ATF3 Represses 72-kDa Type IV Collagenase (MMP-2) Expression by Antagonizing p53-dependent trans-Activation of the Collagenase Promoter*

The murine homologue of the ATF3 transcription factor increases tumor metastases but, surprisingly, represses 72-kDa type IV metalloproteinase (MMP-2) expression. The current study describes a novel mechanism by which ATF3 regulates transcription. Progressive deletions of the MMP-2 promoter indicated a 38-base pair region (~1659/~1622) necessary for the ATF3-mediated repression. This region lacked CREB/AP-1 motifs but contained a consensus p53 motif shown previously to regulate MMP-2 expression. The activity of a p53 response element-driven luciferase reporter was reduced in ATF3-expressing HT1080 clones. Although MMP-2 promoter activity was not repressed by ATF3 in p53-deficient Saos-2 cells, p53 re-expression increased MMP-2 promoter activity and restored the sensitivity to ATF3. The activity of a GAL4-driven reporter in HT1080 cells co-expressing the full-length p53 sequence fused to the GAL4 DNA binding domain was diminished by ATF3. p53-ATF3 protein-protein interactions were demonstrated both in vivo and in vitro. Cell cycle analysis, performed as an independent assay of p53 function, revealed that γ-irradiation-induced slowed G2/M cell cycle progression (attributable to p53) was countered by ATF3. Thus, ATF3 represses MMP-2 expression by decreasing the trans-activation of this gene by p53.

ATF3 (the human homologue of Ti241) is a member of the ATF/CREB subfamily of bZIP transcription factors (1) and encoded by a four-exon gene spanning 15 kb. Transcription of this gene yields a 2-kb mRNA (1), or an alternatively spliced isoform ATF3Zip2 (2), the former transcript encoding the full-length protein product (~22 kDa) (2). ATF3 homo- and heterodimers bind specifically to the ATF/CREB and AP-1 motifs (3, 4) and regulate the expression of several genes (2, 4–6) as well as gluconeogenic enzymes in transgenic mice as shown recently (7).

In a previous study comparing gene expression in metastatic and non-metastatic tumors, Ishiguro and co-workers (8) identified the murine homologue of ATF3 (Ti241) as overexpressed in metastatic tumors, concomitantly with tumor invasion. The Ti241 induced this behavior. Considering the established role of type IV metalloproteinases in tumor metastases (9–13), we were interested in identifying which, if any, type IV collagenase(s) are regulated by ATF3 (the human homologue of Ti241). We report herein the unexpected finding that the 72-kDa type IV collagenase gene (MMP-2) is transcriptionally down-regulated by this transcription factor. More importantly, this transcriptional repression is achieved by ATF3 interfering with p53-dependent trans-activation of MMP-2 gene expression via a mechanism in which p53 transcriptional activity, but not DNA binding, is attenuated. Thus, our findings reveal a novel transcriptional mechanism in which ATF3 interferes with p53-dependent gene expression.

EXPERIMENTAL PROCEDURES

DNA Constructs and Antibodies—pGL2 reporters containing a luciferase reporter driven by full-length or 5’-deleted MMP-2 promoter fragments were as described elsewhere (14, 15). p53-luc and pFR-luc were purchased from Stratagene (La Jolla, CA). p53-luc contains 15 tandem repeats of the p53 response element flanking a luciferase reporter whereas the pFR-luc comprises the luciferase coding sequence downstream of five tandem-repeated GAL4 binding sites. The plasmid encoding the chimeric GAL4-p53 fusion protein has been described previously (16). The rabbit anti-human ATF3 antibody and mouse monoclonal antibody to human p53 (DO-1) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The antibody to human MMP-2 was supplied by Chemicon (Temeecula, CA). The pRc-p53 expression plasmid bearing the human p53 cDNA or its control vector (pRc-CMV) (17) has been described previously.

Cell Culture and Stable Transfections—Human fibrosarcoma HT1080 cells contain the wild-type p53 gene and were routinely cultured in McCoy’s 5A medium supplemented with 10% fetal bovine serum and antibiotics. To obtain ATF3-overexpressing clones, pCG/ATF3, an expression plasmid encoding the full-length human ATF3 cDNA (18) (kindly provided by Dr. T. Hai, The Ohio State University, Columbus, OH), was transfected into HT1080 cells using poly-L-ornithine as described previously (19). The transfectants were selected with 600 µg/ml G418, and resistant clones were isolated, expanded, and screened for ATF3 gene expression. Human osteosarcoma p53-deficient Saos-2 cells, or a derivative Saos-2/p53 made to express wild type p53 (20), were cultured in Dulbecco’s modified Eagle’s medium supplemented with 15% serum.

