ERKs and p38 Kinase Phosphorylate p53 Protein at Serine 15 in Response to UV Radiation*

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Running Title: UV-induced Phosphorylation of p53 by ERKs and p38 Kinase

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Phosphorylation of the p53 tumor suppressor protein is likely to play an important role in regulating its activity. Serine 15 phosphorylation of p53 leads to a stabilization of p53 by reducing its interaction with MDM2, a negative regulatory partner. Recently, p53 was reported to be activated and phosphorylated at serine 15 following UV radiation. However, the signaling pathway that mediates UV-induced phosphorylation is less well characterized. Here, we provide evidence that UVB-induced phosphorylation of p53 at serine 15 is mediated directly by ERKs and p38 kinase. We find that in a mouse JB6 epidermal cell line, ERKs and p38 kinase form a complex with p53 following UVB radiation. Inhibition of ERKs or p38 kinase activity by the use of a dominant negative mutant of ERK2 or p38 kinase or their respective specific inhibitor, PD98059 or SB202190, results in abrogation of UVB-induced phosphorylation of p53 at serine 15. Strikingly, incubation of UVB-activated ERKs or p38 kinase immunoprecipitated complex with exogenous p53 shows serine 15 phosphorylation of both exogenous and co-precipitated endogenous p53 protein. Additionally, active recombinant ERK1/2 and p38 kinase but not JNKs are also able to phosphorylate p53 at serine 15 in vitro. Furthermore, pretreatment of cells with PD98059 or SB202190 blocks p53-dependent transcription activity but increases the level of p53 co-precipitated MDM2. These results strongly suggest that both ERKs and p38 kinase have a direct role in UVB-induced phosphorylation of p53 at serine 15 in vivo.
The p53 tumor suppressor protein is a potent transcription factor that is activated in response to signals arising from DNA-damaging agents including ultraviolet (UV) radiation (1-4). Following activation, p53 coordinates a change in the balance of gene expression leading to growth arrest or apoptosis, which is thought to prevent the proliferation of genetically damaged cells (5, 6). The regulation of p53 activity is through multiple mechanisms, one of which is phosphorylation (7, 8). p53 is phosphorylated at multiple sites in vivo and by several different protein kinases in vitro (7). Many p53 phosphorylation sites are clustered in the N-terminal activation domain (amino acids 1-42), whereas the others are more dispersed in the C-terminal region of the molecule (7). Recent studies have shown that phosphorylation of p53 protein at serine 15 may play a critical role in the stabilization, up-regulation and functional activation of p53 during cellular stress (3, 4, 9-11). The phosphorylation of p53 at serine 15 in vitro by DNA-dependent protein kinase (DNA-PK) leads to reduced binding to its negative regulator, the oncoprotein MDM2 (3). However, cells with defective DNA-PK still accumulate p53 protein normally and undergo G1 arrest in response to DNA damage (12, 13). Two recent reports demonstrated that ATM (ataxia telangiectasia-mutated), which encodes a 370-kD protein with a C-terminal domain similar to the catalytic subunit of phosphoinositide 3-kinase, mediates the phosphorylation of p53 at serine 15 in response to γ-radiation (14) and radiomimetic agents (15). However, the kinase activity of ATM is not increased in UV-radiated normal cells (14). On the other hand, in cell lines derived from AT patients, who bear loss-of-function mutations in ATM, p53 induction (16) and serine 15 phosphorylation (4, 14) are observed after UV radiation. These results suggest that other cellular kinases must also phosphorylate p53 at serine 15 in vivo in response to UV.
One of the major UV responsive pathways is the *ras/mitogen-activated protein (MAP)* kinases cascade (17). In mammalian cells, this pathway has three distinct components: extracellular-signal-regulated protein kinases (ERKs), p38 kinase, and the stress-activated c-Jun N-terminal kinases (JNKs). These kinases participate in the regulation of cell proliferation, differentiation, stress responses and apoptosis (18-24). The activation of MAP kinases may be by translocation to the nucleus, where these kinases phosphorylate target transcription factors (25, 26). Previously, we and others found that exposure of cells to UV radiation causes activation of MAP kinases and a subsequent increase in the activity of transcription factors such as AP-1 (27-30) and p53 (31). These data led us to test whether MAP kinases might interact with p53 and whether p53 may be a target for phosphorylation at serine 15 by MAP kinases. Here, we demonstrate by immunoprecipitation of UVB-treated cells that ERKs or p38 kinase binds to p53 *in vivo*. Using a phospho-specific antibody against p53 at serine 15 (11), a dominant negative mutant of ERK2 or p38 kinase, and the MEK1 or p38 kinase inhibitor, PD98059 or SB202190 respectively, we provide evidence that in a mouse JB6 epidermal cell line, UVB-induced p53 phosphorylation at serine 15 (numbering according to Soussi *et al.* (32)) is directly mediated by ERKs and p38 kinase both *in vitro* and *in vivo*. We conclude that in response to UV radiation, ERKs or p38 kinase plays an important role in regulating p53 activity through phosphorylation of serine 15.
EXPERIMENTAL PROCEDURES

