Response to UV irradiation is important for a cell to maintain its genetic integrity when challenged by environmental genotoxins. An immediate early response to UV irradiation is the rapid induction of activating transcription factor 3 (ATF3) expression. Although emerging evidence has linked ATF3 to stress pathways regulated by the tumor suppressor p53 and the histone acetyltransferase Tip60, the role of ATF3 in the UV response remains largely unclear. Here, we report that ATF3 mediated dichotomous UV responses. Although UV irradiation enhanced the binding of ATF3 to Tip60, knockdown of ATF3 expression decreased Tip60 stability, thereby impairing Tip60 induction by UV irradiation. In line with the role of Tip60 in mediating UV-induced apoptosis, ATF3 promoted the death of p53-defective cells in response to UV irradiation. However, ATF3 could also activate p53 and promote p53-mediated DNA repair, mainly through altering histone modifications that could facilitate recruitment of DNA repair proteins (such as DDB2) to damaged DNA sites. As a result, ATF3 rather protected the p53 wild-type cells from UV-induced apoptosis. Our results thus indicate that ATF3 regulates cell fates upon UV irradiation in a p53-dependent manner.

The DNA damage response is essential for the maintenance of genetic integrity in the face of intrinsic and environmental genotoxins. In addition to γ irradiation (IR), which often induces DNA double strand breaks, UV irradiation represents another major genotoxic challenge that can cause bulky chemical modifications of single DNA strands, such as cyclobutane pyrimidine dimers (CPDs) and (6–4)-phophoproducts, and cross-link DNA. Mammalian cells mobilize a mechanism referred to as nucleotide excision repair (NER) whereby a repair complex composed of up to 30 proteins is assembled at damaged sites, unwinds, and excise DNA adducts from damaged strands. UV-induced DNA damage also provokes cellular signaling mediated by the sensor kinase ATM and Rad3-related (ATR)/ataxia telangiectasia mutated (ATM) and, notably, the tumor suppressor p53, leading to rapid induction of cell cycle arrest to allow repair of damaged DNA or apoptosis for the removal of irreparable cells. Intriguingly, although p53 induced by UV irradiation transactivates genes that can drive cell cycle arrest (e.g. p21) or apoptosis (e.g. Bax), it can also directly engage in NER by inducing the expression of genes (i.e. DDB2 and XPC) responsible for sensing and binding DNA adducts to prime the repair of cross-linked DNA. p53 can also regulate the helicase activity of transcription factor II H (TFIIH) and promote UV-induced histone H3 acetylation and global chromatin relaxation required for the access of damaged sites to NER proteins, thereby promoting UV damage repair independent of its transcriptional activity. Contrary to the general view that p53 is proapoptotic, p53 is often prosurvival in the UV response. Indeed, it has been shown that p53 protects cells from UV-induced apoptosis and that p53 induced by a small molecule, Nutlin-3a, can effectively block apoptosis induced by UV irradiation via a mechanism involving p21-mediated repression of BRCA1 expression.

The MYST histone acetyltransferase Tip60, or KAT5, is another important regulator of the cellular UV response. Although it can acetylate both histones and non-histone proteins to regulate gene expression, Tip60 is best known for its roles in regulating the cellular response to DNA double strand breaks. Tip60 not only senses double strand breaks but promotes damage repair through altering chromatin structure, increasing the deoxynucleoside triphosphate pool, and acetylating ATM for its activation. Tip60 also regulates the helicase activity of TFIIH and promotes UV-induced histone H3 acetylation and global chromatin relaxation required for the access of damaged sites to NER proteins, thereby promoting UV damage repair independent of its transcriptional activity. Contrary to the general view that p53 is proapoptotic, p53 is often prosurvival in the UV response. Indeed, it has been shown that p53 protects cells from UV-induced apoptosis and that p53 induced by a small molecule, Nutlin-3a, can effectively block apoptosis induced by UV irradiation via a mechanism involving p21-mediated repression of BRCA1 expression.

The MYST histone acetyltransferase Tip60, or KAT5, is another important regulator of the cellular UV response. Although it can acetylate both histones and non-histone proteins to regulate gene expression, Tip60 is best known for its roles in regulating the cellular response to DNA double strand breaks. Tip60 not only senses double strand breaks but promotes damage repair through altering chromatin structure, increasing the deoxynucleoside triphosphate pool, and acetylating ATM for its activation. Tip60 can also selectively promote the expression of proapoptotic genes (e.g. PUMA) by acetylating p53 at lysine 120 in response to genotoxic stresses, including UV irradiation. It thus comes as no surprise that Tip60 was shown to be indispensable for UV-induced apoptosis. However, recent evidence indicates that Tip60-mediated apoptosis upon UV irradiation does not require p53 but, rather, is involved in prosurvival signaling mediated by JNK. Notably, although Tip60 stability was
ATF3 and the UV Response

shown to be controlled by the E3 ubiquitin ligase MDM2 (16), how Tip60 is regulated during the UV response is poorly understood.