Transient Transfections and Luciferase Activity Assays—Transient transfections were performed using the LipofectAMINE 2000 reagent (Invitrogen, Grand Island, NY) according to the manufacturer’s instructions. Cells were harvested and lysed 30 h post-transfection, and luciferase activity was assayed using the Dual-luciferase Reporter Assay System (Promega, Madison, WI) as instructed. For each transfection, 1 ng of Renilla luciferase reporter pRL-TK was included to normalize for differences in transfection efficiency. Unless indicated otherwise, transfections

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1 The abbreviations used are: MMP-2, 72-kDa Type IV collagenase; CMV, cytomegalovirus; DTT, dithiothreitol; EMSA, electrophoretic mobility shift assay; GST, glutathione S-transferase; PBS, phosphate-buffered saline; wt, wild type; IR, ionizing radiation; RLU, relative light units; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
sient transfections with the MMP-2-luciferase construct employed the full-length promoter (1659).

For co-immunoprecipitation experiments, pRe-p53, a plasmid encoding wild type human p53 cDNA (17) or its control vector (pRe-CMV) were transfected into cells using the FuGENE 6 reagent (Roche Molecular Biochemicals, Indianapolis, IN) as instructed by the manufacturer.

**Northern Blotting**—Total cellular RNA isolated from 90% confluent cultures using TRIzol reagent, treated with DNase I and proteinase K, and purified using two cycles of phenol/chloroform extraction. Radioactive cDNAs corresponding to MMP-2 and glyceraldehyde-3-phosphate dehydrogenase were hybridized to nylon-immobilized cDNAs as described previously (21). Nuclei from 6 × 10^6 cells were isolated and incubated in the presence of [α-32P]UTP in transcription buffer (150 mM KCl, 5 mM MgCl2, 1 mM MnCl2, 20 mM Hepes, pH 7.9, 10% glycerol, 5 mM DTT). Total RNA was extracted from nuclei with TRIzol reagent, treated with DNase I and proteinase K, and purified using two cycles of phenol/chloroform extraction. Radioactive RNA (6.7 × 10^6 cpm/mL) was then hybridized to nylon-immobilized cDNAs corresponding to MMP-2 and glyceraldehyde-3-phosphate dehydrogenase.

**Zymography**—These assays were performed as described by us previously (21). Briefly, conditioned medium was electrophoresed in a polyacrylamide gel containing 1 mg/mL gelatin. The gel was then washed at room temperature for 2 h with 2.5% Triton X-100 and, subsequently, at 37°C overnight in a buffer containing 10 mM CaCl2, 150 mM NaCl, and 50 mM Tris-HCl, pH 7.5. The gel was stained with 0.2% Coomassie Blue and photographed on a light box. Proteolysis was detected as a white zone in a dark blue field.

**Western Blotting and Co-immunoprecipitation**—Western blotting was performed as described previously by us (21). Preparation of nuclear and cytosolic extracts were as recently described by this laboratory elsewhere (21). Nuclei from 6 × 10^6 cells were isolated and incubated in the presence of [α-32P]UTP in transcription buffer (150 mM KCl, 5 mM MgCl2, 1 mM MnCl2, 20 mM Hepes, pH 7.9, 10% glycerol, 5 mM DTT). Total RNA was extracted from nuclei with TRIzol reagent, treated with DNase I and proteinase K, and purified using two cycles of phenol/chloroform extraction. Radioactive RNA (6.7 × 10^6 cpm/mL) was then hybridized to nylon-immobilized cDNAs corresponding to MMP-2 and glyceraldehyde-3-phosphate dehydrogenase.

**RESULTS**

ATF3 Expression Diminishes 72-kDa Type IV Collagenase Expression—Because the previous study by Ishiguro et al. (8) had demonstrated the ability of Ti241 (the murine homologue of ATF3) to augment tumor metastases, and considering the wealth of data implicating type IV collagenases as mediators of tumor spread, we were initially interested in determining if ATF3 regulated expression of these collagenases. Toward this end, we stably transfected HT1080 cells with an expression construct encoding the full-length ATF3 cDNA. Endogenous ATF3 protein and mRNA levels in HT1080 cells bearing the empty vector (V1, V3 clones) were below the detection limit of Western blotting (Fig. 1, A and B). In contrast, in four independent clones (A2, A12, A17, A26) stably transfected with the ATF3 expression construct, the ATF3 transcript and protein were readily detectable with a substantial amount of the protein residing in the nuclear compartment (Fig. 1, A–C).