Plasmids and Reagents—CMV-neo vector plasmid and p53 luciferase reporter plasmid (PG13-Luc) were constructed as reported previously (33, 34); dominant negative mutants of ERK2, p38 kinase and JNK1 were generous gifts from Dr. Melanie H. Cobb (35), Dr. Mercedes Rincon (36, 37) and Dr. Roger J. Davis (38, 39), respectively; active recombinant ERK1, p38 kinase and JNK2 were from Upstate Biotechnology; active recombinant ERK2, Elk-1, ATF-2 and e-Jun fusion proteins, phospho-specific p53 (Ser\textsuperscript{15}) antibody, phospho-specific Elk-1 (Ser\textsuperscript{383}), ATF-2 (Thr\textsuperscript{71}) and e-Jun (Ser\textsuperscript{63}) antibodies, PhosphoPlus p44/42 MAP kinase, p38 kinase and JNK antibody kits, p44/42 MAP kinase, p38 kinase and JNK assay kits were purchased from New England Biolabs; monoclonal mouse IgG against p53 (Ab-1) antibody were from Oncogene Research Products; p53 fusion protein, polyclonal rabbit IgG against p53 and MDM2 antibodies, monoclonal mouse IgG against ERK2 and p38 kinase antibodies were from Santa Cruz Biotechnology; MEK1 specific inhibitor, PD98059, was from Biomol; p38 kinase inhibitor, SB202190, was from Calbiochem; Eagle's minimal essential medium (MEM) was from Life Technologies, Inc.; fetal bovine serum (FBS) was from Biowhittaker; and luciferase substrate was from Promega.

Cell Culture—JB6 mouse epidermal cell line Cl 41 and its stable transfectants, Cl 41 CMV-neo, Cl 41 DN-ERK2 B\textsubscript{3} mass\textsubscript{1}, Cl 41 DN-p38 G7, Cl 41 DN-JNK1 mass\textsubscript{1}, and Cl 41 p53 were cultured in monolayers at 37 °C and 5% CO\textsubscript{2} using Eagle's MEM containing 5% FBS, 2 mM L-glutamine, 25 µg/ml gentamicin (21, 33, 40).

Generation of Stable Cotransfectants—The cDNA of dominant negative mutants of ERK2, p38 kinase or JNK1 were subcloned into a mammalian expression vector, CMV-neo, respectively. JB6 Cl 41 cells were transfected with the respective construct by using
LipofecAmine (Gibco BRL) following the manufacturer’s instructors. The stable transfectants were obtained by selection for G418 resistance (300 µg/ml) and further confirmed by assay of respective activity as described (24, 31, 41).

Coimmunoprecipitation Assay—To study the effect of UVB radiation on the interaction of ERKs or p38 kinase with p53 as well as the phosphorylation of p53 at serine 15 on p53-MDM2 interaction in vivo, p53 protein, ERKs or p38 kinase was first immunoprecipitated with a specific antibody against p53, ERKs or p38 kinase, respectively. The immunocomplex was then analyzed by SDS-PAGE and immunoblotted with the appropriate antibodies. Briefly, JB6 Cl 41 cells or transfectants were cultured in 100-mm dishes with 5% FBS MEM until they were 80-90% confluent. Then, the cells were starved by culturing them in 0.1% FBS MEM for 24 h. The cells were exposed to UVB radiation to induce p53 phosphorylation at serine 15 and then were lysed on ice for 30 min in lysis buffer (20 mM Tris pH 7.4, 150 mM MaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na3 VO4, 1 mg/ml leupeptin and 1 mM phenylmethylsulfonyl fluoride) and centrifuged at 14,000 rpm for 10 min in a microcentrifuge. The lysates were immunoprecipitated using monoclonal mouse IgG against p53, ERK2 or p38 antibodies and protein A/G plus-agarose. The beads were washed extensively to eliminate nonspecific binding, and levels of phosphorylated proteins of p53 at serine 15, p53 and MDM2 proteins, ERKs and p38 kinase, as well as phosphorylated ERKs and p38 kinase, were selectively measured by western immunoblotting using a specific antibody and chemiluminescent detection system.

