Ultraviolet Irradiation Blocks Cellular Responses to Transforming Growth Factor-β by Down-regulating Its Type-II Receptor and Inducing Smad7*

Received for publication, November 30, 2000, and in revised form, April 29, 2001
Published, JBC Papers in Press, April 24, 2001, DOI10.1074/jbc.M010835200

Taihao Quan‡, Tianyuan He‡, John J. Voorhees, and Gary J. Fisher§

From the Department of Dermatology, University of Michigan Medical School, Ann Arbor, Michigan, 48109-0609

Transforming growth factor-β (TGF-β) is a multi-functional cytokine that regulates cell growth and differentiation. Cellular responses to TGF-β are mediated through its cell surface receptor complex, which activates transcription factors Smad2 and Smad3. Here we report that UV irradiation of mink lung epithelial cells causes near complete inhibition of TGF-β-induced Smad2/3-mediated gene expression. UV irradiation inhibited TGF-β-induced phosphorylation of Smad2 and subsequent nuclear translocation and DNA binding of Smad2/3. Specific cell surface binding of TGF-β was substantially reduced after UV irradiation. This loss of TGF-β binding resulted from UV-induced down-regulation of TGF-β type II receptor (TβRII) mRNA and protein. UV irradiation significantly inhibited TβRII promoter reporter constructs, indicating that UV reduction of TβRII expression involved transcriptional repression. In contrast to its effects on TβRII, UV irradiation rapidly induced Smad7 mRNA and protein. Smad7 is known to antagonize activation of Smad2/3 and thereby block TGF-β-dependent gene expression. UV irradiation stimulated Smad7 promoter reporter constructs, indicating that increased Smad7 expression resulted, at least in part, from increased transcription. Overexpression of Smad7 protein to the level induced by UV irradiation inhibited TGF-β-induced gene expression 30%. Maintaining TβRII levels by overexpression of TβRII prevented UV inhibition of TGF-β responsiveness. Taken together, these data indicate that UV irradiation blocks cellular responsiveness to TGF-β through two mechanisms that impair TGF-β receptor function. The primary mechanism is down-regulation of TβRII, and the secondary mechanism is induction of Smad7.

Transforming growth factor-beta (TGF-β) family members are multifunctional cytokines whose cellular effects are dependent on cell type and cellular context. For example, TGF-β stimulates proliferation of fibroblasts in connective tissue and inhibits growth of epithelial cells (1). The TGF-β play important roles in cellular differentiation and biosynthesis of extracellular matrix (2, 3). Impairment of TGF-β responsiveness occurs in a variety of cancer cells and contributes to loss of growth control (4–7).

TGF-β signal transduction is mediated by a complex of three transmembrane receptors, Type I (TβRI), Type II (TβRII), and Type III (TβRIII) TGF-β receptors. TβRI and TβRII possess intrinsic serine/threonine kinase activity. TβRIII is a membrane proteoglycan that is thought to facilitate ligand binding to TβRII (8–11). Binding of ligand to TGF-β receptors induces formation of a heteromeric complex of TβRI and TβRII receptors (2, 12–15). Formation of this heteromeric complex enables the TβRII to phosphorylate TβRI, resulting in activation of TβRII kinase (2, 13, 16–18). TβRI phosphorylates and thereby activates transcription factors Smad2 and Smad3 (1, 2, 19, 20). Phosphorylated Smad2 and/or Smad3 then bind their common partner, Smad4, to form a heteromeric complex, which then translocates to and accumulates in the nucleus (19), where it acts as a transcription factor (2, 15). The actions of TGF-β are antagonized by Smad7, which interacts stably with TβRII to prevent phosphorylation and activation of receptor-regulated Smad2/3, thereby blocking TGF-β signaling (21, 22).

Solar ultraviolet (UV) irradiation is a potent environmental hazard capable of damaging cellular DNA and causing mutations (23, 24). Recent studies demonstrate that UV irradiation of cells causes ligand-independent rapid activation and clustering of many different growth factor and cytokine cell surface receptors (24–26). The resulting stimulation of multiple signal transduction pathways leads to induction and activation of transcription factors, notably activator protein-1 and NF-κB, which results in transcription of their target genes (25, 27–29).

