

p53-Degradation by HPV-16 E6 preferentially affects the removal of cyclobutane pyrimidine dimers from non-transcribed strand and sensitizes mammary epithelial cells to UV-irradiation

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Abstract

Nucleotide excision repair (NER), the most versatile and ubiquitous mechanism for DNA repair, operates to remove many types of DNA base lesions. We have studied the role of p53 function in modulating the repair of DNA damage following UV irradiation in normal and p53-compromised human mammary epithelial cells (HMEC). The effect of UV-induced DNA damage on cellular cytotoxicity and apoptosis was determined in conjunction with global, gene- and strand-specific repair. Cytotoxicity studies, using clonogenic survival and MTT assays, showed that HPV-16 E6-expressing HMEC were more UV sensitive than p53-WT cell lines. High apoptotic index obtained with p53-compromised cells was in conformity to both the low clonogenic survival and the low cellular viability. No discernible differences in the formation of initial UV-induced cyclobutane pyrimidine dimers (CPD) were observed in the cell lines of varying p53 functional status. However, the extent and the rate of damage removal from genome overall were highest for p53-WT cells. Further examination of strand-specific repair in the *p53* gene revealed that the removal of CPD in the non-transcribed strand (NTS) was slower in p53-compromised cells compared to the normal p53-WT cell lines. These results suggest that loss of p53 function, in the absence of other genetic alterations, decreased both overall amount of CPD repaired and their removal rate from the genome. Additionally, normal function of p53 is required for the repair of the NTS, but not of the transcribed strand (TS) in genomic DNA in human epithelial cells. Thus, failure of quantitative removal of CPD by global genomic repair (GGR), due to loss of p53 function, causes the enhanced UV sensitivity and increased damage-induced apoptosis via a p53-independent pathway. Nevertheless, recovery of cells from UV damage requires normal p53 function and efficient GGR. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: p53; Cyclobutane pyrimidine dimers; DNA repair; Human mammary epithelial cells; Apoptosis

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1. Introduction

A wide variety of endogenous and exogenous agents cause DNA damage, and complex biochemical processes exist to repair these lesions by diverse mechanism [1]. Nucleotide excision repair (NER) is the most versatile and ubiquitous mechanism which enables cells to remove many types of lesions, including UV-induced cyclobutane pyrimidine dimers (CPD) [2–4]. Such lesions may pose as structural blocks to transcription and replication and result in mutations through translesional replication or faulty repair [5]. Mutational inactivation of key genes has been found in a wide range of human diseases, including cancer, and have been linked to defective DNA repair or defective cellular response to DNA damage [6].

Genotoxins target key cellular growth and differentiation controlling genes leading to certain genomic alterations that play a major role in the multi-stage carcinogenesis pathway [7–9]. Among these, p53 mediates specific responses following DNA damage in mammalian cells as demonstrated by its ability to (a) initiate DNA repair via transcriptional induction of its downstream genes, i.e., p48, PCNA, etc. [10], (b) interact with ERCC3 [11] and the TFIIH-associated NER [12]; (c) cause G1 arrest [13]; and (d) induce apoptosis in some cell types [14]. The fact that p53 interacts physically and functionally with p62, XPD and XPB, three components of TFIIH, suggests that it could have multiple effects on NER and other repair processes. Experimental evidence for the role of p53 in regulating the global genomic repair (GGR) is becoming stronger [5,15–18]. It has been reported that Li–Fraumeni syndrome (LFS) fibroblasts homozygous for p53 mutations are deficient in GGR, but exhibit normal transcription-coupled repair (TCR) and greater UV resistance through suppression of apoptosis [5,17]. However, our recent study shows the lack of repair of GGR in p53 null LFS cells accompanied with enhanced sensitivity to UV and increased apoptosis [15]. This observation is supported by demonstration of decreased GGR and UV sensitivity in primary human fibroblasts expressing HPV *E6* gene [19].

