

Decreased DNA Repair Efficiency by Loss or Disruption of p53 Function Preferentially Affects Removal of Cyclobutane Pyrimidine Dimers from Non-transcribed Strand and Slow Repair Sites in Transcribed Strand*

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The tumor suppressor protein p53 plays a central role in modulating the cellular responses to DNA damage. Several recent studies, undertaken with the whole genomic DNA or full-length gene segments, have shown that p53 is involved in nucleotide excision repair and it selectively influences the adduct removal from the non-transcribed strand in the genome. In this study, we have analyzed the damage induction at nucleotide resolution by ligase-mediated polymerase chain reaction and compared the repair of ultraviolet radiation-induced cyclobutane pyrimidine dimers within exon 8 of p53 gene in normal and Li-Fraumeni syndrome fibroblasts as well as in normal and human papillomavirus 16 E6 and E7 protein-expressing human mammary epithelial cells. The results demonstrate that (i) loss or disruption of p53 function decreases efficiency of DNA repair, by preferentially affecting the repair of non-transcribed strand and of intrinsically slow repair sites in transcribed strand; (ii) mutant p53 protein affects DNA repair, at least of non-transcribed strand, in a dominant negative manner; and (iii) pRb does not have an effect on the repair of DNA damage within transcribed or non-transcribed strand. The overall data suggest that p53 could regulate excision repair or related events through direct protein-protein interaction.

Mammalian cells have evolved sophisticated DNA repair mechanisms to overcome DNA damaging hazards that threaten the integrity of genome (1–4). The most versatile and thoroughly studied repair system is the excision repair, of which two major pathways, nucleotide excision repair (NER)¹ and base excision repair, have been identified (5, 6). NER removes many types of DNA lesions including cyclobutane pyrimidine dimers (CPDs) by global genomic repair (GGR) and transcription-coupled repair (TCR) (5–8). It is now well estab-

lished that NER along genome is heterogeneous, CPDs are more efficiently removed from transcriptionally active genes, and TCR is generally faster than GGR (2, 9, 10). GGR acts on the elimination of lesions from non-transcribed strand (NTS) and transcriptionally inactive genes, whereas TCR removes lesions from DNA strand transcribed by RNA polymerase II.

In mammalian cells, a variety of cellular responses following genotoxic exposure may contribute to prevent DNA lesions from interfering with essential cellular functions. Of those cellular responses, activation of p53 pathway is well studied and documented (11, 12). p53 is a critical protein for maintaining genomic stability and homeostasis. It is believed that p53 activation signals the G₁ arrest to delay the transit from G₁ to S, thus preventing the effects of DNA lesions on vital cellular functions. Accumulating evidence indicates that p53 plays a role in DNA repair, especially in GGR (13–17). Viral proteins that bind p53, causing p53 inactivation or degradation, interfere with p53-regulated DNA repair (14, 18–20). Conceivably, p53 could be involved in NER by regulating its downstream genes, which are either related to or actively participate in NER. For example, Gadd45 and p21^{waf1}, which are among the many p53-regulated proteins, have been shown to interact with proliferating cell nuclear antigen (21). Additionally, recent evidence has shown that p48, a UV-damaged DNA-binding protein is transcriptionally regulated by p53, linking p53 to NER (22). Besides transcriptional activation, p53 may also be directly involved in repair or repair-related processes. For example, in addition to demonstrated p53 binding to insertion/deletion mismatches (23), p53 has also been found to both physically and functionally interact with p62, XPD, and XPB, three components of basal transcription factor IIH (10, 24).

Despite a plethora of experimental data, the definitive role of p53 in NER and the mechanistic basis of its interaction with repair machinery has not been fully delineated in eukaryotic systems. Several observations, either supporting a pronounced effect of p53 for an efficient repair of NTS alone or its involvement also in the repair of transcribed strand (TS), have been put forth to identify the role of p53 in NER (13–17, 19, 25, 26). These observations are primarily based upon the assessment of an average of the repair events within an entire genome, a specific gene segment, or in some cases an episomally replicating plasmid within mammalian host cells. Thus, in the present study we performed the damage analysis at nucleotide resolution and systematically compared DNA repair of individual UV-induced CPD sites in normal, p53 mutant (p53-Mut), and p53 nullizygous (p53-Null) Li-Fraumeni syndrome (LFS) fibroblasts, as well as in normal, human papillomavirus (HPV) 16 E6 and E7 protein-expressing human mammary epithelial cells (HMEC). Our results show that, compared with the efficiency

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¹ The abbreviations used are: NER, nucleotide excision repair; CPD, cyclobutane pyrimidine dimer; LFS, Li-Fraumeni syndrome; HMEC, human mammary epithelial cell; LMPCR, ligation-mediated polymerase chain reaction; WT, wild type; Mut, mutant; Null, nullizygous; HPV, human papillomavirus; NTS, non-transcribed strand; TS, transcribed strand; TCR, transcription-coupled repair; GGR, global genomic repair.

