UV Induces p21 Rapid Turnover Independently of Ubiquitin and Skp2*

Received for publication, June 5, 2006 Published, JBC Papers in Press, June 27, 2006, DOI 10.1074/jbc.M605366200

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It was previously reported that low doses, but not high doses, of UV trigger the Skp2-mediated proteasomal degradation of the cyclin-dependent kinase inhibitor p21 in mammalian cells. Here we show that both UV-C and UV-B lead to decrease of p21 protein, but not mRNA, level in a dose-dependent fashion in all of six human cell lines and five mouse cell lines tested. Also, high doses of UV reduce the half-life of p21. High doses, but not low doses, of UV induced p21 degradation in both skp2-proficient and -deficient murine embryonic fibroblast cells. UV-induced p21 reduction was rescued by proteasome inhibitors in all human and mouse cell lines tested. Neither a caspase inhibitor nor small interfering RNA against skp2 had an effect on the UV-induced p21 decrease, suggesting that this p21 degradation pathway may not involve caspases, or Skp2. Finally, UV did not induce p21 ubiquitination but still induced its degradation when the E1-activating enzyme was inactivated in an E1 temperature-sensitive murine embryonic fibroblast cell line. Altogether, these results demonstrate that UV induces p21 degradation through an Skp2- and ubiquitin-independent pathway.

The tight cell cycle control is executed through the concerted efforts of cyclin-dependent kinases and their cellular inhibitors (1). One of the inhibitors, p21, is induced at the mRNA level by the tumor suppressor p53, subsequently leading to G1 arrest in response to DNA damage (2–4). Because of the inhibitory effect of this protein on the cell cycle and possibly on DNA repair (5, 6), constantly high levels of p21 would be detrimental to normal cell growth. Thus, a post-translational mechanism has been evolved to adjust the level of this protein.

It has been shown that the p21 protein is unstable due to active proteasome-mediated degradation in the nucleus (7). However, it has been debated whether ubiquitin mediates the proteasomal turnover of p21. On one hand, p21 is ubiquitinated in cells (7–11). This ubiquitination occurred at both the internal lysine residues and the N terminus of this protein (12). Two recent reports showed that it was through the N-terminal ubiquitination that p21 was degraded by the proteasomal system (12, 13). Also, Skp2 was shown to mediate p21 ubiquitination and degradation in response to UV (14). On the other hand, challenging this ubiquitin-dependent model are several independent studies showing that p21 is degraded through a ubiquitin-independent mechanism (15–17). For example, lysine-free p21 was not ubiquitinated but still degraded through the proteasomal system (7). In addition, the 20 S proteasome directly bound to p21 and led to its degradation in vitro (18).

Moreover, p21 degradation was promoted by MDM2 independently of ubiquitin (15, 17). Further supporting the non-ubiquitin pathway is that p21 was found to be acetylated at its N terminus in cells, suggesting that N-terminal ubiquitination is unlikely. Consistently, inactivation of the E1 or Skp2 components in the ubiquitination-proteasome system did not change the stability of p21 (16). These results suggest a ubiquitin-independent mechanism for p21 turnover. Our study as described here further shows that UV induces p21 degradation independently of Skp2 and ubiquitin, consistent with the ubiquitin-independent model for p21 degradation.

EXPERIMENTAL PROCEDURES

Cell Culture—Human retinal pigment epithelial RPE cells, human lung adenocarcinoma H1299 cells, human osteosarcoma U2OS, human breast cancer MCF7, human embryonic fibroblast WI38, human embryonic kidney epithelial 293 cells, mouse embryonic testicular carcinoma F9 cells, human epidermal keratinocyte HEK, ts20TG6 ME and -deficient murine embryonic fibroblast cells. UV-induced p21 reduction was rescued by proteasome inhibitors in all human and mouse cell lines tested. Neither a caspase inhibitor nor small interfering RNA against skp2 had an effect on the UV-induced p21 decrease, suggesting that this p21 degradation pathway may not involve caspases, or Skp2. Finally, UV did not induce p21 ubiquitination but still induced its degradation when the E1-activating enzyme was inactivated in an E1 temperature-sensitive murine embryonic fibroblast cell line. Altogether, these results demonstrate that UV induces p21 degradation through an Skp2- and ubiquitin-independent pathway.

