERT cDNA was expressed in the transformed cells at levels approaching that of the HTERT in HL60 cells used as a control (Figure 1a). The population doubling time from 11 independent experiments for HTERTG was 0.47 ± 0.08 population doubling/day and 0.38 ± 0.15 population doublings/day for HF57 cells. Based on the error, the doubling times of the HF57 and HTERTG are not significantly different. The normal (HF57) and telomerase-transformed (HTERTG) human skin fibroblasts were cytogenetically evaluated at various passage levels. This shift in telomere length from ~5.7 to

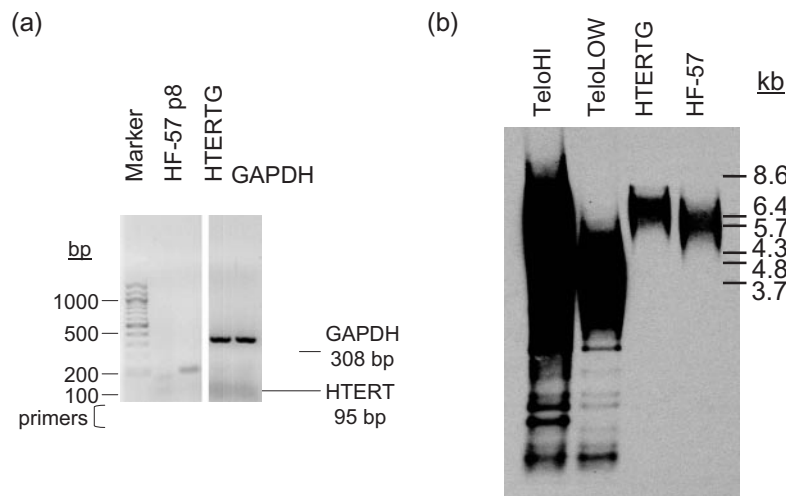


Figure 1. Characterization of telomerase expression and telomere lengthening in human cell lines. (a) Agarose gel electrophoretic separation of RT-PCR products demonstrating the expression of the telomerase reverse transcriptase in HF57 p8, HTERTG p8, HEK-293, HL-60 and BJ-1. The expression of GAPDH was used as a control in all isolates. The lengths of the HTERT and GAPDH RT-PCR products are indicated on the right hand side of the figure in base pairs (bp). The markers are a 100 bp ladder with several of the bands indicated on the figure. (b) Telomere length assay for HF57 and HTERTG with long (TeloHI) and short telomere (TeloLOW) controls. The size marker indicates the position of the bacteriophage λ HindIII digestion controls.

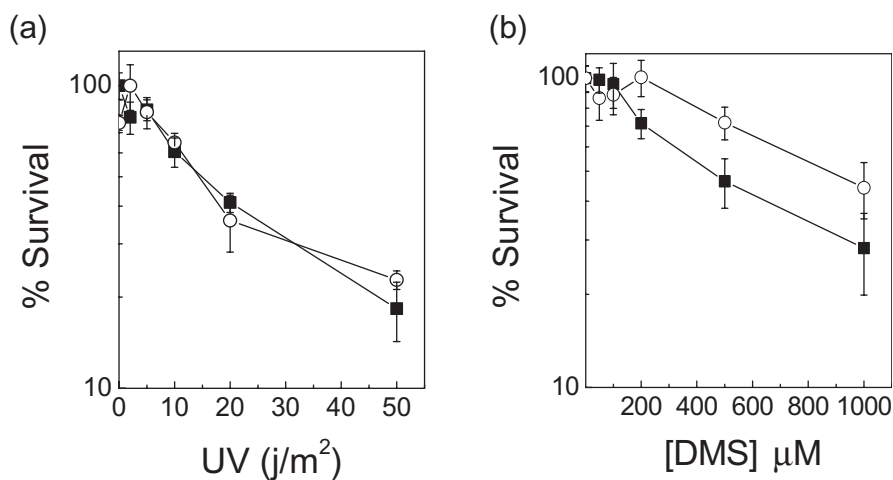


Figure 2. Survival curves of HF57 and HTERTG following exposure to UV or DMS. Cells were seeded and exposed to either UV or DMS treatment, cultured for 48 h, harvested and counted for trypan blue exclusion. (a) UV treatment of HF57 (filled squares) or HTERTG (open circles). (b) DMS treatment of HF57 (filled squares) or HTERTG (open circles).

7 kb is indicative of an increased expression of the HTERT as demonstrated in Figure 1b. To characterize the HTERTG cell line further, chromosome counts of at least 100 metaphases were made for each cell line, and both showed $2N = 46$ (90%) and $4n = 92$ (10%). No aneuploidy was observed. GTG banding was performed, and chromosomes of each cell line exhibited normal banding patterns to a resolution level of 400 bands per haploid genome. In culture, both HF57 and HTERTG cell lines exhibited contact inhibition at confluence and did not form mounds or foci.

