Role and Regulation of p53 during an Ultraviolet Radiation-induced G1 Cell Cycle Arrest

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Abstract
p53 can play a key role in response to DNA damage by activating a G1 cell cycle arrest. However, the importance of p53 in the cell cycle response to UV radiation is unclear. In this study, we used normal and repair-deficient cells to examine the role and regulation of p53 in response to UV radiation. A dose-dependent G1 arrest was observed in normal and repair-deficient cells exposed to UV. Expression of HPV16-E6, or a dominant-negative p53 mutant that inactivates wild-type p53, caused cells to become resistant to this UV-induced G1 arrest. However, a G1 to S-phase delay was still observed after UV treatment of cells in which p53 was inactivated. These results indicate that UV can inhibit G1 to S-phase progression through p53-dependent and independent mechanisms. Cells deficient in the repair of UV-induced DNA damage were more susceptible to a G1 arrest after UV treatment than cells with normal repair capacity. Moreover, no G1 arrest was observed in cells that had completed DNA repair prior to monitoring their movement from G1 into S-phase. Finally, p53 was stabilized under conditions of a UV-induced G1 arrest and unstable when cells had completed DNA repair and progressed from G1 into S-phase. These results suggest that unrepaird DNA damage is the signal for the stabilization of p53, and a subsequent G1 phase cell cycle arrest in UV-irradiated cells.

Introduction
The tumor suppressor protein p53 plays a critical role in the cellular response to DNA damage by functioning as a cell cycle checkpoint determinant (1). Wild-type p53 levels are usually quite low because of a short protein half-life (2, 3). In contrast, p53 levels increase and the protein is stabilized in response to IR,3 and the cells undergo a G1-phase cell cycle arrest (2–4). No G1 arrest is observed in IR-treated cells that lack p53, indicating an essential role for p53 in the arrest response (4–6). The p53-dependent G1 arrest is thought to allow cells time to repair the damaged DNA before proceeding into S-phase, thereby preventing an accumulation of mutations that could occur from replicating a damaged genome. Consistent with this hypothesis are reports that loss or inactivation of p53 causes cells to accumulate mutations at a higher rate (7, 8). p53 can also trigger apoptosis (programmed cell death) in certain cell types after irradiation treatment (9, 10). For example, thymocytes from p53 knock-out mice were more susceptible to radiation-induced apoptosis than were thymocytes from cells expressing p53 (9). On the basis of these results and others, it has been proposed that the normal function of p53 is to monitor the integrity of the genome and protect cells from accumulating genetic damage. p53 carries out this function by temporarily halting cell proliferation to allow DNA repair or by eliminating DNA damaged cells through apoptosis.

In contrast to IR, a role for p53 in response to UV radiation has not been clarified. p53 levels increase in UV-irradiated cells as they do after IR treatment, and the cells undergo a G1 arrest. However, in some cases this G1 arrest was observed in normal cells and in cells in which p53 was inactivated by expression of either SV40 large T-antigen or the E6 oncoprotein of human papillomavirus (11, 12). These results suggested that the UV-induced G1 arrest occurs in a p53-independent fashion. In contrast, a moderate G1 arrest that appeared to be p53 dependent was observed recently in cells exposed to low doses of UV radiation (12). Furthermore, a transient G1 arrest was observed in UV-irradiated human oral keratinocytes that expressed wild-type p53 but not in keratinocytes that lacked wild-type p53 expression (13). These results suggest that, at least in some cases, p53 can play a role in the establishment of a G1 arrest after UV radiation treatment.