Only limited studies have utilized mammary epithelial cells to study the nature and role of different repair modulatory factors. The study of repair in

epithelial cells is particularly relevant since most solid human tumors are of epithelial cell origin [20]. Therefore, in this report, we have used normal human mammary epithelial cells (HMEC) and cognate transfectants expressing either human papilloma virus (HPV)-16 E6 or E7 to study the modulatory role of p53 on cellular responses to UV irradiation. The effect of DNA damage on cellular cytotoxicity and apoptosis was determined in conjunction with the repair at global, gene- and strand-specific levels. The results demonstrate that failure of removal of CPD by GGR due to loss of p53 function causes the enhanced UV sensitivity and increases damage-induced apoptosis via a p53-independent pathway. Recovery of cells from UV damage requires normal p53 function and efficient GGR, suggesting the possibility of targeting NER for clinically responsive efficient treatment of breast cancer.

2. Materials and methods

2.1. Cell culture and treatment

The normal HMEC were established in culture according to Stampfer [21] and the actively replicating early passage (< 15 population doublings) were used in all the experiments. Immortalized HMEC transfectants, 76E6 and 76E7, were used as a model in which p53 and Rb is selectively degraded by the influence of human HPV proteins E6 or E7, respectively. These two cell lines were kindly provided by Dr. Vimla Band (Tufts University School of Medicine, Boston, MA). The cells were grown in a humidified atmosphere of 5% CO₂ in DFCI medium supplemented with suitable nutrient and growth factors [22]. For UV exposure, the cells were washed and replaced with a thin layer of prewarmed PBS, irradiated with varying UV doses and incubated in suitable medium for varying post-exposure times. The irradiation was carried from a germicidal lamp at a dose rate of 0.5 J/m² as measured by a Kettering model 65 radiometer (Yellow Springs Instruments).

2.2. Western blot analysis of proteins

Exponentially growing cells were exposed to 20 J/m² and maintained in fresh medium for 2 to 24 h

following the irradiation. At the indicated time points, the cells were lysed by boiling for 10 min in sample buffer (2% sodium dodecyl sulfate, 10% glycerol, 1 mM dithiothreitol in 62 mM Tris–HCl, pH 6.8, 10 mg/ml pepstatin and 10 mg/ml leupeptin), snap frozen and kept at -20°C until further processing. Western blot analysis was performed as described earlier [23]. For p53 detection, a mixture of anti-p53 antibodies (Neomarkers, Ab-2 and Ab-6 from hybridoma clones 1801 and DO-1, respectively) was used at a 1:200 dilution. The antibody for the detection of p21 and Rb were Ab-3 (clone DCS60.2) and Ab-1 (clone 1F8), respectively. Following incubation with the corresponding enzyme-conjugated secondary antibody (Boehringer Mannheim), the filter-bound enzymatic activity was detected using the enhanced chemiluminescence substrate reaction (Pierce, Rockford, IL).

2.3. Cytotoxicity measurement

UV-induced cytotoxicity was evaluated using clonogenic survival [15] and methylthiozole tetrazolium (MTT) assays [24]. For clonogenic assay, exponentially growing cells were plated in 100-mm dishes at densities appropriate for each individual exposure dose. After 24 h, the cells were washed once with PBS, exposed to varying doses of UV and cultured in fresh medium for 10 days. The growth medium was removed, cells fixed in cold ethanol and stained with 0.1% crystal violet. Colonies of > 50 cells were counted and the survival calculated as a percentage of the non-irradiated cells. For MTT assay, cell viability was assessed by the ability of cells to convert the soluble salt of MTT into an insoluble formazan precipitate. Cells were seeded in 96-well plates at an initial density of 1×10^4 cells/well. After 24 h, the monolayers were washed once with PBS, exposed to increasing doses of UV and maintained in fresh medium for additional 22 h. The cells were again washed with PBS, and medium containing MTT (1 mg/ml) was added for 2 h. The intracellular formazan complex formed was dissolved in DMSO and the color formed was quantitated by a colorimetric plate reader (*EL* \times 800 BIO-TEK, Winooski, VT, USA). The cell viability was expressed relative to the set of unexposed control cells.

