[CANCER RESEARCH 60, 2273–2280, April 15, 2000]

Enhanced Sensitivity to *anti*-Benzo(*a*)pyrene-diol-epoxide DNA Damage Correlates with Decreased Global Genomic Repair Attributable to Abrogated p53 Function in Human Cells[1]

Manzoor A. Wani, Qianzheng Zhu, Mohammed El-Mahdy, Sundaresan Venkatachalam,[2] and Altaf A. Wani[3]

Department of Radiology [M. A. W., Q. Z. Z., M. E-M., S. V., A. A. W.], Biochemistry Program [S. V., A. A. W.], and James Cancer Hospital and Research Institute [A. A. W.], The Ohio State University, Columbus, Ohio 43210

ABSTRACT

DNA damage from exposure to environmental chemical carcinogens and failure of repair systems to eliminate these lesions from the genome are considered as the crucial initial steps in the development of various human malignancies. Many cellular proteins are known to play vital roles to overcome the effects of DNA damage. Among such proteins, p53 is known to respond to DNA damage by accumulating in the nucleus and inhibiting cell cycle progression to facilitate DNA repair and the maintenance of genomic stability. In this study, we have investigated the role of p53 protein in modulating nucleotide excision repair of *anti*-benzo(*a*)pyrene-diol-epoxide (BPDE)-DNA adducts and related effects using human fibroblasts with normal (p53-WT) and altered p53 protein (p53-Mut and p53-Null). Interestingly, irrespective of the presence or absence of p53, the *anti*-BPDE dose-dependent p21 protein induction response was qualitatively comparable in all of the three cell lines. However, cells with defective p53 function were deficient for the removal of *anti*-BPDE-DNA adducts from the overall genome compared to cells with wild-type p53 activity. Strand-specific repair analysis within the individual strands of the p53 gene revealed decreased repair of adducts from the nontranscribed strand in p53-Mut and p53-Null cells. However, the repair of the transcribed strand appeared to be identical in all of the three cell lines. Furthermore, p53-Mut and p53-Null cells were more sensitive than p53-WT cells and displayed increased levels of *anti*-BPDE-induced apoptosis. Thus, wild-type p53 is required for the efficient global genomic repair of *anti*-BPDE-induced DNA adducts from the overall genome, but not for transcription-coupled repair of actively transcribed genes. These findings indicate that inefficient DNA repair of potentially cytotoxic and mutagenic lesions from the nontranscribed strand due to the loss of p53, but not the loss of p21, function might be responsible for enhanced cytotoxicity and apoptosis in human cells upon DNA damage.

INTRODUCTION

Mutational spectra and signature mutations in the p53 tumor suppressor gene have revealed many useful clues regarding the etiology of cancer (1–3). Loss of tumor-suppressor functions of p53 can occur as a result of a genetic rearrangements (4) or somatic (5) and hereditary mutations (6). The p53 protein plays a vital role in the regulation of various DNA damage response pathways within the cell. After DNA damage, p53 accumulates in the nucleus and shuts off DNA replication to allow the opportunity for repair of damaged DNA (7, 8). This rapid induction of the tumor suppressor protein occurs in cells containing wild-type p53 but not in cells with the mutated p53 gene (9). If DNA repair fails, p53 may trigger cell death by apoptosis (7, 10–12). However, p53-deficient cells have also been shown to undergo apoptotic cell death, indicating the presence of other p53-independent pathways (8, 13). Besides these functions, p53 can act as a transcriptional activator of repair-related genes like gadd45 and associate with NER[4] proteins XPB, XPD, and CSB *in vitro* and *in vivo* (14, 15). In addition, earlier studies with p53-deficient cells have implicated a role of p53 in the excision repair process (16, 17). Thus, normal p53 acts as a "molecular policeman" guarding genomic integrity by virtue of its ability to mediate a wide range of interrelated processes that ensure the faithful propagation of parental genomic sequences and the avoidance of mutations. The importance of p53 in maintaining cellular homeostasis has been reiterated in p53 knockout mice, which show an increased susceptibility to tumorigenesis and have provided a useful model for cancer research (18).

It is well known that living cells rely on NER to remove a wide range of DNA damage caused by different physicochemical agents (19). This defense system allows cells to cope with the harmful effects of various DNA-damaging agents (20). An interesting feature of the excision repair process is its damage repertoire that allows different types of DNA lesions to be recognized and repaired (21, 22). The importance of DNA repair for maintaining normal cellular integrity and prevention of neoplastic phenotype is underscored by the fact that defective repair is linked to increased susceptibility of cells to toxic, mutagenic, and carcinogenic effects of genotoxin exposure (22, 23).

Benzo(*a*)pyrene is a major environmental pollutant and an ubiquitous carcinogen present in automobile exhaust, cigarette smoke, various foods, and industrial waste (24). The diol-epoxide, (±)-*anti*-BPDE (racemic 7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene), is the highly reactive electrophilic metabolite of benzo(*a*)pyrene and is reported to cause mutations, cytotoxicity, and inhibition of DNA synthesis in both prokaryotic and eukaryotic cells (25). *anti*-BPDE reacts at several nucleophilic sites in DNA, and the covalent N^2-dG-*anti*-BPDE adduct is most abundant (26). It has been well established that the guanine adducts are the major adducts formed in cultured cells or DNA treated with racemic mixtures of BPDE, and 80–90% of the guanine adducts are the (+) optical isomers (27, 28). The DNA base alterations arising from exposure to (±)-*anti*-BPDE have been implicated in carcinogenesis and cellular transformation (29, 30). In the present report, we have used (±)-*anti*-BPDE as a model DNA-damaging agent to study the modulation of p53 induction response and its related effects in human cells having wild-type, mutant, or no p53 protein. We have shown that human fibroblast cells derived from normal individuals with wild-type p53 protein are more resistant to the cytotoxic action of *anti*-BPDE than cells with mutant p53 or no p53 protein derived from LFS patients. We have also shown the wild-type p53 dependence for efficient global genomic DNA repair as well as the removal of *anti*-BPDE-DNA adducts from the NTS. However, expression of wild-type p53 was not

Received 11/18/99; accepted 2/17/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by National Institute of Environmental Health Sciences Grant ES2388.

[2] Present address: Division of Molecular Virology, Baylor College of Medicine, Houston, TX 77030.

[3] To whom requests for reprints should be addressed, at 103 Wiseman Hall, 400 W. 12th Avenue, Columbus, OH 43210. Phone: (614) 292-9375; Fax: (614) 292-7237; E-mail: wani.2@osu.edu.

[4] The abbreviations used are: NER, nucleotide excision repair; BPDE, benzo(*a*)pyrene-diol-epoxide; GGR, global genomic repair; LFS, Li-Fraumeni syndrome; MTT, methylthiozole tetrazolium; NTS, nontranscribed strand; p53-Null, p53-nullizygous; p53-Mut, p53-mutant; p53-WT, p53-wild type; TCR, transcription-coupled repair; TS, transcribed strand.

required for TCR of the transcribed strand, apoptosis, and the induction response of its signal transducer protein, p21.

MATERIALS AND METHODS

Cell Culture and BPDE Treatment. Normal human (p53-WT) fibroblasts (OSU-2) were established in culture as described earlier (31). LFS fibroblast strains, MDAH087 (p53-Mut), harboring a codon 248 single base substitution, and MDAH041 (p53-Null), harboring a codon 184 frameshift mutation, both >200 population doubling, postcrisis p53 homozygous cell strains, were kindly provided by Dr Michael Tainsky (M.D. Anderson Cancer Center, Houston, TX). The position 248 substitution mutation, like that in MDAH087 cells, can inactivate the transactivation function of p53 (32). On the other hand, a single base deletion, like that in MDAH041 cells, causes a translational frameshift, resulting in the production of a smaller protein from a cryptic message (33, 34). All fibroblast cell lines were grown under standard conditions as described (17). For the assessment of DNA damage and repair in the above mentioned cell lines, the monolayer cells were grown to confluence in 150-mm dishes, and cells were starved by placing them in serum-deficient medium for 24 h. Modifications with different doses of (±)-*anti*-BPDE (prepared from a fresh 100× stock in 95% ethanol) were done by exposing monolayer cells in Hank's balanced salt solution for 30 min (pH 7.0) and 37°C. The exposed cells were washed with PBS and further incubated in serum-deficient medium for varying posttreatment periods. No DNA replication was observed under these conditions (31).