It has also been suggested that p53 may play a direct role in DNA repair after UV radiation treatment. UV radiation causes pyrimidine dimer formation and generates (6-4) photoproducts in DNA, both of which are repaired through a process called NER (14). Expression of wild-type p53 was reported to be necessary for efficient NER in UV-irradiated human fibroblasts, suggesting that p53 may play a role in the NER process (15). The best characterized NER components are the XP factors, of which there are seven, designated XP-A to XP-G. XP-B and XP-D are DNA helicases and critical

1 This work was supported by USPHS Grant 1R01CA80918 and by a breast cancer research grant from the Massachusetts Department of Public Health (both to C. G. M.).
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Received 9/22/99; revised 1/24/00; accepted 1/24/00.
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3 The abbreviations used are: IR, ionizing radiation; NER, nucleotide excision repair; XP, xeroderma pigmentosum; HPV, human papillomavirus; UDS, unscheduled DNA synthesis; MRD, minimum required UV dose; FCS, fluorescence-activated cell sorting.
components of the NER pathway (16, 17). p53 can interact directly with XP-B and XP-D and inhibit their helicase activities in vitro (18). These results raise the possibility that p53 may function during NER by modulating the activities of these two helicases. In contrast, Wang et al. (19) reported that XP-B and XP-D are required components of a p53-mediated apoptosis pathway (19). Therefore, the interaction between p53 and either XP-B or XP-D may mediate an apoptotic function of p53, without affecting DNA repair. Given the role of p53 in cell cycle control and its potential role in NER, it is important to determine the relationship between UV radiation, p53, and DNA repair. In this study, we used normal and repair-deficient cell lines to examine the role and regulation of p53 in response to UV radiation. Toward this end, normal human fibroblasts (GM6419 cells) were infected with control retroviruses or retroviruses that express the HPV-16 E6 oncoprotein. HPV-16 E6 promotes the rapid degradation of p53 through the ubiquitin-proteolysis pathway (20–22), and cells that express E6 are therefore similar to cells that lack p53. The effect of UV radiation on the progression of these cells from G1 into S-phase was then assessed. Cells were maintained at confluence for 48 h to obtain G1-phase cell populations. The cells were then treated with increasing doses of UV radiation and replated at low density to stimulate their movement from G1 to S-phase. Progression from G1 into S-phase was monitored by FACS analysis. As shown in Fig. 1, A and B, >90% of the cells had a G1 DNA content at the zero time point. The decrease in the percentage of G1 is attributable to the movement of cells from G1 into S-phase. C, cells were either nonirradiated or exposed to UV (8 J/m2) and plated as described above. At the indicated time points after plating, protein extracts were prepared. One hundred μg of each extract were examined by Western blot analysis with the p53 antibody Ab-6 (Oncogene Science) or the p21 antibody 15431E (PharMingen).

Fig. 1. GM6419 cells that were infected with a retrovirus expressing HPV-16 E6 or a control retrovirus (LXSN) were maintained at confluence for 48 h. The cells were then UV irradiated (0, 4, or 8 J/m2) and plated at low density to stimulate their movement from G1 into S-phase. Cell cycle distribution was determined by FACS analysis at various time points after plating. A, representative FACS data from a single experiment is illustrated and shows a complete G1 arrest in control virus-infected cells exposed to 8 J/m2 and a G1 to S-phase delay in E6-expressing cells exposed to 6 J/m2. B, the percentage (%) of cells with a G1 DNA content at each time point from an experiment similar to that in A is plotted. The decrease in the percentage of G1 is attributable to the movement of cells from G1 into S-phase. C, cells were either nonirradiated or exposed to UV (8 J/m2) and plated as described above. At the indicated time points after plating, protein extracts were prepared. One hundred μg of each extract were examined by Western blot analysis with the p53 antibody Ab-6 (Oncogene Science) or the p21 antibody 15431E (PharMingen).