Normal diploid skin fibroblasts express a senescent phenotype by passage 30 in our hands. HTERTG cells were derived by electroporation from HF57 at passage 8, selected with Hygromycin B, allowed to clone for 5 weeks and were then grown to passage 20. If we assume, conservatively, that the cells experience 2 population doubling/passage, then since selection, the HTERTG population has undergone 2^{40}

population doublings. The population doublings during selection are not calculable, but must be significant. Thus, the sum of the population doubling level of the parental line at electroporation plus the cumulative population doubling level of the HTERTG line after selection and growth has exceeded the Hayflick senescence limit (50). Other cell lines expressing telomerase (HTERTB and HTERTC) were also characterized with similar properties.

Survival of normal human fibroblasts and HTERT-immortalized fibroblasts to UV and DMS

To determine whether there is an advantage of either the normal human fibroblasts or HTERT-immortalized fibroblasts following exposure to UV or DMS, we constructed survival curves (Figure 2). The survival with respect to UV for HF57 and HTERTG is identical (Figure 2a), showing that

no advantage with respect to survival is conferred on the HERTERT-immortalized fibroblasts. Similarly, survival with respect to DMS for both HF57 and HERTERTG is within the experimental error (Figure 2b). It should also be noted that the survival curves were determined using the 48 h controls. Cells that are untreated do not undergo replication arrest and at 48 h approximately double in number compared with the initial seeding. When exposed to even low levels of UV or DMS (even 5 J/m^2 UV or $200 \mu\text{M}$ DMS), no mitosis is observed within 30 min post-treatment even at low levels of DNA-damaging agents (5 J/m^2 UV or 1 mM DMS). Therefore, the number of control cells is almost always greater than that of the treated cells. However, replicating cells are observed at 48 h post-treatment even with 20 J/m^2 UV or 1 mM DMS. Thus, using 20 J/m^2 UV and 1 mM DMS for the repair studies, at least 85% of the initial number of cells are viable.

Global repair of CPDs is identical in normal and telomerase-immortalized cells

To test the repair of CPDs at the global level, we treated both HF57 and HERTERTG with 20 J/m^2 of UV radiation and either harvested the cells immediately or allowed for repair of DNA at various times. Following DNA isolation, it was treated with T4 DNA UV endonuclease followed by *E.coli* photolyase and analyzed by denaturing alkaline gel electrophoresis. Figure 3a shows the progressive repair observed in both cell lines from 0 to 48 h, with no significant difference in the repair rates. Figure 3b demonstrates that quantitatively, the M_w values for repair in the two lines have no significant differences. The same experiment in the HERTERTC cell line also provided results indistinguishable from those of HERTERTG and HF57.

Repair of CPDs at nucleotide resolution is identical in normal and telomerase-immortalized cells

To follow repair at nucleotide resolution for both normal and telomerase-immortalized cells, the DNA treated with T4 UV endonuclease and DNA photolyase was subjected to LMPCR analysis in exon 5 of the human P53 gene on both the translated strand (TS) and the non-translated strand (NTS). The results shown in Figure 4 demonstrate that the repair patterns obtained in both cell lines on both strands are virtually identical. Similar results were obtained using the HERTERTB cell line.

Repair of CPDs on the TS and NTS is identical in normal and telomerase-immortalized cells

To further demonstrate that NER has the same characteristics in both HF57 and HERTERTG, some of the CPD band intensities in these cell lines are shown. Figure 5 shows repair at some individual nucleotide positions based on data obtained using RFLPScan. Bands representing slow, fast or intermediate rates were chosen to illustrate the similarity of repair at nucleotide resolution at the individual time points. At least six time points were analyzed for the different clones examined. Figure 5 shows that only one point is substantially different in its repair rate for CPDs. That time point is found at 12 h in the position labeled as 'Fast'. The other points are within the experimental error.

Data collected for the repair half-lives of CPDs in exon 5 of the P53 gene are shown in Figure 6. There are only slight