Cell Survival Assays. Cell survival was measured using colony formation assay and MTT metabolic viability assay. Exponentially growing cells were plated at appropriate densities in 100-mm dishes and exposed to varying doses of *anti*-BPDE after overnight attachment. The cells were then allowed to grow for 14 days in growth medium, washed with PBS, fixed with methanol:acetic acid (3:1), and stained with 0.1% crystal violet. Colonies of >50 cells were counted after normalizing for plating efficiency of cell types. For MTT assay, asynchronously growing cells (5×10^3) were transferred into 96-well tissue culture plates (Corning) in 200 μl of complete medium. After 24-h incubation, the medium was removed, the attached cells were washed once with PBS, and the columns of 8 wells were exposed to increasing doses of *anti*-BPDE. After 30-min carcinogen treatment, cells were once again washed with PBS, replaced with fresh medium, and incubated for another 24–72 h. Cell viability in octuplicate wells was determined by the ability of live cells with intact mitochondria to convert the soluble salt of MTT into an insoluble formazan precipitate (35). Aliquots of 50-μl MTT (1 mg/ml) were added to each well for 2 h, and the color formed was quantitated by a spectrophotometric plate reader (Biotek) after solubilization in DMSO. Cell viability was expressed relative to the untreated control wells.

Western Blot Analysis. Exponentially growing cells were washed with PBS and exposed to varying doses of *anti*-BPDE. Cells were recovered by gentle trypsinization after incubation for indicated time periods. The cells were immediately lysed by boiling for 10 min in sample buffer [2% SDS, 10% glycerol, 10 mM DTT in 62 mM Tris-HCl (pH 6.8), 10 μg/ml pepstatin, and 10 μg/ml leupeptin]. Western blot was performed with aliquots from an equivalent number of cells, as described earlier (36). Briefly, protein extracts from $\sim 1.2 \times 10^5$ cells were separated in 8 or 12% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes using a semidry electroblotter (Hoeffer, San Francisco, CA). Equal protein loading was confirmed from visualization of the membranes stained for 15–20 min with fast green (0.1% fast green, 5% glacial acetic acid, and 20% methanol). For p53 protein detection, a mixture of *anti*-p53 protein antibodies [p53 Ab-2 and p53 Ab-6 from hybridoma clones 1801 and DO-1, epitope maps within the NH_2 terminus (amino acid 32–79) and (amino acid 37–45) of p53, respectively] were used at 1:200 dilution. Antibodies for the detection of p21 (clone DC60.2) were also used at a 1:200 dilution. All these antibodies were obtained from Neomarkers (Freemont, CA). The membranes were developed after treatments with goat antimouse IgG horseradish peroxidase conjugate (Boehringer Mannheim, Indianapolis, IN). The specific proteins were visualized using the enhanced chemiluminescence substrate reaction (Pierce, Rockford, IL). All experiments were repeated at least thrice, and the results of representative experiments are presented.

Quantitation of BPDE-DNA Adducts by Immunoslot Blot Assays. The extent of *anti*-BPDE-DNA adduct formation and repair was quantitated using noncompetitive immunoslot blot assay, as described earlier (31). Briefly, cells were exposed to different doses of *anti*-BPDE for 30 min. Cells were then washed with PBS and either harvested immediately or maintained in serum-free medium for varying posttreatment times. Cells were recovered by trypsinization, and genomic DNA was isolated after immediate cell lysis. DNA concentration was determined spectrophotometrically at 260 nm and by the microdiphenylamine assay (14). For determining the extent of *anti*-BPDE-DNA adduct formation and repair, increasing concentrations of unmodified, modified, and repaired DNA samples were immobilized to nitrocellulose filters. The filters were sequentially exposed to primary Pab BP1 and developed against *anti*-BPDE-modified single-stranded DNA and enzyme-labeled secondary antibody. The specificity of BP1 antibodies against various modified DNA base adducts has been thoroughly characterized (37), and their use in quantitative assessment of *anti*-BPDA-treated cellular DNA has been described earlier (38). The damage levels were calculated by comparing the band intensities of the samples with *anti*-BPDE-modified DNA standard samples run in parallel with all of the blots. The experiments were repeated three times to ensure reproducibility of results.

DNA Fragmentation Analysis. DNA fragmentation assays were performed essentially as described earlier (17). Briefly, at various posttreatment times, the attached cells were gently trypsinized, mixed with any unattached cells, removed with the medium, and pelleted by centrifugation. DNA was isolated from the cell pellets by a differential cellular lysis procedure that separates fragmented DNA released only by apoptotic cells from native intact DNA of unaffected cells by centrifugation. The 13,000-g supernatant was treated with RNase-A for 30 min at 37°C followed by Proteinase K digestion for 1 h at 45°C. Supernatant sample aliquots, equivalent to $\sim 1 \times 10^6$ cells, were separated by agarose gel electrophoresis (1.2%) and visualized by ethidium bromide staining. The apoptotic index was calculated based on the ratio of the fraction of released fragmented DNA:the total DNA in the initial pellet (13).

Strand-specific Repair Analysis by UvrABC Excision Reaction. Strand-specific repair of *anti*-BPDE-DNA adducts was examined within the TS and NTS of the 16-Kb *Eco*RI restriction fragment containing all of the exons of the p53 gene except exon-1. The UvrA protein was purified from *Escherichia coli* SURE host strain harboring a UvrA expression vector, pUNC45, as described (39). UvrB and UvrC proteins were purified from *E. coli* XL-1 Blue and C41(DE3) strains containing vector pUTG97 and pUTG98 respectively, using the intein-mediated purification with an affinity chitin-binding tag system. Approximately 40 μg of DNA, from cells, treated with 2 μM *anti*-BPDE and allowed different periods of time to remove BPDE-DNA adducts, was digested with *Eco*RI (5 units/μg of DNA) at 37°C for 4–6 h. Complete digestion was verified by electrophoresis of sample aliquots on agarose minigels. The digested DNA was purified by extraction with Phenol/Chloroform (3.5:6), precipitated with ethanol, and quantitated. Five pg of linearized plasmid DNA containing the p53 cDNA sequence was added into each DNA sample as an internal standard. One-half of the mixture was exposed to UvrABC excinuclease, and the other half was exposed to reaction buffer alone under conditions of excinuclease digestion. The reaction mixture contained 20 μg of *Eco*RI-digested genomic DNA plus 10 pmol of UvrA, 50 pmol of UvrB, and 50 pmol of UvrC in a final volume of buffer containing 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 75 mM KCl, 2 mM ATP, 1 mM DTT, and 1 μg/μl BSA. The samples were incubated at 37°C for 1 h, and the reaction was stopped by adding 0.1 μg/μl Proteinase K and 0.1% SDS to each sample followed by an additional incubation at 42°C for 1 h. The UvrABC-treated DNA was purified by extraction with phenol/chloroform (3.5:6) and precipitated with ethanol. The samples were then dissolved in denaturation buffer [90% formamide, 0.025% bromphenol blue] and incubated at 37°C for 30–45 min to denature the DNA. After incubation, the samples were electrophoresed at 1.5 V/cm for 18–20 h in a 0.7% neutral agarose gel. The DNA in the gel was stained, acid-depurinated, neutralized, and transferred to a nylon membrane (40). Hybridization experiments were performed in 10 ml of solution containing 50% (v/v) formamide, 6× SSC, 0.5% SDS, 5% dextran sulfate, denatured salmon sperm DNA (100 μg/ml), and $1–2 \times 10^8$ cpm ^{32}P-labeled single-stranded exon 8-specific probes generated by PCR (41). After hybridization for 20–22 h, the filters were washed to final stringency at 62°C in 1× SSC/1% SDS and exposed to a phosphorimager screen, and the individual band intensities were quantitated upon imaging and processing by Imagequant software (Molecular Dynamics). The rate of removal of BPDE-DNA adducts within the

individual strands of the p53 gene was determined by quantifying the reappearance of the full-length restriction fragment bands. The average number of UvrABC-sensitive sites per fragment was calculated by Poisson distribution equation (42). The filters were also used to expose Kodak X-OMAT film for autoradiography. The strand-specific repair experiments were repeated with two independent samples.