Previously, we reported that activating transcription factor 3 (ATF3) is a major Tip60 regulator that can bind Tip60 and promote Tip60-mediated activation of ATM signaling upon IR (17). ATF3 achieves this function partly through stabilizing Tip60 as a consequence of promoting its deubiquitination mediated by the deubiquitinase USP7 (17). ATF3 is a member of the ATF/cAMP response element-binding protein transcription factor family and can regulate gene expression through binding the consensus ATF/cAMP response element-binding protein cis-regulatory element via its basic region leucine zipper domain (18). ATF3 can also regulate cellular functions independent of its transcriptional activity. ATF3, for instance, can directly interact with key cancer-associated proteins (e.g. p53, E6, androgen receptor, and p63) and alter their interactions with DNA or other proteins (19–22). Although emerging evidence has linked ATF3 to several important human diseases, including cancer (23, 24), the exact biological function of ATF3 remains largely unknown and sometimes controversial (25). Because ATF3 can be rapidly induced by a wide range of cellular stresses, including DNA damage (26), it is often assumed that ATF3 is required for a cell to maintain homeostasis upon cellular stresses (18). Indeed, our findings that ATF3 can activate p53 by blocking MDM2-mediated ubiquitination while regulating Tip60 and ATM activation (17, 27) argue for the notion that ATF3 contributes to the maintenance of genetic stability in the face of genotoxic challenges. Because ATF3 is one of the few genes immediately induced by UV irradiation in various cell types (28, 29), ATF3 might also regulate the cellular response to UV-induced DNA damage. However, although ATF3 was shown to mediate UV-mediated cell death through transcriptional control (30), an early study also suggests that ATF3 induces p15AF expression required for eliminating UV-induced DNA adducts and thus protect cells from UV-induced damage (31). This apparent paradox warrants further investigations into the precise role ATF3 plays in the UV response.

Here we provide evidence demonstrating that ATF3 mediated the dichotomous cellular response to UV irradiation. Although ATF3 was found to regulate Tip60 and promote UV-induced death of p53-defective cells, this stress-responsive gene could rather protect p53 wild-type cells from UV-induced apoptosis by promoting p53-mediated DNA repair. ATF3 thus determined cell fates upon UV irradiation in a p53-dependent manner.

Experimental Procedures

Cell Culture—HCT116, U2OS, DU145, and PC3 cells were cultured in McCoy’s 5A medium (HCT116 cells), DMEM (U2OS and DU145 cells), and RPMI 1640 medium (PC3 cells) supplemented with 10% FBS, respectively, and routinely maintained in our laboratory. HCT116-F-Tip60 cells were genetically modified from HCT116 cells to express the endogenous Tip60 protein fused with a 3× FLAG tag (32). This modification allowed us to detect endogenous Tip60 using the well characterized FLAG antibody. To generate ATF3 knockout (ATF3−/−) cells, HCT116 cells were infected with adeno-assoc-

iat ed vir uses carrying a vector targeting exon 2 of the ATF3 gene. After removal of the targeting vector, a 22-bp deletion was generated within the exon.4

Knockdown by shRNA, siRNA, or Single Guided RNA—The pShIH-H1 shRNA cloning and lentivector expression system (System Biosciences) was used to knock down ATF3 and p53 expression in HCT116 and U2OS cells as described previously (20). The targeted sequences for ATF3 and p53 were 5′-GCCAAGTGCCGAAAACAAGA-3′ and 5′-GACTCCAGTTGTAATCTAC-3′, respectively. The Tip60 siRNA was synthesized on the basis of an earlier publication (13), and the targeted sequence was 5′-ACGGGAAGGTGGAGTGGTT-3′. To knock down ATF3 expression in PC3 and DU145 cells, a single guided RNA (sgRNA) (5′-AAAAATGATGCTTCAACCACCC-3′) targeting a region immediately downstream of the ATF3 start codon was co-expressed with hCas9. Clones with sgRNA-guided ATF3 knockdown were isolated as described previously (24).

Western Blotting, GST Pulldown, and Co-immunoprecipitation Assays—For Western blotting, cells irradiated with UV light were lysed in modified radioimmune precipitation assay buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 0.5% sodium deoxycholate, 1 mM EDTA, and protease inhibitor mixtures. Cytosolic, nucleoplasic, and chromatin-bound proteins were separated from UV-treated cells following a protocol described recently (33). Briefly, cells were suspended in low-salt buffer (10 mM HEPES (pH 7.4), 25 mM KCl, 10 mM NaCl, 1 mM MgCl2, 0.1 mM EDTA, 0.5% Nonidet P-40, and protease inhibitors) at 4 °C for 10 min to release cytosolic proteins. After centrifugation, pellets were suspended in high-salt buffer (50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 10 mM MgCl2, 300 mM KCl, and protease inhibitors) and immediately centrifuged at 10,000 rpm for 5 min to extract nucleoplasmic proteins. Pellets were further suspended in MNase buffer (10 mM HEPES (pH 7.9), 10 mM KCl, 1 mM CaCl2, 1.5 mM MgCl2, 0.35 mM sucrose, 10% glycerol, 0.1% Triton X-100, and 1 mM DTT) containing MNase (New England Biolabs) and incubated at 37 °C for 10 min before an equal volume of solubilization buffer (MNase buffer plus 2% Nonidet P-40, 2% Triton X-100, and 600 mM NaCl) was added to extract chromatin-bound proteins (33). For GST pulldown assays (27), GST or GST fusion proteins (1 μg) immobilized on 25 μl of glutathione-agarose (Sigma) were incubated with cell lysate containing equal amounts of Tip60 or ATF3 (adjusted on the basis of pre-run Western blotting results) at 4 °C overnight, followed by extensive washes. Bound proteins were eluted and detected by Western blotting. For the co-immunoprecipitation assay, cell lysates (1–2 mg) were incubated with 25 μl of anti-FLAG M2 affinity gel (Sigma) at 4 °C overnight. After extensive washes, precipitated proteins were detected by Western blotting. The antibodies were purchased from Santa Cruz Biotechnology (ATF3 (sc-188) and p53 DO-1 (sc-126)), Cell Signaling Technology (PARP (9542) and cleaved caspase 3 (9661)), Abcam (DDB2 (ab51017)), and Sigma (FLAG (F3165) and β-actin.