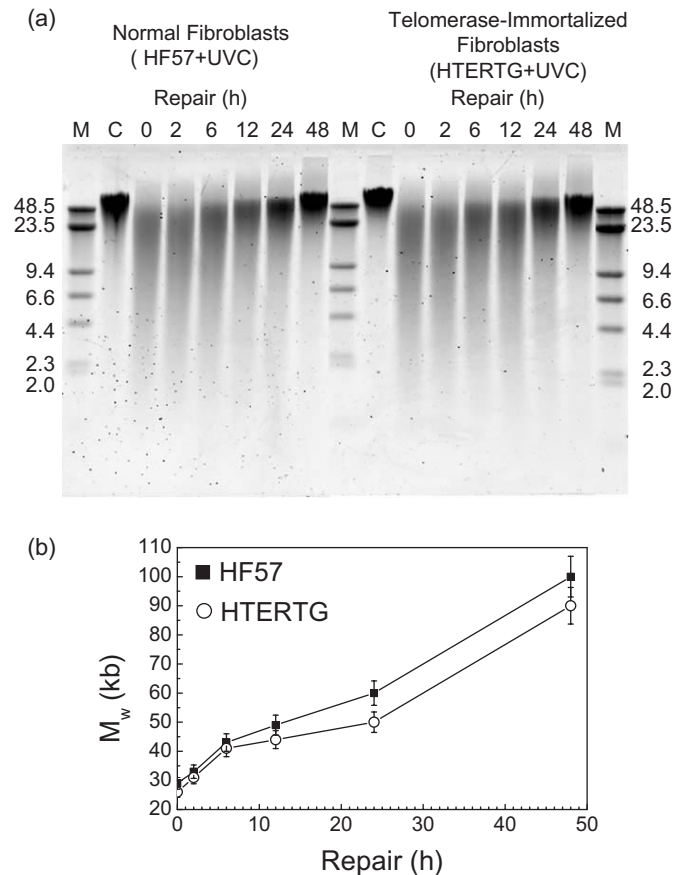


Figure 3. Global repair of CPDs induced in normal and telomerase-immortalized human skin fibroblasts following UV repair. (a) Denaturing alkaline agarose gel showing restoration of global genome weight average molecular weight, M_w , with time after irradiation in normal and telomerase-immortalized human skin fibroblasts. The molecular weight standards correspond to a bacteriophage λ HindIII digest. The times in hours for each of the points are indicated on the figure. (b) M_w plotted against repair time. The M_w values increase as a function of time indicating repair. The M_w values were calculated as described in Materials and methods. The average error in the determination is 7%.

alterations in the repair rates in the HF57 and HERTERTG lines that are observed at nucleotide resolution. There are few positions of CPD sites in exon 5 on the TS. To obtain an indication of the repair on the TS and NTS overall, the average values for repair half-lives (20 and 22 h for CPD bands on the NTS in HF57 and HERTERTG and 16 and 19 h for CPD bands on the TS for those two lines, respectively) were calculated based on the number of CPDs and their repair rates as indicated in Figure 6. It should be emphasized that this number is not a number weighted on the intensity of each band, but only a simple average of the repair half-lives; therefore, the difference in CPD repair on the TS is faster than on the NTS, but not as fast as might be expected based on a weighted average. There is less than half the number of CPDs on the TS compared with the NTS and that reduces the difference in the repair rates on the NTS and the TS. The fact that the repair rates at all the nucleotide positions are extremely similar is another demonstration that NER in the telomerase-immortalized cells is identical to that observed for normal human fibroblasts.