RESULTS

***anti*-BPDE Induction Response of p53 and p21 Proteins.** To establish a carcinogen dose and DNA damage response in fibroblasts with distinct p53 status, human cells were treated with increasing doses of *anti*-BPDE. Normal human fibroblasts, expressing wild-type p53 and the two homozygous cell lines with altered p53 protein, showed clear and distinguishable differences in their *anti*-BPDE induction response of p53 and p21 proteins (Fig. 1*A*). A brief 30-min exposure to doses as low as 0.3 μM *anti*-BPDE resulted in a significant increase in p53 protein levels in p53-WT cells. Time course experiments indicated that the rapid induction response, detectable within a few hours and peaking at about 8–24 h, was well sustained beyond a period of 24 h (Fig. 1*B*). A severalfold increase in the induction of p53 protein was observed with higher doses of *anti*-BPDE. Consistent with the wild-type p53 status of p53-WT cells, the levels of p21, a known transcriptional target of p53, were also induced severalfold and sustained up to 24 h. Homozygous p53-Mut cells, harboring a missense Arg→Trp base substitution mutation at codon 248 of the p53 gene (43), showed constitutively elevated levels of the mutant p53 protein in carcinogen treated as well as untreated cell populations. No p53 protein was detectable upon *anti*-BPDE treatment in homozygous p53-Null cells containing a frameshift mutation at codon 184 of p53 gene (43). Surprisingly, both the p53-Mut and p53-Null cells showed a clear induction of p21 protein as a function of *anti*-BPDE dose. Time course experiments with 1.2 μM of *anti*-BPDE demonstrated that levels of p21 peaked at ~8 h in all three cell lines. However, the induction of p21 was not sustained up to 24 h in the p53-Mut and p53-Null cells. Nevertheless, it was obvious that *anti*-BPDE induction of p21 expression was not dependent upon the presence of p53-WT activity in human fibroblast cells.

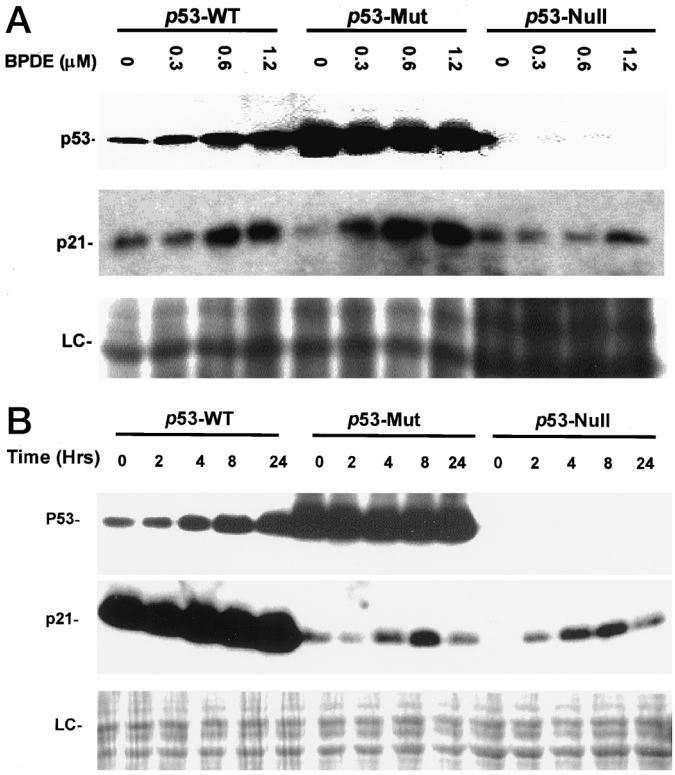

Fig. 1. Carcinogen dose-dependent increase of p53 and p21 proteins in human. *A*, exponentially growing fibroblasts of varying p53 status were treated with the indicated doses of (±)-*anti*-BPDE for 30 min and lysed at 8 h. Cellular extracts (equivalents of 1.2×10^5 cells/lane) were separated by SDS-PAGE, transferred to polyvinylidene difluoride membrane, and visualized by enhanced chemiluminescence as described in "Materials and Methods." *B*, time-dependent increase of p53 and p21 upon 1.2 μM (±)-*anti*-BPDE treatment. Fast green staining of the bottom portion of protein blots was used as the loading control (*LC*).

Global Genomic DNA Repair of *anti*-BPDE Adducts. To ascertain the role of wild-type and mutant p53 proteins on the NER process, we measured the rates of DNA repair in the above mentioned cell lines. The relative extent of *anti*-BPDE-DNA damage induction and its repair in genomic DNA were determined by noncompetitive immunoslot blot assays using lesion-specific polyclonal antibodies (37). In these experiments, human fibroblasts of varying p53 functional status were exposed to increasing doses of *anti*-BPDE, and the amount of *anti*-BPDE-DNA adducts was determined. The three cell lines did not show any significant difference in the formation of initial *anti*-BPDE-DNA adducts in their genomic DNA as indicated by the identical linear dose-response curves (Fig. 2*A*). For repair experiments, the cells lines were treated with a dose of 1.2 μM *anti*-BPDE, which gave an initial DNA damage level of $\sim 40 \pm 5$ adducts/10^6 nucleotides. Cells exposed to the carcinogen for 30 min (0 h) and incubated for increasing periods of time (up to 24 h) were lysed and analyzed for *anti*-BPDE-DNA adducts. Normal human fibroblasts containing wild-type p53 showed an efficient DNA repair response, removing a greater percentage of adducts at earlier time points than p53-Mut and p53-Null cells, which exhibited significantly reduced rates of NER and overall loss of *anti*-BPDE-DNA adducts (Fig. 2*B*). Quantitative comparisons at 24 h after exposure to *anti*-BPDE showed that p53-WT cells removed 70% of *anti*-BPDE-DNA adducts followed by 50% and 35% by p53-Mut and p53-Null cells, respectively. A similar differential response in repair pattern was apparent in the three cell lines at earlier posttreatment times.

Strand-specific Repair of *anti*-BPDE-DNA Adducts in p53 Gene. To further determine the effect of p53 loss or mutation on strand-specific DNA repair, the removal of *anti*-BPDE-DNA adducts was measured from TS and NTS of the p53 gene in p53-WT, p53-Mut, and p53-Null cells. All of the three cell lines were exposed to 2 μM of *anti*-BPDE, which was harvested immediately or after different time periods to allow removal of *anti*-BPDE-DNA adducts. The initial and residual *anti*-BPDE-induced DNA lesions formed within the individual strands of the 16-kb *Eco*RI fragment of the p53 gene were determined upon adduct-specific cleavage by UvrABC excinuclease. The selected fragment contains all of the exons of the p53 gene except exon-1 and is thereby entirely within the full transcription unit. Representative autoradiograms of the repair experiments with the three cell lines are presented in Fig. 3*A*. The band intensities were quantitated using a Molecular Dynamic PhosphorImager by analyzing the fragment images left on phosphor cassettes by the probe hybridized blots. The average number of UvrABC-sensitive sites per fragment were calculated by the Poisson distribution equation, and these calculations took into consideration the UvrABC excinuclease nonspecific cleavage, which was in the range of 0.03–0.16 incisions/16 kb. Under the conditions of our treatment, the initial frequency of *anti*-BPDE adducts formed by 2 μM of *anti*-BPDE was in the range of 1–1.3/16-kb fragment in the DNA of all of the three cell lines. The normal human fibroblasts containing the wild-type p53 gene efficiently repaired both TS and NTS of the p53 gene showing 80% repair within 8 h, and the repair was virtually complete within 24 h (Fig. 3*B*). However, slightly reduced initial repair of the NTS could be seen in p53-WT cells at 4 h posttreatment. Both the homozygous p53-Mut