Despite widespread interest in mechanisms of action of UV irradiation, relatively little is known regarding the effect of UV irradiation on TGF-β receptor activation or on TGF-β/Smad signaling. We report here that in contrast to growth factor- and cytokine receptor-mediated signal transduction, UV irradiation impairs TGF-β receptor-mediated signal transduction. This impairment of TGF-β responsiveness results from down-regulation of TβRII and induction of Smad7 by UV irradiation.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dulbecco’s modified Eagle’s medium, fetal bovine serum, trypsin solution, penicillin, streptomycin, l-glutamine, and G418 (geneticin) were purchased from Life Technologies, Inc. TβRI, TβRII, Smad2, Smad3, and Smad7 primary and secondary antibodies for Western analysis were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-phospho-Smad2 was obtained from Upstate Biotechnology (Lake Placid, NY). Fluorescein isothiocyanate-conjugated anti-rabbit IgG and anti-goat IgG secondary antibodies for immunofluorescence confocal laser microscopy were purchased from Roche Molecular Biochemicals. Human recombinant TGF-β 1 was purchased from R&D Systems (Minneapolis, MN). [32P]dCTP and [α-32P]dCTP were obtained from PerkinElmer Life Sciences. The cross-
obtain a crude membrane fraction, the membrane pellet was re-sus-
3
pended in buffer (20 mM Tris, pH 7.5, 10 mM NaCl, 1.5 mM MgCl,
2
0.5 mM KCl, 0.5% BSA) and then incubated in Krebs-Ringer/Heps/BSA
3
buffer (pH 7.5, 10 mM NaCl, 1.3 mM CaCl, 5 mM KCl, 31 mM
3
NaCl, 1.5 mM MgCl, 0.2 mM EDTA, 0.1% Triton X-100, 0.5 m M
dithio-
3
sulfoxide, 0.1 mM Na3VO4, 2
mM phosphoenolpyruvate) for 30 min of incubation on ice, the protein-DNA complexes were electro-
3
phoresed on 4% polyacrylamide gel at 200 V in 0.5 × TBE (1.0 M Tris,
3
0.9 M boric acid, 0.01 M EDTA). The gel was transferred to Whatman No.
3
MM paper, vacuum-dried, and scanned by STORM PhosphorImager.
UV Irradiation and TGF-β/Smad Signaling

RESULTS

UV Represses Transactivation by TGF-β—We initially investigated the effect of UV irradiation on TGF-β-induced gene expression using two well characterized TGF-β-regulated luciferase reporter constructs: 1) an 800-base pair fragment (−799/+71) of the human PAI-1 gene (45) and 2) four repetitions of the GTCTAGAC Smad3/4 binding element upstream of a SV40 minimal promoter (39). As shown in Fig. 1, TGF-β treatment activated these reporter constructs more than 10-fold. Pretreatment of cells with UV irradiation 8 h before the addition of TGF-β1 repressed activation of both reporter genes almost completely (Fig. 1). UV treatment alone had no significant effect either on the PAI-1 reporter gene or on the Smad binding element reporter construct (Fig. 1). Taken together, these data indicate that UV irradiation inhibits TGF-β responsiveness.

UV Irradiation Inhibits Smad2 Phosphorylation and Nuclear Translocation—Binding of TGF-β to its receptors results in phosphorylation of Smad2 and Smad3, which then associate with Smad4 and translocate into the nucleus. The reduced responsiveness of TGF-β-regulated reporter constructs to TGF-β after exposure of cells to UV irradiation could result from decreased Smad2/3 activation. To examine this possibility, we next examined whether UV irradiation inhibits phosphorylation of Smad2 and nuclear translocation of Smad2 and Smad3. In untreated cells, the basal level of phosphorylated Smad2 was minimally detectable by Western analysis. Treatment of cells with TGF-β1 increased Smad2 phosphorylation ~4.5-fold (Fig. 2). Treatment of cells with UV irradiation alone did not alter the level of phosphorylated Smad2 (data not shown).

However, pretreatment of cells with UV (20 mJ/cm²) before the addition of TGF-β1 resulted in a time-dependent inhibition of Smad2 phosphorylation. Although TGF-β1-induced Smad2 phosphorylation was not sufficiently changed at 1 and 3 h post-UV, it was almost completely blocked at 8 and 16 h post-UV. In contrast, total Smad2 levels were not affected by either TGF-β or UV treatment (Fig. 2).

