Wild Type and Mutant p53 Differentially Regulate the Gene Expression of Human Collagenase-3 (hMMP-13)*

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Yubo Sun‡, Jamie M. Cheung§, Joanne Martel-Pelletier§, Jean P. Pelletier§, Leonor Wenger‡, Roy D. Altman‡, David S. Howell‡, and Herman S. Cheung‡*

From the ‡The Research Service and the Geriatric Research, Education, and Clinical Center, Veterans Administration Medical Center, Miami, Florida 33103, the §Department of Medicine, University of Miami School of Medicine, Miami, Florida 33101, and the ¶Osteoarthritis Research Unit, Centre Hospital, University of Montreal, Quebec H2L 4M1, Canada

Matrix metalloproteinases (MMPs) are a family of secreted or transmembrane proteins that can degrade all the proteins of the extracellular matrix and have been implicated in many abnormal physiological conditions including arthritis and cancer metastasis. Recently we have shown for the first time that the human MMP-1 gene is a p53 target gene subject to repression by wild type p53 (Sun, Y., Sun, Y. I., Wenger, L., Rutter, J. L., Brinckerhoff, C. E., and Cheung, H. S. (1999) J. Biol. Chem. 274, 11555–11560). Here, we report that cotransfection of fibroblast-like synoviocytes with p53 expression and hMMP13CAT reporter plasmids revealed that (i) hMMP13, another member of the human MMP family, was down-regulated by wild type p53, whereas all six of the p53 mutants tested lost the wild type p53 repressor activity in fibroblast-like synoviocytes; (ii) this repression of hMMP-13 gene expression by wild type p53 could be reversed by overexpression of p53 mutants p53–143A, p53–248W, p53–273H, and p53–281G; (iii) the dominant effect of p53 mutants over wild type p53 appears to be a promoter- and mutant-specific effect. An intriguing finding was that p53 mutant p53–281G could conversely stimulate the promoter activity of hMMP13 up to 2–4-fold and that it was dominant over wild type p53. Northern analysis confirmed these findings. Although the significance of these findings is currently unknown, they suggest that in addition to the effect of cytokines activation, the gene expression of hMMP13 could be dysregulated during the disease progression of rheumatoid arthritis (or cancer) associated with p53 inactivation. Since hMMP13 is 5–10 times as active as hMMP1 in its ability to digest type II collagen, the dysregulation or up-modulation of MMP13 gene expression due to the inactivation of p53 may contribute to the joint degeneration in rheumatoid arthritis.

Rheumatoid arthritis (RA)† is a systemic disease of unknown etiology characterized by severe inflammation, abnormal immune response, synovial hyperplasia, and extensive destruction of the articular cartilage (2). The progressive destruction of articular cartilage in RA is partly mediated by matrix metalloproteinases (MMPs) (3, 4). Collagenase-1 (MMP-1), stromelysin (MMP-3), gelatinase A and B (MMP-2 and MMP-9), and collagenase-3 (MMP-13) are all present at significantly elevated levels in cartilage, synovial membranes, and synovial fluid of patients with RA (5–7). MMP-13, a newly discovered human collagenase (6–9), besides cleaving type II collagen more efficiently than MMP-1 (10), is also active against aggrecan, the major proteoglycan of the hyaline articular cartilage (11). Therefore, it has been suggested that MMP-13 may represent a particularly significant mediator of tissue destruction in arthritis.

The p53 tumor suppressor gene has been implicated in the malignant progression of cancers, and the mutational inactivation of p53 is the most frequent genetic alteration in human cancers. Recent studies have linked this powerful tumor suppressor to RA. It has been demonstrated independently by two groups that p53 protein is overexpressed in RA synovium and in synoviocytes cultured from RA patients (12, 14), and that mutant p53 transcripts previously identified in human tumors are present in these tissues and cells (13, 14).