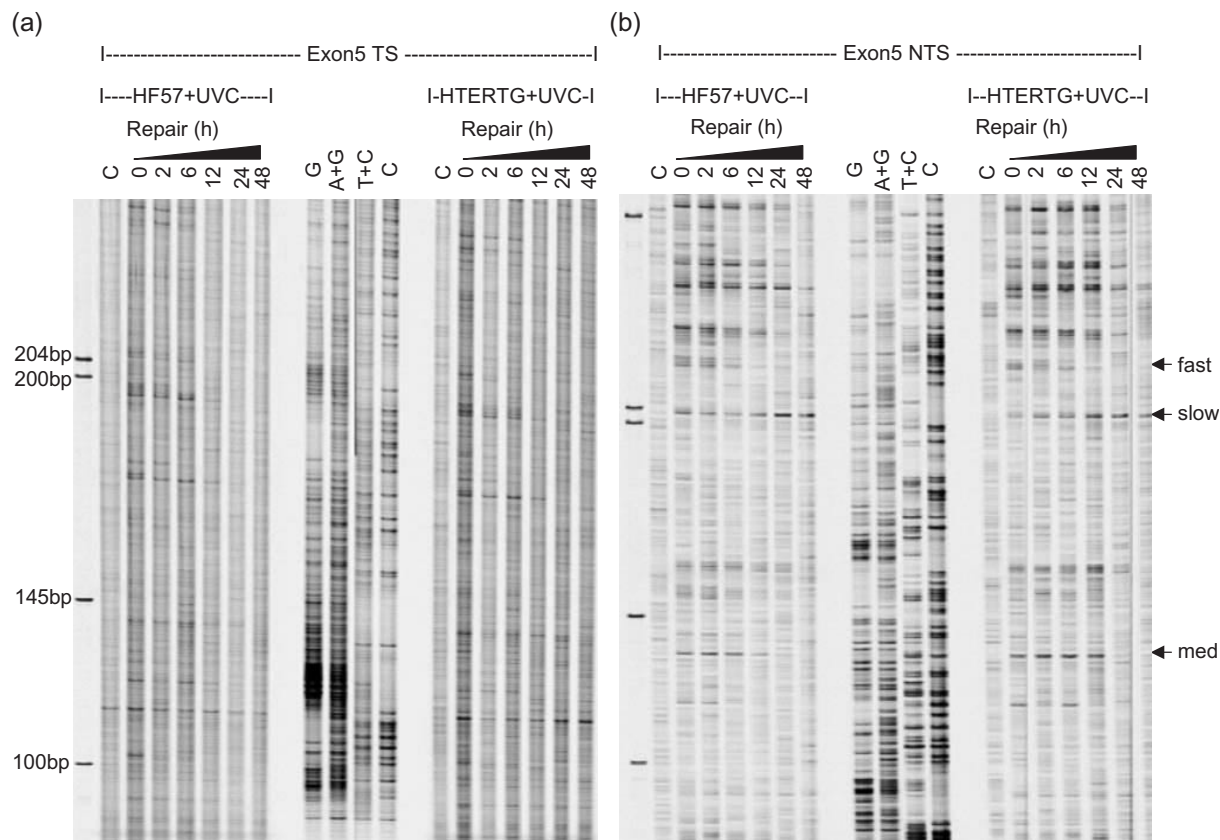


Figure 4. Repair at nucleotide resolution of the TS and NTS of exon 5 of the human P53 gene in normal HF57 and HTERTG telomerase-immortalized skin fibroblasts. Markers obtained from the Li-Cor Corporation are indicated in nucleotide length. Chemical sequencing standards are in the lanes marked G, A+G, T+C and C. Positions used in Figure 4 are indicated by arrows and bracketed by lines. (a) Repair on the TS in exon 5 of the P53 gene. (b) Repair on the NTS in exon 5 of the P53 gene.

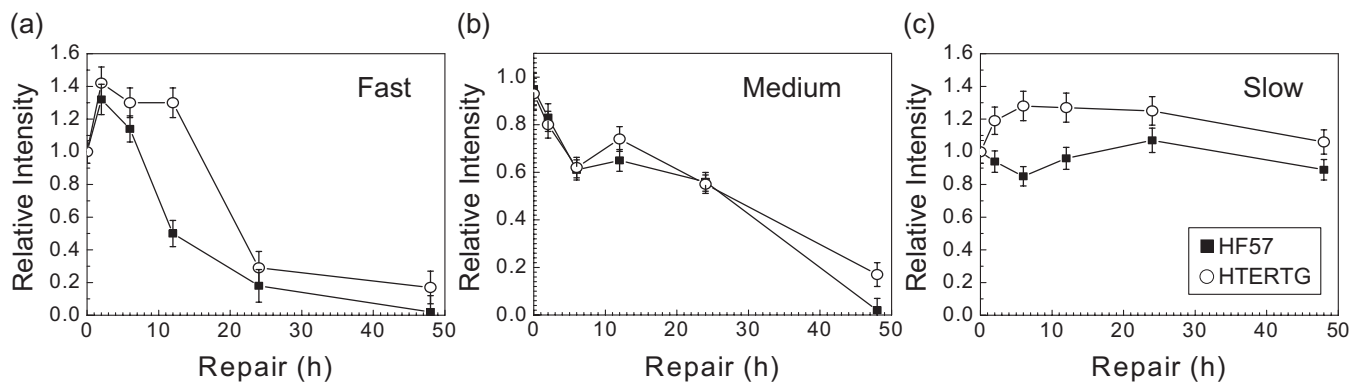


Figure 5. Relative intensity as a function of repair time for CPDs at selected positions on the NTS of exon 5 in the P53 gene. Relative intensity is inversely proportional to % repair. The relative intensity is calculated using the 0 h point as the reference time. The closed squares are relative intensities from HF57 and the open circles from HTERTG. The error for the measurements is 7% for bands >0.5 relative intensity and 10% for bands <0.5. (a) Repair in P53 at amino acid 130. (b) Repair in P53 at amino acid 134. (c) Repair in P53 at amino acid 157.

Global repair of *N*-methylpurines is identical in normal and telomerase-immortalized human skin fibroblast cells

DNA damage inflicted by methylating agents is removed to a large extent by BER. To investigate the effect of telomerase immortalization on BER, HF57 and the telomerase-immortalized lines were treated with DMS and allowed to

repair. The DNA was isolated from the cells and subjected to treatment with AlkA protein and mild alkali. The AlkA/alkali-treated DNA was separated on an alkaline gel as shown in Figure 7a. The M_w values progressively increase as a function of time indicating repair (Figure 7b). These data indicate that there is no significant difference between the DNA repair observed in HF57 and HTERTG. Similar results were obtained with the HTERTB cell line.

