DNA Strand Bias in the Repair of the p53 Gene in Normal Human and Xeroderma Pigmentosum Group C Fibroblasts

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Abstract

We have measured the gene-specific and strand-specific DNA repair of UV-induced cyclobutane pyrimidine dimers in the p53 tumor suppressor gene in a normal, repair-proficient human fibroblast strain and in fibroblasts from a patient with the repair deficient disorder xeroderma pigmentosum, complementation xeroderma pigmentosum group C (XP-C). In both cell strains, repair was measured in the p53 gene and in its individual DNA strands. For comparison, the repair also was measured in other genomic regions in these human fibroblast strains, including the housekeeping gene dihydrofolate reductase, and two inactive genomic regions, the δ-globin gene, and the 754 locus of the X chromosome. In both cell strains, we find that the p53 gene is repaired faster than the dihydrofolate reductase gene and much more efficiently than the inactive genomic regions. Selective repair of the transcribed DNA strand of p53 is observed in both human cell strains; the strand bias of repair is particularly distinct in XP-C. Mutations specific to the nontranscribed strand may occur due to replication errors at the sites of unrepairedor DNA damage. Therefore, our results predict that the majority of mutations in skin cancers, especially those from patients with XP-C, would occur on the nontranscribed strand of the p53 gene. Indeed, Dumas et al. (Proc. Natl. Acad. Sci. USA, in press, 1993) report such a strand bias of p53 mutation in skin cancers from XP-C patients.

Introduction

p53 is the most commonly mutated gene found in human tumors including lung, breast, gastrointestinal, hepatic, bladder, ovarian, bone, and brain tumors (1, 2). Germline p53 mutations have been identified in patients with a hereditary predisposition to the development of sarcomas and carcinomas (Li Fraumeni syndrome) (3). The animal model of germline loss of function, the p53−/− knockout mouse, is cancer prone (4).

The p53 protein is a transcription factor (5-7) and has been demonstrated to play a role in the cellular response to DNA damage. In normal mouse cells, treatment with UV and UV-mimetic agents induces a rapid increase in p53 protein levels (8). Normal hematopoietic human cells increase p53 protein levels after treatment with either γ-irradiation or actinomycin D (9). A temporal correlation between the DNA damage induced G1 arrest and the increase in wild type p53 protein in normal cells suggests that p53 plays a role in inhibiting DNA synthesis after damage, thus preventing replication on a damaged or unrepared template. Furthermore, severe DNA damage can lead to programmed cell death (10-12). These data suggest that normal p53 function is necessary for the maintenance of genomic stability following DNA damage.


Recent work from different laboratories including our own has suggested that a strand bias of mutations in housekeeping genes can be due to a strand bias of repair (21, 22). In controlled experiments where the strand bias of repair is measured concomitantly with the strand bias of premutagenic lesions, there is selective repair of the transcribed strand and mutations occur due to replication errors at unrepaired DNA damage in the nontranscribed strand. These observations suggest that aspects of DNA repair are a more important determinant than previously thought for the distribution of mutations.

In this report, we have measured the gene-specific and strand-specific repair in the p53 gene in human fibroblasts. Gene-specific repair of UV dimers was analyzed in the p53 gene, in the housekeeping gene DHFR, and in the inactive δ-globin gene. Repair has also been examined in the locus 754, an untranscribed region of the X chromosome located near the Duchenne’s muscular dystrophy locus. In addition, we have also examined the strand specific repair of p53 in both normal and DNA repair-deficient cell strains.

The characterization and understanding of this level of repair not only provides information about the mechanisms of DNA repair but also allows us to make predictions about the expected distribution of mutations. The characteristics of DNA repair are particularly important in the XP-C cells because these patients are highly cancer prone and develop skin tumors in which mutations might arise in the p53 gene.

Materials and Methods

Fibroblast Cell Strains. The normal cell strain GM00038A and the XP complementation group C strain (XP2BE) GM000677 were obtained from the Coriell Institute for Medical Research, Camden, NJ. The GM00038A cell line was grown in minimal essential medium 1× supplemented with 10% fetal bovine serum, 2 mM l-glutamine, essential and nonessential amino acids, vitamins, and antibiotics. The XP cell strain was grown in minimal essential medium 1× supplemented with 10% fetal bovine serum, 2 mM l-glutamine, and antibiotics. All cell culture materials were obtained from Advanced Biotechnologies, Inc., Columbia, MD. Cells were grown at 37°C in humidified 5% CO2 and 95% air.