We next examined whether UV irradiation affects TGF-β-induced nuclear translocation of phospho-Smad2 and Smad3. As shown in Fig. 3, phospho-Smad2 and Smad3 were predominantly located in the cytoplasm of untreated cells or cells treated with UV alone (data not shown). Treatment of cells with TGF-β1 induced nuclear translocation of phospho-Smad2 and Smad3. Pretreatment of cells with UV 8 h before the addition of TGF-β1 substantially blocked TGF-β-induced translocation of phospho-Smad2 and Smad3 (Fig. 3).

UV Irradiation Inhibits DNA Binding of Smad Proteins—UV inhibition of Smad2 and Smad3 activation by TGF-β would be expected to reduce Smad protein DNA binding. To investigate this possibility, we performed electrophoretic mobility shift assays with two well characterized Smad3/4 DNA binding probes, one containing the TGF-β response element in the PAI-1 promoter (36), and a second probe, identified by random oligonucleotide screening, as a Smad3 DNA binding element (37). MLECs treated with TGF-β alone exhibited enhanced protein binding to both probes (Fig. 4). In contrast, treatment of cells with UV irradiation before the addition of TGF-β blocked protein binding to both probes in a time-dependent manner (Fig. 4). These results indicate that UV inhibits TGF-β-induced DNA binding of Smad proteins.

UV Irradiation Inhibits Binding of TGF-β1 to Its Cell Surface Receptors—The above data indicate that UV irradiation interferes with TGF-β activation of Smad2 and Smad3. This interference could occur as a result of physical and/or func-

Fig. 1. UV irradiation impairs TGF-β-induced gene transcription. MLECs stably expressing a PAI promoter-luciferase reporter gene (open bars) or MLECs transiently co-transfected with a luciferase reporter construct containing four repetitions of the GTCTAGAC Smad3/4 binding element and an expression vector for β-galactosidase (hatched bars) were exposed to UV irradiation (20 mJ/cm²) 8 h before addition of TGF-β1 (1 ng/ml) for 16–24 h. Cell lysates were normalized to cell number for stable transfectants or β-galactosidase activity for transient transfectants and assayed for luciferase activity. Bar heights show representative Western blots for phospho-Smad2 and total Smad2 in whole cell extracts (filled bars) and total Smad2 in nuclear extracts (open bars) (MLECs stably expressing a PAI promoter-luciferase reporter gene). As shown in Fig. 1, TGF-β treatment activated these reporter constructs more than 10-fold. Pretreatment of cells with UV irradiation 8 h before the addition of TGF-β1 repressed activation of both reporter genes almost completely (Fig. 1). UV treatment alone had no significant effect either on the PAI-1 reporter gene or on the Smad binding element reporter construct (Fig. 1). Taken together, these data indicate that UV irradiation inhibits TGF-β responsiveness.

UV Irradiation Inhibits Smad2 Phosphorylation and Nuclear Translocation—Binding of TGF-β to its receptors results in phosphorylation of Smad2 and Smad3, which then associate with Smad4 and translocate into the nucleus. The reduced responsiveness of TGF-β-regulated reporter constructs to TGF-β after exposure of cells to UV irradiation could result from decreased Smad2/3 activation. To examine this possibility, we next examined whether UV irradiation inhibits phosphorylation of Smad2 and nuclear translocation of Smad2 and Smad3. In untreated cells, the basal level of phosphorylated Smad2 was minimally detectable by Western analysis. Treatment of cells with TGF-β1 increased Smad2 phosphorylation ~4.5-fold (Fig. 2). Treatment of cells with UV irradiation alone did not alter the level of phosphorylated Smad2 (data not shown).

However, pretreatment of cells with UV (20 mJ/cm²) before the addition of TGF-β1 resulted in a time-dependent inhibition of Smad2 phosphorylation. Although TGF-β1-induced Smad2 phosphorylation was not sufficiently changed at 1 and 3 h post-UV, it was almost completely blocked at 8 and 16 h post-UV. In contrast, total Smad2 levels were not affected by either TGF-β or UV treatment (Fig. 2).

We next examined whether UV irradiation affects TGF-β-induced nuclear translocation of phospho-Smad2 and Smad3. As shown in Fig. 3, phospho-Smad2 and Smad3 were predominantly located in the cytoplasm of untreated cells or cells treated with UV alone (data not shown). Treatment of cells with TGF-β1 induced nuclear translocation of phospho-Smad2 and Smad3. Pretreatment of cells with UV 8 h before the addition of TGF-β1 substantially blocked TGF-β-induced translocation of phospho-Smad2 and Smad3 (Fig. 3).