Assay for ERKs and p38 Kinase Activities—Assays of ERKs and p38 kinases were carried out as described in the protocol of New England Biolabs. In brief, JB6 Cl 41 cells or transfectants were starved for 24 h in 0.1% FBS MEM at 37 °C, in a 5% CO2 atmosphere
incubator. The cells were exposed to UVB (4 KJ/m²) and cultured for an additional 30 min. Then, the cells were washed once with ice-cold phosphate-buffered saline and lysed in 300 µl of the lysis buffer. The lysates were sonicated and centrifuged and the supernatant fraction was incubated with the specific ERKs, p38 kinase or p53 antibody with gentle rocking for 6-10 h at 4 °C and then the protein A/G plus-agarose was added and the incubation continued for another 4 h. The beads were washed twice with 500 µl of lysis buffer and twice with 500 µl of kinase buffer (25 mM Tris pH 7.5, 5 mM β-glycerolphosphate, 2 mM DTT, 0.1 mM Na₃VO₄ and 10 mM MgCl₂). The kinase reactions were carried out in the presence of 200 µM ATP at 30 °C for 30 min using 2 µg of Elk-1 or ATF-2 as substrate for ERKs or p38 kinase, or 5 µg of p53 as substrate for ERKs and p38 kinase. The phosphorylated proteins were detected by immunoblotting using phospho-specific antibodies.

**Protein Phosphorylation Assay in Vitro**—Phosphorylation of p53, Elk-1, ATF-2, or c-Jun by active recombinant ERK1, ERK2, p38 kinase or JNK2 was carried out at 30 °C for 60 min in the presence of the kinase buffer with 200 µM ATP and p53, Elk-1, ATF-2 or c-Jun as substrate. The phosphorylated proteins were detected by immunoblotting using phospho-specific antibodies.

**Assay for p53-dependent Transcription Activity**—p53-dependent transcription activity was assayed by using a Cl 41 cell line stably expressing a luciferase reporter gene controlled by p53 DNA binding sequences (34, 40). Confluent monolayers of Cl 41 p53 cells were trypsinized and 8 × 10³ viable cells suspended in 100 µl of 5% FBS MEM were seeded into each well of a 96-well plate. Plates were incubated at 37 °C in a humidified atmosphere of 5% CO₂ until the cells were 80-90% confluent. The cells were starved by culturing them in 0.1% FBS MEM for 24 h. Then the cells were treated with different concentrations of PD98059 or SB202190 for 1 h,
followed by exposure to UVB (4 KJ/m²) to induce p53 activity, and cultured for an additional 24 h. The cells were extracted with lysis buffer (100 mM K₂HPO₄ pH 7.8, 1% Triton, 1 mM DTT and 2 mM EDTA) and luciferase activity was measured using a luminometer (Monolight 2010). The results were expressed as relative p53 activity (40).

RESULTS

ERKs and p38 Kinase Associate with p53 Phosphorylation at Serine 15 in Response to UVB Radiation—Previous studies indicated that JB6 Cl 41 cells contain high levels of wild-type p53 protein that could activate p53-dependent transcription in response to UV exposure (34, 40, 42). To identify whether p53 was phosphorylated at serine 15 in cells treated with UV radiation in vivo, a phospho-specific antibody against p53 at serine 15 was used in western blot analysis (11). We found that the level of p53 phosphorylation at serine 15 reached a maximum 4-6 h following UVB radiation (Fig. 1A). A dose response study indicated that 4 KJ/m² was the optimal dosage for induction of p53 phosphorylation at serine 15 (Fig. 1A). These results are in agreement with previous findings that UV radiation leads to increased phosphorylation of p53 at serine 15 (3, 4). Phosphorylation of both tyrosine and threonine residues in the activation segment of the kinases domain on MAP kinases is known to be essential for full kinase activity (25, 43). Using phospho-specific antibodies against MAP kinases (24, 31), we also found that activation of MAP kinases, including ERKs and p38 kinase as well as JNKs, was observed following exposure of cells to UVB radiation (Fig. 1B). These data suggest a possible role of MAP kinases acting upstream of p53 in a signal transduction pathway initiated by UVB radiation. This possibility is supported by studies in which ERKs or p38 kinase was co-precipitated with p53 in response to UVB stimulation (Fig. 2A). ERKs or p38 kinase and their
activities could be detected in the p53 immunoprecipitated complex while no activity was observed in JNKs (data not shown). Conversely, serine 15 phosphorylation of p53 was detected in the ERKs or p38 kinase immunoprecipitates from UVB-treated JB6 Cl 41 cells (Fig. 2B). The kinetics of ERKs or p38 kinase binding to p53 coincided with the kinetics of their activation induced by UVB (Fig. 1B), and the total level of ERKs or p38 kinase in all the immunoprecipitation samples was almost the same (Fig. 2A). Taken together, these data show that UVB induces the formation of a complex between p53 and ERKs or p38 kinase, suggesting that the activated ERKs or p38 kinase may be responsible for p53 phosphorylation at serine 15.