To determine whether the expressed ATF3 regulated type IV collagenase expression, conditioned medium from the vector and the ATF3 clones was harvested and analyzed by gelatin zymography (Fig. 2A). A band that was indistinguishable in size from the 72-kDa type IV collagenase (MMP-2) was evident in conditioned medium from the clones harboring the empty vector (V1, V3). In contrast, the intensity of this band (Fig. 2A) was reduced in all four clones (A2, A12, A17 and A26) shown to be positive for ATF3 expression. The modest differences in MMP-2 amounts among the ATF3-expressing clones might rep-
and GAPDH.

The decreased MMP-2 levels in the conditioned medium was a direct consequence of diminished transcription as evident by Northern blotting and nuclear run-on experiments (Fig. 2, C and D). Densitometric analysis (using Quantity One software, Bio-Rad, Hercules, CA) of the Northern blot indicated a 70–85% reduction in the amount of MMP-2 mRNA in the ATF3-expressing clones. To rule out the possibility that reduced MMP-2 transcription achieved with ATF3 expression was a general phenomenon, we transiently co-transfected HT1080 cells with a luciferase reporter regulated by the promoter of a separate collagenase gene (92-kDa type IV collagenase promoter-MMP-9) and varying amounts of the ATF3 expression construct (Fig. 2B). The ATF3 expression construct caused an increase in MMP-9 reporter activity (square symbol) in contrast to the repression evident with the MMP-2-driven luciferase reporter (diamond symbol). These data argue against the possibility that the repressive effect of ATF3 on MMP-2 transcription is a generalized phenomenon.

To further address the role of ATF3 in regulating MMP-2 expression, we determined the effect of MG-132 (an agent known to increase endogenous ATF3 expression (23), Calbiochem, San Diego, CA) on the expression of this collagenase. Predictably, MG-132 treatment of HT1080 cells caused a strong induction of ATF3 protein as evident in Western blotting (Fig. 3A). The increase in ATF3 mRNA preceded the decrease in MMP-2 mRNA levels as shown in Northern blotting (Fig. 3B). Thus, although elevated ATF3 transcript could be detected as early as 4 h after MG-132 treatment, MMP-2 levels started to decline only at 8 h (Fig. 3B). Parallel experiments (Fig. 3C) using a MMP-2 promoter-driven luciferase reporter also clearly showed a reduction in MMP-2 promoter activity in MG-132-treated HT1080 cells thus further supporting the notion that ATF3 expression diminishes MMP-2 transcription.

ATF3 Interferes with p53 Trans-activation of the MMP-2 Promoter—Previous mapping of the MMP-2 promoter (14, 15) revealed putative CREB and AP-1 motifs (located at −301 and −1270, respectively), which could potentially bind ATF3 and mediate its repressive effects on expression of this collagenase. Thus, we considered the possibility that the repression of MMP-2 expression was achieved through these binding sites. Toward this end, we first determined whether ATF3 could bind to these motifs. ATF3 was in vitro translated and reacted with an oligonucleotide spanning the CREB or AP-1 motifs in the MMP-2 promoter. As a control, in vitro translated ATF3 was reacted with a consensus CREB motif (Santa Cruz Biotechnology, Santa Cruz, CA). As expected, the in vitro translated ATF3 bound the consensus CREB motif (Fig. 4A, lane 1) and was clearly supershifted with an anti-ATF3 antibody (Fig. 4A, lane 2). In contrast, this transcription factor was not recognized by oligonucleotides spanning the MMP-2 promoter-derived CREB or AP-1 motifs (Fig. 4A, lanes 4 and 7). Likewise, nuclear extract from the ATF3-expressing HT1080 clones failed to show specific complex formation with these same MMP-2-derived oligonucleotides (data not shown). Presumably the inability of ATF3 to bind to the putative CREB and AP-1 sites reflects the presence of inhibitory flanking sequences in the MMP-2 promoter.