UV Irradiation Inhibits DNA Binding of Smad Proteins—UV inhibition of Smad2 and Smad3 activation by TGF-β would be expected to reduce Smad protein DNA binding. To investigate this possibility, we performed electrophoretic mobility shift assays with two well characterized Smad3/4 DNA binding probes, one containing the TGF-β response element in the PAI-1 promoter (36), and a second probe, identified by random oligonucleotide screening, as a Smad3 DNA binding element (37). MLECs treated with TGF-β alone exhibited enhanced protein binding to both probes (Fig. 4). In contrast, treatment of cells with UV irradiation before the addition of TGF-β blocked protein binding to both probes in a time-dependent manner (Fig. 4). These results indicate that UV inhibits TGF-β-induced DNA binding of Smad proteins.

UV Irradiation Inhibits Binding of TGF-β1 to Its Cell Surface Receptors—The above data indicate that UV irradiation interferes with TGF-β activation of Smad2 and Smad3. This interference could occur as a result of physical and/or func-
UV irradiation and TGF-β/Smad Signaling

UV Irradiation Down-regulates TβRII but Not TβRI—Reduced binding of TGF-β to its receptors after UV irradiation could occur as a result of decreased expression of TGF-β receptors. To investigate this possibility, we next examined the effect of UV on TGF-β receptor mRNA and protein levels. UV irradiation reduced TβRII mRNA (Fig. 6A) and protein levels (Fig. 6B) in a time-dependent manner, as measured by Northern and Western analysis, respectively. TβRII mRNA was reduced within 3 h post-UV and was progressively reduced during the succeeding 13 h (Fig. 6A). By 16 h after UV, TβRII mRNA was reduced ~50% relative to control. TβRII protein expression was also reduced after UV exposure. TβRII protein was maximally reduced (80%) at 16 h post-UV (Fig. 6B). In contrast, TβRII mRNA levels were modestly increased between 1 and 3 h post-UV and then returned to their initial values (Fig. 6C). UV had no significant effect on TβRI protein levels (Fig. 6D).

Reduction of the TβRII mRNA after UV irradiation could reflect inhibition of TβRII gene transcription. To examine this possibility, we transiently transfected MLECs with two different TβRII promoter/luciferase constructs, a longer construct (~1640 bp) and a shorter construct (~137 bp). Both promoter constructs were active in non-irradiated cells (Fig. 7).

However, the shorter TβRII promoter/luciferase construct (~137 to +62) was markedly more active than the longer promoter constructs, consistent with the observed presence of a negative regulatory element between ~1240 to ~250 bp. UV irradiation inhibited the activity of both TβRII promoter constructs nearly 70% (Fig. 7).

UV Irradiation Induces Smad7 mRNA and Protein—Recent evidence indicates that cytokines and growth factors can induce Smad7, which inhibits TGF-β signaling (49). Since UV irradiation activates cytokine and growth factor receptors in a variety of cell types (25, 50), we examined whether UV inhibition of the TGF-β responsiveness involves induction of Smad7 in addition to loss of TβRII. As shown in Fig. 8A, Smad7 mRNA increased 2-fold between 1 and 3 h post-UV and then returned to basal levels between 8 and 24 h post-UV. Smad7 protein levels rose steadily between 1 and 8 h post-UV and remained elevated (3-fold) for at least 24 h post-UV (Fig. 8B). We also examined the effect of UV on Smad7 gene promoter activity by
transiently transfecting MLECs with long (2420 to 112) and short (2408 to 112) Smad7 promoter/luciferase constructs. As shown in Fig. 8C, the two promoter constructs were equally active in non-irradiated cells and equally induced (2-fold) after UV irradiation. These data indicate that the induction of Smad7 by UV irradiation is mediated at least in part by increased transcription.