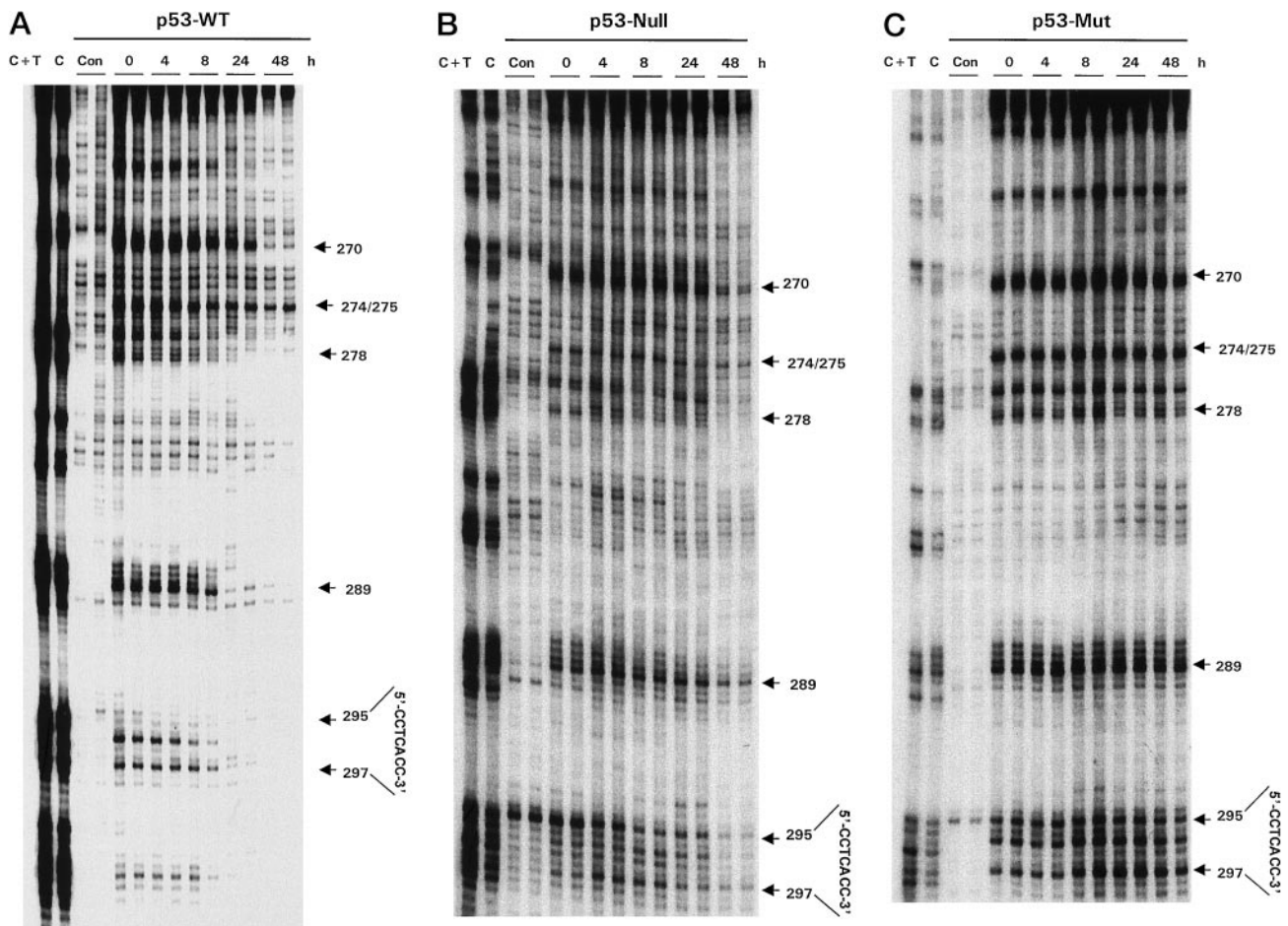


FIG. 1. Repair of CPDs in non-transcribed strand of exon 8 of p53 in genomic DNA. Human fibroblasts were irradiated with an UV dose of $20 \text{ J}\cdot\text{m}^{-2}$ and DNA isolated at indicated times after irradiation. CPDs in non-transcribed strand of genomic DNA were determined by LMP-PCR using upper strand specific primers as described under "Materials and Methods." Maxam-Gilbert-derived sequencing lanes, lanes C+T and C, are shown on the left of each autoradiogram. Formation or remaining amount of CPDs is shown at indicated postirradiation time points. Lanes marked Con represent LMP-PCR profiles of DNA sample from cells without UV treatment. A, p53-WT normal human fibroblasts; B, p53-Null LFS fibroblasts; C, p53-Mut LFS fibroblasts.

of TS repair, a significantly reduced rate of CPD removal was observable at all the dipyrimidine target sites of exon 8 NTS in both p53-Null and p53-Mut LFS cell lines. A close comparison of the extent of damage removal at 24 and 48 h after UV irradiation indicated that the repair of NTS was more severely affected in p53-Mut cells than in p53-Null cells. Relatively slight decrease in the initial rate of repair within TS, discernible at 24 h after treatment, was fully apparent at the inherently slow repair sites, e.g. codons 286 and 294 of p53 gene. This selective effect of p53 protein on the slow repair site of TS was much more clearly demonstrable at the same site in HPV 16 E6-expressing HMEC in which p53 is functionally inactivated by E6 protein. Like LFS cells, HPV 16 E6-expressing HMEC show a reduced rate of repair of CPDs in the NTS than in the TS. Both normal HMEC, having wild-type p53 and pRb proteins, and the isogenic HPV 16 E7-expressing HMEC, having wild-type p53 and compromised pRb protein expression, failed to show the deficiency of CPD repair in NTS as seen clearly due to the absence of p53 within human cells. Thus, pRb, another important cell cycle regulatory protein, does not seem to influence the NER process to a meaningful extent.

MATERIALS AND METHODS

Cell Culture and Treatment—The normal human (p53-WT) fibroblasts (OSU-2) were established in culture as described earlier (27). LFS fibroblast strains, MDAH087 (p53-Mut, harboring a codon 248 single-base substitution) and MDAH041 (p53-Null, harboring a codon