4 H. Cui, X. Li, C. Han, Q. E. Wang, H. Wang, H. F. Ding, J. Zhang, and C. Yan, unpublished data.
Partial ATFi3 and the UV Response

Quantitative RT-PCR (qRT-PCR)—Total RNA was extracted from cells using TRIzol (Invitrogen), reverse-transcribed using the RevertAid cDNA synthesis kit, and subjected to real-time PCR assays using SYBR Green reagents (Qiagen) essentially as described previously (35). The sequences of the primers were as follows: Tip60, 5′-GGGAGATAATCGAGGGCTG-3′ and 5′-TCCAGAGTTTGTGAATCAT-3′; p15ΔAF, 5′-ATGGTCCGACTAAGCAGAC-3′ and 5′-CCTCGATGAAC-TGATGTCAAT; DDB2, 5′-CTCTCATCAAAGGTTTGTGAATCAT-3′ and 5′-TTGAGGCGCTTCTCTTGGAGAAAGCAGAC-3′; and GADPH, 5′-CAGCCTCAAGATCATCAGCA-3′ and 5′-TTGTGTCGATGTCCTTCCTCAA-3′.

 Colony Formation Assays—For colony formation assays, 200 cells plated in 6-well plates were irradiated with UV light, and surviving colonies were stained with crystal violet 10 days later and counted as described previously (27).

CPD Quantitation—The cellular CPD level was measured using the OxiSelect UV-induced DNA damage ELISA kit (Cell Biolabs, STA-322) according to the protocol of the manufacturer. Briefly, genomic DNA was prepared using lysis buffer (10 mM Tris-HCl (pH 7.5), 10 mM EDTA, 10 mM NaCl, 0.5% SDS, and 0.5 mg/ml protease K) at 50 °C overnight, followed by phenol extraction (1:1) and ethanol precipitation. The DNA dissolved in Tris-EDTA (TE) buffer was then treated with 0.2 mg/ml RNase A at 37 °C for 2 h and purified by phenol extraction. 500 ng of DNA was then denatured and absorbed into the wells of a DNA high-binding plate for ELISA using an anti-CPD antibody. To ensure that equal amounts of DNA were used for ELISA, 50 ng of DNA was also subjected to real-time PCR using the primers 5′-CGCGAGGAGGAGCAACTG-3′ and 5′-AGGAGCTCACATCCCCATT-3′, which amplified a 63-bp region in the human genome (chr9:8091735–80912222, hg19).

Immunofluorescence Staining—This was carried out as described previously (33). Essentially, cells cultured on coverslips were washed with PBS, UV-irradiated at 40 J/m² through a polycarbonate filter containing 5-μm pores (Millipore), and then double-stained with a mouse anti-DDB2 (1:50) (ab51017, Abcam) and a rabbit anti-CPD antibody. Fluorescence images were obtained with a Nikon E801 fluorescence microscope and processed with SPOT software (Diagnostic Instruments).

Transfections and Reporter Assays—Transfections were carried out using Lipofectamine 2000 (Invitrogen) according to the protocol of the manufacturer. pCMV-Luc was constructed by inserting a CMV promoter into pGL3 (35) and irradiated with 500 J/m² of UV light before transfections. Cells in 24-well plates were co-transfected with 100 ng of UV-damaged or intact pCMV-Luc and 5 ng of pRL-CMV. 48 h later, cells were lysed for Dual-Luciferase activity assays (Promega).