Inactivated ERKs and p38 Kinase Block UVB-induced p53 Phosphorylation at Serine 15—To further determine whether phosphorylation of p53 at serine 15 requires ERKs or p38 kinase in vivo, we used two approaches to inactivate ERKs or p38 kinase. First, PD98059, a specific inhibitor of MEK1 that acts by inhibiting activation of ERKs, and SB202190, a specific inhibitor of p38 kinase, were tested for their effect on UVB-induced serine 15 phosphorylation of p53. Pretreatment with 25-100 µM of PD98059 or 1-4 µM of SB202190 markedly inhibited UV-induced activation of ERKs or p38 kinase (data not shown) and significantly inhibited the phosphorylation of p53 at serine 15 (Fig. 3A). In contrast, pretreatment with 100-400 nM of wortmannin, which effectively inhibits DNA-PK (44) or ATM (15), did not show any inhibition of p53 phosphorylation at serine 15 (Fig. 3B). These data suggest that the intrinsic kinase activities of ERKs and p38 are required for UVB-induced serine 15 phosphorylation of p53. The second strategy used to inactivate ERKs or p38 kinase was to use dominant negative (DN) mutants of these MAP kinases. The Cl 41 cells stably expressing the genes were reported previously (23, 27, 31) and described in “Experimental Procedures.” Overexpression of DN-ERK2 or DN-p38 kinase specifically blocked UVB-induced ERKs or p38 kinase activity (Fig. 4).
Overexpression of DN-ERK2 has been shown to have no effect on activation of JNK and p38 kinase in DN-ERK2 tranfectants; overexpression of DN-p38 kinase had no effect on activation of ERKs and JNKs in DN-p38 kinase transfectants (23, 27, 31). The expression of DN-ERK2 or DN-p38 markedly abrogated p53 phosphorylation at serine 15 up to 4 h following UVB radiation (Fig. 5). In contrast, overexpression of dominant negative JNK1 did not result in significant inhibition of p53 phosphorylation at serine 15. These two experiments provide strong evidence that in Cl 41 cells, UVB-induced phosphorylation of p53 at serine 15 is mediated through ERKs and p38 kinase, but not other kinases such as JNKs, DNA-PK, or ATM.

**Serine 15 in p53 is a Phosphorylation Site for ERKs and p38 Kinase**—To test whether ERKs and p38 kinase phosphorylated p53 at serine 15 directly, we performed immune complex kinase assays of UVB-activated ERKs, p38 kinase, and JNKs using a full length GST-p53 fusion protein as the exogenous substrate. This experiment revealed that the exogenous p53 protein was phosphorylated at serine 15 by UVB-activated ERKs or p38 kinase but not by activated JNKs (Fig. 6A). Intriguingly, co-precipitated endogenous p53 was found in the anti-phospho-ERKs or anti-phospho-p38 kinase immunoprecipitates and could be phosphorylated at serine 15 at a level substantially above that observed *in vivo* at 30 min after UVB radiation (Fig. 1A). To confirm that phosphorylation of p53 was mediated by ERKs or p38 kinase, rather than a contaminating protein kinase, we incubated the exogenous GST-p53 protein with one of several pure and activated recombinant MAP kinase family members in the presence of 200 μM ATP. The results showed that active ERK1/2 or p38 kinase could directly phosphorylate p53 protein at serine 15 *in vitro*, but active JNK2 did not result in phosphorylation (Fig. 6B). Active JNK2, however, did exert its activity to phosphorylate c-Jun (Fig. 6B). These results, taken together with the other
results from this study, strongly demonstrate that ERKs and p38 kinase are direct mediators of UVB-induced p53 phosphorylation at serine 15.