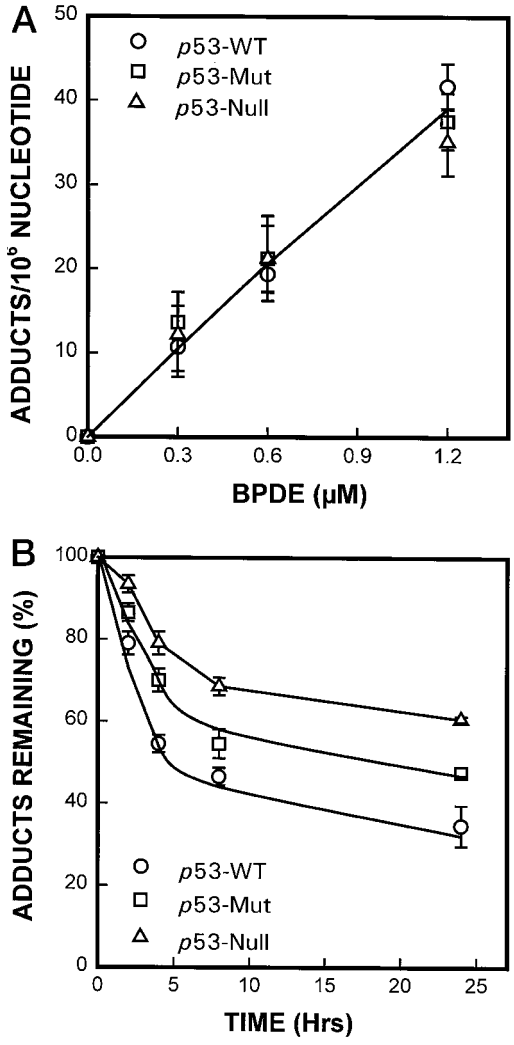

Fig. 2. DNA damage and repair of *anti*-BPDE-DNA adducts in human fibroblasts. *A*, monolayer cells were exposed to increasing doses of *anti*-BPDE, and adduct levels were quantitated from duplicate analysis of two to three different concentrations falling within the linear response range of the modified standard. The data shown is an average of three independent experiments. *B*, global NER of (±)-*anti*-BPDE-DNA adducts in human fibroblast cells. Confluent cultures of human fibroblast cells were treated with 1.2 μM (±)-*anti*-BPDE for 30 min and incubated for the indicated posttreatment times before harvesting and DNA isolation. Immunoslot blot analysis of constant amounts of isolated DNA samples was performed within the linear response range of the modified standard. DNA repair was determined from the decrease in the immunoslot blot band intensities due to as the loss of adduct antibody binding sites from DNA obtained from indicated cell types at varying times after *anti*-BPDE exposure. Extent of DNA repair is expressed as the percent of initial damage remaining at the indicated time points.

and p53-Null cells also displayed efficient repair of TS of the p53 gene, showing repair rates almost comparable to that of p53-WT cells. Moreover, the repair of the NTS of the p53 gene in these cell types was considerably less than that observed with p53-WT cells. Homozygous p53-Mut cells showed about 30 and 40% repair within 8 and 24 h, respectively. Homozygous p53-Null cells exhibited the least repair, showing about 20% repair of NTS within 24 h. Consistent with their inefficient capacity for GGR, these results indicated that the p53-deficient cells lacked the ability to repair chemical carcinogen-induced DNA adducts from NTS while their TCR activity remained unaffected by the absence of p53 protein.

Cellular Sensitivity to *anti*-BPDE Treatment. Clonogenic survival assays were performed to determine the effect of wild-type p53 protein on the inherent cellular sensitivity to carcinogen exposure. The three cell lines exhibited marked differences in the colony-forming ability after exposure to *anti*-BPDE. Normal human fibroblasts (p53-WT) containing wild-type p53 protein showed higher resistance to *anti*-BPDE than fibroblasts with mutant or no p53 protein (Fig. 4*A*). The cells lacking p53 protein (p53-Null) exhibited lowest survival, indicating greater reproductive cell death. The short-term viability assessments of cell lines were in concordance with the results of survival assays. The cell lines exposed to 1.2 μM of *anti*-BPDE showed significant differences in carcinogen cytotoxicity at 24, 48, and 72 h (Fig. 4*B*). Although all of the three cell lines exhibited a carcinogen-induced decrease in the extent of dye metabolizing functional cell population, the p53-WT cells were relatively more resistant than p53-Mut and p53-Null cells. The differences in the cytotoxic response, as indicated by cell viability, was more pronounced when cells were analyzed after 72 h than at earlier time points. The homozygous p53-Null cells showed the highest cytotoxicity in comparison to p53-Mut and p53-WT cells. In addition, there was a significant recovery in viable p53-WT cells upon maintaining in growth medium for 72 h after chemical carcinogen exposure.

Cellular Apoptosis after *anti*-BPDE Treatment. To determine whether the increased cytotoxicity of carcinogen-treated p53-Mut and p53-Null cells was due to p53-independent apoptosis, we performed DNA fragmentation analysis of the *anti*-BPDE-treated cells. All of the three cell lines exhibited a time-dependent increase in the amount of fragmented DNA after 1.2 μM of *anti*-BPDE exposure. DNA gel electrophoresis showed that even after 48 h of *anti*-BPDE exposure, the released DNA from p53-WT cells retained the molecular size in excess of 50 Kb (Fig. 5*A*). On the other hand, p53-Mut and p53-Null cells demonstrated significant DNA fragmentation, and the size of DNA fragments was greatly reduced, reaching the expected 180-bp nucleosome monomer size for the p53-Null cells at 48 h. Quantitative assessment of apoptotic index in the treated cell populations indicated a gradual increase in the released DNA (apoptotic fraction) as the incubation time of cells was increased. The level of DNA fragmentation was 15% for p53-WT cells followed by 30% for p53-Mut cells (Fig. 5*B*). The highest level of apoptosis was seen with p53-Null cells reaching up to 43% at 48 h, and these cells also showed a rapid DNA fragmentation response after *anti*-BPDE exposure. Furthermore, cytological examination of fluorescent dye-stained p53-Mut and p53-Null cells also revealed morphological changes typical of apoptosis *i.e.*, DNA condensation, membrane blebbing, cell shrinkage, and apoptotic bodies. No such changes were prominent in the population of treated p53-WT cells.

DISCUSSION

The processing of DNA damage is a complex cellular phenomenon involving various biochemical factors responsible for adduct recognition, incision, and excision (44). Emerging evidence suggests that DNA repair may be intimately related to the interplay of several cellular processes, including transcription (45), cell cycle arrest (46), tumor suppressor gene induction, and apoptosis (8, 19). Attempts to define functional links between these processes have implicated various proteins that are directly and indirectly affected upon genotoxic damage. The p53 tumor suppressor protein, one of the key effector molecules that is activated in response to a variety of physicochemical agents (47, 48), regulates a number of diverse cellular processes. Several investigations have provided evidence suggesting a role of p53 in affecting NER activity (49–51). The aim of this investigation was to gain further insights into the possible involvement of p53 in processing DNA damage induced by a chemical carcinogen, *anti*-BPDE, known to cause bulky base modifications. In an attempt to address several important interrelated questions regarding the consequences of genotoxin damage, we used human cells of varying p53