Results

ATF3 Stabilizes Tip60 in the UV Response—We previously reported that ATF3 can stabilize Tip60 and activate ATM in response to IR (17). Given that ATF3 can be rapidly induced by UV irradiation (28, 29), we sought to determine whether ATF3 also regulates Tip60 in the UV response. We thus knocked down ATFi3 expression by shRNA or sgRNA in three genetically diversified cancer cell lines (U2OS, PC3, and genetically modified HCT116 cells), treated the cells with UV light (20 J/m²), and measured the Tip60 expression level by Western blotting. UV irradiation elevated the Tip60 protein level at early time points (4 and 8 h), but the Tip60 expression level was decreased to the basal level 24 h after UV irradiation (Fig. 1, A–C). Importantly, not only the basal Tip60 level but also the UV-induced increase in Tip60 expression was decreased in all three cell lines when ATFi3 expression was knocked down (Fig. 1, A–C). We also measured the Tip60 mRNA level by qRT-PCR. Although UV irradiation rather repressed Tip60 transcription at 4 and 8 h after irradiation, ATFi3 knockdown did not alter Tip60 transcription in both quiescent and UV-treated cells (Fig. 1D). These results suggest that UV irradiation likely triggered an ATFi3-dependent mechanism that could increase Tip60 protein stability as an early response to UV irradiation. Indeed, we found that Tip60 stability was decreased in UV irradiation-treated ATFi3 knockdown cells, as measured by cycloheximide chase experiments (Fig. 1E). These results indicate that ATFi3 can stabilize Tip60 in response to UV radiation.

Interestingly, although it has been shown that ATFi3 can stabilize Tip60 by binding the latter protein and promoting its deubiquitination (17), we found that UV irradiation could enhance the ATFi3-Tip60 interaction. Thus, when cell lysates containing similar amounts of Tip60 were incubated with immobilized GST-ATFi3, the amount of Tip60 pulled down by GST-ATFi3 from UV-treated samples was much more than that from quiescent cells (Fig. 1F, lane 6 versus lane 5). This effect was also apparent 8 h after UV irradiation but appeared to be diminished 24 h after irradiation (Fig. 1F). Co-immunoprecipitation assays confirmed that more ATFi3 bound by Tip60 in UV-irradiated cells (Fig. 1G, lane 2 versus lane 1). However, immobilized GST-ATFi3 failed to pull down more ATFi3 from lysates of UV-treated cells (data not shown), suggesting that it was UV-induced modification of Tip60, but not of ATFi3, that led to the increase in ATFi3-Tip60 binding affinity.

Knockdown of ATFi3 Expression Impairs UV-mediated Apoptosis in a Tip60-dependent Manner—Because it has been shown that Tip60 can mediate apoptosis in the UV response (9, 15), we determined whether the regulation of Tip60 by ATFi3 contributes to UV-mediated cell death. Consistent with impaired Tip60 induction, UV-induced cleavage of PARP and caspase 3, two well established apoptosis markers, was significantly inhibited in ATFi3 knockdown PC3 cells that were null for p53 (Fig. 2A). Similar results were also obtained with DU145 cells harboring a p53 mutation (Fig. 2B). Consistent with these results, ATFi3-down-regulated PC3 cells were resistant to UV-induced cell death, as determined by colony formation assays (Fig. 2C). Interestingly, although Tip60 siRNA (siTip60) impaired UV-induced apoptosis as expected, ATFi3 knockdown was less effective in suppressing apoptosis in siTip60-expressing cells (Fig. 2D, lane 8 versus lane 4). These results argue for the notion that ATFi3 promoted UV-mediated apoptosis by regulating Tip60.
Knockdown of ATF3 Enhances UV-mediated Apoptosis in HCT116 and U2OS Cells—To our surprise, UV irradiation induced more cell death in ATF3-down-regulated U2OS and HCT116 cells, as evidenced by higher levels of UV-induced cleaved PAPR and caspase 3 in shATF3-expressing cells (Fig. 3, A and B). Consistent with these observations, shATF3-expressing U2OS cells were rather sensitive to UV-induced cell death (Fig. 3, C). Importantly, although siTip60 retained its capability to impair UV-mediated apoptosis in HCT116 cells (Fig. 3, D), shATF3 expression could efficiently enhance apoptosis in Tip60 knockdown cells (Fig. 3, E), suggesting that these unexpected apoptosis-promoting effects were likely independent of Tip60.