_**Serine 15 Phosphorylation of p53 Mediated by ERKs and p38 Kinase Associates with p53-dependent Transcription Activity and MDM2 Expression**—To test the functional requirement of ERKs and p38 kinase in mediating the phosphorylation of p53 at serine 15 in response to UV radiation, we determined the inhibitory effects of PD98059 or SB202190 on p53-dependent transcription activity and expression of MDM2 protein, a p53 target gene product. Our results showed that UVB-induced p53-dependent transcription activity was blocked by pretreatment of cells with PD98059 or SB202190 (Fig. 7A), indicating that phosphorylation of p53 at serine 15 is functionally required for p53-dependent transcription. MDM2, through complex formation with the N-terminus of p53 (amino acids 19-26), both represses p53 transcriptional activity (45) and mediates the degradation of p53 through the ubiquitin pathway (46, 47). The MDM2 gene possesses two p53 response elements in its first intron and can be activated by p53 (48). Thus, p53 increases MDM2 at the transcriptional level but MDM2 suppresses p53 activity at the post-translational level. Here, we found that p53 was induced by UVB radiation within 2 h and the level of p53 protein peaked at 4-6 h (Fig. 7B). The increased levels of p53 protein correlated with the observed increase in p53 phosphorylation at serine 15 (Fig. 1A), confirming a previous report that the phosphorylation of p53 at serine 15 could result in accumulation of p53 protein (4). Increased MDM2 was first noted at 2 h after UVB radiation and continued to accumulate at 6 h, adding further support that MDM2 is induced in response to p53. However, the amount of MDM2 in the p53 immunoprecipitates remained at an almost constant and low level throughout the time course and was increased by pretreatment with ERKs
or p38 kinase inhibitor (Fig. 7C). These data demonstrate that serine 15 phosphorylation of p53 mediated by ERKs and p38 kinase prevents the interaction of MDM2 and p53.

DISCUSSION

The p53 protein plays a critical role in cellular response to environmental stress, including various types of genomic damage. Activation of p53 in response to stress involves post-translational modification, including specific phosphorylation at serine residues within the N-terminal transactivation domain (7, 8, 49). Phosphorylation of serine 15, a key target during p53 activation, is critical for p53-dependent transactivation (11). Biochemical data indicate that stimulation of p53-dependent transactivation by serine 15 phosphorylation occurs through decreased binding of p53 to its negative regulator MDM2 (3) and increased binding to the p300 coactivator protein (10). Mutation at serine 15 impaired the apoptotic activity of p53 (9), suggesting a pivotal role of phosphorylation at this site in p53 activation and induction of apoptosis. Therefore, identifying the kinase(s) that phosphorylates serine 15 will help to delineate the signaling cascade leading to functional activation of p53. UV radiation has been previously shown to induce p53 phosphorylation at serine 15. The critical kinase mediating this UV-responsive phosphorylation is, however, less well documented. ERKs have been shown to phosphorylate p53 at threonine residues 73 and 83 (50), which lie outside the transactivation domain of p53, and serine 34 is a target for phosphorylation by JNKs (51, 52). Very recently, we reported that p38 kinase mediates UV-induced p53 phosphorylation at serine 389 (31), and another group demonstrated that p38 kinase phosphorylates human p53 at serines 33 (serine 34 of mouse p53) and 46 in vitro (53). However, the p53 phosphorylation sites mediated by MAP kinases are not completely identified. In this study, we further determined the role of MAP.
kinases in UVB-induced p53 phosphorylation at serine 15. Exposure of Cl 41 cells to UVB radiation led to activation of MAP kinases including ERKs, JNKs and p38 kinase, and the phosphorylation of p53 at serine 15. Interestingly, we found that ERKs and p38 kinase were present in the p53 immunoprecipitates, while serine 15 phosphorylation of p53 was detected in the ERKs or p38 kinase immunoprecipitates from UVB-treated Cl 41 cells. Pretreatment of cells with PD98059 or SB202190, a specific inhibitor of MEK1 or p38 kinase respectively, or expression of dominant negative mutant of ERK2 or p38 kinase impaired the phosphorylation of p53 at serine 15 and p53-dependent transactivation while increasing the level of MDM2 in the p53 immunoprecipitates in response to UVB. Most importantly, UVB-activated or active recombinant ERK1/2 or p38 kinase was shown to phosphorylate the p53 protein at serine 15 in vitro. All these data clearly demonstrate that ERKs and p38 kinase play a critical role in UVB-induced phosphorylation of p53 at serine 15, suggesting that induction of p53 transcription activity by UVB depends on the activities of ERKs and p38 kinase and their phosphorylation of p53 at serine 15.