To examine the functional consequences of this induction of Smad7 by UV irradiation, we determined the relationship between Smad7 protein levels and inhibition of TGF-β-induced gene expression. For these studies, MLECs were transfected with varying amounts of Smad7 expression vector, and the levels of Smad7 protein and TGF-β-induced luciferase reporter activity were determined. Transfection of MLEC cells with increasing amounts of Smad7 cDNA resulted in dose-dependent increases in Smad7 protein expression and inhibition of TGF-β-induced luciferase activity (Fig. 9). Transfection of MLEC cells with 2 µg of Smad7 cDNA resulted in an approximate 3-fold induction of Smad7 protein and 30% inhibition of TGF-β-induced luciferase activity (Fig. 9). As described above (Fig. 8B), UV irradiation resulted in a 3-fold induction of Smad7 protein at 8–24 h post-UV. Together, these data indicate that UV induction of Smad7 could account for ~30% of the total inhibitory effect of UV on TGF-β-induced gene expression.

Overexpression of TβRII Overcomes UV Inhibition of TGF-β-Induced Gene Expression—The finding that UV-induced Smad7 only modestly inhibits TGF-β responsiveness suggests that the majority of UV inhibition results from down-regulation of TβRII. To examine this possibility, we determined representative Northern analysis. Bars heights indicate means ± S.E. of TβRII mRNA levels (normalized to 36B4 mRNA levels) relative to levels in non-irradiated control cells (Ctrl, 100%). *p < 0.05 versus Ctrl. n = 4. B, TβRII protein in the membrane fraction of MLECs was quantified by Western analysis. The inset shows a representative Western blot. Bar heights indicate the means ± S.E. of receptor protein levels relative to receptor protein levels in non-irradiated control cells (Ctrl, 100%). *p < 0.05 versus Ctrl. n = 6. C, TβRI and 36B4 mRNA were analyzed by Northern analysis. Representative Northern blots for TβRI and 36B4 mRNA are shown in the inset. Bar heights indicate means ± S.E. for fold change in TβRI mRNA levels (normalized to 36B4 mRNA levels) relative to levels in non-irradiated control cells (Ctrl, 100%). *p < 0.05 versus Ctrl. n = 3. D, TβRI protein in the membrane fraction of MLEC cells was quantified by Western analysis. The inset shows representative Western blots. Bar heights indicate the means ± S.E. of receptor protein levels in UV-irradiated cells relative to receptor protein levels in non-irradiated control cells (Ctrl). n = 3.
UV Irradiation and TGF-β/Smad Signaling

on TGF-β-induced (or basal, data not shown) PAI-1 promoter activity (Fig. 10), indicating that TβRII levels are not limiting for TGF-β responsiveness in non-irradiated cells. As expected, exposure of mock-transfected cells to UV before the addition of TGF-β repressed TGF-β-induced reporter gene 80%. In contrast, in cells overexpressing TβRII, UV did not inhibit TGF-β responsiveness. Complete prevention of UV-induced loss of TGF-β-induced gene expression was observed with 4 μg of TβRII expression vector. These data demonstrate that down-regulation of TβRII by UV is critical for UV inhibition of TGF-β responsiveness.

UV Induction of c-Jun/AP-1 Does Not Correlate with Inhibition of TGF-β Responsiveness—Accumulating evidence indicates that transcription factor AP-1 or its component c-Jun can both positively and negatively modulate TGF-β/Smad-dependent gene expression (51–53). In addition, we and others show that UV irradiation induces c-Jun and AP-1 activity (23, 25, 50, 54–56). These observations raise the possibility that UV induction of c-Jun/AP-1 could contribute to reduced TGF-β responsiveness. To examine this issue, we determined the kinetics of UV induction of c-Jun protein and AP-1 DNA binding in MLECs. UV irradiation induced c-Jun protein 3.0 ± 0.7-fold (n = 3) within 1 h. Maximum induction (5.8 ± 0.9-fold, n = 3) occurred within 5 h post-UV. c-Jun protein levels returned to baseline by 24 h post-UV (1.3 ± 0.3-fold, n = 3). Electrophoretic mobility shift assays using oligonucleotides containing the consensus AP-1 binding element as probe revealed nearly identical kinetics of induction (data not shown). These kinetics of c-Jun/AP-1 activation substantially differ from the kinetics of UV inhibition of TGF-β responsiveness described above. At early times post-UV (1–5 h), c-Jun/AP-1 is maximally induced, whereas TGF-β responsiveness is not altered. At later times post-UV (8–24 h) c-Jun/AP-1 activation subsides, whereas TGF-β responsiveness is inhibited. These data indicate that UV inhibition of TGF-β responsiveness does not correlate with c-Jun/AP-1 activation.