Because it was unlikely that ATF3 was repressing MMP-2 expression via its cognate binding sequences, we undertook a systematic search using sequentially deleted MMP-2 promoter fragments to define the position of the regulatory element. Toward this end, HT1080 cells stably expressing the ATF3 expression construct (clone A12) or the empty vector (clone V1) were transfected with a luciferase reporter regulated by 5’ deletions of the MMP-2 promoter (Fig. 4B). Repression of luciferase activity was apparent with the reporter regulated by 1659 base pairs of upstream sequence (wt). However, deletion of 38 base pairs from the 5’-end of the promoter (construct D1) reduced the activity of the reporter in the vector clone and completely ablated the repression evident in the ATF3-expressing clone. ATF3 had no further effect on luciferase reporter activity using shorter MMP-2 promoter fragments progressively deleted from the 5’-end. Note that the inability of ATF3 to further repress the activity of the shorter MMP-2 promoter...
fragments (−1622 and shorter) cannot be attributed to a low basal activity, because these reporter plasmids yielded at least 30,000 relative light units (RLU) as measured with a Moonlight 2010 luminometer. These data would suggest that repression of MMP-2 transcription by ATF3 is mediated through a promoter sequence residing between −1659 and −1622. Interestingly, although this region is devoid of CREB or AP-1 motifs (5, 14), it contains a conserved consensus p53 binding site (−1649/−1630) previously shown to up-regulate MMP-2 expression in HT1080 cells (14).

We were therefore intrigued by the possibility that ATF3 regulates MMP-2 expression by way of interfering with the trans-activation of MMP-2 promoter by p53. HT1080 cells express wild type p53 (24, 25), which binds to its recognition motif in the MMP-2 promoter (14), and our EMSA data (Fig. 5A) were consistent with these previous reports. To explore the possibility that ATF3 down-regulated p53-dependent gene expression, a luciferase reporter driven by 15 tandem repeats of the p53 response element was transfected into HT1080 cells stably expressing the ATF3 expression construct (clones A12, A26) or the empty vector (V1, V3). Transfection efficiencies were normalized by co-transfection with a tk-driven Renilla luciferase construct. Remarkably, the activity of the reporter driven by the tandem p53 repeats (Fig. 5B) was down-regulated over 4-fold in the ATF3-expressing clone (A12) or the vector-only clone (V1) along with pRL-TK to normalize for differences in transfection efficiencies. After 30 h, cells were harvested, and promoter activity was determined with the Dual-luciferase Reporter Assay System. A value of 1 on the Relative Luciferase Activity scale represents 30,000 RLU as measured using a Moonlight 2010 luminometer (Analytical Luminescence Laboratory). Representative data of more than three experiments are shown with values in B representing average ± S.D.

If ATF3 represses MMP-2 expression by way of blocking trans-activation of the gene by p53, one would predict that this repressive activity would be lost in p53-deficient cells. Toward this end, Saos-2 cells, which lack p53 protein (26), were co-transfected with the MMP-2 promoter-driven luciferase reporter and an expression vector bearing the coding ATF3 sequence (pCG/ATF3). The expression of the MMP-2 promoter in the p53-deficient cells was substantially lower than in the HT1080 cells, which bear the wt p53 (Fig. 6). More importantly, the expression of the exogenous ATF3 plasmid had no effect on
MMP-2 promoter activity in the Saos-2 cells (Fig. 6). Note that this absence of ATF3-dependent repression in Saos-2 cells does not reflect the detection limit of the luciferase assays. In contrast, Saos-2 cells made to express p53 (Saos-2/p53) (20), demonstrated increased MMP-2 promoter activity (compared with Saos-2) and showed ATF3-dependent repression of the MMP-2 promoter. Taken together, these data suggest that ATF3 expression interferes with p53-dependent transcriptional activation of the MMP-2 gene.

**ATF3 Blocks p53 Trans-acting Activity but Not DNA Binding.**—How then does ATF3 interfere with trans-activation of gene expression by p53? We first considered the possibility that ATF3 either directly, or indirectly, interferes with DNA binding of p53. Indeed, Hai and co-workers (27) reported that nuclear export is modulated by ATF3. To address this issue, nuclear extracts were generated for ATF3-expressing clones (A12, A26) and clones bearing the empty vector (V1, V3). Differences in transfection efficiency were accounted for using Renilla luciferase activity. Panel C is identical to Panel B with the exception that a human β-actin promoter-driven Renilla luciferase reporter was co-transfected into the cells with plasmid pRSV-luc, the latter encoding firefly luciferase. Transfection efficiencies were normalized using the firefly luciferase activity. The experiment was performed at least twice. Data in Panels B and C reflect average values ± S.D. of at least three experiments.