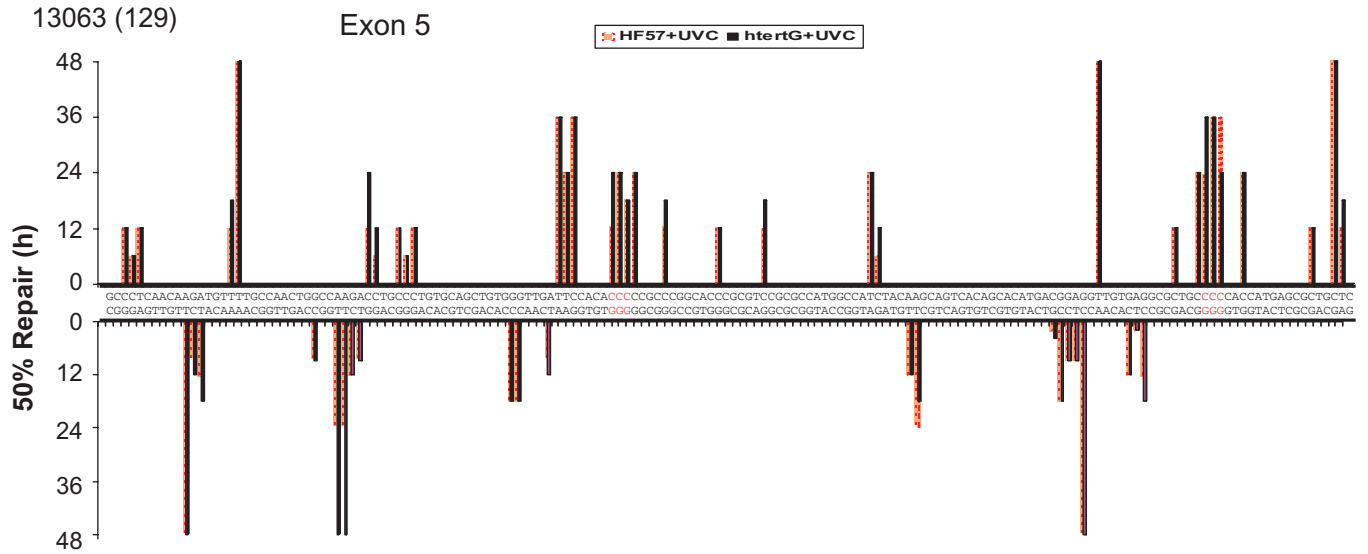


Figure 6. CPD half-lives for repair at individual nucleotide positions along exon 5 of the P53 gene for the normal and telomerase-immortalized human skin fibroblasts. Repair half-lives. The top strand represents NTS and the bottom the TS. HF57 is represented by the red striped lines and HTERTG by the solid black lines.

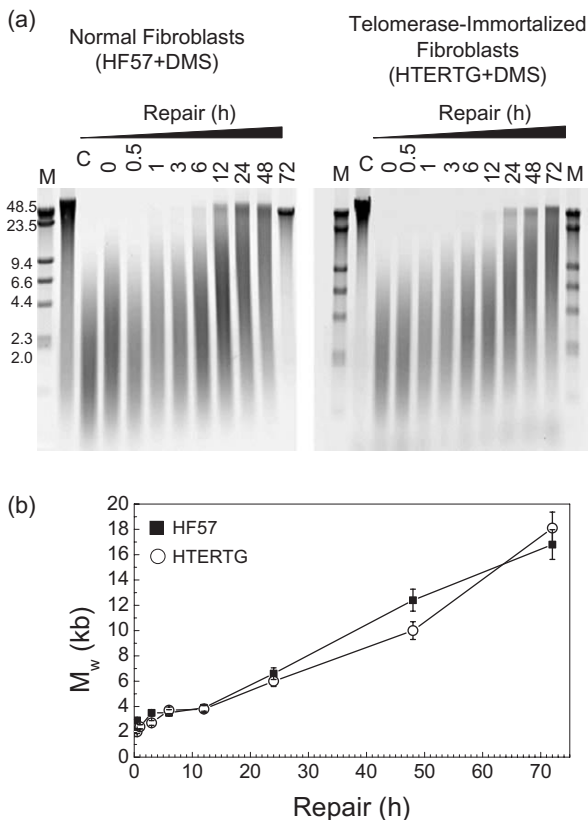


Figure 7. Global repair of DMS damage induced in normal and telomerase-immortalized human skin fibroblasts following DMS treatment. (a) Denaturing alkaline agarose gel showing restoration of global genome weight average molecular weight, M_w , with time after irradiation in normal and telomerase-immortalized human skin fibroblasts. The molecular weight standards correspond to a bacteriophage λ HindIII digest. The times in hours for each of the points are indicated on the figure. (b) M_w plotted against repair time. The M_w values increase as a function of time indicating repair. The closed squares are M_w values from HF57 and the open circles from HTERTG. The M_w values were calculated as described in Materials and methods. The average error in the determination is 7%.