184 frameshift mutation, resulting in premature termination of translation of p53 protein), both >200 population doubling, post-crisis p53 homozygous cell strains, were kindly provided by Dr. Michael Tainsky (M. D. Anderson Cancer Center, Austin, TX). These fibroblast cells were grown in DMEM supplemented with 10% fetal calf serum and antibiotics at 37°C in a humidified atmosphere of 5% CO_2 . Normal HMEC were established in culture according to Stampfer (28). HPV 16 E6 protein-expressing HMEC 76E6 and HPV 16 E7 protein-expressing HMEC 76E7 were kindly provided by Dr. Vimla Band (Tufts University School of Medicine, Boston, MA). These cells were grown in DFCI medium supplemented with required nutrients and growth factors as described (29). For experiments to assess DNA damage and repair, the monolayer cells were grown to confluence in 150-mm dishes and then placed in serum-free medium for 12 h. Under these growth and maintenance conditions, the index of cellular DNA replication, as measured from changes in the specific radioactivity of DNA pre-labeled with ^3H dThd, does not show significant genome duplication during the test periods of repair analysis (27). The medium was removed, and cells were washed with prewarmed phosphate-buffered saline and irradiated with desired doses of UV (254-nm) light at a rate of $0.5 \text{ J}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ as measured by Kettering model 65 radiometer (Cole-Palmer Instrument Co., Vernon Hill, IL). After irradiation, fresh serum-free medium was added to cell culture and incubation continued for desired periods.

DNA Isolation and Conversion of CPDs to Ligatable DNA Strand Breaks—Briefly, after UV exposure, desired incubation periods, and washes to remove any floating dead cells, the adherent cells were recovered by trypsinization and immediately lysed for DNA isolation by a salt precipitation procedure as described (13, 30). The CPDs were cleaved and converted to single-strand breaks by digestion with T4 endonuclease V (31). The ligation-inhibiting 5'-pyrimidine overhang

TABLE I
Repair of CPDs of exon 8 of p53 genomic DNA in normal and LFS fibroblasts