Stress activation is complex, and important differences exist in the sites and kinetics of p53 phosphorylation after UV radiation compared with γ-radiation (4, 6, 14). DNA-PK has been shown to phosphorylate serine 15 of p53 in vitro (3) and ATM mediates the phosphorylation of serine 15 in response to γ-radiation (14). Cells lacking DNA-PK or ATM, however, do not exhibit aberrant p53 stabilization or defects in serine 15 phosphorylation after UV radiation (4, 13, 14). In addition, our data also shows that no inhibition of p53 phosphorylation at serine 15 was observed in cells treated with a selective inhibitor of DNA-PK or ATM (Fig. 3B). Recently, ATM-Rad3-related protein (ATR) has been reported to also phosphorylate p53 at serine 15 in vitro (54, 55), but the level of intrinsic ATR is still low following UV radiation (54). On the
other hand, ATR does not mediate serine 15 phosphorylation induced by the topoisomerase I inhibitor, CPT (54). Serine 15 phosphorylation of p53 is induced in response to a variety of DNA-damaging agents (1-5). However, we also noted that this phosphorylation could be induced by tumor promoters such as TPA or growth factors such as EGF, which both are strong activators of ERKs (21, 41), while overexpression of DN-ERK2 could substantially inhibit the phosphorylation (data not shown). Thus, our results indicate that ERKs and p38 kinase are primarily responsible for serine 15 phosphorylation after UV radiation.

We explored the possible involvement of other MAP kinases such as JNKs, which were shown not to be involved in phosphorylation of serine 15 of p53. The role of JNK1 appears to be more than phosphorylation, as it was recently reported to bind to and degrade p53 in an MDM2-independent fashion when this kinase is in an inactive (dephosphorylated) form (56). Following activation by UV radiation, JNK1 inversely stabilizes and activates p53, probably by phosphorylating it at site(s) other than serine 15 (57). The evidence from this study using JB6 cells demonstrates that activation of both ERKs and p38 kinase by UVB radiation is required for serine 15 phosphorylation of p53 and its activation. Although the significance of both ERKs and p38 kinase being required for p53 activation is not presently known, some evidence indicates that crosstalk between ERKs and p38 kinase signaling may play an important role in determining cell survival or death (18, 58). Overall, we demonstrate for the first time that ERKs and p38 kinase can physically interact with and phosphorylate p53 at serine 15 in response to UVB radiation, both in vivo and in vitro. We propose a model in which activated ERKs and/or p38 kinase bind to p53 molecules to form a complex, leading to phosphorylation of p53 at serine 15 or other phosphorylation sites and reduced binding to MDM2, thereby enhancing its functional activities. Collectively, our data identify ERKs and p38 kinase as direct signal mediators of p53.
phosphorylation at serine 15 following UVB radiation, and both ERKs and p38 kinase are functionally required for UV-induced p53-dependent transcription.

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REFERENCES


   (1997) Genes Dev. 11, 3471-3481


   (1999) Oncogene 18, 3205-3212

    Biol. Chem. 273, 33048-33053


    57, 68-74

13. Jimenez, G. S., Bryntesson, F., Torres-Ardayus, M. I., Priestley, A., Beeche, M., Saito, S.,
    (1999) Nature 400, 81-83

14. Canman, C. E., Lim, D. S., Cimprich, K. A., Taya, Y., Tamai, K., Sakaguchi, K., Appella,


FOOTNOTES

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1 The abbreviations used are: UV, ultraviolet; ERKs, extracellular-signal-regulated protein kinases; JNKs, c-Jun N-terminal kinases; MAP, mitogen-activated protein; MEK1, mitogen-activated protein kinase kinase-1; DNA-PK, DNA-dependent protein kinase; ATM, ataxia telangiectasia-mutated; ATR, ATM-Rad3-related protein; GST, glutathione S-transferase; MDM2, murine double minute; MEM, Eagle's minimum essential medium; FBS, fetal bovine serum; CMV, cytomegalovirus; DTT, dithiothreitol.
FIG. 1. UVB radiation induces p53 phosphorylation at serine 15 and activates MAP kinases. (A) Induction of p53 phosphorylation at serine 15. Serum-starved Cl 41 cells were exposed to UVB (4 KJ/m²) and then cultured for the times designated (top), or were treated with different doses of UVB and then cultured for 4 h (bottom). Lysates were prepared from these cells and immunoprecipitated using monoclonal antibodies against p53 and phosphorylation of p53 at serine 15 was measured as described under “Experimental Procedures.” (B) Activation of ERKs, p38 kinase and JNKs. Serum-starved Cl 41 cells were exposed to UVB (4 KJ/m²) and cultured for the times designated. The cells were extracted and phosphorylated and total proteins of ERKs, p38 kinase, as well as JNKs were immunodetected as described previously (21,24,28).