MMP-2 promoter activity in the Saos-2 cells (Fig. 6). Note that this absence of ATF3-dependent repression in Saos-2 cells does not reflect the detection limit of the luciferase assays. In contrast, Saos-2 cells made to express p53 (Saos-2/p53) (20), demonstrated increased MMP-2 promoter activity (compared with Saos-2) and showed ATF3-dependent repression of the MMP-2 promoter. Taken together, these data suggest that ATF3 expression interferes with p53-dependent transcriptional activation of the MMP-2 gene.

**Repression of MMP-2 Promoter Activity by ATF3 Requires p53.** The MMP-2 promoter reporter construct (0.4 μg) and an expression vector encoding ATF3 (pCG/ATF3) (0.1 μg) or the empty vector (pCG) were co-transfected into the indicated cells. pRL-TK was also included. Two days after transfection, the promoter activity was measured by Dual-luciferase Reporter Assay System after normalization for differences in transfection efficiency. The data show average values ± S.D. of at least three experiments.

**ATF3 Blocks p53 Trans-acting Activity but Not DNA Binding.** How then does ATF3 interfere with trans-activation of gene expression by p53? We first considered the possibility that ATF3 either directly, or indirectly, interferes with DNA binding of p53. Indeed, Hai and co-workers (27) reported that nuclear export is modulated by ATF3. To address this issue, nuclear extracts were generated for ATF3-expressing clones (A12, A26) and clones bearing the empty plasmid (V1, V3) and compared for p53 amounts. ATF3 did not alter the amount of nuclear p53 (Fig. 7A) arguing against the possibility that ATF3 is repressing p53-dependent gene expression by accelerating p53 nuclear export. An alternate possibility is that the DNA binding affinity of p53 is reduced in response to ATF3 expression. To test this possibility, EMSA was performed using nuclear extract from HT1080 clones expressing either the exogenous ATF3 or the empty vector (Fig. 7B) and an oligonucleotide spanning the p53 motif in the MMP-2 promoter. A DNA-protein complex (Fig. 7B, asterisk) competed by an excess of a wt p53 consensus sequence (but not by a mutated p53 consensus) was apparent using nuclear extract from pooled clones V1/V3 bearing the empty plasmid. However, no difference in the amount of this complex was observed between the ATF3-expressing clones (A12, A26) and clones bearing the empty vector (V1, V3). These data suggest that ATF3 does not interfere with either import/export of p53 or the binding of this transcription factor to its recognition motif in the MMP-2 promoter.

Because we could find no evidence that ATF3 was repressing p53-dependent gene expression by way of interfering with the DNA binding of the p53 transcription factor, we considered the alternate possibility that the ability of p53 to trans-activate promoters might represent an ATF3 target. To test this contention, cells were co-transfected with an expression construct
bearing the full-length p53 protein fused to the GAL4 DNA binding domain (16) and a luciferase reporter regulated by tandem GAL4 DNA binding sites. Interestingly, the activity of the GAL4-regulated luciferase reporter was reduced (Fig. 7C) 6-fold in the ATF3-expressing clones (A12, A26) when compared with the vector clone (V1) lacking ATF3. To corroborate these findings, the experiment was repeated using parental HT1080 cells treated with MG-132 to induce endogenous ATF3 expression. Again, trans-activation of the GAL4 synthetic promoter by the p53-GAL4 chimeric protein was reduced (Fig. 7D) in cells induced for ATF3 expression. All together, these data strongly suggest that ATF3 represses the trans-acting activity of p53. 

**ATF3 and p53 Proteins Interact in Vitro and in Vivo—**Our data imply that ATF3 represses MMP-2 expression by way of blocking the trans-acting activity of p53. This block could possibly involve a direct interaction of the proteins. To test this contention, two independent experiments were undertaken. In the first experiment, we tested whether p53 and ATF3 interact in vitro. HT1080 cells were transfected with a p53 expression construct (pRe-p53), and the success of the transfection was confirmed by Western blotting for p53 (Fig. 8A). The exogenous p53 protein has a slightly slower migration compared with the endogenous protein. We then determined if p53 was complexed with ATF3 in these cells. Cells were lysed, extracts were immunoprecipitated with, or without, an anti-ATF3 antibody, and the immunoprecipitated material was subjected to Western blotting using an anti-p53 monoclonal antibody. 