DNA	Codon	Sequence (5'-3')	CPD remaining ^a after					
			p53-WT		p53-Mut		p53-Null	
			24 h	48 h	24 h	48 h	24 h	48 h
Non-transcribed	270	TΔTT	22	6	65	60	58	38
		TTΔT	49	12	73	60	60	44
	274/275	GTTΔT	68	48	80	56	60	42
	276/277	CΔTG	22	11	100	53	50	22
	277/278(M)	TΔCCT	36	34	100	53	65	40
	278(M)	CΔCT	32	18	100	97	65	40
	289	CΔTC	10	9	100	80	34	20
		CTΔC	17	10	100	80	54	22
	289/290	CTCΔC	41	15	100	84	60	30
	Transcribed	285/286(M)	CΔCTC	44	23	62	31	40
286(M)		TTΔC	41	30	55	31	52	32
291		CTΔT	30	18	40	20	47	22
292		TTΔT	10	8	34	8	30	14
293		CΔCC	32	17	47	20	34	8
294(M)		CTΔC	36	20	75	50	42	20

^a Repair rates were measured at mutation hotspots (M) and at various surrounding sites. Repair at each position is described as average percentage derived from time *versus* repair plots of three independent experiments. Percentage of CPDs remaining was calculated from band intensities at 24 and 48 h in reference to the band intensity at 0 h and normalized for any intensity observed at same sites in control lanes.

was removed by *Escherichia coli* photolyase (31) (generous gift from Dr. Aziz Sancar, University of North Carolina, Chapel Hill, NC). DNA was then recovered and quantitated as described previously (13). The same amount of DNA (1–2 μg) was used for each reaction set of LMPCR.

LMPCR—LMPCR is an extremely sensitive genomic sequencing method used for the detection of DNA damage (31, 32). DNA, specifically cleaved at CPDs, was used to create blunt end DNA fragment by extension of primer specific to p53 with DNA sequenase 2.0. Blunt-ended DNA was then ligated to a double-strand linker and followed by amplification with the longer oligonucleotide of the linker and a second nested p53-specific primer. After 20–21 cycles of polymerase chain reaction, DNA fragments were size-fractionated on an 8% urea-polyacrylamide sequencing gel, electroblotted onto a nylon filter, and hybridized to a single-stranded p53-specific ³²P-labeled probe generated by polymerase chain reaction using a third p53-specific primer. The filter was used to expose a PhosphorImager screen and the individual band intensities quantitated upon imaging and processing by Imagequant software (Molecular Dynamics). The filters were also used to expose Kodak X-Omat film for autoradiography and documentation by image scanning. LMPCR analysis of each sample was carried out in duplicate, and results described are from at least three independent experiments.

RESULTS

Loss or disruption of normal p53 function generally results in decreased efficiency of repair of CPDs of non-transcribed DNA strand. To compare repair of CPDs in p53-WT containing normal human fibroblasts, p53-Mut, and p53-Null LFS fibroblasts, formation and removal of CPDs induced by UV at a dose of 20 J·m⁻² were mapped by LMPCR, in both strands of exon 8 of p53 from genomic DNA. Under such an exposure condition, no discernible differences in formation of CPDs in genomic DNA could be seen in these three cell lines as measured by immunoslot blot assay (13). However, repair of CPDs by LMPCR could be seen at almost all the potential dimer sites of NTS, albeit with a clearly demonstrable variation in the rates of their repair. For example, repair at many sites in p53-WT normal human fibroblasts was apparent after 8 h, followed by an approximately 30–70% repair after 24 h and 60–95% repair after 48 h (Fig. 1A, Table I). Higher efficiency of removal of CPDs was seen at 5' side of adjacent pyrimidine sites, *e.g.* codons 270, 274, and 289. Consistent with earlier observation by Tornaletti and Pfeifer (32), repair of CPDs at codon 278, which is one of the frequently mutated in human skin cancers, was slower than those of surrounding positions. However, repair of CPDs (5'-TCTCΔC) at codon 289/290 was also found to be slower. It may be noted that this dimer is located at the 3'