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**UV Irradiation**—UV Cross-linker (Fisher Scientific, FB-UVXL-1000) was used to for UV C irradiation. Cells were irradiated with UV-C or UV-B at about 60–80% confluence with 6 ml of medium in a 100-mm Petri dish or with 2 ml of medium in a 60-mm Petri dish with the lid removed. This approach was adapted from that as described previously (14).

**Buffers**—Lysis buffer consisted of 50 mM Tris-HCl, pH 8.0, 0.5% Nonidet P-40, 1 mM EDTA, 150 mM NaCl, and 1 mM phenylmethylsulfonyl fluoride.

**Cell Culture**—Human retinal pigment epithelial RPE cells, human lung adenocarcinoma H1299 cells, human osteosarcoma U2OS, human breast cancer MCF7, human embryonic fibroblast WI38, human embryonic kidney epithelial 293 cells, mouse embryonic testicular carcinoma F9 cells, human epidermal keratinocyte HEK, ts20TG6 ME and -deficient murine embryonic fibroblast cells.

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The same results as that shown in Figs. 1–2 were obtained when cells were irradiated with UV in the absence of media. The latter was our original protocol (20).

The on-line version of this article (available at http://www.jbc.org) contains a supplemental Fig. S1.

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2 The abbreviations used are: MEF, mouse embryonic fibroblast; E1, ubiquitin-activating enzyme; WB, Western blotting; RT, reverse transcription; RPE, retinal pigment epithelium; ALLN, N-acetyl-leucinyl-leucinyl-norleucinal; Z, benzoyloxycarbonyl.

* This work was supported by National Institutes of Health Grants CA095441, CA93614, and CA079721 (to H. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Antibodies and Plasmids—Monoclonal anti-α-tubulin antibodies were purchased from Sigma. Anti-p21 antibodies were purchased from NeoMarker Biotech (AB-11 for human), Oncogene Science (Ab-6 for mouse), and Santa Cruz Biotechnology (H-164 and C19 for mouse), respectively. Monoclonal anti-MDM2 antibodies 4B11 and 2A10 were described previously (21). The anti-c-Myc antibody was purchased from Santa Cruz Biotechnology. The monoclonal anti-p53 antibody DO-1 was purchased from Santa Cruz Biotechnology. The polyclonal anti-skp2 antibody was a generous gift from Stephan Lanker. The polyclonal anti-p27 antibody was a gift from Mihail Iordanov. pCMV-p53 was described previously (21). The anti-c-Myc antibody was purchased from Santa Cruz Biotechnology. The monoclonal anti-p53 antibody was a generous gift from Stephen Lanker. The polyclonal anti-skp2 antibody was a generous gift from Stephen Lanker. The polyclonal anti-p27 antibody was a gift from Mihail Iordanov. pCMV-p53 was described previously (21), and pCEP4-p21 waf1/cip1 was described (2). PCDNA3-2X FLAG-p21 was cloned using BamH1 and EcoRI sites. 2X FLAG-p21 was cloned using BamH1 and EcoRI sites. (21), and pCEP4-p21 waf1/cip1 was described (2). PCDNA3-

Transient Transfection and WB Analysis—H1299 cells (60% confluence in a 60-mm plate) were used for transient transfection (see figure legends to Figs. 4 and 6A for the amounts and types of plasmids used) using the Lipofectamine reagent (Invitrogen Corp.). At 48 h post-transfection, cells were harvested for preparation of whole-cell lysates. Whole-cell lysates containing 75 μg of protein were loaded directly onto an 12% SDS gel, and proteins were detected by ECL reagents (Bio-Rad) after WB using antibodies, as indicated in the figure legends.