Gene-specific Damage and Repair of UV-induced Pyrimidine Dimers. In this approach, we create strand breaks in specific restriction fragments using a CPD-specific endonuclease, T4 endonuclease V. The samples are then resolved in alkaline agarose gels followed by qualitative Southern blotting, probing, analysis, and calculations to provide the number of lesions in a certain

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1 The abbreviations used are: DHFR, dihydrofolate reductase; TS, transcribed strand; NTS, nontranscribed strand; XP, xeroderma pigmentosum; CPD, cyclobutane pyrimidine dimer; XP-C, xeroderma pigmentosum group C.
The methodology for the examination of gene-specific repair has been previously described in detail (23). Cells were prelabelled with $[^3]H$thymidine, and logarhythmically growing cell cultures were irradiated at 10 J/m² using a germicidal UV lamp (254 nm). The repair occurred in the presence of bromodeoxyuridine. The DNA was extracted, purified, and restricted using HindIII or EcoRI restriction endonucleases purchased from Bethesda Research Labs. Following enzymatic restriction, samples were sedimented in a neutral CsCl gradient, and parental DNA was separated from bromodeoxyuridine-labeled replicated DNA and then dialyzed (23, 25).

The pyrimidine dimer-specific T4 endonuclease V was prepared as previously described (24). Two 5-µl aliquots of DNA from each sample were analyzed; one aliquot from each sample was treated with 2 µl of T4 endonuclease V per 5 µg of DNA. The other aliquots were treated with endonuclease buffer alone. All samples were incubated for 15 min at 37°C. After incubation, samples were loaded on a 0.7% alkaline agarose gel and electrophoresed under alkaline conditions at 16 V for 18 to 22 h on a Protech apparatus (ONCOR). The DNA was transferred to Sureblot nylon membranes (Oncor, Inc.) in a transfer solution of 1 N NaOH.

**Probes.** Gene-specific repair was studied in fragments of the housekeeping DHFR gene, the inactive 754 locus on the X chromosome, and the p53 gene. The maps of these genes are presented in Fig. 1. The 1.8-kilobase DHFR probe obtained from Giuseppe Attardi, California Institute of Technology, detects a 21.5-kilobase fragment. Leon Mullenders, University of Leiden, kindly provided the plasmid containing the 2.0-kilobase HindIII fragment of locus 754 cloned into pAT153 which detects a 14-kilobase fragment in EcoRI-restricted DNA. The 754 locus, located on the X chromosome, is transcriptionally silent in primary human fibroblasts (25). The p53 complementary DNA probe and pBsp53 plasmid construct was obtained from Bert Vogelstein, Johns Hopkins University. The 1.8-kilobase probe detects a 20-kilobase fragment in EcoRI-restricted DNA. The riboprobes were prepared from a pBluescript KS phagemid. Labeled riboprobe is generated using the SP6-T7 transcription kit from Boehringer Mannheim (substituting T3 polymerase for the SP6 polymerase).

The Random Prime labeling Kit (Boehringer-Mannheim) was used to label all double-stranded DNA probes. The specific activity of both DNA and RNA was $10^9$ cpm/µg of DNA. After hybridization, the membranes were washed under standard conditions for double stranded DNA probes (23) and riboprobes (24).

**Data Collection.** Two UV-irradiation gene-specific repair experiments were performed for each cell strain. For the normal control two replicate gels were prepared for each biological experiment. In the XP group C cell strain, one gel was prepared for the first experiment and two gels were prepared for the second experiment. For both cell strains, autoradiograms were prepared for each hybridization of each gel for the genes and loci studied. In each experiment, for each gene, the percentage repair at each time point is the average of the values obtained for that time point on each of two autoradiograms prepared from that blot. The average repair for each cell strain is the average of both biological experiments performed on that cell strain.

Quantitation of the resulting autoradiographic bands was done by scanning densitometry using Shimadzu or Molecular Dynamics densitometers. The frequency of restriction fragments containing no CPDs is equal to the ratio of full length fragments in the T4 endonuclease V-treated samples to that of untreated samples. The CPD frequency was calculated from the zero class measurement by using the Poisson expression (average number of dimers/fragment of the zero class frequency).