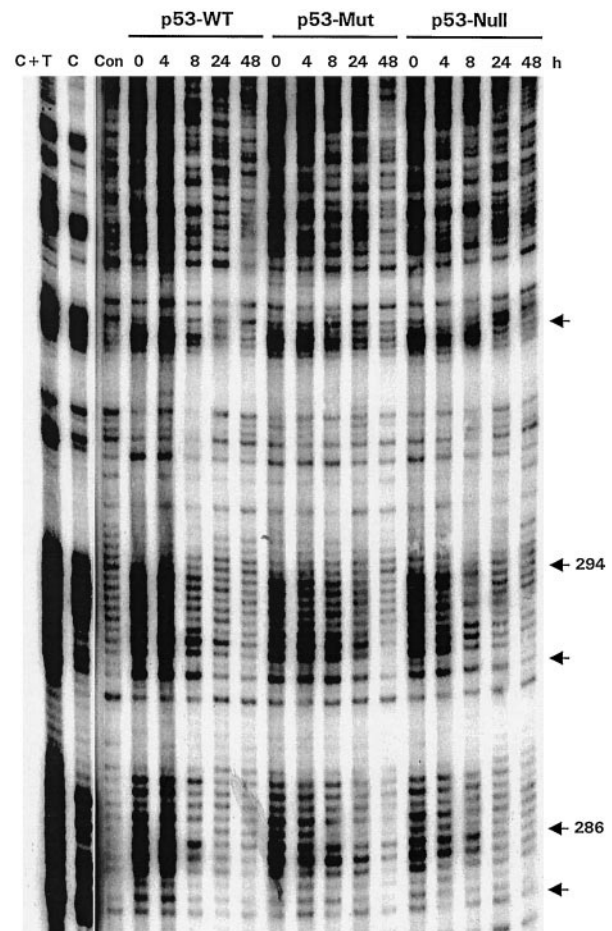


FIG. 2. Repair of CPDs in transcribed strand of exon 8 of p53 genomic DNA in p53-WT, p53-Null, and p53-Mut LFS fibroblasts. The LMPCR processing of the sample was as described under "Materials and Methods." The composite is a representative autoradiogram of data from several independent experiments.

end of four adjacent potential pyrimidine sites.

In p53-Null cells, repair of CPDs at most of the dipyrimidine sites of NTS was slower compared with that of the same CPDs at same position in p53-WT cells. Moreover, slow repair sites