RT-PCR Analysis—Total RNA was isolated using Trizol reagent (Invitrogen) from RPE cells and other cells. RT-PCR was conducted as described previously (19). PCR products were analyzed on a 1% agarose gel followed by ethidium bromide staining. The following primers were used: human p21, 5'-ATGTCAGAACC-GGCTGGGGATG-3' and 5'-TTAGGGCTTCCTCTTGGAGGAG-3'; β-actin, 5'-ATG TGG CAC CAC ACC TTC TAC AAT GAG CTG CG-3' and 5'-CTG CAT ACT CTT GCT TGC TGA TCC ACA TCT GC-3'; mouse skp2 primers, 5'-ATGCA-TATGGAGGACCCCTCAG-3' (forward) and 5'-GTACCACCCTCTTGCAAGACCC-3' (reverse); mouse p21, 5'-ATGTCACATACCTGGTATGTCACC-3' and 5'-TCAAGGTTTTCTCTT-GCAGAAG-3'; human p53, 5'-GGCGGATCCGAGGAGCCCGACATCC-3' and 5'-CCGGAAATTCTCAGTCTCTGTCAGGCCCCTTC-3'. p21 in Vivo Ubiquitination Assay—H1299 cells in 100-mm plates were transfected with Hisα-ubiquitin (2 μg), p21mut/cip-1 (2 μg), and p53 expression plasmids using the Lipofectamine reagent (Invitrogen). 48 h after transfection, cells from each plate were harvested and split into two aliquots, one for straight WB and the other for in vivo ubiquitination assays using nickel-nitrilotriacetic acid beads (Qiagen) as described previously (22). The eluted proteins from the beads were analyzed by WB to detect ubiquitinated p21 using Ab11 and ubiquitinated p53 using DO-1.

RNA Interference Transfection—pSUPER-RNA interference-SKp2 constructs were generously provided by Stephen Lanker, where 21 nucleotide RNA targeted the N terminus of skp2 5'-GAGGAGCAGCGAGTCTGAGA-3', the C terminus of skp2 5'-GGTCGTGAGCTGATGTC-3', and scrambled siRNA 5'-GAGAAGCGAGUGAAGGATdTdT-3' (control siRNA) were cloned into a siRNA vector PS (23). The siRNA encoding (si-skp2) vector or a scrambled siRNA vector were transfected (3 μg each) into H1299 or 293 cells following the transient transfection protocol as described above.

Results

UV Reduces p21 Level in Human Cells in a Dose-dependent Fashion—In our initial attempt to understand how the p53 pathway in human RPE cells is regulated in response to UV irradiation, we surprisingly found that despite p53 induction by irradiation of RPE cells with 20 J/m² UV-C, one of its targets, p21, was conversely reduced at the protein level in a time-dependent manner. Within 3 h post-irradiation, p21 protein almost completely disappeared (Fig. 1A). Unlike p21, MDM2 slightly increased at the 3-h point after irradiation and then decreased in a timely fashion afterward (Fig. 1A). The reduction of MDM2 was due to the repression of its transcription by UV as shown previously (20). However, this was not the case for
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UV decreases the level of p21 in various human cell lines. A and B, cells as indicated at the bottom of each panel were harvested 6 h after either UV-C (A) or UV-B (B) irradiation with increasing doses (as indicated). WB (75 µg of protein) was conducted using antibodies as indicated on right. * indicates nonspecific bands detected by the p53 antibody.

p21, as its mRNA level was induced by UV, consistent with the idea that p21 transcription is activated by p53 (24–26). Also, the UV-induced reduction of p21 in this cell line was dose-dependent, as the protein level of p21 was inversely proportional to the dose of UV used for irradiation (Fig. 1, A and C). This was true for both UV-C (>40 J/m²) and UV-B (>562 J/m²), but UV-C was more effective than UV-B, regardless of the induction of p53 (Fig. 1, A and C). The reduction of the p21 level by high doses of UV was due to the decrease of p21 stability, but not the change of its mRNA, as this reduction was rescued by the proteasome inhibitor MG132 (supplemental Fig. S1), and again the p21 mRNA level was conversely induced by high doses of UV (Fig. 1B). These results indicate that UV-C and UV-B can reduce p21 protein, but not mRNA, levels independently of p53 activity in RPE cells.