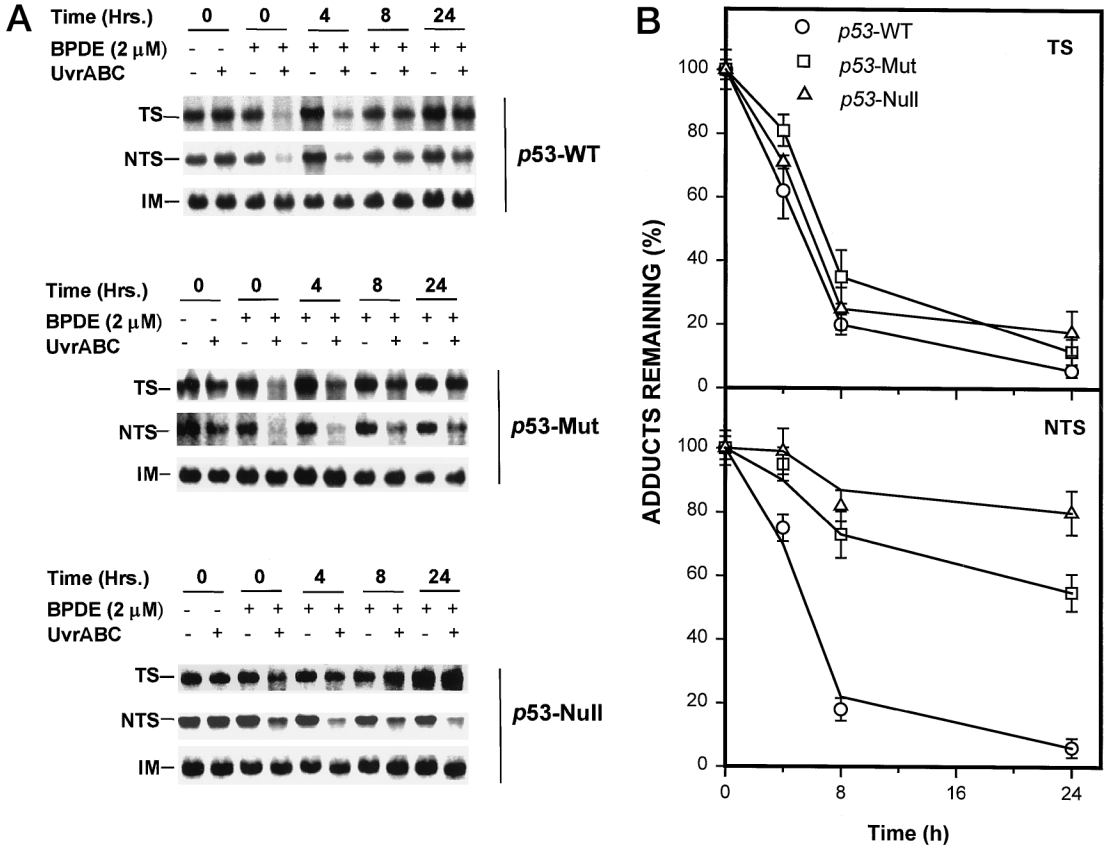

Fig. 3. Repair within the TS and NTS of the p53 gene. Human fibroblast cells with varying p53 status were exposed to the carcinogen as described in "Materials and Methods." *A*, autoradiograms illustrating the extent of repair in the TS and NTS of the p53 gene. DNA was isolated from untreated cells (first two lanes of each panel) or from cells incubated for the indicated time periods after 2 μM (±)-*anti*-BPDE treatment. DNA fragments containing the sequences to be probed were included in each DNA sample as internal markers. The samples were then treated (+) or mock-treated (−) with UvrABC excinuclease and subjected to electrophoresis and Southern hybridization with ^{32}P-labeled probes. Bands marked *TS* and *NTS* correspond to the 16-kb fragment of the p53 gene, and the bands marked as *IM* correspond to the DNA fragments serving as internal markers. *B*, repair profile for the rate of removal of *anti*-BPDE adducts from the TS and NTS of the p53 gene. Bands were quantitated by phosphorimager analysis, and the percentage of repair was determined upon comparison with the intensity from bands at 0 h. The nonspecific incision ranging from 0.03–0.10 was subtracted from each point. The data shown represent mean ± SE from two separate experiments.

status to study the effects of DNA damage on repair processing and programmed cell death from exposure to *anti*-BPDE.

We have previously reported that p53-Mut and p53-Null cells are deficient in GGR as well as the repair of UV-induced pyrimidine dimers from the NTS compared to cells having a functional wild-type p53. To extend these findings to chemical genotoxin, the present study used (±)-*anti*-BPDE as the model DNA-damaging agent. Demonstration of an association between *anti*-BPDE-induced DNA adducts and an increase in wild-type p53 protein suggested a direct cause-effect relationship between *anti*-BPDE-DNA damage and p53 response. Constitutive high level expression of mutant p53 protein was detected in p53-Mut cells, and a complete absence of p53 protein in p53-Null

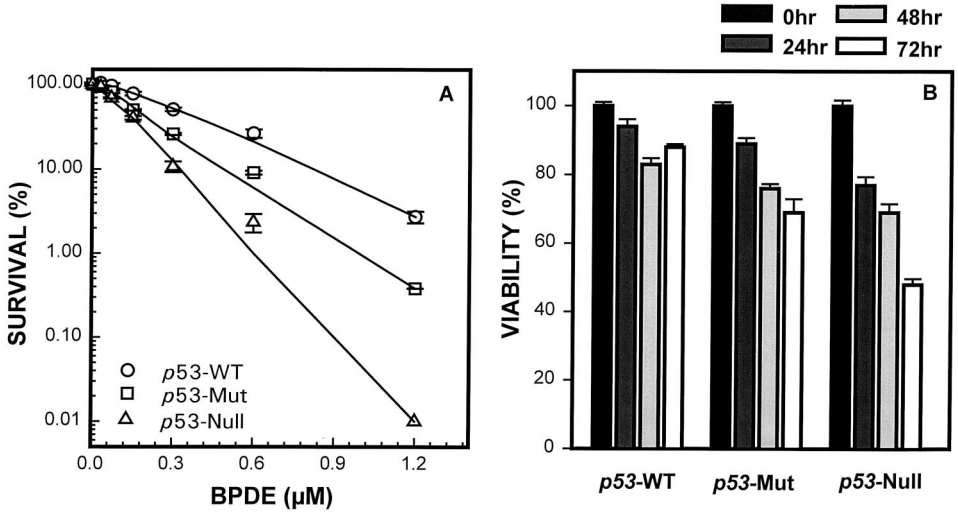

Fig. 4. Clonogenic cellular survival and viability of (±)-*anti*-BPDE-treated cells. Human fibroblast cells of varying p53 status were exposed to increasing doses of the carcinogen, and cell survival and viability were determined as described in "Materials and Methods." *A*, exponentially growing cells were exposed to *anti*-BPDE, and cell survival was estimated by assessing colony formation after 14–21 days. The values shown are mean ± SE of 3–4 individual dishes. *B*, cellular sensitivity to 1.2 μM (±)-*anti*-BPDE at 24, 48, and 72 h posttreatment. The values shown are mean ± SE of eight replicate samples.