2.4. DNA fragmentation and apoptotic index

Actively dividing HMEC were exposed to 20 J/m² and maintained in fresh medium for 4 to 48 h. At each time point, the attached as well as any detached cells were pooled together, centrifuged and differentially lysed in ice cold hypotonic lysis buffer (0.1% Triton X-100 in TE, pH 8) as described earlier [23,25]. Fragmented DNA was separated from native intact DNA by centrifugation at 14,000 rpm. An aliquot of fragmented DNA, in the supernatant, was quantitated fluorimetrically for DNA content. An equivalent of 1.5×10^6 cell extract was resolved by electrophoresis in 1.2% agarose gel and DNA visualized by ethidium bromide staining. Native DNA, left in the pellet from initial hypotonic lysis, was also quantitated. The percentage of apoptotic cells was determined from the amount of fragmented DNA/amount of total DNA \times 100.

2.5. GGR analysis by immuno-slot blot assay

The initial CPD formed after DNA damage and the cellular repair rate were quantitated using the non-competitive immuno-slot blot assay as described earlier [23,25–27]. Confluent cells were maintained overnight in nutrient-free medium and exposed to increasing doses of UV. DNA was isolated immediately or at different time points after exposure. For CPD estimation, several increasing concentrations of unirradiated, irradiated and repaired DNA samples were evaluated by standard immunoassay using dimer specific polyclonal antibody [27,28]. Band color intensities were evaluated using LKB laser densitometry and the data were transferred to an interfaced computer and analyzed using LKB Gelscan XL software. In vitro UV-modified DNA standard was run in parallel on each filter.

2.6. Strand-specific repair analysis by endonuclease sensitivity site assay

Repair of CPD was determined within the transcribed (TS) and non-transcribed (NTS) of the 16-kb *EcoR1* fragment of the *p53* gene by quantitative strand specific Southern hybridization according to Chen et al. [29,30]. Isolated DNA (50 μg), from

cells exposed to indicated doses and time points, was extensively digested with *Eco*R1 and aliquots of digests (20 μ g) were treated or mock-treated with T4 endonuclease and electrophoresed in parallel under formamide-denaturing conditions. The resulting fragments were Southern transferred to a nylon membrane and hybridized with 32 P-labeled strand-specific DNA probes generated by asymmetric PCR [31]. The frequency of induction of CPD and their rate of removal from the TS and NTS of the human *p53* gene was measured by quantifying the reappearance of the full-length restriction fragments in DNA from cells allowed various times to remove CPD from their DNA. The ratio of full-length restriction fragments in the T4-treated and untreated samples was determined by phosphorimager analysis of integrated signals (Molecular Dynamics). The average numbers of lesions (T4-sensitive sites) per fragment were calculated using the Poisson expression as described earlier [32]. Each experiment was independently repeated at least three times.

3. Results

3.1. UV response of epithelial cell regulatory proteins

HMEC, expressing either HPV-16 E6 or E7 proteins and parental normal cells showed distinct induction responses for various regulatory proteins upon exposure to 20 J/m² UV-irradiation (Fig. 1). As expected, HMEC and 76E7, containing p53-WT function showed induction of both p53 and p21 with higher basal levels of both proteins in 76E7 than the parental HMEC. No detectable level of Rb protein could be seen in 76E7 cells due to the expression of E7 protein. The induction of p53 and p21 in HMEC and 76E7, observed within a few hours, peaked at 8 h and was sustained up to 24 h post UV-irradiation. On the other hand, 76E6 showed no expression of p53 or p21 proteins up to 24 h following the UV exposure. However, these cells showed several fold increase in both the hypophosphorylated and hyperphosphorylated forms of Rb protein within 2 h post treatment.

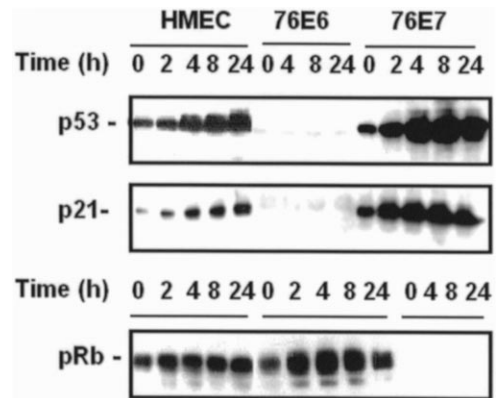


Fig. 1. UV-induced response of cellular regulatory proteins in mammary epithelial cells. Exponentially growing cells were irradiated with 20 J/m² and maintained further in fresh medium at the indicated times, protein extracts from normal (HMEC), E7-expressing (76E7) and E6-expressing (76E6) epithelial cells were separated by SDS-PAGE electrophoresis, transferred onto PVDF membrane, developed with monoclonal antibody specific to p53, p21 and pRb and visualized by enhanced chemiluminescence as described in Section 2. The representative results presented were independently repeated, at least, three times.