DISCUSSION

Accumulating evidence indicates that UV irradiation activates cytokine and growth factor signal transduction pathways (25, 50). In contrast, we report here that UV irradiation impairs TGF-β/Smad signaling. This impairment results primarily from reduction of TβRII expression and, to a lesser extent, from induction of Smad7. This UV-induced loss of TβRII and increase in Smad7 impairs TGF-β signal transduction as man-

Fig. 8. UV irradiation induces Smad7 mRNA, protein expression, and promoter activity. MLECs were exposed to UV irradiation (20 mJ/cm²). At the indicated times post-UV, cells were collected, and either total RNA or protein were extracted. A, Smad7 and 36B4 (used as an internal control) mRNA levels were analyzed by Northern analysis. The inset shows representative Northern blot for Smad7 and 36B4 (normalized to 36B4 mRNA levels). The bars indicate the means ± S.E. for fold change in Smad7 mRNA relative to levels in non-irradiated control cells (Ctrl). *, p < 0.05 versus control, n = 3. B, Smad7 protein was quantified by Western analysis. The inset shows a representative Western blot for Smad7 protein. The bar heights indicate the means ± S.E. for fold change in Smad7 protein relative to levels in non-irradiated control cells (Ctrl). *, p < 0.05 versus control, n = 3. C, UV irradiation increases Smad7 promoter activity. MLECs were transiently transfected with Smad7 promoter (−4200 to +112 or −408 +112)/luciferase constructs. Twenty-four hours after transfection, cells were irradiated with UV (20 mJ/cm²). Cell lysates were prepared 48 h after transfection and normalized by β-galactosidase activity. Promoter activities were determined by luciferase assay. The bar heights represent the means ± S.E. *, p < 0.05 versus non-irradiated cells, n = 3.

whether maintaining TβRII levels by forced overexpression of TβRII could overcome UV inhibition of TGF-β-induced gene expression. Overexpression of TβRII had no significant effect
UV Irradiation and TGF-β/Smad Signaling

Overexpression of TβRII overcomes UV inhibition of TGF-β induced gene expression. MLECs stably expressing a PAI promoter-luciferase reporter gene were transiently transfected with 2 or 4 μg of TβRII expression vector. Twenty-four hours after transfection, cells were irradiated with UV (20 mJ/cm²) and then 8 h later treated with TGF-β 1 (1 ng/ml) for 16 h. Cells lysates were normalized to cell numbers, and luciferase activity was determined by luciferase assay. The bar heights are the means ± S.E. of fold change in luciferase activity relative to activity in control (Ctrl) cells. n = 3, * p < 0.05 versus mock-transfected, non-treated control cells; † p < 0.05 versus mock-transfected, no UV + TGF-β-treated cells. UV irradiation had no significant effect on luciferase activity in cells over-expressing TβRII.

Reduced levels of TβRII protein could result from reduced synthesis attributable to reduced transcription and/or to increased degradation. UV irradiation may cause internalization of TβRII, which could accelerate degradation of TβRII protein. Future studies are needed to clarify the precise molecular mechanism(s) by which UV irradiation reduces TβRII expression.

In contrast to its effect upon TβRII, UV irradiation did not alter TβRI protein levels. However, UV transiently induced TβRI mRNA, indicating that UV regulates TβRI and TβRII differently. Although cDNA sequences of TβRI and TβRII share partial homology, there are significant differences in their promoter regions (40, 62, 63), consistent with our finding that these two genes are differentially regulated by UV irradiation. The 5′-flanking region of the TβRI gene promoter is extremely GC-rich and contains multiple Sp1 sites, which are essential for basal and maximal promoter activity (62, 63).

Smad7 antagonizes TGF-β/Smad signaling by interacting with TβRI to interfere with phosphorylation and activation of Smad2 and -3. TGF-β induces Smad7 expression, which serves as a negative feedback loop to limit the intensity and duration of the TGF-β response (21, 22). UV transiently induced Smad7 mRNA and elevated Smad7 protein levels for at least 24 h post-UV. There was a significant correlation between Smad7 protein levels and inhibition of TGF-β-induced gene expression. We estimate that UV induction of Smad7 (3-fold) could account for 30% of the observed inhibition of TGF-β-induced gene expression.