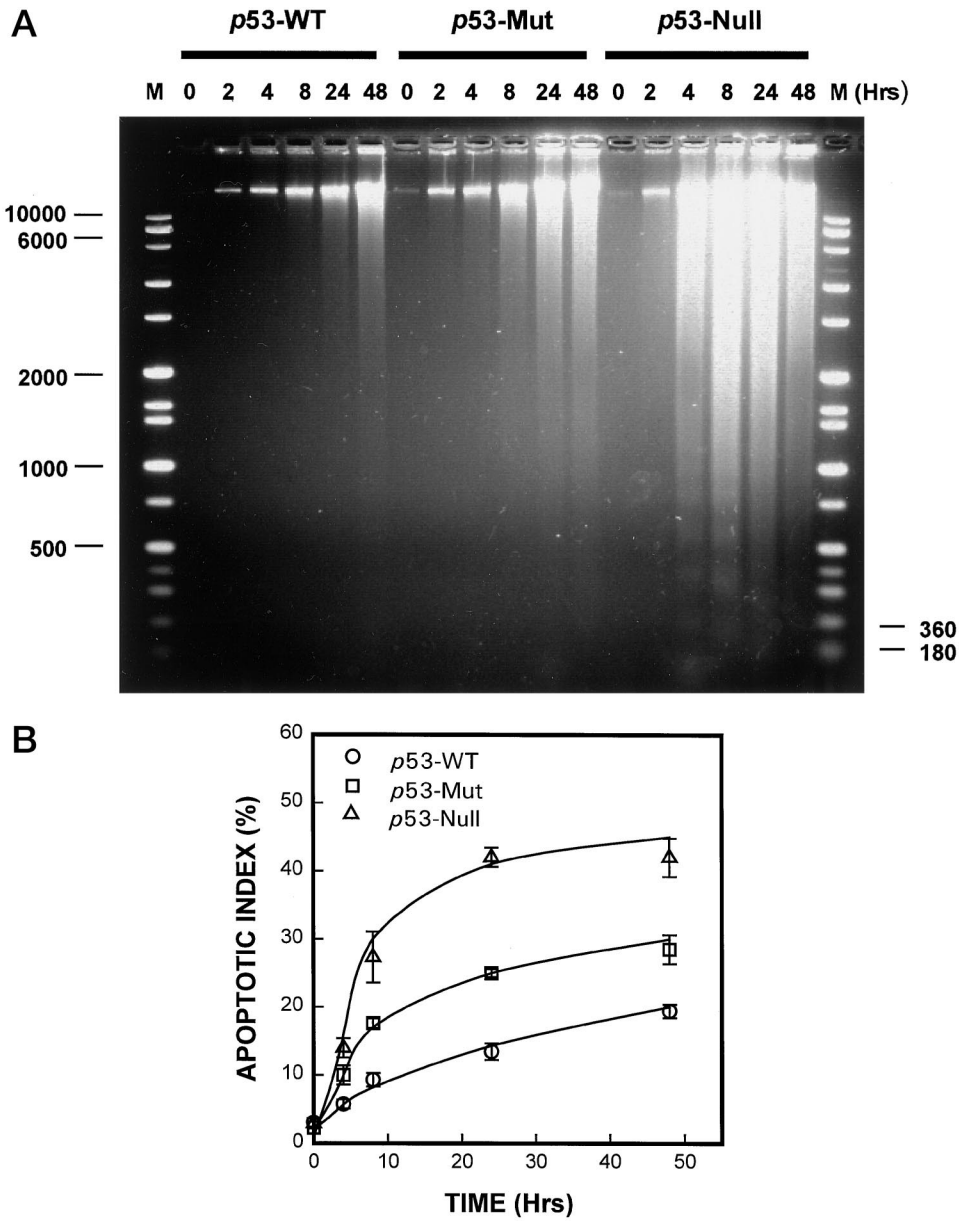

Fig. 5. Effect of (±)-*anti*-BPDE treatment on the induction of apoptosis. Human fibroblast cells with distinct p53 status were exposed to 1.2 μM (±)-*anti*-BPDE and evaluated for apoptosis as described in "Materials and Methods." *A*, fragmented DNA from apoptotic cells was separated and analyzed by agarose gel electrophoresis and ethidium bromide staining. Lanes marked *M* were loaded with DNA size markers. *B*, extent of apoptosis was quantitatively determined by measuring the fraction of cells releasing the fragmented DNA. The data shown represent three individual experiments.

cells was seen irrespective of the level of *anti*-BPDE-induced DNA damage. In addition, our results indicate that wild-type p53 is not an absolute requirement for the expression of p21, implicating the presence of additional pathways. This is further strengthened by the fact that the p21 induction response was qualitatively comparable in all of the three cell lines despite the presence or absence of wild-type p53 function. Although initial reports have shown that the p21 protein induction is p53-mediated (52, 53), recent studies with p53 null and LFS cells have shown that p53 is dispensable for p21 induction mediated by UV exposure and serum-starvation (46, 47). Furthermore, the now well established ability of the p53 homologue, p73, to induce p21 and apoptosis also suggests the presence of additional p53-independent mechanisms (54, 55).

Several investigations based upon assessing the effect of p53-WT on the efficiency of repairing genotoxic damage have suggested a direct participation of p53 protein in the damage excision process. Transient transfection of RKO cells containing wild-type p53 function, with vectors expressing genes that abrogate wild-type p53 activity, resulted in decreased host-cell reactivation of exogenously UV-irradiated reporter plasmid and NER (56). Decreased repair in the absence of wild-type p53 function has also been reported by directly measuring the repair of UV-induced lesions in LFS fibroblasts of different p53 status (16). The results presented here are in agreement with these studies that show a significant difference in NER rates of *anti*-BPDE-induced bulky DNA adducts in human LFS fibroblasts. Besides, all of the three human fibroblasts maintained the preferential and rapid repair within TS of the p53 gene irrespective of p53 status. However, the repair of the NTS was faster and virtually complete within 24 h in p53-WT cells in comparison to reduced repair in p53-Mut cells. No such enhancement in repair was apparent within the NTS of the p53 gene in p53-Null cells even at 24 h (Fig. 3). These results suggest that p53 may not be a necessary component of the active TCR complex, but may be involved in global repair that is also responsible for the lesion elimination within NTS. Our results are further strengthened by the fact that p53 can interact with the repair factors, ERCC-3 and p62, present in transcription factor IIH, which is essential for NER and transcription initiation (18, 37, 43, 50). The intermediate levels of repair seen in p53-Mut cells suggest that the

mutant p53 protein with codon-248 mutation might retain partial function. X-ray crystallography studies have shown that the Arg at codon 248 present in the central domain of the protein is critical for p53 protein-DNA contacts and in turn the transcriptional activation function of p53 (57). However, functional studies indicate that this mutation does not confer a dominant negative function of the mutant protein (over the p53-WT protein) like other mutations seen in Li-Fraumeni patients (58, 59). In addition, protein-protein interactions of p53 with the transcriptional complex and various repair proteins map to the NH_2 terminus and the COOH terminus of the protein, giving rise to the possibility that the mutant protein may be active, albeit inefficiently, for the repair function(s) of p53. Thus, the enhanced DNA repair of p53-Mut cells over that of p53-Null cells could very well be attributed to the maintenance of p53-repair protein interactions. Because inefficient repair of NTS has been implicated in mutagenesis and carcinogenesis, one would expect an increase in the mutation frequency in the absence of p53. However, a similar mutation frequency of the *lacI* transgene in p53 wild type as well as deficient backgrounds has been reported in an earlier study (60). Although the above mentioned study had used a different model system and determined different end points of DNA damage (DNA repair rate *versus* mutations), additional studies are required to dissect the complete role of p53 in the molecular mechanisms of DNA repair and biological consequences of altered p53 function.

In conjunction with the effect of p53 on NER, we also observed that the p53-Null and p53-Mut cells were more sensitive to the cytotoxic effects of *anti*-BPDE-induced DNA lesions than the cells expressing wild-type p53. Although the p53-WT cells showed a dose-dependent decrease in clonogenic survival and viability, they were relatively more resistant and able to promptly recover under full growth conditions. The cells with nonfunctional p53 do not seem to possess a capacity to recover and produce a higher fraction of cells in the survival assays. The ability of p53-WT cells to show such a recovery might be attributed to their capacity for the efficient removal of potentially cytotoxic lesions from their genome, and it could be argued that the presence of p53-WT function confers *anti*-BPDE resistance through a DNA repair related mechanism. It is well documented that the relatively high cytotoxic and mutagenic potential of *anti*-BPDE adducts results from their slower repair as well as their interference on DNA replication and transcription (61). In addition, this cellular recovery phenomenon exhibited by normal human fibroblasts could very well depend on proficient DNA excision repair because NER deficient XP cells are incapable of such recovery (62, 63).

Presence of wild-type p53 also enables cells to arrest at the G_1-S stages of the cell cycle and undergo apoptotic cell death after DNA damage. The apoptotic response is generally cell-type-dependent, and human fibroblast cells are known to show resistance to apoptosis upon genotoxic insult (36, 64, 65). In addition, p53-independent apoptotic pathways have been observed in normal proliferating lymphoid cells (66) and in a variety of other tumor cell types (67). The data presented here are in agreement with a clear lack of apoptosis in normal human fibroblast cells containing wild-type p53. On the contrary, cells with mutant p53 or no p53 protein readily and rapidly undergo apoptosis after genotoxic exposure. The induction of apoptosis recognized by DNA fragmentation was apparent within 4–6 h (Fig. 5*A*). Quantification of apoptotic process demonstrated that in contrast to p53-Mut and P53-Null cells, the p53-WT cells are resistant to the induction of apoptosis (Fig. 5*B*). The results also indicate that *anti*-BPDE-induced damage invokes Phase I fragmentation, resulting in an early release of high molecular weight DNA in both the cell types (36, 68). The data presented here are consistent with our previous findings and with recent reports, whereas wild-type p53 function abrogated by HPV E6 in human fibroblast cells resulted in decreased GGR, enhanced cytotoxicity and apoptosis upon UV-irradiation (69, 70). Based on the overall data and our previous findings, we suggest that the persistent cytotoxic lesions within the NTS that escape repair are responsible for the induction of apoptosis and cytotoxicity seen in p53-deficient cells.

In summary, the data show that the loss of p53-WT function is associated with a decrease in the repair of genotoxic lesions within overall genome and the inefficient removal of *anti*-BPDE-induced DNA lesions from NTS without any significant effect on TS support a clear role of p53 tumor suppressor gene in GGR. Although the mechanism by which p53 activity affects GGR remains unknown, our results demonstrate that cell death can be a p53-independent response, but dependent on DNA damage of the genome that evades repair. Further unraveling of damage response, cell cycle, and the apoptotic pathway and identification of key determinants of eukaryotic repair complex is expected to lead to more innovative approaches for understanding the intricacies of DNA damage processing at various levels of genomic organization.