Repair of CPDs at nucleotide resolution is identical in normal and telomerase-immortalized human skin fibroblast cells

The DNA analyzed by alkaline gel electrophoresis was analyzed at nucleotide resolution using LMPCR (Figure 8). The bands in Figure 8 result mainly from 3-meA and 7-meG modifications. DMS modifies almost every G or A position, so a large number of bands are observed. Qualitative examination of the repair rates suggests that there is little difference in the repair rates observed between the two cell lines.

Repair of N-methylpurines on the TS and NTS is identical in normal and telomerase-immortalized cells

The bands in Figure 8 were analyzed for their relative intensity compared with the point of repair time at 0 h. Figure 9 shows that quantitatively the differences observed in the bands are statistically insignificant. Therefore, repair is observed at the same rate in both HF57 and HTERTG.

The data obtained from exon 5 for repair half-lives are accumulated in Figure 10 for repair in HF57 and HTERTG. The average repair half-life for N-methylpurines on the NTS is 51 h and for N-methylpurines on the TS, 45 h for both cell lines. Therefore, repair of N-methylpurine damage in normal and telomerase-immortalized fibroblasts is identical.

DISCUSSION

Our results indicate that telomerase overexpression does not alter the repair of UV damage in normal human foreskin fibroblasts. The extension of life span by the HTERT reverse transcriptase does not alter NER at any level, thus suggesting that these human skin fibroblasts are an excellent model for the study of repair in human cells. These experiments demonstrate that at every level of NER, no differences are observed between the normal and telomerase-immortalized lines. BER for repair of N-methylpurines is also identical for HTERT-immortalized fibroblasts and normal human skin fibroblasts.