ATF3-mediated Suppression of UV Irradiation-induced Apoptosis Is Dependent on p53—In addition to Tip60, ATF3 can increase p53 stability in response to genotoxic stresses (27). Indeed, the p53 level was significantly lower in shATF3-expressing U2OS cells upon UV treatment (Fig. 3, F). Because HCT116 and U2OS cells differ from PC3 and DU145 cells in that they carry wild-type p53, we tested the possibility that the suppression of UV-mediated death of HCT116 and U2OS cells by ATF3 was a consequence of ATF3-mediated p53 activation. Although counterinstinctual, both in vitro and in vivo evidence demonstrated that p53 activation can protect cells from UV-mediated apoptosis (7–9). Employing isogenic p53 wild-type and p53-null (p53−/−) HCT116 cells, we confirmed that p53 deficiency promoted apoptosis induced by UV light (Fig. 4, A). Similarly, knockdown of p53 expression by shRNA in U2OS cells also increased the amounts of cleaved PARP and caspase 3 (Fig. 4, B). To test our hypothesis, we generated isogenic HCT116 cells either null for ATF3 (ATF3−/−) or expressing shATF3 in the p53-null background (p53−/−;shATF3) and treated them, along with wild-type and p53−/− HCT116 cells, with UV light. Knockout of ATF3 impaired UV-mediated p53 activation in HCT116 cells as expected (Fig. 4, C).
Importantly, although UV-mediated apoptosis appeared to be more profound in ATF3-deficient, p53 wild-type cells (Fig. 4C, lanes 5 and 6 versus lanes 2 and 3), defective ATF3 expression concurrently suppressed apoptosis induced by UV light in p53-null cells (Fig. 4C, lanes 11 and 12 versus lanes 8 and 9). Colony formation assays confirmed that the ATF3 defect sensitized p53 wild-type cells to, but prevented p53-deficient cells from, UV-mediated cell death (Fig. 4D and E). Similar effects were observed when isogenic cells were treated with higher dosages of UV radiation (Fig. 4F). These results thus demonstrated that ATF3 can mediate dichotomous UV responses in a p53-dependent manner.
ATF3 Promotes p53-mediated DNA Repair—p53-mediated protection of UV-induced cell death is attributable to its ability to promote DNA damage repair (Fig. 5A) (6). To understand the mechanism by which ATF3 protected p53 wild-type cells from UV-mediated death, we tested whether ATF3 promotes p53-mediated DNA repair. Because CPDs are major DNA adducts induced by UV light, we measured the CPD level in quiescent and UV-treated U2OS cells by ELISA. As expected, UV irradiation induced an increase in the CPD level that was largely eliminated because of DNA repair 4 and 24 h after irradiation in U2OS cells (Fig. 5B). Intriguingly, significantly higher levels of CPDs were detected in ATF3 knockdown cells than in shLuc cells 4 and 24 h after UV irradiation (Fig. 5B). Of note, qPCR results confirmed that the samples subjected to ELISA contained an equal amount of DNA (Fig. 5C). Moreover, it is unlikely that the observed difference between shATF3 and shLuc cells was due to off-target effects of shATF3 because we obtained similar results in U2OS cells engineered to knock out ATF3 expression with the CRISPR/Cas9 system (Fig. 5D, U2OS-KO). Furthermore, ATF3 knockout HCT116 cells (HCT116-KO) engineered with a different genome-editing strategy, i.e., adeno-associated virus-mediated homologous recombination, also maintained higher CPD levels after UV irradiation (Fig. 5E). These results thus indicate that ATF3 could promote DNA repair in response to UV irradiation.

To confirm that ATF3 is involved in repairing UV-damaged DNA, we transfected cells with a firefly luciferase reporter construct (pCMV-Luc) preirradiated with 500 J/m² of UV light and measured the relative luciferase activity 2 days after transfection. Consistent with the CPD ELISA results, the level of luciferase expressed from the UV-damaged DNA was significantly lower in ATF3 knockdown cells than in control U2OS cells (Fig. 5F). Such a decrease was not due to direct repression of reporter expression by the transcription factor ATF3 because the cells transfected with an intact, undamaged construct expressed luciferase at the same level (Fig. 5F). We also carried out similar experiments using HCT116 isogenic cells (Fig. 5G). Although p53 deficiency resulted in impaired DNA repair as expected, the ATF3-null cells again repaired UV-damaged DNA less efficiently (Fig. 5G, ATF3—/— versus WT). Interestingly, ATF3 deficiency did not lead to a further decrease in DNA repair efficiency in p53-null cells (Fig. 5G, comparing ATF3—/—; p53—/— with p53—/—).
These results are in line with the notion that ATF3 can promote p53-mediated DNA repair in the UV response.

**ATF3 Promotes p53-mediated H3 Acetylation for DNA Repair**—It was reported previously that ATF3 can promote DNA repair by inducing p15PAF expression upon UV irradiation (31). However, p15PAF expression was only marginally induced by UV light in both p53 wild-type and p53-null HCT116 cells (Fig. 7A). Because p53 can transactivate genes involved in NER (e.g., DDB2, XPC, and p21) (Fig. 6A), we determined the effects of ATF3 on p53 target gene expression for an understanding of how ATF3 promoted p53-mediated DNA repair. Although it was shown that expression of DDB2 and XPC is UV-inducible (2, 3), we found that UV light only slightly and transiently induced DDB2 and XPC expression in HCT116 cells (Fig. 6B). However, p21 expression was strongly induced by UV light (Fig. 6C), indicating that UV-induced p53 was functional in these cells. Consistent with decreased p53 expression (Fig. 6E, lanes 5 and 6 versus lanes 2 and 3), ATF3 deficiency caused a slight but significant decrease in DDB2 and XPC expression (Fig. 7B) and a more profound decrease in p21 expression upon UV irradiation (Fig. 7C). However, it was unlikely that these transcriptional changes were the main mechanism by which ATF3 promoted DNA repair because knockout of ATF3 expression did not significantly alter the total DDB2 protein level (Fig. 6D), whereas UV irradiation caused p21 degradation (Fig. 6E). Indeed, it has been demonstrated that p21 degradation is required for efficient DNA repair in response to UV irradiation (36).