Results
Role of p53 in a UV-induced G1 Block or Delay. The purpose of this study was to examine the role and regulation of p53 during the cell cycle response to UV radiation. Toward this end, normal human fibroblasts (GM6419 cells) were infected with control retroviruses or retroviruses that express the HPV-16 E6 oncoprotein. HPV-16 E6 promotes the rapid degradation of p53 through the ubiquitin-proteolysis pathway (20–22), and cells that express E6 are therefore similar to cells that lack p53. The effect of UV radiation on the progression of these cells from G1 into S-phase was then assessed. Cells were maintained at confluence for 48 h to obtain G1-phase cell populations. The cells were then treated with increasing doses of UV radiation and replated at low density to stimulate their movement from G1 to S-phase. Progression from G1 into S-phase was monitored by FACS analysis. As shown in Fig. 1, A and B, >90% of the cells had a G1 DNA content at the zero time point. The percentage of nonirradiated G1 phase cells decreased between 12 and 18 h after growth stimulation because of the movement of cells from G1 into S-phase. A UV dose of 4 J/m2 caused a delay in the movement of control virus-infected cells into S-phase, and a UV dose of 8 J/m2 caused a complete G1 arrest up to 42 h after irradiation. Eight J/m2 appeared to be the minimum dose that could cause a complete G1 arrest in control virus-infected GM6419 cells (not shown). Cells expressing HPV-16
E6 were resistant to a UV-induced delay at 4 J/m², and their movement into S-phase was delayed, although not completely inhibited, at a UV dose of 8 J/m². p53 and p21 protein levels were also monitored in the nonirradiated and irradiated cells (Fig. 1C). In control virus-infected cells that were not irradiated, p53 and p21 levels were unchanged or slightly decreased after growth stimulation. In contrast, p53 and p21 levels were increased in cells treated with 8 J/m² prior to plating and growth stimulation. Furthermore, p53 and p21 levels were low in cells expressing HPV-16 E6, and neither p53 nor p21 were induced upon UV treatment. These results are consistent with the UV-induced arrest resulting, at least in part, from activation of the p53-p21 growth arrest pathway.

The ability of E6 to overcome a UV-induced G₁ arrest could have resulted from inactivation of p53 or from other E6 activities. To confirm the involvement of p53 in this UV-induced G₁ arrest, GM6419 cells were infected with a retrovirus encoding a dominant-negative p53 mutant (p53-CTF) capable of inactivating the wild-type p53 protein (10). The effect of UV radiation on the progression of these cells from G₁ into S-phase was then assessed (Fig. 2A). As with E6 expression, cells that expressed p53-CTF were resistant to a UV-induced G₁ phase arrest after exposure with 8 J/m² (Fig. 2A). These results indicate that inactivation of p53 by either the dominant-negative p53 mutant or HPV-16 E6 can overcome a UV-induced G₁ arrest. A G₁ to S-phase delay was still observed after exposure to 8 J/m² in p53-CTF-expressing cells, indicating that UV can also induce a G₁ to S-phase delay that is independent of p53. Steady-state levels of p53 were increased in cells expressing p53-CTF, attributable to the fact that the p53-CTF mutant can stabilize the endogenous p53 protein by sequestering it in inactive complexes (10). Nonetheless, levels of full-length p53 and p53-CTF were unchanged after UV treatment of the p53-CTF-expressing cells, and p21 protein levels were undetectable even after UV exposure (Fig. 2B). It should also be noted that p21 as well as MDM2 protein levels were low and not increased after IR treatment of the p53-CTF-expressing cells (not shown). Taken together, these results indicate that the p53-CTF mutant functionally inactivated the endogenous p53 protein.