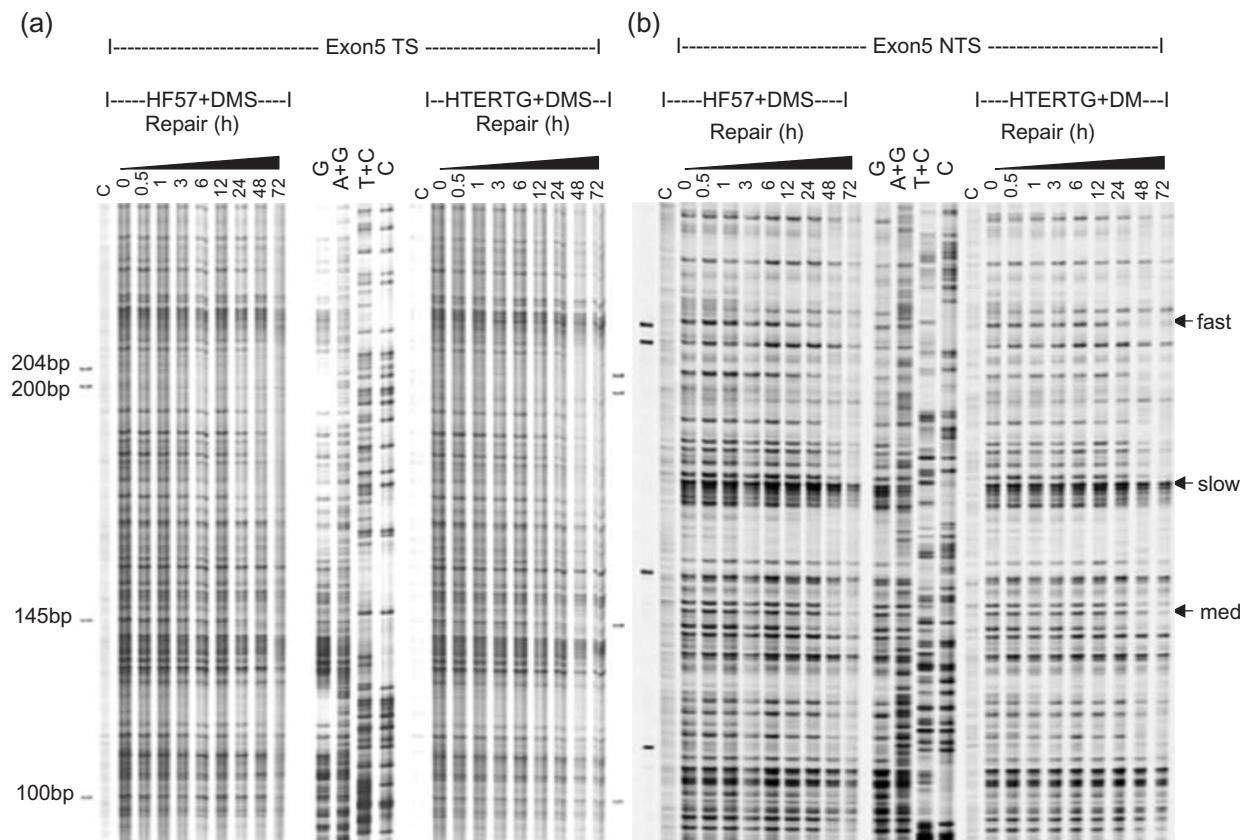


Figure 8. Repair of DMS damage at nucleotide resolution of the TS and NTS of exon 5 of the human P53 gene in normal and telomerase-immortalized skin fibroblasts. Markers obtained from the Li-Cor Corporation are indicated in nucleotide length. Chemical sequencing standards are in the lanes marked G, A+G, T+C and C. Positions used in Figure 4 are indicated by arrows and bracketed by lines. (a) Repair on the NTS in exon 5 of the P53 gene. (b) Repair on the TS in exon 5 of the P53 gene.

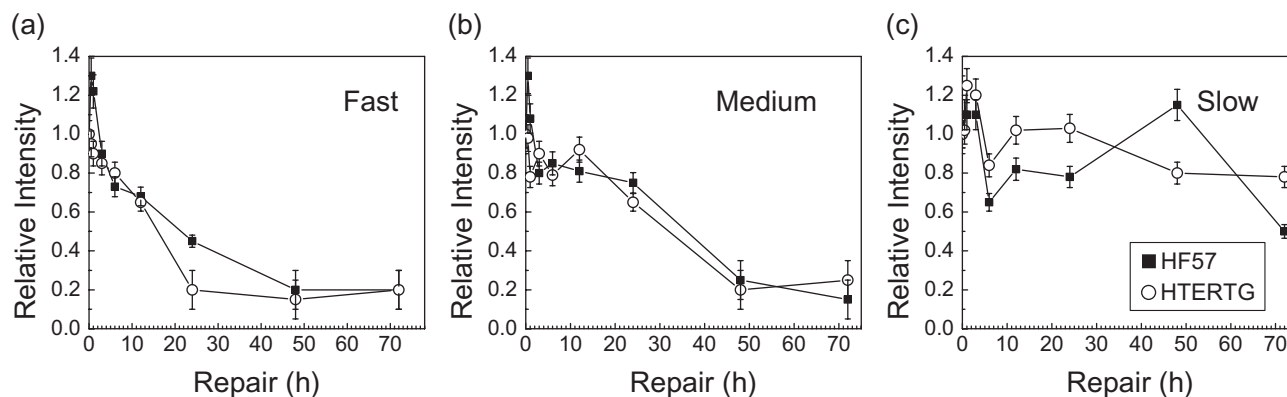


Figure 9. Relative intensity as a function of repair time for *N*-methylpurines at selected positions in exon 5 on the NTS of the P53 gene. Relative intensity is inversely proportional to % repair. The relative intensity is calculated using the 0 h point as the reference time. The closed squares are relative intensities from HF57 and the open circles from HTERTG. The error for the measurements is 7% for bands >0.5 relative intensity and 10% for bands <0.5. (a) Repair in P53 at amino acid 133. (b) Repair in P53 at amino acid 146. (c) Repair in P53 at amino acid 157.

However, while the BER of *N*-methylpurines is the same in both normal and HTERT-immortalized fibroblasts, it is possible that BER of other adducts, e.g. induced by oxidative damage, could be different.

Originally, telomerase-immortalized human skin fibroblast cells (BJ-1) were derived from a pre-senescent culture, a strategy that clearly demonstrated the transforming capability of

HTERT (51,52). However, since these cells are not far from the point of crisis, they proliferate poorly compared with skin fibroblasts at a lower population doubling level. Although they overexpress telomerase and possess long telomeres, we found BJ-1 difficult to use in our experiments. Clearly, although senescence is delayed in BJ-1 telomerase-transformed cells, the proliferative properties of the cell line are not close to that