Because p53 also contributes to DNA repair by inducing H3 acetylation, which can cause an increase in the global DNA accessibility to NER proteins (Fig. 6A), we tested whether ATF3 affects this transcription-independent event. As expected, UV irradiation caused a dramatic increase in the global H3 acetylation level that was largely impaired in p53-null cells (Fig. 6F, lanes 8 and 9 versus lanes 2 and 3). Importantly, ATF3 deficiency almost completely abolished UV-induced H3 acetylation (Fig. 7E, lanes 5 and 6 versus lanes 2 and 3). In line with the notion that these chromatin changes could lead to impaired recruitment of NER proteins to damaged DNA sites, we found that the UV-induced increase of DDB2 binding to the chromatin (Fig. 6G, lane 6 versus lane 3) (33) was largely abolished in ATF3-deficient cells (Fig. 6G, lane 12 versus lane 9). Moreover,
the amount of DDB2 recruited to CPD foci caused by UV microirradiation was largely decreased in a majority of ATF3-deficient U2OS cells and HCT116 cells (Fig. 6G), indicating that ATF3 deficiency indeed impeded the recruitment of DDB2 to damaged DNA sites. Therefore, our results strongly suggest that ATF3 can facilitate p53-mediated DNA repair by promoting UV-induced H3 acetylation and a subsequent increase of accessibility of damaged DNA to NER proteins.

Discussion

The common stress-responsive transcription factor ATF3 is one of the few immediate early genes induced by UV irradiation...
FIGURE 7. A model whereby ATF3 mediates dichotomous UV response. In response to UV radiation, ATF3 can bind and stabilize Tip60 to promote cell death while promoting p53-mediated DNA repair to evade apoptosis upon UV radiation. The outcome depends on whether p53 is functional in the cells. ATF3 protects p53 wild-type cells from UV-induced death but promotes apoptosis in cells defective in p53.

(28, 29), but its role in the UV response remains largely unknown. Two earlier studies report seemingly conflicting results, i.e. ATF3 mediates UV-induced apoptosis while promoting DNA repair upon UV irradiation in the same cells (30, 31). They also report that ATF3 achieves these different functions through transactivating Hif-2α and p15PAF expression, respectively (30, 31). However, neither Hif-2α (data not shown) nor p15PAF expression (Fig. 6A) were noticeably induced by UV light in our experimental settings. Rather, we found in this study that ATF3 mediated dichotomous UV responses in a manner independent of its transcriptional activity but dependent on cellular contexts. In this regard, ATF3 could bind and stabilize Tip60 to promote cell death while promoting p53-mediated DNA repair to evade apoptosis upon UV irradiation. Because the latter effect can outcompete Tip60-mediated apoptosis in cells defective in p53 after UV irradiation (Fig. 7). It is important to note that ATF3 mediated dichotomous UV responses in isogenic cell lines differing only in p53/ATF3 status, and thus these different responses were not likely caused by the difference in other genetic contexts. Interestingly, the early report indicating that ATF3 is proapoptotic in the UV response employed cells either carrying a mutant p53 gene (i.e. HaCaT) or expressing inactivated p53 protein (i.e. HeLa cells) (30). Our results thus provide evidence arguing for the notion that p53 functionality dictates the role of ATF3 that plays in the UV response. As therapeutic agents (e.g. cisplatin) often induce ATF3 expression and cause DNA damage, our findings also suggest that ATF3 might mediate different cellular responses resulting in either sensitization of, or resistance to, therapies in cancer cells with different p53 mutation status. Indeed, although ATF3 is often regarded as a proapoptotic molecule (22), it was also shown to suppress apoptosis induced by cisplatin in T98G glioblastoma cells (37). Although the p53 status in T98G cells remains controversial, our results support targeting ATF3 as an effective strategy for treating p53-mutated cancers (22).

Although Tip60 is required for UV-induced apoptosis (9, 15), how this histone acetyltransferase is regulated in the UV response remains unclear. Previously, we found that ATF3 is a major Tip60 regulator and required for the Tip60-mediated cellular response to double strand breaks (17). Here we show that ATF3 could also bind and stabilize Tip60 in the UV response. Interestingly, although IR does not affect the ATF3-Tip60 interaction, UV enhanced the binding of Tip60 to ATF3 (Fig. 1, F and G). Such an increase in ATF3-Tip60 binding appeared to be important for cells to sustain and increase the Tip60 protein level in the early periods when UV-induced DNA damage dramatically inhibited Tip60 transcription (Fig. 1D). Indeed, we previously showed that the binding of ATF3 to Tip60 can promote the removal of ubiquitin chains by the deubiquitinase USP7, thereby preventing Tip60 from proteasomal degradation (17). Because ATF3 can interact with MDM2 (38) and the latter E3 ubiquitin ligase was suggested to be involved in UV-induced Tip60 expression (16), there is also a possibility that ATF3 stabilized Tip60 by regulating MDM2 in the UV response. However, we did not find evidence that MDM2 could mediate the degradation of Tip60 (data not shown). On the other hand, our results suggest that UV irradiation might cause posttranslational modifications of Tip60, thereby altering its conformation in a way that favors its interaction with ATF3. Although it remains elusive whether UV can indeed induce Tip60 posttranslational modifications, it was recently shown that IR can induce Tip60 phosphorylation to promote its binding to methylated histones (39).