p53 Mediates a UV-induced G₁ Block in UV Repair-deficient Cells. G₁ to S-phase progression was delayed in GM6419 cells exposed to 4 J/m² and completely blocked at a UV dose of 8 J/m², indicating that the extent of G₁ arrest after UV treatment was dose dependent. We predicted, based on these results, that cells deficient in the repair of UV-induced DNA damage would be more susceptible to a UV-induced G₁ arrest than normal cells. Patients with XP cannot efficiently repair UV-induced DNA damage (14, 23). XP cells from complementation group C (XPC cells) repair damage to actively transcribed DNA strands normally but are defective in the repair of nontranscribed DNA regions (24). XP cells from complementation group D (XPD cells) are defective in the repair actively transcribed DNA regions (25). XPC and XPD cells were infected with control retroviruses or retroviruses that express HPV-16 E6 or p53-CTF, and the effect of UV on their movement from G₁ to S-phase was assessed. Immunoblot analyses similar to that shown in Fig. 2 demonstrated p53-CTF expression in the XPC and XPD cells infected with the p53-CTF-expressing retrovirus (not shown). The minimum dose that caused a complete G₁ arrest up to 60 h after irradiation was ~1.5 J/m² in the XPD cells and 5–6 J/m² in XPC cells (Fig. 3). It is important to note that similar results were obtained with one other XPC and XPD cell line (not shown). Expression of either HPV-16 E6 or p53-CTF abolished the UV-induced G₁ arrest in these repair-deficient cells, indicating that the arrest was mediated in part by p53 (Fig. 3A). As in GM6419 cells, inactivation of p53 in these repair-deficient cells did not completely overcome the effects of UV, because a UV-induced G₁ to S-phase delay was still observed in cells expressing HPV-16 E6 or p53-CTF. Immunoblot analyses (Fig. 3B) indicated that p53 and p21 levels were induced by UV radiation in control cells but not induced in cells infected with either the HPV-16 E6 or p53-CTF retroviruses, consistent with the UV-induced G₁ arrest resulting in part from activation of the p53-p21 growth arrest pathway.

Fig. 2. A, GM6419 cells that were infected with a retrovirus expressing a dominant-negative mutant form of p53 (p53-CTF) were grown to confluence to obtain G₁-phase cells. The cells were then untreated or exposed to a UV dose of 8 J/m² and plated at low density to stimulate their movement from G₁ to S-phase. Cell cycle distribution was determined by FACS analysis at various time points after plating. The percentage of cells with a G₁ DNA content at each time point is indicated. B, Cells were either nonirradiated or exposed to UV (8 J/m²) and plated as described above. At the indicated time points after plating, protein extracts were prepared. Thirty μg of each extract were examined by immunoblotting using the p53 antibody Ab-6 for full-length p53 or the p53 antibody Ab-1 for p53-CTF, and 100 μg of extract were examined by Western blotting using the p21 antibody 15431E. The positive control for the p21 blot was 100 μg of extract from control retrovirus-infected GM6419 cells treated with 8 J/m² UV and harvested 21 h after plating.
Unrepaired DNA Damage Mediates a UV-induced G1-Phase Block. Because the repair-deficient cells were more susceptible to a UV-induced arrest than normal cells, we suspected that unrepaired DNA damage may be the signal for a UV-induced arrest. To examine this possibility, cell cycle progression was analyzed in UV-irradiated cells that were first allowed to repair their DNA before being stimulated to move from G1 into S-phase. DNA repair activity (UDS) was assessed in UV-irradiated GM6419 cells as described previously (26). Briefly, G1 phase cells were UV irradiated and maintained in G1 for 24 h. At various time points after UV treatment, the cells were pulse labeled with [3H]thymidine. Because the cells were in G1, the uptake of [3H]thymidine was attributable to DNA repair synthesis only and not because of replicative DNA synthesis. The uptake of radiolabeled nucleotide at each time point was monitored by fixing the cells directly to the culture dish and exposing them to a photographic emulsion prior to autoradiographic development. The average number of silver grains precipitated from the emulsion per cell nucleus was determined by microscopic examination and is a measure of DNA repair activity (UDS). The data are plotted in Fig. 4 as % UDS at various time points after plating.