Our finding was surprising as a previous report showed that lower doses, instead of higher doses, of UV-C irradiation triggered p21 degradation in several mammalian cell lines (14). Because the RPE cells used here were not used in that study (14), it is likely that this difference might be cell type-specific. To check this possibility, we carried out a set of experiments similar to that in Fig. 1 by employing five more human cell lines including U2OS, MCF7, HeLa, WI-38, and HEK cells. These cells are known to harbor wild-type p53 (Fig. 2 and data not shown). These cells were irradiated with different doses of UV-C (0–100 J/m²) (Fig. 2A). Consistent with the result of Fig. 1, but different from the previously reported result (14), UV-C also induced reduction of p21 protein levels to different degrees in a dose-dependent fashion in these cells regardless of the induction of p53 by UV (Fig. 2A and data not shown). UV-C at the doses higher than 80 J/m² resulted in almost complete degradation of the p21 protein in most of the cells tested (Fig. 2A).

This result is in striking contrast with the previously published result, which showed that UV-C at the doses higher than 40 J/m² had no effect on p21 levels in several mammalian cell lines including U2OS cells (14). This difference would not be due to possible variations in defining the dose of UV-C between our laboratory and the other laboratory (14). Our 100 J/m² could not be the same as their 30 J/m², as most of the cells treated with 80 or 100 J/m² underwent drastic death within 10 h post-irradiation (data not shown). Also, as expected (20), MDM2 levels also decreased in response to different doses of UV-C (Fig. 2A). The level of MDM2 in HEK cells was not shown because...
MDM2 was hardly detectable in this cell line (data not shown). To test whether UV-B can also affect p21 levels, we irradiated U2OS and MCF7 with different doses of UV-B (0–843 J/m²).

To determine whether UV-induced p21 decrease is due to proteasome-mediated degradation, RPE cells were incubated with or without 10 μM of the proteasome inhibitor MG132 immediately after being exposed to different doses of UV-C for 6 h and then harvested for WB analyses with antibodies against p21 and tubulin. Consistent with the results in Fig. 1 and 2, UV induced p21 reduction in a dose-dependent manner (Fig. 3A and supplemental Fig. S1). This reduction was completely rescued by MG132 (Fig. 3A and supplemental Fig. S1), suggesting that UV may induce proteasomal turnover of p21. To confirm this result, a similar set of experiments was conducted using RPE, U2OS, and MCF7 cells. These cells were treated with or without another proteasome inhibitor ALLN or the caspase inhibitor Z-VAD besides MG132 prior to being exposed to UV irradiation (20 J/m²). Again, MG132 rescued UV-induced reduction of p21 in all three cell lines (lane 3 of Fig. 3B). Similarly, but to a lesser degree, ALLN also prevented p21 reduction by UV irradiation (lane 5). By contrast, the caspase inhibitor Z-VAD was without any effect on UV-induced reduction of p21 (lane 4). Z-VAD was effective in inhibiting caspase 3- and 7-mediated cleavage of SSRP1, a nuclear transcriptional regulator, as shown in our recent report (27). The reason for checking whether caspases are involved in p21 degradation is that UV-induced p21 reduction appeared to be less apparent in caspase 3-deficient MCF7 cells in comparison with other cell lines (Fig. 2A).

**FIGURE 4.** UV-responsive proteosome-mediated degradation of p21 is independent of ubiquitination. A, exogenous and endogenous p21 proteins were degraded upon UV irradiation. H1299 cells were transfected with FLAG-p21 (2 μg), and the p21 level was shown by WB with the Ab11 antibody. B, the in vivo ubiquitination assay was carried out as described under “Experimental Procedures.” RPE cells in 100-mm plates were transfected with His6-ubiquitin (2 μg), wild-type p21 (2 μg), and wild-type p53 expression plasmids (2 μg). Transfected cells were treated with a 10 μM concentration of the proteasome inhibitor MG132 at the time of UV-C irradiation at 30 J/m² followed by a 6-h incubation. Polyubiquitinated p21 or p53 as well as the level of p21 and p53 were detected by WB with AB11 and DO-1, respectively. * indicates the possibly shorter band of exogenous p53. # denotes the exogenous p21 that co-migrated with the endogenous p21 as Ab-11 can recognize both of them. Ubi, indicates polyubiquitinated proteins.

As shown in Fig. 2B, UV-B also led to p21 decrease in a dose-dependent fashion to a lesser degree in comparison with UV-C, although p53 was induced. Taken together, these results, which were also repeated in other human cell lines tested regardless of the presence of p53 or not (Figs. 1 and 2 and Figs. 4A and 6A; data not shown), demonstrate that UV irradiation leads to a decrease of p21 protein in human cells in a dose-dependent fashion.