3.2. UV-induced cytotoxicity in HMEC

The effect of increasing doses of UV (0–20 J/m²) on the survival of HMEC is shown in Fig. 2A. The clonogenic survival assay showed significant differences in the UV cytotoxicity in cells with fully functional or degraded p53. Whereas all three cell lines showed a dose-dependent loss in their colony forming ability, HMEC and 76E7 were relatively more resistant. A very low number of colonies was observed with 76E6, thus indicating greater reproductive death by UV. The cellular viability following 24, 48 and 72 h of exposure to 20 J/m² of UV showed distinct differences in the genotoxin-induced cytotoxicity in all cells with functional or degraded p53 (Fig. 2B). A time-dependent decrease in cell viability was noted in 76E6 cells compared to the HMEC parental cells. However, there was not a notable difference in viability between 76E7 and HMEC following the UV exposure. While the viability of 76E6 continued to drop up to 72 h post-irradiation, a significant recovery in the viable p53-WT cells, HMEC and 76E7, was observed at both 48 and 72 h post-incubation times. The percent inhibition of cell viability of HMEC, 76E6 and 76E7 at 72 h

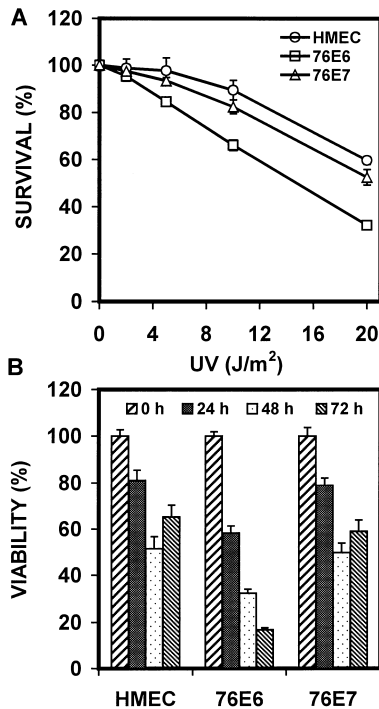


Fig. 2. Effect of p53 function on UV sensitivity of normal HMEC. (A) Overnight seeded cells were irradiated with increasing doses of UV light and maintained in fresh medium for 12–14 days. Cell survival was determined by scoring the colony formation and normalizing the values to unirradiated controls. The points shown are the mean \pm S.E.M. of six individual dishes. (B) Cells were seeded in 96-well plates, incubated for 24 h, exposed to increasing doses of UV and maintained in fresh medium for various periods. Cell viability was colorimetrically measured at 0, 24, 48 and 72 h post irradiation using MTT–Microculture Tetrazolium Assay. Each point represents mean \pm S.E.M. of six experiments.

following 20 J/m², was calculated to be 37.7 \pm 5.1, 8.19 \pm 0.8 and 35.1 \pm 4.9, respectively.

3.3. Induction of cellular apoptosis following UV-irradiation

DNA fragmentation analysis showed a direct relationship of cellular sensitivity and apoptosis, resulting from exposure of epithelial cells to UV irradiation. A time-dependent induction of apoptosis in all cell lines was clearly observed upon exposure to 20 J/m² of UV (Fig. 3a). However, cells expressing HPV-16 E6 were more sensitive to the genotoxic

damage than the control HMEC, as well as those expressing HPV-16 E7. The quantitative assessment of apoptotic index of non-irradiated populations showed a basal level of approximately 3% cells that failed to retain the DNA upon differential lysis. The fraction of the cells undergoing apoptosis increased only slightly, up to 14.9 \pm 1.8% and 17.1 \pm 1.4% in case of HMEC and 76E7 cells after 24 h post-irradiation. While the cells having degraded p53 reached up to 34.8 \pm 1.1% at 24 h following 20 J/m² of UV irradiation. The highest level of apoptosis was seen with 76E6 cells, which not only showed an increased overall extent, but also a rapid induction of this response following irradiation. As expected, the quantitative values of apoptotic index fully comple-