In vitro translated radioactive ATF3 was mixed with p53-GST fusion proteins immobilized on glutathione-agarose. After extensive washing, the material was analyzed for ATF3 protein "pulled down" by the p53-GST fusion proteins. The detection of ATF3 in the protein complexes (~10% of the ATF3 was pulled down by the full-length p53, similar in amount to that of another p53-interacting protein-mSin3A (28)) clearly indicated interactions between the two proteins. No binding was observed with p53 peptides spanning residues 1–160, 160–318, or 1–42. In contrast, the p53 peptide 160–393 clearly interacted with ATF3 suggesting the requirements of sequences residing between 318 and 393. The full-length p53 protein showed greater binding of ATF3 presumably reflecting the necessity of non-interacting p53 sequences for establishing affinity for the ATF3 protein. Nevertheless, these in vitro and in vivo data strongly support the contention that ATF3 and p53 proteins interact.

**ATF3 Abrogates the G2 Arrest Induced by Ionizing Radiation—**Thus far, our molecular and biochemical data point to interference of p53-dependent gene activation by ATF3. To further corroborate these findings, we employed a functional assay. Because p53 prolongs G2 arrest in response to ionizing radiation (29, 30), we argued that, if p53 represents an ATF3 target, this cell cycle block should be attenuated in the γ-irradiated ATF3-expressing cells. Toward this end, HT1080 cells expressing ATF3 (clone A12) or bearing the empty vector (clone V1) were exposed to 12.6 Gy of ionizing radiation and, after varying periods, the cells were fixed and subjected to cell cycle analysis (Fig. 9). Over 70% of cells of both clones accumulated in G2/M 16 h after exposure to ionizing radiation (compared with less than 25% of non-irradiated cells from either clone). As expected, progression out of G2/M was slowed in HT1080 cells bearing the empty vector, and this block was sustained up to 48 h post-ionizing radiation (V1 IR). In contrast, the ATF3-expressing clone progressed through G2/M with <30% of the cells remaining at this checkpoint 48 h later (A12 IR). Thus, these functional data support the contention that ATF3 interferes with p53-dependent gene regulation.
We report herein that ATF3 represses 72-kDa type IV collagenase (MMP-2) expression and that this effect is achieved by interference with the p53-dependent trans-activation of this gene. To our knowledge, this is the first report to demonstrate ATF3-regulated gene expression through motifs other than the well-characterized CREB/AP-1 binding motifs. ATF3 regulates the expression of several genes, including thrombospondin, decorin, E-selectin, gluconeogenic enzymes, gadd153/Chop10, and osteocalcin (4–7) via CREB/AP-1 motifs (31). Indeed, binding sites (CREB/AP-1) for ATF3 are present in the MMP-2 promoter, and a study by Hasan and Nakajima (32) suggested that the synergy of retinoic acid and dibutyryl cAMP on MMP-2 expression was mediated via the corresponding motif. Additionally, the role of ATF/CREB family members in regulating MMP-2 expression was further indicated in a separate report by Jean and Bar-Eli (33), which documented a repressive effect of a dominant negative CREB on MMP-2 mRNA levels. Notwithstanding these studies, it is unlikely that diminished MMP-2 transcription by ATF3 involve the CREB/AP-1 motifs in the MMP-2 promoter for two reasons. First, repression by ATF3 was not evident with 5′-deleted MMP-2.
Repression of MMP-2 Expression by ATF3 Is p53-dependent

Our findings that ATF3 regulates gene expression via an unrelated transcription factor bears resemblance to a previous study by Hirano and co-workers. In that work, the authors reported Sp1 regulation of NF-kB-dependent gene expression (49). However, one notable difference exists between the two studies. Although Sp1 regulated gene expression by binding to NF-kB recognition motifs, we could find no evidence of ATF3 binding directly to the p53 motif in the MMP-2 promoter (data not shown). Rather, our data suggest that ATF3, which interacts with the p53 protein, interferes with p53-dependent gene expression by reducing transcriptional activity of the latter.

We were initially surprised by the observation of repressed MMP-2 synthesis by ATF3, because the present study was initiated by the observation that Ti241 (the mouse homologue of ATF3) augments metastases and with the knowledge that, for some cancers, MMP-2 contributes to tumor spread (50–52). However, the HT1080 cells used in the current study were derived from a separate organ system (fibrosarcoma) to the B16 melanoma (8) the latter in which Ti241 was implicated in metastases. It may be that the repression of MMP-2 expression evident in the HT1080 cells reflects organ system differences or is related to other physiological processes such as cytokine activation (e.g. interleukin 1β) (53).

Thus, in conclusion we have demonstrated a novel transcriptional mechanism in which ATF3 represses MMP-2 expression by interfering with p53-dependent trans-activation of this collagenase gene. Moreover, the repressive effect of ATF3 on gene expression reflects a reduced trans-acting activity of p53 as opposed to diminished DNA binding of the latter transcription factor.

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