FIG. 2. ERKs and p38 kinase associate with p53 phosphorylation at serine 15 in response to UVB radiation. (A) Association of p53 with ERKs and p38 kinase. Serum-starved Cl 41 cells were exposed to UVB (4 KJ/m²) and then cultured for the times designated. Lysates were prepared from these cells and immunoprecipitated using monoclonal antibodies against p53. The p53 immunoprecipitates were immunodetected with phospho- or total ERKs and p38 kinase antibodies, or subjected to kinase assay by adding Elk-1 or ATF-2 as ERKs or p38 kinase substrate, respectively. The phosphorylated protein was immunodetected with phospho-Elk-1 (Ser383) or phospho-ATF-2 (Thr71) as described under “Experimental Procedures.” (B) Association of ERKs and p38 kinase with serine 15 phosphorylation of p53. Serum-starved Cl 41 cells were exposed to UVB (4 KJ/m²) and cultured for the times designated. Lysates were prepared from these cells and immunoprecipitated using monoclonal antibodies against ERKs or
p38 kinase. The ERKs or p38 kinase immunoprecipitates were immunodetected with phospho-p53 (Ser 15) antibody as described under “Experimental Procedures.” IP, immunoprecipitate.

FIG. 3. Inhibition of UVB-induced p53 phosphorylation at serine 15 by MEK1 inhibitor, PD98059, and p38 kinase inhibitor, SB202190, but not by DNA-PK or ATM inhibitor, wortmannin. Serum-starved Cl 41 cells were pretreated with PD 98059 or SB202190 (A) or wortmannin (B) for 1 h at the concentration as indicated followed by treatment with UVB (4 KJ/m²) radiation and then cultured for 4 h. Lysates were prepared from these cells, and the phosphorylation of p53 at serine 15 was measured as described under “Experimental Procedures.”

FIG. 4. Expression of dominant negative ERK2 or p38 kinase blocks the activation of ERKs or p38 kinase induced by UVB radiation. Serum-starved Cl 41 cell stable transfectants, Cl 41 CMV-neo, Cl 41 DN-ERK2 B₃ mass₁ or Cl 41 DN-p38 G7 were exposed to UVB (4 KJ/m²) and then cultured for 30 min. Lysates were prepared from these cells, and the ERKs or p38 kinase activity was determined as described under “Experimental Procedures.”

FIG. 5. Blocking UVB-induced p53 phosphorylation at serine 15 by dominant negative mutant of ERK2 or p38 kinase. Serum-starved Cl 41 cell stable transfectants as indicated were exposed to UVB (4 KJ/m²) and cultured for the times designated. Lysates were prepared from these cells, and the phosphorylation of p53 at serine 15 was measured as described under “Experimental Procedures.”
FIG. 6. p53 is phosphorylated at serine 15 in vitro by UVB-activated or active ERKs and p38 kinase, but not by active JNKs. (A) Serine 15 phosphorylation of exogenous and endogenous p53 by UVB-activated ERKs and p38 kinase. Serum-starved Cl 41 cells were exposed to UVB (4 KJ/m²) and cultured for 30 min. Lysates were prepared from these cells, and the immunoprecipitated phospho-ERKs, p38 kinase or JNKs was subjected to kinase assay by adding purified GST-p53 as exogenous substrate. Serine 15 phosphorylation of exogenous and endogenous p53 was detected as described under “Experimental Procedures.” IB, immunoblotting. (B) top, serine 15 phosphorylation of exogenous p53 by active recombinant ERK1/2 and p38 kinase but not by active JNK2. bottom, phosphorylation of Elk-1 (Ser383), ATF-2 (Thr71) and c-Jun (Ser63) by active recombinant ERK1/2, p38 kinase and JNK2, respectively. Phosphorylation of p53 at serine 15 or the phosphorylation of Elk-1 at serine 383, ATF-2 at threonine 71 and c-Jun at serine 63 was carried out at 30 °C for 60 min in the presence of specific substrate, kinase buffer, 200 µM ATP, and one of the MAP kinases family. The phosphorylated proteins were immunodetected using phospho-specific antibodies.