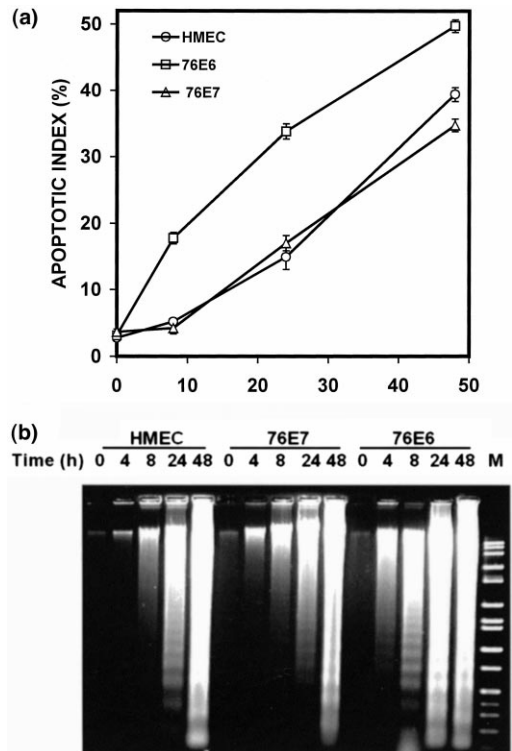


Fig. 3. Induction of apoptosis in HMEC following UV-irradiation. Exponentially dividing cells were exposed to 20 J/m² of UV and maintained in fresh medium for varying time periods. (a) Fragmented DNA obtained upon differential lysis was resolved by agarose gel electrophoresis, ethidium stained and photographed. (b) Apoptotic index was quantitatively determined as the percentage of the fragmented DNA to the total DNA in the initial cell pellets. Points represent mean \pm S.E.M. of five individual samples.

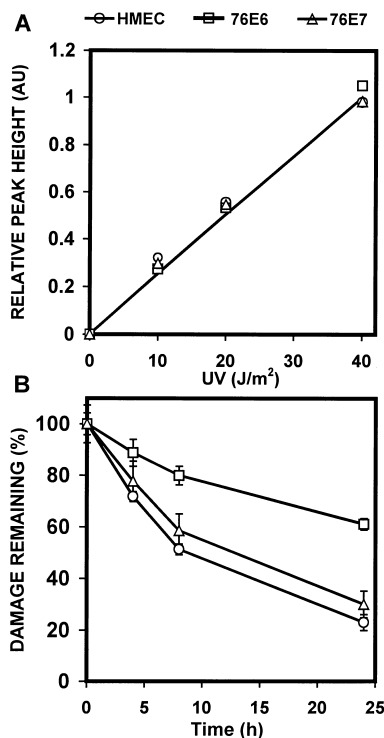


Fig. 4. Formation and repair of CPD in HMEC. (A) Dose-dependent induction of CPD. Confluent culture of HMEC were exposed to increasing doses of UV and immediately lysed for DNA isolation. The extent of CPD formation was immunologically determined by the antibody-binding sites. (B) Global NER of UV-induced CPD. Following the exposure to 20 J/m² of UV light, cells were incubated for varying times (0–24 h). The repair was determined as the loss of antibody binding sites from DNA obtained from p53-WT function (HMEC and 76E7) and p53-compromised epithelial cells at different times after irradiation. For each point, five different DNA concentrations were used and the extent of DNA damage was determined from the linear portion of the curve. The amount of damage was calculated upon comparison with a standard reference and the repair expressed as the percentage of initial damage remaining at the indicated time. Data represents the mean \pm S.E.M. of three independent experiments.

mented the results of gel electrophoresis analysis (Fig. 3b). The size of DNA fragments was greatly reduced, reaching the expected 180 bp nucleosome monomer size. Unlike fibroblast cells, the typical ladder pattern for apoptosis was clearly seen in all the epithelial cells. The most prominent response was seen in 76E6 having degraded p53. In these cells apoptosis could be detected as early as 4 h post-irradiation. Flow cytometric analysis also showed a

significant increase of sub-G1 apoptotic population in 76E6 cells treated with UV (data not shown).