Smad7 gene promoter activity was increased after UV irradiation, suggesting that up-regulation of Smad7 mRNA was due at least in part to increased gene transcription. Recently, Bitzer et al. (49) reported that pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β suppress TGF-β/Smad signaling through up-regulation of Smad7 gene transcription. This induction of Smad7 is mediated by the NF-κB/RelA pathway. UV irradiation activates NF-κB and induces proinflammatory cytokines such as interleukin-1α and tumor necrosis factor-α (64–71). Interestingly, we have recently observed that interleukin-1α and tumor necrosis factor-α reduce expression of TβRII and induce Smad7 (2). These raise the possibility that UV induction of NF-κB and/or NF-κB-regulated cytokines may coordinate TβRII and induce Smad7. We are currently investigating this possibility.

Finally, we utilized Smad7 and TβRII expression vectors to determine the relative contributions of Smad7 induction and TβRII down-regulation to UV inhibition of TGF-β responsiveness. Our data indicate that UV-induced Smad7 accounts for ~30% of the total inhibitory effect of UV on TGF-β responsiveness. In contrast, overexpression of TβRII completely prevented UV inhibition of TGF-β responsiveness, indicating that down-regulation of TβRII plays a major role in UV inhibition of TGF-β signaling.

In addition, we examined the possibility that UV induction of c-Jun/AP-1 could contribute to reduced TGF-β responsiveness since UV irradiation induces c-Jun/AP-1 and overexpression of c-Jun inhibits Smad3-dependent transcription (51, 52). However, we found there was no correlation between the kinetics of UV induction of c-Jun/AP-1 and the kinetics of UV inhibition of TGF-β responsiveness. Furthermore, inhibition of TGF-β-induced gene expression by c-Jun/AP-1 does not involve inhibition of Smad2/3 nuclear translocation (51), whereas we found that UV irradiation blocked Smad2/3 nuclear translocation, presumably due to down-regulation of TβRII. These results indicate that UV-induced c-Jun/AP-1 is not a major contributing factor to UV-inhibition of TGF-β responsiveness in MLEC.

2 T. Quan, T. He, J. J. Voorhees, and G. J. Fisher, unpublished data.
Alternatively, c-Jun/AP-1 may cooperate with Smad2/3 to induce TGF-β-regulated genes (53, 72). If so, then UV activation may make c-Jun/AP-1 unable to function as a co-factor in TGF-β-induced gene expression. Our data indicate that this scenario is unlikely because return of c-Jun/AP-1 to its basal state 24 h post-UV did not restore TGF-β responsiveness.

In summary, UV irradiation blocks cellular responsiveness to TGF-β through two mechanisms that impair TGF-β receptor function. The primary mechanism is down-regulation of TβRII, and the secondary mechanism is induction of Smad7.

Acknowledgments—We thank YueXian Hu for technical assistance, Laura VanGoor for the preparation of graphic material, Ted Hamilton for statistical analysis, and Anne Chapple for editorial assistance.

References

Primary references

10. Zhao, J., and Buick, R. N. (1995) Nature 374, 381–386

Secondary references

14. Kadin, M. E., Cavailles-Coll, M. W., Gertz, R., Massague, J., Cheifetz, S., and Weinberg, R. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1081–1091

Footnotes

16. Okadome, T., Yamashita, H., Franzen, P., Moren, A., and Heldin, C. H. (1994) Mol. Cell. Biol. 14, 2578–2580

5. Park, K., Kim, S. J., Bang, Y. J., Park, J. G., Kim, N. K., Roberts, A. B., and Weinberg, R. A. (1991) Cell 67, 797–805

26. Kulms, D., Poepelmann, B., Yarosh, D., Luger, T. A., Krutmann, J., and Rahmsdorf, H. J. (1997) J. Invest. Dermatol. 111, 57–66

8. Wang, X. F., Ling, H. Y., Ng-Eaton, E. Downward, J., Lodish, H. F., and Weinberg, R. A. (1991) Cell 67, 797–805

17. Akiyoshi, S., Inoue, H., Hanai, J., Kusanagi, K., Nemoto, N., Miyazono, K., and Kawabata, M. (1999) J. Biol. Chem. 274, 35269–35277

19. Quinn, T., and Fisher, G. J. (1999) J. Biol. Chem. 274, 28566–28574

24. Bender, K., Blattner, C., Knebel, A., Iordanov, M., Herrlich, P., and Rahmsdorf, H. J. (1997) J. Photochem. Photobiol. B Biol. 37, 1–17

23. Fisher, G. J., Datta, S. C., Talwar, H. S., Wang, Z. Q., Varani, J., Kang, S., and Voorhees, J. J. (1999) J. Biol. Chem. 274, 21260–21267