**FIGURE 5.** UV irradiation leads to p21 degradation independently of the E1-dependent ubiquitination system. A, ts20TGR MEF cells were incubated at 35°C (lanes 1 and 2) and 40°C (lane 3). MG132 (10 μM) was added for 6 h (lane 2). The same amounts of protein from harvested cell lysates were used for WB with antibodies indicated. B, ts20TGR MEF cells were incubated at 35°C (lanes 1–6) and 40°C (lanes 7–9). Cells were irradiated with different doses of UV-C as indicated, and MG132 (10 μM) was added at the time of UV-C treatment (lanes 4–6). Six hours after UV-C irradiation, harvested cell lysates were used for WB with antibodies indicated on the right.
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A. Vector (PS) + -- + -- Si-skp2 -- + -- + UV 40 J/m² -- + -- +

![Image of gel electrophoresis with bands labeled p21, tubulin, and skp2]

B. RT-PCR

![Image of RT-PCR gel with bands labeled skp2, p21, and β-Actin]

C. CHX (50 μg/ml) 0 15 35 105 180 p21

![Image of Western blot with bands labeled p21 and tubulin]

D. UV C 0 10 40 80 100 J/m² Mdm2 p53 p21 Tubulin β-Actin

![Image of Western blot with bands labeled Mdm2, p53, p21, and β-Actin]

**FIGURE 6.** A, UV-induced p21 proteasomal turnover is independent of Skp2. H1299 cells were transfected with 3 μg each of skp2 siRNA-encoding (Si-skp2) vector or a scrambled siRNA vector (PS). 48 h after transfection, cells were irradiated with UV-C at 40 J/m² and harvested 6 h post-irradiation. WB (75 μg of protein per lane) was conducted with antibodies as indicated. B, RT-PCR products of mouse skp2 in skp2+/+ and skp2−/− MEF cells, which were used for the experiments in C and D. C, p21 displays the same half-life in skp2+/+ and skp2−/− MEF cells. Cycloheximide (CHX, 50 μg/ml) was added to cultured cells and harvested at different time points. Equal amounts of protein from cell lysates were used for WB with the p21 antibody (AB6). The intensity of the bands was quantified using Adobe Photoshop and plotted on a graph. D, high dose of UV-C induce p21 degradation in skp2+/+ and skp2−/− MEF cells. Cells were treated with different doses of UV-C as indicated and harvested after 6 h. The same amounts of protein from cell lysates were used for WB with antibodies as indicated. The mRNA level of p21 was assessed by RT-PCR with β-actin as a control.

suggest that UV may lead to p21 degradation through the proteasomal, but not the caspase, pathway.

To confirm that UV indeed promotes p21 proteasomal degradation, we determined the effect of UV irradiation on the p21 half-life. As shown in Fig. 3C, the half-life of p21 was drastically reduced from ~90 min without UV irradiation to ~25 min after UV irradiation of RPE cells. To determine whether this UV-induced degradation of p21 is due to enhancement of p21 ubiquitination, we performed an in vivo p21 ubiquitination assay. p53 null H1299 cells were transfected with the plasmid encoding p21 or p53 alone or together with the His-ubiquitin expression vector. The transfected cells were irradiated with 30 J/m² UV-C and harvested 6 h afterward for in vivo ubiquitination and straight WB assays. As shown in Fig. 4, UV irradiation resulted in the reduction of both endogenous and exogenous p21 levels (A) but did not apparently affect the ubiquitination of p21 (compare lane 4 with lane 5 of Fig. 4B). This result is consistent with the previous report showing that endogenous p21 ubiquitination is not affected by UV-C irradiation (9). In contrast with this result, p53 ubiquitination was markedly reduced by UV, which was also as expected (9, 28, 29), largely due to the reduction of MDM2 levels (20) (Fig. 1). These results indicate that UV induces the proteasome-mediated p21 degradation, but not ubiquitination, in human cells.