3.4. Global genomic DNA repair of CPD following UV irradiation

A dose-dependent formation of CPD was observed following in vivo exposure of cells to increas-

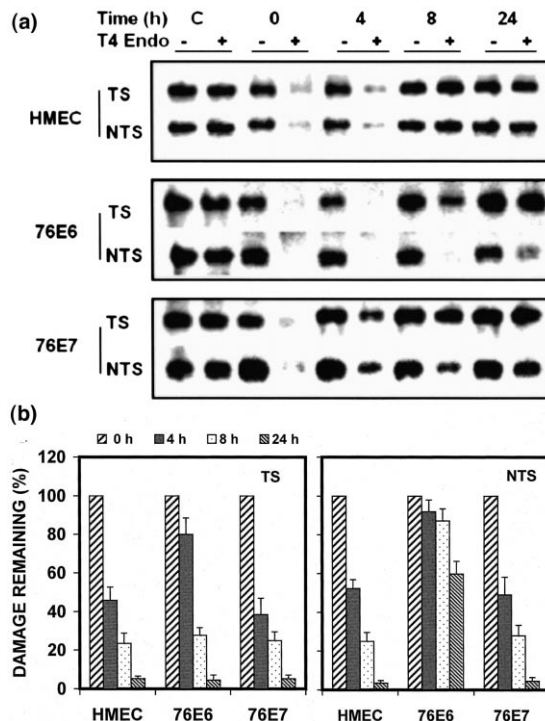


Fig. 5. Strand specific repair of UV damage in *p53* gene. (a) Autoradiograms representing extent of repair in the TS and NTS strand of the *p53* gene. DNA was isolated from unirradiated confluent culture of indicated cell type (first two lanes of each panel) or from cells incubated for the indicated repair period after exposure to 20 J/m². Samples of 20 μ g *Eco*R1-digested DNA were treated (+) or mock-treated (-) with T4 endonuclease, electrophoresed, under formamide-denaturing conditions, Southern transferred to a membrane, and hybridized with ³²P-labeled strand-specific DNA probes. Bands represent the 16-kb *p53* gene fragment. (b) Repair profiles of CPD within TS and NTS in DNA of cells having different p53 status. The frequency of induction of CPDs and their removal rate was determined as reappearance of full-length restriction fragments in the T4-treated and untreated samples. The average number of lesions (T4-sensitive sites) per fragment was calculated using the Poisson expression. The points shown represent the mean \pm S.E.M. of three independent experiments.

ing doses of UV up to 40 J/m² (Fig. 4A). The three cell lines did not seem to exhibit any discernible difference in the formation of initial damage within their genomic DNA from varying UV doses. However, the total amount of CPD repaired and their removal rate was highest for control HMEC, which did not show a significant difference compared to 76E7. On the contrary, 76E6 cells showed decreased rates of NER and extent of CPD loss from the overall genome compared to HMEC (Fig. 4B). A quantitative comparison of CPD removal showed that HMEC, 76E6 and 76E7 cells, respectively, repaired $77.1 \pm 3.1\%$, $38.9 \pm 5.1\%$ and $70.0 \pm 2.2\%$ of initial CPD within 24 h following irradiation.

3.5. Strand-specific repair of CPD within the *p53* gene

The repair rate of CPD within the TS and NTS of the 16-kb *Eco*R1 restriction fragment of the human *p53* gene was determined in all of the cell lines using endonuclease sensitivity site assay (Fig. 5). The three cell lines exhibited comparable rate and extent of CPD removal from the TS of *p53* gene. However, in comparison to both HMEC and 76E7, the 76E6 cells were distinctly slower in removing the lesions from the NTS. For example, compared to the initial damage, the amount of CPD repaired at 8 h post-irradiation within the TS of *p53* gene in HMEC, 76E6 and 76E7 was calculated to be 77%, 73% and 75%, respectively. A similar extent of overall repair was observed within the NTS in both HMEC and 76E7. However, at 8 h post-irradiation, only 13% of the CPD were removed from the NTS of *p53* gene in 76E6 compared with 75% and 72% in the parental and 76E7 HMEC, respectively. Unlike fibroblast cells, the results with HMEC show that repair of CPD in NTS is comparable to that of TS. This could be a unique feature of epithelial cells that show much faster rate of CPD repair than fibroblastic cells.