The results that ATF3 knockdown promotes UV-induced apoptosis in HCT116 and U2OS cells (Fig. 4, A and B) came initially as a surprise because Tip60 expression was significantly suppressed in these cells (Fig. 1, A and B). Although our results confirmed the previous observations that ATF3 can activate p53 in response to UV irradiation (Figs. 4C and 6F) (27), it is counterintuitive that p53, the widely regarded proapoptotic molecule, protects cells from UV-induced apoptosis (7–9). However, the roles of p53 in promoting NER for the repair of UV-damaged DNA have been well established (6). Although UV irradiation only slightly/modestly induced DDB2 and XPC expression in our experiments, it dramatically induced histone H3 acetylation to promote access of damaged DNA sites to NER proteins in a p53-dependent manner (5) (Fig. 6F). Accordingly, ATF3 appeared to promote DNA repair mainly through regulating p53-mediated H3 acetylation. Indeed, ATF3 did not appear to promote DNA repair in p53-null HCT116 cells (Fig. 5G) but could facilitate the recruitment of DDB2 to CPD foci (Fig. 6H). Because p53 serves as a chromatin accessibility factor.
ATF3 and the UV Response

by recruiting the histone acetyltransferase p300 to the damaged sites (5), ATF3 might regulate this p53-dependent function by promoting the interaction between p53 and p300. In support of this notion, we previously showed that p300 mediated acetylation of p53 was increased in ATF3-expressing cells (27).

Author Contributions—H. C. performed the experiments shown in Figs. 1–4. X. L. performed the experiments shown in Figs. 5 and 6 except for those shown in Fig. 6H, which were carried out by C. H. Figs. 1–4. X. L. performed the experiments shown in Figs. 5 and 6 for the HCT116 p53-null and F-Tip60 cells and Dr. Bruno Amati for the Tip60 antibody.

Acknowledgments—We thank Drs. Bert Vogelstein and Zhenghe Wang for the HCT116 p53-null and F-Tip60 cells and Dr. Bruno Amati for the Tip60 antibody.

References

1. Latonen, L., and Laiho, M. (2005) Cellular UV damage responses: functions of tumor suppressor p53. Biochim. Biophys. Acta 1755, 71–89
2. Hwang, B. J., Ford, J. M., Hanawalt, P. C., and Chu, G. (1999) Expression of the p48 xeroderma pigmentosum gene is p53-dependent and is involved in global genomic repair. Proc. Natl. Acad. Sci. U.S.A. 96, 424–428
3. Adimoolam, S., and Ford, J. M. (2002) p53 and DNA damage-inducible expression of the xeroderma pigmentosum group C gene. Proc. Natl. Acad. Sci. U.S.A. 99, 12985–12990
4. Wang, X. W., Yeh, H., Schaeffer, L., Roy, R., Moncollin, V., Egly, J.-M., Wang, Z., Freidberg, E. C., Evans, M. K., Taffe, B. G., Bohr, V. A., Weeda, G., Hoeijmakers, J. H. J., Forrester, K., and Harris, C. C. (1995) p53 modulation of TFIIF-associated nucleotide excision repair activity. Nat. Genet. 10, 188–195
5. Rubbi, C. P., and Milner, J. (2003) p53 is a chromatin accessibility factor for nucleotide excision repair of DNA damage. EMBO J. 22, 975–986
6. Sengupta, S., and Harris, C. C. (2005) p53: Traffic cop at the crossroads of the molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor 3 activates p53 by preventing E6-associated protein from binding to the histone acetyltransferase Tip60.