To demonstrate that ubiquitination is not required for UV-induced p21 degradation, we employed an E1 temperature-sensitive ts20TGR MEF cell line (30). As shown in Fig. 5A, c-Myc was stabilized when ts20TGR cells were switched from 35 to 40 °C, indicating that E1 was inactivated, as c-Myc has been shown to be degraded through a ubiquitin-dependent proteasome pathway (31). As a control, c-Myc was also markedly stabilized by the proteasome inhibitor MG132 when the ts20TGR cells were switched from 35 to 40 °C (middle lane in Fig. 5A). Using this cell line, we then tested whether UV would induce p21 degradation when E1 is inactivated. Indeed, this was the case, as the p21 protein level was still drastically reduced by high doses of UV-C when the ts20TGR cell line was irradiated with 30 J/m² UV-C (compare the top left panel with the top right panel). The reason for that both inactivation of E1 by switching culture temperature and inhibition of the proteasome by MG132 failed to completely rescue the
level of MDM2 (top panels) was because the transcription of this mdm2 gene was inhibited by UV (20). These results indicate that UV-induced p21 turnover is proteasome-dependent but ubiquitination-independent.

UV-induced p21 Proteasomal Turnover Is Independent on Skp2—It was previously shown that p21 degradation induced by low doses of UV (5–30 J/m²) is dependent upon the F box protein Skp2 (14). Because here we showed that higher doses (>40 J/m²), instead of lower doses, of UV irradiation induced p21 degradation in all human cell lines and the ts20TG⁸ MEF cell line tested (Figs. 1–5 and data not shown), we wanted to determine whether Skp2 is also required for UV-triggered p21 degradation. To this end, H1299 cells were transfected with the vector encoding Skp2 siRNA or scrambled siRNA (23). At 40-h post-transfection, the transfected cells were irradiated with 40 J/m² UV-C and harvested at 6 h post-irradiation for WB. As shown in Fig. 6A, siRNA designed to deplete all the variants of Skp2 as described previously (23) remarkably reduced the expression of endogenous Skp2 in H1299 cells. As a result, one of the Skp2 targets, p27 (32–34), was induced (lower panel). Consistent with the results in Figs. 1–5, the high dose of UV irradiation caused drastic degradation of p21 in the cells. However, surprisingly, ~90% reduction of the Skp2 level by siRNA did not rescue the UV-induced p21 degradation in this cell line (middle panel of Fig. 6A). This result, which was also reproduced in 293 cells (data not shown), suggests that UV-induced p21 proteasomal turnover may not require the Skp2-dependent ubiquitination pathway.

To further verify this possibility, Skp2 null MEF cells (35), as also evident in Fig. 6B, were employed in the following experiments. First, we checked the half-life of p21 in both wild-type and skp2²⁻/⁻ MEF cells without UV irradiation to see if the status of Skp2 would affect the stability of p21. Surprisingly, endogenous p21 in both of the cell lines displayed a similar half-life of ~2 h (Fig. 6C). Next, both of the MEF cell lines were irradiated with different doses of UV-C and harvested for WB 6 h after irradiation. As shown in Fig. 6D, UV-C induced the decrease of MDM2 in both the wild-type and skp2 null MEF cells in a dose-dependent fashion (top panel), similar to what was seen in human cells (Fig. 1A). However, the p53 level was not changed (second top panel), suggesting that p53 was not functional in these cells probably due to the transformation process-mediated inactivation of p53 during the establishing the cell lines. Consistent with this result, the p21 mRNA level was not induced either by UV-C (bottom panel). However, the p21 protein level was clearly reduced by extremely high doses (80–100 J/m²) but not low doses (10–40 J/m²) of UV-C in both wild-type and skp null MEF cells (third top panel). This result, consistent with the result using Skp2 siRNA in Fig. 6A, demonstrates that Skp2 is not required for UV-induced proteasomal degradation of p21.