4. Discussion

Since DNA repair pathways can no longer be considered in isolation of cell biology and physiol-

ogy, studies are beginning to address the interaction between the vital process involved in the maintenance of genomic stability and normal cellular function. It is well known that p53 plays a critical role in the maintenance of genomic stability. Growing evidence suggests that p53 also plays a role in DNA repair, while it modulates the response of mammalian cells to DNA damaging agents. The present study is an integrated approach utilizing mammary epithelial cell model to correlate between absence of p53-WT function and some important cellular responses that occur as a consequence of genotoxic damage. In support of our early observation in LFS fibroblast, we demonstrate that disruption of p53-WT activity is associated with a decrease in total amount and the rate of removal of CPD from the overall genome of HMEC [15]. Like LFS cells, functional p53 is required for NER of NTS but not for the TS of *p53* gene. Additionally, loss of p53-WT function in epithelial cells also resulted in a significant enhancement of UV-induced cytotoxicity and apoptosis.

The modulatory role of p53 protein on cell survival has been evaluated by many studies using diverse test system and genotoxic agents. The results have so far been equivocal. The resulting controversy may be because of the difficulty in separating the effect of *p53* loss from the acquired genetic abnormalities in cancer cells. It has been reported that transfection with HPV-16 E6 or E7 selectively inactivates p53 and Rb, respectively, and transfectants do not display gross chromosomal abnormalities [33,34]. A number of reports, particularly those using cancer cell lines, have shown that loss of p53 function resulted in increased resistance to DNA damaging agents [35,36]. The present results show a dose-dependent decrease in the colony forming ability in all three cell lines. However, the lowest D₁₀ was observed in the case of cells having degraded p53 protein. The parental cells and those expressing HPV-16 E7 were relatively resistant to UV-induced damage and able to promptly recover under full growth conditions. This observation does not seem to support the suggestions that (i) wild-type p53 eliminates the genetically non-restorable and critically damaged cells, (ii) the clinical observations that positively correlate altered p53 function with enhanced cellular resistance to chemotherapeutic agents, and

(iii) loss of p53 function results in decreased sensitivity to chemotherapeutic agents [14,35–37] and UV-irradiation [17]. Nonetheless, these observations are not universal as other studies report a greater sensitivity to different DNA damaging agents in the presence of disrupted p53 function [14,34,37,38]. Our earlier study reported a similar type of response using LFS fibroblast cells having either mutant or lost p53 function [15]. Using clonogenic assay, Ford et al. [19] have reported that primary fibroblasts expressing HPV E6 show a greater sensitivity to UV-irradiation after 24 h. E6 mediated disruption of p53 function enhancing the cellular sensitivity has also been observed in human breast cancer-derived MCF-7 and colon cancer-derived RKO cells [34,39]. Several reports have tried to explain these conflicting results [34,40–42]. It seems that this is not a cell-type but a p53 status and p21 expression related phenomenon. As discussed below, it may also depend on the capability of cells to perform DNA repair. Alternatively, HPV E6 protein, besides disrupting p53 function, may interfere with other cellular factors that regulate cellular response like apoptosis by DNA damage.

Wild type p53 function has been shown to enable genotoxin-exposed cells to arrest at G1/S phase of cell cycle leading to apoptotic cell death [14,36]. However, p53-independent apoptosis has also been reported [43–45]. Our results show that 76E6 cells underwent apoptosis faster and more extensively than those expressing p53-WT and this response was clearly evident as a function of both genotoxin dose and exposure time. The typical DNA ladder pattern of apoptosis was distinctly observed at 8 h post-irradiation in these cells. These observations are consistent with our early report in which LFS fibroblasts lacking p53 function showed enhanced apoptosis following UV-irradiation. However, both normal HMEC and 76E6 cells were more sensitive to UV-induced DNA damage than normal human and LFS fibroblasts. More interestingly, in both LFS fibroblasts and mammary epithelial cells having degraded p53, UV-induced apoptosis occurred via p53-independent mechanism, while p53 independent expression of p21 could only be seen in LFS fibroblasts [15]. Expression of p21 seemed to confer an increase in resistance to UV irradiation [46,47]. Xu et al. [48] have reported that mammary epithelial cells express-