FIG. 7. Pretreatment of cells with PD98059 or SB202190 impairs the UVB-induced p53-dependent transcription activity through the increased level of MDM2 binding to p53. (A) p53-dependent transcription activity. Cl 41 p53 transfectants were seeded in 96-well plates and cultured until 80-90% confluent. The cells were starved in 0.1% FBS MEM for 24 h. Then the cells were pretreated with PD98059 or SB202190 for 1 h at the concentration indicated. The cells were exposed to UVB (4 KJ/m²) and then cultured for 24 h. Activity was measured by the luciferase assay, and results were expressed as relative p53 activity (40). Data from four independent experiments were averaged and are presented as means ± SEM. (B) Association of...
p53 with MDM2. Serum-starved Cl 41 cells were exposed to UVB (4 KJ/m²) and cultured for the times designated. Lysates were prepared from these cells. One-tenth of the Cl 41 lysates was immunodetected with MDM2 antibody, while the rest was used to immunoprecipitate p53, and the p53 immunoprecipitates were immunodetected with p53 and MDM2 antibodies. (C) Association of ERKs or p38 kinase with p53-MDM2. Conditions were as in Fig. 3A, except immunodetection was with MDM2 antibody.
Figure 1

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B

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- phospho-ERKs
- ERKs
- phospho-p38 kinase
- p38 kinase
- phospho-JNKs
- JNKs
**Figure 2**

**A**

IP: anti-p53

<table>
<thead>
<tr>
<th>UVB (h)</th>
<th>0</th>
<th>0.5</th>
<th>2</th>
<th>4</th>
</tr>
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<tbody>
<tr>
<td>phospho-ERKs</td>
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<tr>
<td>ERKs</td>
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<tr>
<td>phospho-Elk-1</td>
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<tr>
<td>phospho-p38 kinase</td>
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<tr>
<td>p38</td>
<td></td>
<td></td>
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<tr>
<td>phospho-ATF-2</td>
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</table>

**B**

IP: anti-ERKs, anti-p38 kinase

<table>
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<tr>
<th>UVB (h)</th>
<th>0</th>
<th>0.5</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>phospho-p53 (ser15)</td>
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</tbody>
</table>
### Table A

<table>
<thead>
<tr>
<th>SB202190 (µM)</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>-</th>
<th>1</th>
<th>2</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD98059 (µM)</td>
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<td>-</td>
<td>25</td>
<td>50</td>
<td>100</td>
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<td>-</td>
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<tr>
<td>UVB</td>
<td>-</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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**Phospho-p53 (Ser15)**

### Table B

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<th>Wortmannin (nM)</th>
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<th>25</th>
<th>50</th>
<th>100</th>
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<tbody>
<tr>
<td>UVB</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Phospho-p53 (Ser15)**
Figure 4

UVB

Cl 41
CMV-neo

Cl 41
DN-ERK2
B_3 mass_1

phospho-Elk-1

UVB

Cl 41
CMV-neo

Cl 41
DN-p38 G7

phantom-ATF-2
Figure 5

UVB (h) 0 0.5 2 4

phospho-p53 (Ser15)

Cl 41 CMV-neo
Cl 41 DN-ERK2 B\textsubscript{3} mass\textsubscript{1}
Cl 41 DN-p38 G7
Cl 41 DN-JNK1 mass\textsubscript{1}
Qing-Bai She et al., Fig. 6

A

<table>
<thead>
<tr>
<th>IP:</th>
<th>anti-phospho-ERKs</th>
<th>anti-phospho-p38 kinase</th>
<th>anti-phospho-JNKs</th>
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</thead>
<tbody>
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<td>UVB kDa</td>
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</tr>
</tbody>
</table>

- exogenous GST-p53
- endogenous p53

IB: anti-phospho-p53 (Ser15)

B

| p53 (5µg) | + | + | + | + | + |
| ERK2 (ng)  | - | - | 10| 20|    |
| ERK1 (ng)  | - | 50| 100| -|    |
| p38 kinase (ng) | - | 50| 100|    |
| JNK2 (µg)  | - | 1| 2|    |

- phospho-p53 (Ser15)

Elk-1 (2µg) | + | + | + |
| ERK2 (ng)  | - | 10|    |
| ERK1 (ng)  | - | 50|    |
| p38 kinase (ng) | - | 50|    |
| JNK2 (µg)  | - | 1|    |

- phospho-Elk-1
- phospho-ATF-2
- phospho-c-Jun
Figure 7

A

![Relative p53 activity graph with bars showing the effect of UVB, PD98059, and SB202190 on p53 activity over time.]

B

![Western blot analysis showing MDM2, p53, and MDM2 protein levels with time (0, 0.5, 2, 4, 6 hours) and UVB treatment.

C

![IP: anti-p53 Western blot showing the effect of SB202190, PD98059, and UVB on MDM2 protein levels.]}
ERKs and p38 kinase phosphorylate p53 protein at serine 15 in response to UV radiation
Qing-Bai She, Nanyue Chen and Zigang Dong

J. Biol. Chem. published online April 25, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M001020200

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