32. Kielba, A., Rahmsdorf, H. J., Ulrich, A., and Herrlich, P. (1996) EMBO J. 15, 5314–5325

33. Dumont, N., O’Connor-McCourt, M. D., and Philip, A. (1995) Mol. Cell. Endocrinol. 111, 57–66

34. Wakefield, L. M., Smith, D. M., Masui, T., Harris, C. C., and Sporn, M. B. (1987) J. Cell Biol. 105, 965–975

35. Wells, R. G., Yankelev, H., Lin, H. Y., and Lodish, H. F. (1997) J. Biol. Chem. 272, 11444–11451

36. Denzler, S., Ishi, S., Vivien, D., ten Dijke, P., Huet, S., and Gauthier, J. M. (1998) EMBO J. 17, 3091–3100

37. Miller, J. H. (1972) Expression in Molecular Genetics, pp. 17:34–17:35, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

38. Gorman, C. M., Moffat, L. F., and Howard, B. H. (1982) Mol. Cell. Biol. 2, 1044–1051

39. Chen, R., and Derynck, R. (1994) J. Biol. Chem. 269, 22868–22874

40. Abe, M., Harpel, J. G., Metz, C. N., Nunes, I., Loskutoff, D. J., and Rifkin, D. B. (1994) Anal. Biochem. 216, 276–284

41. Sekiguchi, T., Ohtsuka, H., and Takeda, K. (1999) J. Biol. Chem. 274, 35269–35277

42. Bae, H. W., Geiser, A. G., Kim, D. H., Chung, M. T., Burmester, J. K., Sporn, M. B., Roberts, A. B., and Kim, S. J. (1995) J. Biol. Chem. 270, 29460–29468

43. Nagarajan, R., Zhang, J., Li, M., and Chen, Y. (1999) J. Biol. Chem. 274, 33412–33418

44. Fisher, G. J., and Loeb, H. F. (1996) J. Biol. Chem. 271, 1103–1104

45. Udoff, P. A., Haery, T., Abdollahi, S., Stapleton, M., and O’Connor, M. B. (1996) Cell 85, 489–500

46. Zhang, Y., Peng, X. H., Wu, R. Y., and Derynck, R. (1996) Nature 383, 168–172

47. Poss, K. D., and Massague, J. (1996) Nature 387, 710–713

48. Topper, J. N., Cai, X., Qiu, Y., Anderson, K. R., Xu, Y. Y., Deeds, J. D., Feeley, R., Gimeno, C. J., Woolf, E. A., Tayber, O., Mays, G. G., Sampson, B. A., Gao, J., Kirzner, I. I.,Gimbrele, M. A., and Faib, D. P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 9314–9319

49. Zawel, L., Dai, J. L., Buckhaults, P., Zhou, S., Kirzner, K. W., Vogelstein, B., and Kern, S. E. (1998) Mol. Cell. Biol. 1, 611–617

50. Quan, T., and Fisher, G. J. (1999) J. Biol. Chem. 274, 28566–28574

51. Bae, H. W., Geiser, A. G., Kim, D. H., Chung, M. T., Burmester, J. K., Sporn, M. B., Roberts, A. B., and Kim, S. J. (1995) J. Biol. Chem. 270, 29460–29468

52. Nakao, A., Afraikhite, M., Moren, A., Nakayama, T., Christian, J. I., Heuchel, R., Itoh, S., Kawabata, M., Heldin, C. H., and Ten Dijke, P. (1997) Nature 389, 631–637

53. Fisher, G. J., Talwar, H., Xiao, J., Datta, S., Reddy, A., Gaub, M., Rochette-Egly, C., Chambron, P., and Voorhees, J. (1994) J. Biol. Chem. 269, 20629–20635
Ultraviolet Irradiation Blocks Cellular Responses to Transforming Growth Factor-\(\beta\) by Down-regulating Its Type-II Receptor and Inducing Smad7
Taihao Quan, Tianyuan He, John J. Voorhees and Gary J. Fisher

J. Biol. Chem. 2001, 276:26349-26356. doi: 10.1074/jbc.M010835200 originally published online April 24, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M010835200

Alerts:
- When this article is cited
  - When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 71 references, 35 of which can be accessed free at http://www.jbc.org/content/276/28/26349.full.html#ref-list-1