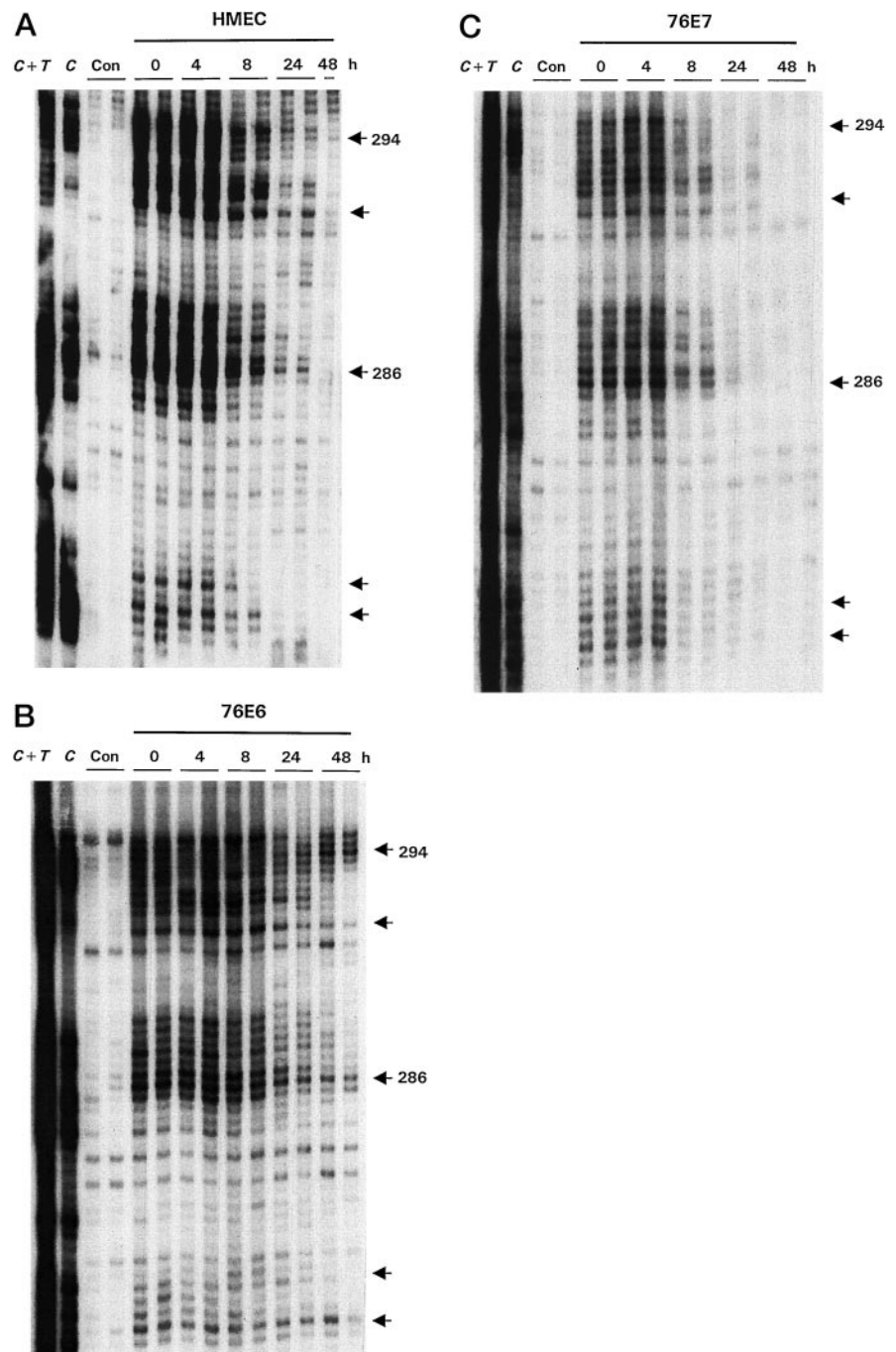


FIG. 3. Repair of CPDs in transcribed strand of exon 8 of p53 genomic DNA in normal, HPV 16 E6 and E7 protein-expressing HMEC. A, normal HMEC; B, HPV 16 E6 protein-expressing HMEC, 76E6; C, HPV 16 E7 protein-expressing HMEC, 76E7. The LMPCR processing of the sample was as described under "Materials and Methods."

were more prominently affected (Fig. 1, A and B, Table I). For example, dimer C[^]CT, at codon 278, was 68% repaired at 24 h and 82% repaired at 48 h in p53-WT cells, whereas in p53-Null cells, there was only 35% and 60% repair observed at these sites within 24 and 48 h, respectively. Repair of CPDs (5'-TCTC[^]C) at codon 289/290 was also drastically affected by the loss of p53 function (Fig. 1B). These observations were further confirmed by comparison of normal HMEC with HPV 16 E6 protein-expressing HMEC. In the case of 76E6 HMEC, in which p53 protein is degraded by E6 protein-mediated ubiquitin proteolysis pathway (33), the overall p53 modulation of DNA repair events appeared exactly like that of p53-Null fibroblast cells described above (redundant data not shown).

Among the cell lines tested, repair of CPDs in NTS was most dramatically affected in p53-Mut fibroblasts (Fig. 1C and Table I). In this cell line, repair of CPDs at all dipyrimidine sites was significantly slow. Approximately 80–100% of CPDs remained

after 24 h, and 50–100% of CPDs remained 48 h after UV irradiation. These results, in conjunction with the data from p53-Null cells, indicate that mutant p53 protein affects DNA repair in a dominant negative manner. This would seem to suggest that p53 protein regulates DNA repair, at least of the non-transcribed strand, by direct protein-protein interaction. Such a dominant effect could be exerted by interaction with the proteins of NER assembly or by altered transcription of proteins essential for optimal assembling and targeting of the damage recognition complex.

At the position of one potential dipyrimidine site, (5'-C[^]CTCACC) at codon 295, an abnormal signal was distinctly and reproducibly detected in p53-Null and p53-Mut cells, but not in p53-WT cells (Fig. 1, A–C). Accordingly, a distinct LMPCR generated band could be seen in the control unirradiated sample lane. We surmise that this band could not arise due to nonspecific or enzymatic cleavage as the signal gradually decreased between

TABLE II
Repair of CPDs of exon 8 of p53 genomic DNA in normal, HPV 16 E6 and E7 protein-expressing HMEC

DNA	Codon	Sequence (5'–3')	CPD remaining after					
			HMEC		76E6 ^a		76E7 ^a	
			24 h	48 h	24 h	48 h	24 h	48 h
Transcribed			%					
	285/286(M)	TTC^C	5	1	30	18	10	2
	286(M)	TT^C	14	2	40	20	8	0
		T^TC	26	6	50	16	20	0
	287	CT^C	16	0	24	6	12	2
	291	CT^T	10	1	13	0	7	0
	292	TT^T	16	2	35	10	16	3
	293	C^CC	8	0	40	30	14	2
	294(M)	CT^C	10	0	40	22	12	1

^a 76E6 and 76E7 represent HPV 16 E6 and E7 protein-expressing HMEC, respectively. Extent of repair at each site was quantitated at indicated times as described in Table I.