ACKNOWLEDGMENTS

We thank Dr. Aziz Sancar (University of North Carolina) for providing UvrA plasmid construct, Drs. Yue Zou and Ben Van Houten (University of Texas) for UvrB and UvrC plasmid constructs and protein purification protocols, and Dr. Michael Tainsky (University of Texas) for Li-Fraumeni syndrome cell lines.

REFERENCES

1. Levine, A. J., Wu, M. C., Chang, A., Silver, A., Attiyeh, E. F., Lin, J., and Epstein, C. B. The spectrum of mutations at the p53 locus. Ann. NY Acad. Sci., *768:* 111–128, 1995.
2. Greenblatt, M. S., and Harris, C. C. Molecular genetics of lung cancer. Cancer Surv., *25:* 293–313, 1995.
3. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. p53 mutations in human cancers. Science (Washington DC), *253:* 49–53, 1991.
4. Cullinane, C., Mazur, S. J., Essigmann, J. M., Phillips, D. R., and Bohr, V. A. Inhibition of RNA polymerase II transcription in human cell extracts by cisplatin DNA damage. Biochemistry, *38:* 6204–6212, 1999.
5. Yoon, J. H., Swiderski, P. M., Kaplan, B. E., Takao, M., Yasui, A., Shen, B. H., and Pfeifer, G. P. Processing of UV damage *in vitro* by FEN-1 proteins as part of an alternative DNA excision repair pathway. Biochemistry, *38:* 4809–4817, 1999.
6. Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Jr., Nelson, C. M., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z., and Tainsky, M. A. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas and other neoplasms. Science (Washington DC), *250:* 1233–1238, 1990.
7. Price, B. D., and Park, S. J. DNA damage increases the levels of *MDM2* messenger RNA in wtp53 human cells. Cancer Res., *54:* 896–899, 1994.
8. Nelson, W. G., and Kastan, M. B. DNA strand breaks: the DNA template alterations that trigger p53-dependent DNA damage response pathways. Mol. Cell. Biol., *14:* 1815–1823, 1994.
9. Fritche, M., Haessler, C., and Brandner, G. Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging proteins. Oncogene, *8:* 307–318, 1993.
10. Olive, P. L. DNA damage and repair in individual cells: applications of the comet assay in radiobiology. Int. J. Radiat. Biol., *75:* 395–405, 1999.
11. Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, A. J. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. Cell, *71:* 587–597, 1992.
12. Kastan, M. B., Onyekwere, O., Sidransky, D., Vogelstein, B., and Craig, R. W. Participation of p53 protein in the cellular response to DNA damage. Cancer Res., *51:* 6304–6311, 1991.
13. Venkatachalam, S., Denissenko, M. F., Alvi, N., and Wani, A. A. Rapid activation of apoptosis in human promyelocytic leukemia cells by (±)-*anti*-benzo[a]pyrene-diol epoxide induced DNA damage. Biochem. Biophys. Res. Commun., *197:* 722–729, 1993.
14. Wang, X. W., Zhan, Q. M., Coursen, J. D., Khan, M. A., Kontny, H. U., Yu, L. J., Hollander, M. C., O'Connor, P. M., Fornace, A. J., Jr., and Harris, C. C. GADD45 induction of a G_2/M cell cycle checkpoint. Proc. Natl. Acad. Sci. USA, *96:* 3706–3711, 1999.
15. Wang, X. W., Forrester, K., Yeh, H., Feitelson, M. A., Gu, J-R., and Harris, C. C. Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. Proc. Natl. Acad. Sci. USA, *91:* 2230–2234, 1994.