**Results**

**Initial Dimer Frequency.** Based on the size of the gene fragments studied and our previous data (24, 26, 27), the estimated initial dimer frequency after 10 J/m² for the genes studies is as follows: 1.28 for the 21-kilobase HindIII DHFR fragment; 1.16 for the 18-kilobase δ globin fragment; 1.25 for the 19.5-kilobase p53 fragment; and 0.9 for the 14-kilobase 754 locus fragment. In the 4 separate biological experiments for the XP-C and normal cell strains, we obtained an average initial dimer frequency of 1.5 (range, 1.24–1.7) for DHFR, 1.3 (range, 1.02–1.7) for δ globin, 1.17 (range, 0.93–1.39) for p53, and 1.1 (range, 0.86–1.92) for locus 754. The observed initial dimer frequency for each gene on each membrane was used to calculate the percentage repair for the gene on that membrane.

**Gene-specific Repair of CPDs in the Normal Cell Strain.** The repair experiments were performed after 10 J/m² UV-irradiation with repair time points of 4, 8, and 24 h for the normal cell strain at 8 and 24 h for the XP-C cell strain. Representative blots are shown in Fig. 2.

The gene-specific repair results for the normal strain are shown graphically in Fig. 3, A and B. Fig. 3A displays the gene-specific repair profile of the normal human cell strain studied. As has been previously described for normal human cell strains, there is preferential repair in the active DHFR gene (24). Repair in the p53 tumor suppressor gene is preferential compared to the inactive δ globin and locus 754; in addition, the p53 gene is more efficiently repaired than the DHFR gene.

The δ globin gene is repaired at the level of the overall genome (61% at 24 h) consistent with our previously published results for δ globin in the normal primary fibroblast cell strain, Pat Bru (24). Repair in the 754 DNA fragment is low with maximal repair of 34% at 24 h. Not only is the repair observed in this inactive fragment lower than that seen in the inactive δ globin gene, but it is also lower than the level of repair previously published for the overall genome of normal human cell lines (27).

As shown in Fig. 3B, repair of the p53 gene is strand-specific. Repair in the TS of p53 is rapid with 91% of the CPDs removed at 24 h. The repair in the NTS parallels the repair seen for bulk DNA and the δ globin gene at 4, 8, and 24 h, respectively.

**Gene-specific Repair in XP-C Cells.** Fig. 4A displays the gene-specific repair data in XP-C. There is preferential repair of the DHFR gene which is comparable to the previously published results for the XP2BE cell strain (24). Both inactive regions studied, locus 754 and
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δ globin, have repair levels which are low: 16% at 24 h for the 754 locus; and 9% at 24 h for δ globin. These findings are consistent with our earlier results in this cell strain (24) and with the hypothesis that XP-C cells are unable to remove damage from inactive regions of chromatin. These results are significantly different from those found in the normal cell strain.

The p53 gene is the most efficiently repaired gene we have studied thus far in this repair deficient XP-C cell strain. The repair of the p53 is more efficient than the repair of the DHFR gene. The differences in repair are most pronounced at 24 h; 63% of CPD have been removed from the p53 gene compared with 47% in the DHFR gene. Because of these significant differences in gene specific repair, we also probed DNA from the XP-C cells for a 19-kilobase EcoRI restriction fragment of the active c-myc gene. Repair in the p53 gene was also higher than that seen in this c-myc fragment (data not shown).

As shown in Fig. 4B, the repair of the p53 gene is preferential and strand-specific. Repair in the TS accounts for all of the repair observed in the double-stranded p53 gene. TS repair of p53 also proceeds more slowly in XP-C cells than in the normal cell strain; however, by the 24-h time point the strand-specific repair in the TS of XP-C cells is the same as the normal control, 90 and 91%, respectively. Repair in the NTS of p53 is similar to the level of repair seen in the inactive 754 fragment, the δ globin, and the NTS of the DHFR gene (data not shown).

Discussion

We have examined gene-specific and strand-specific repair of CPDs in the p53 gene in a normal cell strain and in an XP group C cell strain. There is efficient and preferential repair in the p53 and DHFR genes for both cell strains. Interestingly, the repair of the p53 gene is faster than that in the constitutively expressed DHFR gene in both cell strains, with this difference being more pronounced in the XP-C cell strain. In both cell strains, there is a strand bias of repair in p53 with more rapid repair in the transcribed strand than in the nontranscribed strand. This strand bias of repair in the p53 gene is more prominent than the repair bias seen in the DHFR gene in this XP-C cell strain (24). We also found a difference in repair of the two inactive regions in the normal cell strain, but this difference was not observed in the XP-C cell strain. Although the p53 gene is repaired faster in normal cells than in XP-C, the repair efficiency of this gene is particularly high in the XP-C cells. XP-C cells have a distinct repair phenotype (24); the overall genome repair is low, only about 10% of the CPDs are repaired in 24 h, yet active genes are repaired almost as fast as in