4- and 48-h time intervals following irradiation. According to the nature of LMPCR, an assay specialized for detecting nicks in individual DNA strand, this signal could only represent a specific DNA strand break at this site; due to its occurrence within DNA topoisomerase consensus sequence, it could be the result of an arrested cleavable complex. Surprisingly, such an abnormal DNA break signal was also found in HPV 16 E6-expressing HMEC, but not in normal or in HPV 16 E7-expressing HMEC. This further confirmed that the DNA strand break at this codon site is specific and only appears in cells that are rendered deficient for normal p53 function. Interestingly, exposure of cells to UV seemed to induce the repair of this DNA strand break, as was evident from time-dependent gradual disappearance of the band at this site.

Loss or Disruption of Normal p53 Function Affects the Repair of CPDs at Slow Repair Sites of Transcribed DNA Strand—To examine the effects of loss of normal p53 function on the repair of CPDs within TS, repair of CPDs in TS of exon 8 of p53 gene was mapped by LMPCR (Fig. 2). As expected, removal of CPDs from TS was generally faster than from NTS, albeit with a clearly discernible site-specific variation in the removal of CPDs at individual dipyrimidine sites. Consistent with earlier observation (32), repair of CPDs in p53-WT cells at codons 286 and 294 was seen to be slower than that of surrounding positions. A p53-dependent decreased repair of CPDs was found at several sites of exon 8 in both p53-Null and p53-Mut LFS cells at 24 h after UV irradiation. The intrinsically slow repair dipyrimidine sites, e.g. at codons 286 and 294, were preferentially affected by the absence of normal wild type function than surrounding CPD sites (Fig. 2 and Table I). The intrinsically slow repair is suggested to be the basis for mutational predisposition of these p53 gene sites in human cancers (32). An absence of p53 function would be expected to further exacerbate the cellular instability through decreased repair of exogenously or endogenously induced DNA damage.

Since the effect of loss of p53 function on TCR has not been fully resolved, we extensively mapped repair of CPDs in TS of exon 8 of p53 gene in normal, isogenic HPV 16 E6 and E7 protein-expressing HMECs. It may be noted that HPV 16 E7 protein selectively activates ubiquitin proteolysis pathway causing degradation of pRb protein, while it stabilizes p53 protein (29). The data shows that the repair of CPD was faster in HMEC than fibroblast, and dimers at most of the sites were quantitatively removed within 48 h after UV treatment. Furthermore, unlike normal fibroblasts, slow repair of CPDs within sites like codon 286 and 294 was not very obvious in either the normal or HPV 16 E7 protein-expressing HMEC (Fig. 3, A and C). Thus, p53-expressing cells appear to have normal NER despite the absence of a functional pRb protein. On the other hand, a clearly visible slower repair at the same sites was found in p53-compromised HPV 16 E6 protein-ex-

pressing HMEC (Fig. 3B and Table II). This observation further confirmed that loss of functional p53 affects the removal of CPDs from TS by TCR and preferentially affects removal of CPDs at slow repair sites.

DISCUSSION

The biochemical mechanisms of NER involve damage recognition and open complex formation by factors such as XPA, RPA, XPC, and transcription factor IIIH, dual incision of the damaged DNA strand by endonucleases XPF-ERCC1 and XPG, repair synthesis mediated by a proliferating cell nuclear antigen-dependent DNA polymerase and ligation of newly synthesized DNA strand. The precise reaction mechanisms of NER have recently been established to a significant extent (for review, see Refs. 1–6). Nonetheless, how NER is regulated or connected to other cellular functions still remains to be explored. Several investigators have examined the involvement of p53 in regulation of NER. Different systems and approaches have been used for assessment of DNA repair. Immunoassay was mostly used for direct assessment of GGR, whereas endonuclease-sensitive site assay was used for examination of strand-specific repair. It is becoming clear that functional p53 is required for efficient GGR (13–17). However, due to different views being supported by various TCR studies (14–17, 19, 26), precise nature of p53 participation in TCR still remains unclear. In this study, we provide the first detailed analysis of effects of loss of functional p53 on the removal of CPDs in both DNA strands at nucleotide resolution. First, the results confirmed that functional p53 is required for efficient GGR (13, 15, 16, 19). Furthermore, the results show that expression of mutant p53 protein more significantly affects removal of CPDs from non-transcribed strand than loss of p53 protein, indicating that mutant p53 protein affects GGR in a dominant negative manner. The results also show that p53 is involved in TCR and repair of CPDs at slow repair sites is the first target to sustain meaningful effects due to the loss of p53 function.