DISCUSSION

The cyclin-dependent kinase inhibitor p21 is regulated not only at the transcriptional level (36, 37) but also at the post-translational level in response to DNA damage. It is clear that one post-translational regulation of p21 is through the proteasomal system (7–11). However, two apparently conflicting models have been proposed to elucidate the mechanism underlying this regulation: one is ubiquitin-dependent (12–14) and the other is ubiquitin-independent (7, 15–17). Our study as described here is further consistent with the latter. Importantly, our study reveals some findings that are different from what has been shown previously (14). First, unlike the previous report, which showed the p21 degradation by lower doses, but not higher doses, of UV (14), we found that UV induced the decrease of p21 protein in a dose-dependent fashion, indicating that higher doses of UV lead to more efficient p21 degradation in all human and mouse cell lines tested (Figs. 1–7), although consistent with the previous study (14), UV indeed shortened the half-life of p21 (Fig. 3C). Also, different from that study showing that UV-induced p21 degradation occurred in both human and mouse cells (14), our data showed that human p21 is more sensitive to UV-induced degradation than mouse p21 (Figs. 1, 2, 6, and 7). Likewise, we observed that UV did not stimulate p21 ubiquitination (Fig. 4) and still induced its degradation when E1 was inactivated by shifting the culture temperature for the E1 ts20TG⁸ MEF cell line (Fig. 5), while it was previously shown that UV-induced p21 degradation is ubiquitin-dependent (14). Finally, unlike the

![Figure 7. High dose of UV-C decreases p21 in mouse cell lines.](image-url)

Mouse O3C, C2C12, B16, and F9 cells were irradiated with different doses of UV-C up to 100 J/m² and analyzed as described in the legend to Fig. 6D.

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previous report, UV-induced p21 decrease was not altered either by siRNA-directed ablation of Skp2 or in the skp2 null MEF cells (Fig. 6). These dissimilarities might be partially due to different p21 antibodies used by two independent laboratories. We obtained the identical results as shown in this study with three anti-p21 antibodies purchased from Oncogene Science (Ab-6 for mouse), Neomarker Biotech (Ab-11 for human), and Santa Cruz Biotechnology (H-164 for mouse), respectively. These antibodies detected a single polypeptide migrated between the 24- and 16-kDa markers (Figs. 1, 3, and 4). However, when using the polyclonal anti-p21 antibody C19 (Santa Cruz Biotechnology) that was one of that p21 antibodies used by the other group (14), for WB analyses, we detected multiple bands with a predominant one that almost co-migrated with a 16-kDa marker (supplemental Fig. S2). Thus, it remains possible that the C19 antibody-reacting band could be a cleaved form of p21. Taken together, our results demonstrate that UV induces p21 degradation in human and mouse cells independently of ubiquitin and Skp2 in a dose-dependent fashion. This is in agreement with the ubiquitin-independent model for p21 proteasomal turnover.

Our study is not only in line with a recent report showing that p21 is degraded through a Skp2-independent proteasomal pathway (16) but also suggests that this might be true in response to UV. Then, what cellular proteins may mediate p21 degradation in response to UV? We and others (15, 17) have previously shown that MDM2 can promote p21 degradation. However, it is unlikely that MDM2 may be a UV responsive regulator of p21 stability, because 1) MDM2 itself was reduced by UV irradiation (Fig. 1; Ref. 20), and 2) ablation of MDM2 levels by siRNA could not avert UV-elicted drastic p21 turn-over in human cells (data not shown). Thus, MDM2 may be mainly responsible for regulating the basal turnover of p21 or during the cell cycle but not for UV-induced p21 turnover.

ATR has been suggested to play a role in regulating p21 stability in response to UV irradiation (14). It appears true in some human cells, as caffeine at the concentration of inhibiting ATR activity could partially rescue UV-induced p21 degradation (Ref. 14; data not shown). The other possible candidates would be AKT1 (38–41) or p38 MAPK (42). However, these two kinases can be excluded because neither the AKT inhibitor Wortmannin nor the p38 MAPK inhibitor SB203580 prevented UV-triggered p21 degradation (data not shown). Therefore, it is conceivable that UV, especially at high doses, may activate a novel ATR-dependent or -independent mechanism that is potentially conserved in all of human cells and leads to desperate degradation of p21. Unraveling such a mechanism would be crucial for our better understanding the regulation of p21 stability in response to UV. Very recently, a protein called WISp39 was shown to associate with p21 and to stabilize it (43). Is it possible that UV might down-regulate the level of WISp39 and thus result in rapid turnover of p21?

Acknowledgments—We thank Mary MacPartlin for proofreading this manuscript and Rong Wen, Matt Thayer, Stephan Lanker, Mihail Iordanov, Molly Kulesz-Martin, and James Roberts for reagents.

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