16. Legube, G., Linares, L. K., Lemencier, C., Scheffner, M., Khochbin, S., and Trouche, D. (2002) Tip60 is targeted to proteasome-mediated degradation by Mdm2 and accumulates after UV irradiation. EMBO J. 21, 1704–1712
17. Cui, H., Guo, M., Xu, D., Ding, Z.-C., Zhou, G., Ding, H.-F., Zhang, J., Tang, Y., and Yan, C. (2015) The stress-responsive gene ATF3 regulates the histone acetyltransferase Tip60. Nat. Commun. 6, 6752
18. Yan, C., and Boyd, D. D. (2006) ATF3 regulates the stability of p53: a link to cancer. Cell Cycle 5, 926–929
19. Stelzl, U., Worm, U., Lalowski, M., Haenig, C., Brembeck, F. H., Goehler, H., Strodieck, M., Zenkner, M., Schoenherr, A., Koppenn, S., Timp, J., Mintzlaff, S., Abraham, C., Bock, N., Kietzmann, S., Goede, A., Toksöz, E., Droege, A., Krobitsch, S., Korn, B., Birchmeier, W., Lehra, H., and Wanker, E. E. (2005) A human protein-protein interaction network: A resource for annotating the proteome. Cell 122, 957–968
20. Wang, H., Mo, P., Ren, S., and Yan, C. (2010) Activating transcription factor 3 activates p53 by preventing E6-associated protein from binding to E6. J. Biol. Chem. 285, 13201–13210
21. Wang, H., Jiang, M., Cui, H., Chen, M., Buttyan, R., Hayward, S. W., Hai, T., Wang, Z., and Yan, C. (2012) The stress response mediator ATF3 represses androgen signaling by binding the androgen receptor. Mol. Cell Biol. 32, 3190–3202
22. Wei, S., Wang, H., Lu, C., Malmut, S., Zhang, J., Ren, S., Yu, G., Wang, W., Tang, D. D., and Yan, C. (2014) The activating transcription factor 3 protein suppresses the oncogenic function of mutant p53 proteins. J. Biol. Chem. 289, 8947–8959
23. Yuan, X., Yu, L., Li, J., Xie, G., Rong, T., Zhang, L., Chen, J., Meng, Q., Irving, A. T., Wang, D., Williams, E. D., Liu, J. P., Sadler, A. J., Williams, B. R., Shen, L., and Xu, D. (2013) ATF3 suppresses metastasis of bladder cancer by regulating gelsolin-mediated remodeling of the actin cytoskeleton. Cancer Res. 73, 3625–3637
24. Wang, Z., Xu, D., Ding, H.-F., Kim, J., Zhang, L., Hai, T., and Yan, C. (2015) Loss of ATF3 promotes Akt activation and prostate cancer development in a Pten knockout mouse model. Oncogene 34, 4975–4984
25. Hai, T., Woldford, C. C., and Chang, Y.-S. (2010) ATF3, a hub of the cellular adaptive-response network, in the pathogenesis of diseases: is modulation of inflammation a unifying component? Gene Expr. 15, 1–11
26. Hai, T., and Hartman, M. G. (2001) The molecular biology and nomenclature of the activating transcription factor/cAMP responsive element binding family of transcription factors: activating transcription factor protein and homeostasis. Gene 273, 1–11
27. Yan, C., Lu, D., Hai, T., and Boyd, D. D. (2005) Activating transcription factor 3, a stress sensor, activates p53 by blocking its ubiquitination. EMBO J. 24, 2425–2435
28. Amundson, S. A., Birttner, M., Chen, Y., Trent, J., Meltzer, P., and Fornace, A. J. (1999) Fluorescent cDNA microarray hybridization reveals complexity and heterogeneity of cellular genotoxic stress responses. Oncogene 18, 3666–3672
29. Abe, T., Oue, N., Yasui, W., and Ryoji, M. (2003) Rapid and preferential induction of ATF3 transcription in response to low doses of UVA light. Biochem. Biophys. Res. Com. 310, 1168–1174
30. Turchi, L., Aberdam, E., Mazure, N., Pouyssegur, J., Deckert, M., Kitajima, S., Aberdam, D., and Virolle, T. (2008) ATF3 and p15PAF are novel gatekeepers of genomic integrity upon UV stress. Cell Death Differ. 15, 1472–1480
31. Turchi, L., Fareh, M., Aberdam, E., Kitajima, S., Simpson, F., Wicking, C., Aberdam, D., and Virolle, T. (2009) ATF3 and p15PAF are novel gatekeepers of genomic integrity upon UV stress. Cell Death Differ. 16, 728–737
32. Du, Z., Song, J., Wang, Y., Zhao, Y., Guda, K., Yang, S., Kao, H.-Y., Xu, Y., Willis, J., Markowitz, S. D., Sedwick, D., Ewing, R. M., and Wang, Z. (2010) DNMT1 stability is regulated by proteins coordinating deubiquitination and acetylation-driven ubiquitination. Sci. Signal. 3, ra80
33. Wang, Q.-E., Han, C., Zhao, R., Wani, G., Zhu, Q., Gong, L., Battu, A., Raoma, I., Sharma, N., Wani, A.A. (2013) p38 MAPK- and Akt-mediated p300 phosphorylation regulates its degradation to facilitate nucleotide excision repair. Nucleic Acids Res. 41, 1722–1733
34. Gorrini, C., Squatrito, M., Luise, C., Syed, N., Perna, D., Wark, L., Martino, F., Sardella, D., Verrecchia, A., Bennett, S., Confolanieri, S., Cfasaroni, M., Marchesi, F., Gasco, M., Scanziani, E., Capra, M., Mai, S., Nuñoforo, P.,
Crook, T., Lough, J., and Amati, B. (2007) Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response. Nature 448, 1063–1067
35. Yan, C., and Boyd, D. D. (2006) Histone H3 acetylation and H3 K4 methylation define distinct chromatin regions permissive for transgene expression. Mol. Cell Biol. 26, 6357–6371
36. Bendjennat, M., Boulaire, J., Jascur, T., Brickner, H., Barbier, V., Sarasin, A., Fotedar, A., and Fotedar, R. (2003) UV irradiation triggers ubiquitin-dependent degradation of p21WAF1 to promote DNA repair. Cell 114, 599–610
37. Hamdi, M., Popeijus, H. E., Carlotti, F., Janssen, J. M., van der Burgt, C., Cornelissen-Steigler, P., van de Water, B., Hoeben, R. C., Matsuo, K., and van Dam, H. (2008) ATF3 and Fra1 have opposite functions in JNK- and ERK-dependent DNA damage response. DNA Repair 7, 487–496
38. Mo, P., Wang, H., Lu, H., Boyd, D. D., and Yan, C. (2010) MDM2 mediates ubiquitination and degradation of activating transcription factor 3. J. Biol. Chem. 285, 26908–26915
39. Kaidi, A., and Jackson, S. P. (2013) KAT5 tyrosine phosphorylation couples chromatin sensing to ATM signalling. Nature 498, 70–74