In a recent review, McKay *et al.* (17) have strongly argued that p53 plays a definitive role in TCR. The difference of observations by various laboratories may be the result of different assays used to detect strand-specific repair. Using the normal endonuclease-sensitive site assay, we too were unable to demonstrate any detectable differences in TCR between normal and LFS fibroblasts as well as between normal and HPV 16 E6 protein-expressing HMEC for both UV- and benzo[a]pyrene diol epoxide-induced DNA damage.² Repair differences between cells did not become pronounced and meaningful until full mapping of repair of CPDs in normal, HPV 16 E6 and E7 protein-expressing HMEC was conducted within the same gene

² Q. Zhu, M. A. Wani, M. El-mahdy, and A. A. Wani, unpublished results.

segment by LMPCR. The reasons are that (i) LMPCR is a much more powerful assay in demonstrating variations of repair along particular sites and stretches of specific gene sequences, (ii) fully discernible differences were visualized mainly during the initial stages of repair, *i.e.* before 24-h time points, and (iii) slow repair sites were more prominently subjected to the influence exerted by the loss of p53 function.

Several investigations suggest that p53-regulated gene products may participate or be associated with NER. Using host reactivation assay, it has been shown that UV- and heat shock-inducible NER is p53-dependent (14, 17). More recently, it has been shown that the expression of p53-downstream genes, *e.g.* p48 gene, was dependent on p53 and involved in GGR (22). It should be noted that p48 has been suggested to have a role in the repair of DNA in chromatin and damage recognition. If effects of loss of function in p53-Null LFS fibroblasts and HPV 16 E6 protein-expressing HMEC on NER reflect requirements of p53-activated downstream genes in NER, dominant negative effects of mutant p53 protein on DNA repair of non-transcribed strand may reflect direct participation of p53 in NER. It has been shown that p53 binds to three components of basal transcription factor: p62, XPD, and XPB (10). Furthermore, XPB and XPD cells are deficient in repair of non-transcribed DNA and inefficiently repair the transcribed strand including sequences near the transcription start site (34). However, contact with these factors may not be the only mechanism by which p53 directly participates in NER. Given that mutant p53 protein negatively regulates NER by binding XPB and XPD, TCR should be the first target. However, dominant negative effects of mutant p53 protein on DNA repair of non-transcribed strand were more distinctly observed. In fact, wild-type rather than mutant p53 protein has been shown to inhibit DNA helicase activity of XPD and XPB (35). In search for the components of NER complex that could be interacting *in vivo* with p53, we have found that recognition of UV-induced damage links p53 pathway to NER and that HHR23A is involved in regulating transcriptional activity of p53.³ Interestingly, it has been shown that XPC protein, complexed with HHR23A and HHR23B, also plays a role in damage recognition and chromatin unfolding (37). It seems that p53 regulates the early steps of damage recognition of NER or chromatin unfolding during NER processes through both transcription activation and protein-protein interaction. However, no experimental evidence shows that p53 protein recognizes or binds UV-induced CPDs.

In eukaryotic cells, genomic DNA is wrapped around histone octamers forming nucleosomes, which are the repeating units of chromatin. Proteins involved in cellular processes, such as DNA replication, transcription, and DNA repair, require access to DNA within chromatin structural hierarchy. Heterogeneity of NER may partially reflect the accessibility of damaged DNA to NER components. In support of this, very fast repair has been seen in both DNA strands near the transcription initiation site (38). It has also been shown that several sequence positions in 5'-flanking region of the *tRNA^{val}* gene, which lacks TCR, were also repaired more efficiently than the gene itself (38, 39). In the case of a run of potential CPD sites in the non-transcribed DNA strand, if each DNA repair event in GGR is considered as an independent event, as suggested by its biochemical mechanisms, there is no reason for higher efficiency of CPD removal at 5' end. This is only possible if TCR or

transcription somehow helped damaged DNA to become more accessible at 5' end. Thus, besides transcription coupling, accessibility of damaged DNA contributes a very important parameter to the heterogeneity of NER. In this regard, p53 may regulate NER through modulating accessibility of damaged DNA rather than damage recognition. One would expect that CBP/p300, a p53 coactivator that has been shown to have acetyltransferase activity (36), could also be involved in p53-regulated DNA repair. Investigation of such DNA repair participating principles should become an area of active interest in the near future.

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