ing HPV-16 E6 rapidly undergo apoptosis after treatment with the DNA damaging mitomycin C or with staurosporine. It is probable that E6 promotes apoptosis through ubiquitin-mediated destruction of p21.

The observation that high apoptotic index obtained with 76E6 was in full conformity to both the low clonogenic survival and the low cell viability is quite note worthy. Viability of 76E6 cells showed a marked decrease through the entire post UV-incubation of cells. However, a significant recovery in viable cells beginning at 48 h was seen for both normal HMEC and 76E7 cells. These results strongly suggest that normal p53 function is required for cells to recover from UV-induced damage. In support of this, p53 has been shown to be required for recovery of both RNA synthesis and DNA replication following UV-irradiation [49,50]. It is well known that the UV-induced CPD can block both RNA synthesis and DNA replication very efficiently. Thus, it is quite conceivable that NER, which removes UV-induced CPD, contributes significantly to the process of cell recovery.

The involvement of p53 in NER has been reported by several studies [1,5,11,15–17,19,46]. It is becoming clear that p53 and/or p53 regulated gene products contribute to the repair of UV-induced CPD. Whereas it is generally accepted that p53 contributes to GGR, the involvement of p53 in TCR still remains to be clarified. To explore whether p53-degradation sensitizes HPV-16 E6 expressing cells to UV-irradiation due to or related to failure of DNA repair, we further examined both the GGR and strand-specific repair of the *p53* gene. The repair rate as well as overall removal of CPD from the genome were faster and more extensive in cells expressing p53-WT. Disruption of p53, by E6, in 76E6 cells preferentially affected the removal of CPD from the NTS of *p53*. Similar findings have been reported with human fibroblasts expressing E6 [19]. Prima facie these observations seem to suggest that p53 may not be a necessary component of the active TCR complex but might be only involved in GGR. However, endonuclease sensitive site assay reflects the incision and pre-incision steps of NER and does not necessarily indicate that the DNA repair is complete. Therefore, these observations do not exclude the involvement of p53 in TCR. In fact, using ligation-mediated PCR, we have found that disruption of p53 function in

76E6 cells affects removal of CPD from TS at slow repair sites (unpublished data). Thus, it is possible to speculate for a direct and indirect mechanism by which p53 protein could regulate DNA repair. Any of the p53 interacting cellular factors, like p21, GADD45, PCNA, XPB, XPC, XPD and XPE, can be envisioned to have potential involvement [1].

Since disruption of p53 function by E6 causes a decreased efficiency of DNA repair as well as increased sensitivity, it is difficult to underscore the specific role conferred by the loss of p53 on these processes. Nevertheless, comparison of NER deficient xeroderma pigmentosum syndrome (XP) cells with p53-deficient LFS cells indicates that XP cells are more UV sensitive than LFS cells [47]. Therefore, it can be concluded that enhanced sensitivity and apoptosis in 76E6 transfectants is the result of inefficient repair process due to the absence of supporting p53 function.

In summary, the present study shows that degradation of p53, as a result of HPV-16 E6, is associated with decrease in overall extent and rate of removal of CPD from the genome of epithelial cells exposed to UV-irradiation. p53 function seems to be required for GGR. Furthermore, we have found that mammary epithelial cells expressing HPV-16 E6 rapidly and extensively undergo apoptosis following the UV exposure, suggesting that expression of E6 oncoprotein triggers apoptosis by compromising the activity of cellular p53 and p21 proteins. Nevertheless, recovery of cells from UV damage requires normal p53 function and efficient GGR, suggesting the possibility of targeting NER for clinically responsive efficient treatment of breast cancer. Full delineation of the integrated mechanism of action of the p53 and p53-regulated gene products on NER and the related biological responses warrants additional studies.

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