Fig. 3. A, XPC and XPD cells that were either uninfected or infected with a retrovirus expressing HPV-16 E6 or the dominant-negative mutant form of p53 (p53-CTF) were grown to confluence to obtain G1-phase cells. The cells were then either untreated or exposed to the indicated UV dose, followed by plating at low density. Cell cycle distribution was determined by FACS analysis at various time points after plating. The percentage of cells with a G1 DNA content at each time point is indicated. B, cells were either untreated or exposed to UV and plated as described above. At the indicated time points after plating, protein extracts were prepared and examined by Western blotting for p53 and p21. Thirty µg of protein extract from cells expressing the dominant-negative p53 mutant was loaded in each lane for the p53 Western blot. In all other cases, 100 µg of protein were loaded per lane. The positive control (+ ctrl.) for the p21 blot was 100 µg of extract from noninfected XPD or XPC cells treated with UV and harvested 21 h after plating.
time points after UV treatment. The level of UDS was max-
imal immediately after UV treatment (100% UDS) and dimin-
ished to background levels after 24 h of holding in G1 (Fig. 
4A). These results indicate that DNA repair after UV treat-
ment was completed during the 24-h period that the cells 
were held in the G1 phase.

Progression from G1 into S-phase was then monitored in 
UV-irradiated cells that were allowed to repair their DNA prior 
to growth stimulation. As shown in Fig. 4B, GM6419 cells 
that were allowed to complete DNA repair prior to growth 
stimulation (held in G1 for 24 h after UV treatment) were 
resistant to a UV-induced G1 arrest. Furthermore, UV radia-
tion caused a complete G1-phase arrest in XPC cells, regard-
less of whether the cells were held in G1 for 24 h prior to 
growth stimulation (Fig. 4B). These results are consistent with 
unrepaired DNA damage being the signal for a p53-
dependent G1 arrest in UV-irradiated cells.

Stabilization of p53 during a UV-induced G1-Phase Block. 
The increase in p53 levels after UV treatment results, in 
large part, from stabilization of the p53 protein (2, 3). If p53 
is stabilized to halt proliferation and allow DNA repair, then 
p53 stability is expected to decrease when DNA repair is 
complete. To test this possibility, p53 stability was deter-
mined in cells that were either growth stimulated immediately 
after UV exposure or were allowed to complete DNA repair 
onto growth stimulation. The half-life of p53 was ~30 min 
in nonirradiated cells 12 h after release from G1 (Fig. 5, OJ). 
In contrast, p53 was stabilized (half-life extended to ~2 h) in 
cells exposed to a UV dose of 8 J/m² and stimulated imme-
diately after UV treatment. Under these conditions, the cells 
underwent a complete G1-phase cell cycle arrest (Figs. 1 and 
4). Importantly, the half-life of p53 was decreased to that of 
nonirradiated cells in cells that were UV irradiated but held in 
G1 for 24 h prior to plating. Under these conditions, UV-
induced DNA damage was completely repaired, and the cells 
progressed with normal kinetics from G1 into S-phase (Fig. 
4). These results establish an excellent correlation between 
p53 stability and a G1 phase arrest in UV-irradiated cells.

Finally, p53, p21, and MDM2 protein levels were deter-
mined in UV-irradiated cells that were either growth stimu-
lated immediately after UV exposure or were held in G1 for 
24 h prior to growth stimulation (Fig. 6). Levels of all three 
proteins were increased in UV-irradiated cells that were 
plated immediately after UV exposure and were arrested in 
G1. In these experiments, p53 was induced at 5 h after 
release from G1 in the UV-irradiated cells, whereas MDM2 
and p21 protein levels were not increased until 10 h after 
release from G1. The levels of all three proteins decreased in 
UV-irradiated cells that were held for 24 h in G1, prior to plating and were resistant to the UV-induced G1 arrest. It 
should be noted that p53 levels were not decreased in UV-
irradiated cells held in G1 for up to 34 h after treatment (Fig. 
6), despite the fact that DNA repair was complete within 24 h 
of holding in G1 (Fig. 4). This suggests that in addition to the 
completion of DNA repair, destabilization of p53 also re-
quires the release of cells from the G1 phase. The expression 
patterns for p53 and p21 in this experiment are consistent 
with the UV-induced G1 arrest resulting from activation of 
the p53-p21 growth arrest pathway. It was perhaps interesting 
that MDM2 displayed an expression pattern similar to that of 
p53 and p21. MDM2 can bind p53 and promote its rapid 
degradation, and current models suggest that the stabiliza-
tion of p53 in DNA-damaged cells results from an inhibition 
of p53:MDM2 binding (27–29). In Fig. 6B, we examined the 
level of p53:MDM2 binding complexes in this experiment by coimmunoprecipitation. A large amount of MDM2 immuno-
precipitated with p53 from cells, which were plated immedi-
ately after UV exposure and in which p53 was stabilized. 
p53:MDM2 complexes were not observed until 10 h after 
release of the UV-irradiated cells from G1, consistent with the 
increased MDM2 levels observed at this time point. The fact 
that p53 was stabilized with no obvious decrease in p53: 
MDM2 binding suggests that UV radiation may stabilize p53 
through alternative pathways, in addition to inhibiting the 
interaction between p53 and MDM2.