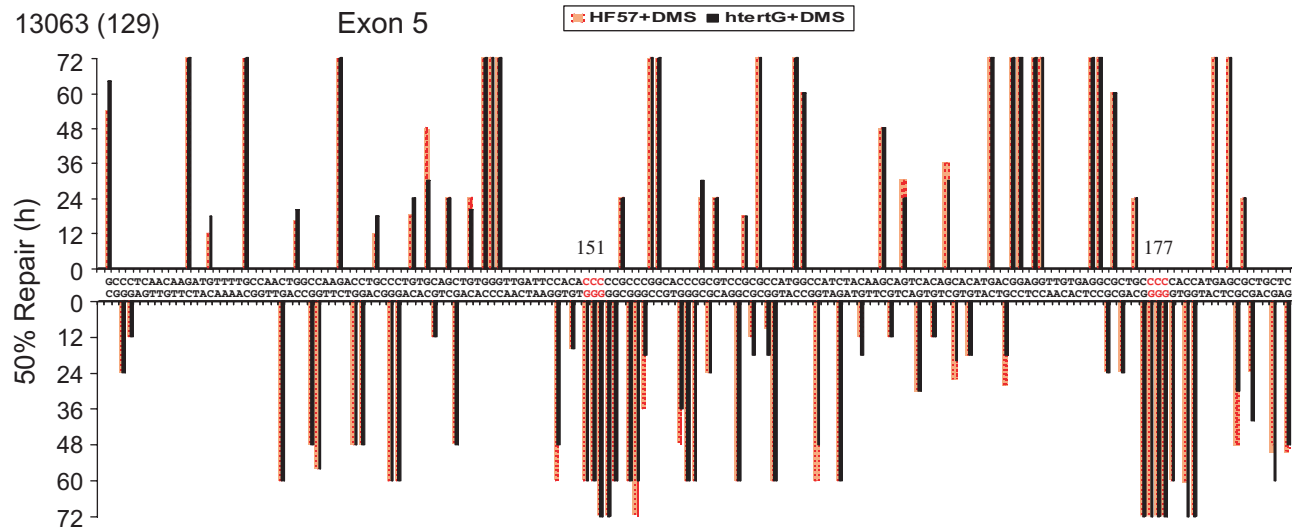


Figure 10. *N*-methylpurine half-lives for repair at individual nucleotide positions along exon 5 of the P53 gene for the normal and telomerase-immortalized human skin fibroblasts. The top strand represents the NTS and the bottom the TS. HF57 is represented by the red striped lines and HTERTG by the solid black line.

of fibroblasts that are at a point of much less population doublings. We chose to transform a much younger cell (with respect to *in vitro* age), reasoning that longer starting telomeres might be more easily maintained. Our cultures consist of small, rapidly dividing cells if maintained optimally. Like their normal counterparts, HTERTG cells held at confluence grow large, polyploid/multinucleate and cease proliferation. Thus, the capacity for contact inhibition is also unaffected by telomerase overexpression.

Telomere lengths are a statistical average not only because the population of cells sampled contains some senescent cells, but also because there is variation in the telomere length on individual chromosomes of a given cell (53). Thus, in a pre-senescent cell, if the telomeres of a linkage group bearing a critical growth control gene are sufficiently short to allow dicentric chromosome formation (54), followed by chromosome breakage in the growth control gene, immortalization and escape from senescence could occur.

Certain proteins associated with DNA repair of double-strand breaks, most notably KU70 and KU80, associate with telomeres and these proteins have a role in telomere maintenance (55–59). Thus, one hypothesis would be that the HTERT expression could alter double-strand break repair. Therefore, it is noteworthy that γ -radiation survival and double-strand break repair are identical in normal and telomerase-immortalized cells as demonstrated in recent papers (60,61). This suggests that although telomerase function is linked to survival after ionizing radiation exposure (62–64), increased HTERT subunit expression does not alter repair of double-strand breaks induced by γ -radiation. In this paper, we have shown that it is the same for NER repair and BER of DMS-induced methylated bases.

A recent publication has indicated that there is some difference in repair in human oral fibroblasts compared with the data we have presented in this paper (44). Several differences are notable between the two studies. First, except for the UV irradiation studies, all the previous work relies on episomal damage and transfection. In the UV study, the authors only had two time points for evaluation. In the two cell lines examined,

one line was almost identical to that of the normal oral fibroblasts; that does not support the conclusion of the paper that there is a difference between normal and telomerase-immortalized oral fibroblasts. It is also possible that there is a difference between skin and oral fibroblasts. One other difference between the two studies is that the previous study has used end points that are not necessarily associated with repair rates and may involve other processes associated with the formation of mutations. A final point of difference between this and the previous study involves the UVC flux. In this study, we used 20 J/m², whereas the other report employed a lower 2.5 J/m² flux. However, in previous reports, repair of UV-induced damage has been shown to maintain repair characteristics until exposed to 30 J/m² flux (65). Therefore, no differences in repair should be observed at 2.5 versus 20 J/m² flux values.

In conclusion, telomerase up-regulation has proved a useful alternative to the use of transforming viruses (SV40, etc.) for the establishment of cell lines from cell types difficult to culture (10). The use of the HTERTG, HTERTB and HTERTC cell lines to study DNA repair is a robust alternative to the use of cell lines, such as its parental HF57 or other human skin fibroblasts, that undergo cellular senescence for the study of NER and BER. These lines will provide researchers with a human cell line at a relatively low passage number that can serve as the basis for studies without dependence on passage number or morphological changes associated with cellular aging. Such alterations could reduce at least one source of differences in the response of cells to DNA damage.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at NAR Online.

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