16. Ford, J. M., and Hanawalt, P. C. Li-Fraumeni syndrome fibroblasts homozygous for p53 mutations are deficient in global DNA repair but exhibit normal transcription-coupled repair and enhanced UV resistance. Proc. Natl. Acad. Sci. USA, *92:* 8876–8880, 1995.
17. Wani, M. A., Zhu, Q. Z., El-mahdy, M., and Wani, A. A. Influence of p53 tumor suppressor protein on bias of DNA repair and apoptotic response in human cells. Carcinogenesis (Lond.), *20:* 765–772, 1999.
18. Donehower, L. A. The p53-deficient mouse: a model for basic and applied cancer studies. Semin. Cancer Biol., *7:* 269–278, 1996.
19. Hanawalt, P. C. Genomic instability: environmental invasion and the enemies within. Mutat. Res., *400:* 117–125, 1998.
20. Ford, J. M., and Hanawalt, P. C. Role of DNA excision repair gene defects in the etiology of cancer. Curr. Top. Microbiol. Immunol., *221:* 47–70, 1997.
21. Sancar, A., and Sancar, G. B. DNA repair enzymes. Annu. Rev. Biochem., *57:* 29–67, 1988.
22. Petit, C., and Sancar, A. Nucleotide excision repair: From *E. coli* to man. Biochimie, *81:* 15–25, 1999.
23. Sancar, A. Excision repair in mammalian cells. J. Biol. Chem., *270:* 15915–15918, 1995.
24. Phillips, D. H. Fifty years of benzo[a]pyrene. Nature (Lond.), *303:* 468–472, 1983.
25. Denissenko, M. F., Cahill, J., Koudriakova, T. B., Gerber, N., and Pfeifer, G. P. Quantitation and mapping of aflatoxin B1-induced DNA damage in genomic DNA using aflatoxin B1–8,9-epoxide and microsomal activation systems. Mutat. Res., *425:* 205–211, 1999.
26. Osborne, M. R., Jacobs, S., Harvey, R. G., and Brookes, P. Minor products from the reaction of (+) and (−) benzo[a]pyrene-*anti*-diolepoxide with DNA. Carcinogenesis (Lond.), *2:* 553–558, 1981.
27. MacLeod, M. C. Interaction of bulky chemical carcinogens with DNA in chromatin. Carcinogenesis (Lond.), *16:* 2009–2014, 1995.
28. MacLeod, M. C., Daylong, A., Adair, G., and Humphrey, R. M. Differences in the rate of DNA adduct removal and the efficiency of mutagenesis for two benzo-[a]pyrene diol epoxides in CHO cells. Mutat. Res., 1991.
29. Harris, C. C. Chemical and physical carcinogenesis: advances and perspectives for the 1990s. Cancer Res., *51:* 5023s–5044s, 1991.
30. Conney, A. H., Chang, R. L., Jerina, D. M., and Wei, S-J. C. Studies on the metabolism of benzo[a]pyrene and dose-dependent differences in the mutagenic profile of its ultimate carcinogenic metabolite. Drug Metab. Rev., *26:* 125–163, 1994.
31. Venkatachalam, S., Denissenko, M. F., and Wani, A. A. DNA repair in human cells: quantitative assessment of bulky *anti*-BPDE DNA adducts by non-competitive immunoassays. Carcinogenesis (Lond.), *16:* 2029–2036, 1995.
32. Kern, S., Kinzler, K., Bruskin, A., Jarosz, D., Friedman, P., Prives, C., and Vogelstein, B. Identification of p53 as a sequence-specific DNA-binding protein. Science (Washington DC), *252:* 1708–1711, 1991.
33. Liu, P. K., Kraus, E., Wu, T. A., Strong, L. C., and Tainsky, M. A. Analysis of genomic instability in Li-Fraumeni fibroblasts with germ-line p53 mutations. Oncogene, *12:* 2267–2278, 1996.
34. Yin, Y., Tainsky, M. A., Bischoff, F. Z., Strong, L. C., and Wahl, G. M. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant *p53* alleles. Cell, *70:* 937–948, 1992.
35. Scudiero, D. A., Shoemaker, R. H., Paull, K. D., Monks, A., Tierney, S., Nofzinger, T. H., Currens, M. J., Seniff, and Boyd, M. R. Evaluation of soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res., *48:* 4827–4833, 1988.
36. Venkatachalam, S., Denissenko, M., and Wani, A. A. Modulation of (+/-)-*anti*-BPDE mediated p53 accumulation by inhibitors of protein kinase C and poly(ADP-ribose) polymerase. Oncogene, *14:* 801–809, 1997.
37. Venkatachalam, S., and Wani, A. A. Differential recognition of stereochemically defined base adducts by antibodies against *anti*-benzo[a]pyrene diol epoxide modified DNA. Carcinogenesis (Lond.), *15:* 565–572, 1994.
38. Venkatachalam, S., Denissenko, M. F., and Wani, A. A. DNA repair in human cells: quantitative assessment of bulky *anti*-BPDE DNA adducts by non-competitive immunoassays. Carcinogenesis (Lond.), *16:* 2029–2036, 1995.
39. Masuda, H., Miller, C., Koeffler, H. P., Battifora, H., and Cline, M. J. Rearrangement of the p53 gene in human osteogenic sarcomas. Proc. Natl. Acad. Sci. USA, *84:* 7716–7719, 1987.
40. Maniatis, T., Fritsch, E. F., and Sambrook, J. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982.
41. Ruven, H. J. T., Seelen, C. M. J., Lohman, P. H. M., Mullenders, L. H. F., and Van Zeeland, A. A. Efficient synthesis of ^{32}P-labeled single-stranded DNA probes using linear PCR; Application of the method for analysis of strand-specific DNA repair. Mutat. Res., *315:* 189–195, 1994.
42. Denissenko, M. F., Venkatachalam, S., Ma, Y. H., and Wani, A. A. Site-specific induction and repair of benzo[*a*]pyrene diol epoxide DNA damage in human H-*ras* proto-oncogene as revealed by restriction cleavage inhibition. Mutat. Res., *363:* 27–42, 1996.
43. Tainsky, M. A., Bischoff, F. Z., and Strong, L. C. Genomic instability due to germ-line p53 mutations drives preneoplastic progression toward cancer in human cells. Cancer Metastasis Rev., *14:* 43–48, 1995.
44. Sancar, A. Mechanisms of DNA excision repair. Science (Washington DC), *266:* 1954–1956, 1994.
45. Hanawalt, P., and Mellon, I. Stranded in an active gene. Curr. Biol., *3:* 67–69, 1994.
46. El-Deiry, W. S. p21/p53, cellular growth control and genomic integrity. Curr. Top. Microbiol. Immunol., *227:* 121–137, 1998.
47. Hall, P. A., McKee, P. H., Menage, H. P., Dover, R., and Lane, D. P. High levels of p53 protein in UV-irradiated normal human skin. Oncogene, *8:* 203–207, 1993.
48. Zhan, Q., Carrier, F., and Fornace, A. J. Induction of cellular p53 activity by DNA-damaging agents and growth arrest. Mol. Cell Biol., *13:* 4242–4250, 1993.
49. Smith, M. L., Chen, I. T., Zhan, Q., Bae, I., Chen, C-Y., Gilmer, T. M., Kastan, M. B., O'Connor, P. M., and Fornace, A. J., Jr. Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. Science (Washington DC), *266:* 1376–1380, 1994.
50. Fan, S., Smith, M. L., Rivet, D. J., II, Duba, D., Zhan, Q., Kohn, K. W., Fornace, A. J., Jr., and O'Connor, P. M. Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. Cancer Res., *55:* 1649–1654, 1995.
51. Wang, X. W., Yeh, H., Schaeffer, L., Roy, R., Moncollin, V., Egly, J-M., Wang, Z., Friedberg, E. C., Evans, M. K., Taffe, B. G., Bohr, V. A., Weeda, G., Hoeijmakers, J. H. J., Forrester, K., and Harris, C. C. p53 modulation of TFIIH-associated nucleotide excision repair activity. Nat. Genet., *10:* 188–195, 1995.
52. El-Deiry, W. S., Harper, J. W., O'Connor, P. M., Velculescu, V. E., Canman, C. E., Jackman, J., Pietenpol, J. A., Burrell, M., Hill, D. E., Wang, Y., Wiman, K. G., Mercer, W. E., Kastan, M. B., Kohn, K. W., Elledge, S. J., Kinzler, K. W., and Vogelstein, B. *WAF1/CIP1* is induced in *p53*-mediated G_1 arrest and apoptosis. Cancer Res., *54:* 1169–1174, 1994.
53. Liu, M., and Pelling, J. C. UV-B/A irradiation of mouse keratinocytes results in p53-mediated WAF1/CIP1 expression. Oncogene, *10:* 1955–1960, 1995.
54. Gong, J. G., Costanzo, A., Yang, H. Q., Melino, G., Kaelin, W. G., Jr., levrero, M., and Wang, J. Y. The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin induced DNA damage. Nature (Lond.), *399:* 806–809, 1999.
55. Jost, C. A., Marin, M. C., and Kaelin, W. G., Jr. p73 is a human p53-related protein that can induce apoptosis. Nature (Lond.), *389:* 191–194, 1997.
56. Smith, M. L., Chen, I-T., Zhan, Q., O'Connor, P. M., and Fornace, A. J., Jr. Involvement of the p53 tumor suppressor in repair of UV-type DNA damage. Oncogene, *10:* 1053–1059, 1995.
57. Cho, Y., Gorina, S., Jeffrey, P. D., and Pavletich, N. P. Crystal structure of p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science (Washington DC), *265:* 346–355, 1994.
58. Milner, J., and Medcalf, E. A. Cotranslation of activated mutant p53 with wild type drives wild type p53 protein into the mutant conformation. Cell, *65:* 765–774, 1991.
59. Srivastava, S., Wang, S., Tong, Y. A., Pirollo, K., and Chang, E. H. Several mutant protein detected in Li-Fraumenic syndrome exhibit transdominant effects on the biochemical properties of wild-type p53. Oncogene, *8:* 2449–2456, 1993.
60. Sands, A. T., Suraokar, M. B., Sanchez, A., Marth, J. E., Donehower, L. A., and Bradley, A. p53 deficiency does not effect the accumulation of point mutations in transgene target. Proc. Natl. Acad. Sci. USA, *92:* 8517–8521, 1995.
61. McGregor, W. G., Mah, M. C. M., Chen, R. H., Maher, V. M., and McCormick, J. J. Lack of correlation between degree of interference with transcription and rate of strand specific repair in the HPRT gene of diploid human fibroblasts. J. Biol. Chem., *270:* 27222–27227, 1995.
62. Maher, V. M., Dorney, D. J., Konze-Thomas, B., and McCormick, J. DNA excision-repair processes in human cells can eliminate the cytotoxic and mutagenic consequences of ultraviolet irradiation. Mutat. Res., *62:* 311–339, 1979.
63. Leadon, S. A. Transcription-coupled repair of DNA damage: Unanticipated players, unexpected complexities. Am. J. Hum. Genet., *64:* 1259–1263, 1999.
64. Agarwal, M. L., Taylor, W. R., Chernov, M. V., Chernova, O. B., and Stark, G. R. The p53 network. J. Biol. Chem., *273:* 1–4, 1998.
65. Canman, C. E., Chen, C-Y., Lee, M-H., and Kastan, M. B. DNA damage responses: p53 induction, cell cycle perturbations, and apoptosis. Cold Spring Harbor Symp. Quant. Biol., *59:* 277–286, 1994.
66. Strasser, A., Harris, A. W., Jacks, T., and Cory, S. DNA damage can induce apoptosis in proliferating lymphoid cells via p53-independent mechanisms inhibitable by Bcl-2. Cell, *79:* 329–339, 1994.
67. Fang, L., Igarashi, M., Leung, J., Sugrue, M. M., Lee, S. W., and Aaronson, S. A. $p21^{Waf1/Cip1/Sdi1}$ induces permanent growth arrest with markers of replicative senescence in human tumor cells lacking functional p53. Oncogene, *18:* 2789–2797, 1999.
68. Walker, P. R., Weaver, V. M., Lach, B., Leblanc, J., and Sikorska, M. Endonuclease activities associated with high molecular weight and internucleosomal DNA fragmentation in apoptosis. Exp. Cell Res., *213:* 100–106, 1994.
69. Ford, J. M., Baron, E. L., and Hanawalt, P. C. Human fibroblasts expressing the human papillomavirus E6 gene are deficient in global genomic nucleotide excision repair and sensitive to ultraviolet irradiation. Cancer Res., *58:* 599–603, 1998.
70. Zhan, Q. M., Antinore, M. J., Wang, X. W., Carrier, F., Smith, M. L., Harris, C. C., and Forance, A. J., Jr. Association with Cdc2 and inhibition of Cdc2/cyclin B1 kinase activity by the p53-regulated protein Gadd45. Oncogene, *18:* 2892–2900, 1999.