Discussion
When normal mammalian cells are exposed to DNA-damag-
ing agents, they undergo a transient G1- and G2-phase cell 
cycle arrest. These arrests allow cells time to repair the 
damaged DNA before proceeding with either replicative DNA 
synthesis or mitosis. Failure to arrest in either G1 or G2 phase 
could lead to an accumulation of mutations because of the 
replication of a damaged genome. IR induces a G1 arrest in 
cells expressing wild-type p53 but not in cells that either lack
p53 expression or in which p53 is inactivated (4–6). These results demonstrate an essential role for p53 in the cell cycle response to IR. In contrast to IR, however, a clear role for p53 in the cell cycle response to certain other DNA-damaging agents has not been established. For example, UV radiation inhibited cell cycle progression in normal embryonic stem (ES) cells and in ES cells homozygous for a targeted deletion of p53 (30). Furthermore, high doses of either UV radiation or actinomycin D were reported to induce a G1 arrest in cells with wild-type p53 and in cells in which p53 was inactivated by expression of the HPV-16 E6 oncoprotein (12). Finally, a p53-independent G1 arrest was reported in murine 3T3 cells exposed to the DNA modifying agent benzo(a)pyrene (31). These findings indicate that certain DNA-damaging agents can signal a G1 cell cycle arrest through mechanisms that are independent of p53.

The purpose of this study was to examine the role and regulation of p53 during a UV-induced G1 arrest. A dose-dependent G1 arrest was observed in normal human fibroblasts as well as in fibroblasts deficient in the repair of UV-induced DNA damage. Expression of HPV16-E6, which promotes the degradation of p53, or a dominant-negative p53 mutant that inactivates wild-type p53, caused the cells to become resistant to this UV-induced arrest. These results clearly demonstrate that p53 can activate a G1 cell cycle arrest in response to UV radiation. Interestingly, however, cells in which p53 was inactivated still underwent a significant G1 to S-phase delay after UV treatment. These findings indicate that UV radiation can also activate a G1 delay that is independent of wild-type p53. On the basis of these findings, we suggest that UV radiation affects multiple pathways to cause a G1-phase arrest or delay, only one of which involves p53.

Our results suggest that the p53-dependent G1 arrest in UV-irradiated cells results from UV damage to actively transcribed genes. This is based on the fact that the minimum UV dose that caused a complete G1 arrest in uninfected or control virus-infected cells was 8 J/m² in cells with normal DNA repair capacity (GM6419 cells), 5–6 J/m² in XPC cells, and 1.5–2.0 J/m² in XPD cells. Thus, XPD cells, which are deficient in the repair of actively transcribed genes, are more susceptible to a UV-induced G1 arrest than are either XPC cells or normal cells, which are not compromised in the repair of transcribed DNA strands. In this regard, it is worth noting the studies of Ford and Hanawalt (15) in which wild-type p53 was required for efficient repair of nontranscribed DNA regions but not for repair of transcribed DNA strands. Insofar as p53 is not required for repair of actively transcribed genes, these results would suggest that the induction of p53 through UV damage to actively transcribed genes is independent of its role in DNA repair. Other studies support our notion that UV radiation signals to p53 through damage to actively transcribed genes. For example, the MRD that stabilized p53 was estimated in normal cells and in cells deficient in various aspects of DNA repair (32). The MRD in cells specifically deficient in the repair of actively transcribed genes was 8-fold lower than the MRD of cells with normal DNA repair capacity. In contrast, the MRD for cells specifically deficient in the repair of nontranscribed DNA regions was as high as that of normal cells. These results suggested that DNA damage to actively transcribed genes is the signal for the stabilization of p53 in response to UV radiation.