Fig. 3. A, average repair in 4 genes/gene regions studied the normal fibroblast strain after treatment with 10J/m² UV. Each repair time point is the average of values obtained for that time point from 2 autoradiograms from each of 2 experiments performed for the cell line in each gene. ■, locus 754; ●, p53; ▲, DHFR; ▼, δ globin. In B, the average repair of the TS (■), double-stranded (DS; ●), and NTS (▲) of the p53 gene is the average of values obtained for that time point from each of 2 experiments performed for each cell line.

Fig. 2. A and B, representative Southern blots of DNA damage and repair in the double-stranded p53 gene as well as in the transcribed and nontranscribed strands in the normal control GM0038A and the XP group C cell strains. The cells were irradiated with UV at 10 J/m². Repair was measured at 4, 8, and 24 h in the normal cell strain and at 8 and 24 h in the XP-C cell line. T4 endo V indicates treatment of sample with T4 endo-nuclease V enzyme.
normal cells, and there is a strand bias of the repair. This means that these repair-deficient cells seem to direct their residual repair capacity toward the active regions of the genome in a manner that resembles the repair phenotype of hamster cells.

The efficiency of gene-specific DNA repair is in many cases associated with the transcriptional activity of a given gene (28, 29); however, it is also becoming evident that transcriptional activity is not the only factor determining the repair level. We speculate that there may be a repair hierarchy in human cells that reflects the “importance” of a given gene for cell survival following DNA damage. The high efficiency of gene-specific repair of the p53 gene in repair-proficient and repair-deficient cell strains suggests that this gene is very important for cell survival. The efficient repair could possibly reflect high transcriptional activity. However, preliminary studies of p53 gene repair in selected tumor cell strains suggests that gene-specific repair is not directly correlated to the level of p53 mRNA expression in the cell strain (30). In addition, our recent studies have uncovered several instances which suggest that gene-specific repair can be independent of transcription. For example, in a study of murine B-cells differences in regional repair do not reflect changes in transcription levels.2

The repair hierarchy exists not only for active genes but also for inactive regions. The differences in repair of the two inactive, transcriptionally silent regions suggest that repair hierarchy is not solely based upon transcriptional activity. In the normal cell strain, we have found that repair in the inactive δ globin gene is comparable to levels of overall genome and bulk DNA repair reported by other investigators (27, 31). However, the repair in the 754 locus is significantly lower than the δ globin gene and the overall genome level in the normal strain. The location of the 754 locus on the X chromosome may be an important factor in determining its repair characteristics. It has been shown that X chromosome inactivation may involve DNA hypermethylation, alteration in DNA-protein interactions, DNA replication, and higher order chromatin structure (32–34). These factors may affect the accessibility of certain genomic regions to DNA repair machinery. This difference in repair for transcriptionally inactive regions is not seen in the XP-C cell strain. The notion that repair in XP-C occurs primarily in transcriptionally active DNA holds true in this XP-C strain as evidenced by the very low levels of repair in both inactive regions studied.

The rapid repair of the p53 gene may relate to the importance of maintaining its genetic fidelity. UV-induced p53 mutations have been implicated in the development of squamous cell carcinoma of the skin in normal individuals (17). This suggests that mutations of p53 may play a role in the high incidence of UV-induced skin cancers manifest in XP patients. As mentioned above, there is increasing evidence for a distinct role of DNA repair in the distribution of mutation. In repair-proficient human fibroblasts, the strand-bias of repair after UV-irradiation results in a strand-bias of mutations on the NTS of the hypoxanthine guanine phosphoribosyltransferase gene (21). The strand-bias of mutations on the NTS of the DHFR gene after treatment of rodent cells with polycyclic aromatic hydrocarbons also correlates with the strand-bias of repair (22). Mutations of p53 found in lung and liver tumors appear to occur preferentially on the nontranscribed strand (2, 13, 15, 16). In the case of UV-induced tumors in XP-C patients, we speculate that there may also be a strand-bias of mutations on the NTS of p53. Indeed, such a finding has just been reported.

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2 E. Beecham et al., submitted for publication.
by Dumasz et al. (35) who examined p53 mutations in skin carcinomas from XP-C patients.

References

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