The mechanism by which UV induces a p53-independent G1 to S-phase delay is unknown. A recent study suggested that high doses of UV radiation can inhibit the expression of E2F-1-transactivated gene products that are required for G1 to S-phase progression (12). Thus, decreased expression of these E2F-1-regulated genes could contribute to the p53-independent G1 to S-phase delay observed in the current report. In a separate study, UV radiation was reported to induce the expression of p21 and a concomitant G1 arrest in Li-Fraumeni cells that lacked both p53 alleles (33). Although this induction of p21 may explain the p53-independent responses to UV radiation in some systems, we did not observe an induction of p21 in UV-irradiated cells in which p53 was inactivated. It is worth noting that in our study, XPD cells in which p53 was inactivated by a p53 dominant-negative mutant remained more sensitive to a UV-induced G1 arrest than either XPC or normal cells in which p53 was similarly inactivated. These results suggest that damage to actively transcribed genes may be the signal for a p53-independent G1-phase delay, in addition to the p53-dependent arrest.

The mechanism by which UV radiation and other DNA-damaging agents stabilize p53 has not been fully clarified. MDM2 can bind p53 and promote its rapid degradation through the ubiquitin proteolysis pathway (27, 28). Current
the immunoprecipitation.

ways for activating a G1 arrest or delay in response to UV irradiated at a dose of 8 J/m². The cells were either plated immediately after UV treatment or were held at confluence for 24 h prior to plating. The indicated time points after plating, protein extracts were prepared and examined by immunoblot analysis with the p53 antibody Ab-421 and examined by immunoblot analysis with the MDM2 antibody SMP-14 to detect p53:MDM2 binding complexes. These results raise the possibility that UV may affect multiple pathways to stabilize p53, in addition to inhibiting the interaction between p53 and MDM2.

models suggest that DNA-damaging agents stabilize p53 by inhibiting p53:MDM2 binding (29). According to this model, one would predict a decreased interaction between p53 and MDM2 under DNA-damaging conditions that stabilize p53. In the current study, MDM2 protein levels were increased under conditions that stabilized p53, and the UV-irradiated cells underwent a G1 cell cycle arrest. Interestingly, the increase in MDM2 levels coincided with a corresponding increased level of p53:MDM2 binding complexes. These results raise the possibility that UV may affect multiple pathways to stabilize p53, in addition to inhibiting the interaction between p53 and MDM2.

Ineffective repair of UV-induced DNA damage can result in a high predisposition to cancer, as well as an increased sensitivity to UV-induced cell death (34, 35). Thus, efficient DNA repair after exposure to UV radiation is essential for maintaining normal cellular homeostasis. The current study indicates that UV can induce a G1 cell cycle arrest or delay through p53-dependent and -independent mechanisms. Furthermore, our results suggest that unrepaired DNA damage to actively transcribed genes is the likely signal for a p53-dependent G1 arrest. The presence of multiple pathways for activating a G1 arrest or delay in response to UV radiation underlies the potential importance of such an arrest in the DNA repair response.

Materials and Methods

Cell Strains and Retroviral Infections. All cell types used in this study were maintained in DMEM containing 15% fetal bovine serum. The human diploid fibroblast strains GM6419, the XPC cell strains GM2995 and GM2996, and the XPD cell strains GM03247 and GM0524 were obtained from the Corielle cell repository in Camden, NJ. GM6419 cells have normal repair capacity for UV-induced DNA lesions. Cell lines producing the HPV-16 E6 or control retrovirus (LXSN) were obtained from Denise Galloway (University of Washington, Seattle, WA). The DNA construct for production of the dominant-negative p53 retrovirus (referred to as p53-CTF) was obtained from Moshe Oren (Weizmann Institute of Science, Rehovot, Israel). p53-CTF encodes the COOH-terminal oligomerization domain of p53 and inactivates wild-type p53 in infected cells (10). The p53-CTF retrovirus-producing cell line was generated by Alan Thompson (Harvard Medical School). Retroviral infection was carried out by incubating exponentially growing GM6419, XPC, or XPD cells in 4 ml of medium containing a 1-ml aliquot of each retrovirus and 4 μg/ml Polybrene for 4 h. The cells were then rinsed with fresh medium once and refed with fresh medium and incubated overnight. The cells were then split at a dilution of approximately 1:4 and maintained in normal medium for an additional 24 h, at which point the cells were refed with medium containing 200 μg/ml G418. The cells were maintained in G418-containing medium for 2 weeks, and pooled populations of selected cells were obtained.

UV Radiation Treatment and Cell Cycle Analysis. UV irradiation was carried out as described previously (2). The UV light exposure apparatus consisted of five UV bulbs in a specially constructed incubator that delivered 254 nm light at a dose of 2.08 J/m²/s. Confluent, G1-phase cells were rinsed with PBS and exposed to the indicated UV dose. The cells were then trypsinized and replated at low density to stimulate their move-ment from G1 into S-phase. At the indicated time after growth stimulation, cells were trypsinized and fixed in 70% ethanol. The fixed cells were suspended in PBS containing 1 mg/ml propidium iodide and 1000 Kunitz units/ml RNase A. Cell cycle distribution was determined by FACS analysis at the Dana-Farber Flow Cytometry Laboratory.

Western Blots, Immunoprecipitations, and p53 Stability Measurements. For Western blot analysis, cells were washed twice with PBS, scraped into 0.5 ml of lysis buffer [50 mM Tris (pH 8.0), 5 mM EDTA, 150 mM NaCl, 0.5% NP-40, and 1 mg/ml phenylmethylsulfonfyl fluoride], and incubated on ice for 15 min with occasional light vortexing. Lysates were spun at 15,000 × g for 15 min to remove cellular debris. Protein extract from the resulting supernatant was resolved by SDS-PAGE and transferred to Immobilon-P membranes (Millipore) for detection with either the p53 antibody Ab-421 (Oncogene Science), the anti-p21 polyclonal antibody 15431E (PharMingen), or the anti-MDM2 antibody SMP-14. For analysis of p53:MDM2 binding, p53 was immunoprecipitated from lysates using the p53 antibody Ab-421 (Oncogene Science) and subsequently examined by immunoblot analysis using the MDM2 antibody SMP-14.

DNA Repair Measurements. DNA repair activity (UDS) was assessed as described (26) in UV-irradiated GM6419 cells in the following manner: G1 phase cells were UV irradiated (8 J/m²) and maintained in G1 for 24 h. At various time points after UV treatment, the cells were pulse labeled with 1 μCi [3H]thymidine. Because the cells were in G1, the uptake of [3H]thymidine in the general cell population was attributable to DNA repair synthesis only and not attributable to replicative DNA synthesis. The uptake of radioc nucleotide at each time point was monitored by fixing the cells directly to the culture dish and subsequently exposing them to a photographic emulsion prior to autoradiographic development. The average number of silver grains precipitated per cell nucleus was determined by microscopic examination and was used as a measurement of DNA repair activity. The experiment was performed in duplicate, and the average number of silver grains precipitated per cell nucleus at each time point was determined. The highest number of silver grains were precipitated from each cell nucleus immediately after UV treatment.

Acknowledgments

We acknowledge Peter M. Howley for guidance and critical reading of the manuscript.

References