p53 is a transcription factor that recognizes a specific consens DNA sequence consisting of two copies of a 10-base pair motif, 5’-RRCA(T/A)T/TA/GY Y-3’ (where R indicates a purine nucleoside and Y indicates a pyrimidine nucleoside), separated by 0–13 base pairs. Wild type (wt) p53 (but not mutants) efficiently binds to this sequence and transactivates expression of the target genes (15–19). p53 can also repress a wide variety of cellular and viral promoters that lack p53-binding sites (20–25). Although wt-p53 can exert its repressive activity on many different genes, most mutants of p53 have lost this wt-p53 repressor activity. This repressive function has been correlated with the ability of p53 to induce apoptosis, whereas the loss of p53 repressive function results in decreased susceptibility to apoptotic stimuli (26). Tumor-derived p53 mutants can also transcriptionally activate a number of gene promoters, whereas wt-p53 inhibits the expression of the corresponding genes (27–30). The detailed mechanism through which this activation occurs is currently unknown. In recent years many genes that are involved in RA have been shown to be regulated by p53, including interleukin-6 (28), basic fibroblast growth factor-13 promoter/chloramphenicol acetyltransferase reporter plasmid; hMMP1luci, human MMP1 promoter/luciferase reporter plasmid; FLS, canine fibroblast-like synoviocytes; CAT, chloramphenicol acetyltransferase; DMEM, Dulbecco’s modified Eagle’s medium; wt, wild type; FBS, fetal bovine serum; MOPS, 4-morpholinepropanesulfonic acid.

‡ To whom correspondence should be addressed: Arthritis Division (D-26), Dept. of Medicine, P. O. Box 016860, Miami, FL 33101. Tel.: 305-324-3646. Fax: 305-324-3365; E-mail: bcheung@mednet.med.miami.edu.

§ The abbreviations used are: RA, rheumatoid arthritis; MMPs, matrix metalloproteinases; hMMP13CAT, human matrix metalloproteinase-13 promoter/chloramphenicol acetyltransferase reporter plasmid; hMMP1luci, human MMP1 promoter/luciferase reporter plasmid; FLS, canine fibroblast-like synoviocytes; CAT, chloramphenicol acetyltransferase; DMEM, Dulbecco’s modified Eagle’s medium; wt, wild type; FBS, fetal bovine serum; MOPS, 4-morpholinepropanesulfonic acid.

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factor (29), and most recently MMP-1 (1) and COX-2 (31). Here we report that MMP-13, another member of the human MMP family, is also a p53 target gene subject to repression. All 6 of the p53 mutants tested have lost this wt-p53 repressor activity, and most of them are dominant over wt-p53 in the regulation of MMP-13 gene expression.

MATERIALS AND METHODS

Dulbecco’s modified Eagle’s medium (DMEM), OPTI-MEM I medium, McCoy’s 5A medium, fetal bovine serum (FBS), stock antibiotic/antimycotic mixture (10,000 units/ml penicillin base, 10,000 μg/ml streptomycin base, and 25 μg/ml Fungizone) were products of Life Technologies, Inc.

DNA Plasmids and Probes—The −1599 human MMP13 promoter/CAT reporter plasmid (hMMP13CAT) used in this study contains the chloramphenicol acetyltransferase (CAT) gene under the transcriptional control of the hMMP13 promoter (32). Wild type p53 and mutant p53 expression plasmids were obtained from Dr. A. J. Levine at Rockefeller University and have been described previously (1). The −4327hMMP1 plasmid/luciferase reporter plasmid (hMMP1luci) contains the firefly luciferase gene under the transcriptional control of the hMMP1 promoter and has been described previously (1). The reporter plasmid p53-luci, which contains the luciferase reporter gene driven by a basic promoter element (TATA box) plus tandem repeats of the consensus binding sequence for p53, was obtained from Dr. P. Mitchell at Pfizer Central Research. Glyceraldehyde-3-phosphate dehydrogenase was an cDNA from a pGEM-T-MMP13 plasmid obtained from Dr. P. Mitchell at Pfizer Central Research. Glyceraldehyde-3-phosphate dehydrogenase was an sence binding sequence for p53, was obtained from Stratagene (La Jolla, CA). The hMMP-13 probe was a 1900-base pair HindIII insert from a pGEM-T-MMP13 plasmid obtained from Dr. P. Mitchell at Pfizer Central Research. Glyceraldehyde-3-phosphate dehydrogenase was an 800-base pair HindIII insert from a PBS-GAPDH plasmid that contains a sequence encoding part of the mouse glyceraldehyde-3-phosphate dehydrogenase cdna.

Cell Culture—Canine fibroblast-like synoviocytes (FLS) were isolated by enzymatic dispersion of canine synovial tissues. Briefly, the tissues were minced and incubated with 1 mg/ml collagenase in serum-free DMEM for 2 h at 37 °C, filtered through a nylon mesh, extensively washed, and cultured in DMEM supplemented with 10% FBS, penicillin, streptomycin, and 1-glutamine in a humidified 5% CO2 atmosphere. After overnight culture, non-adherent cells were removed, and adherent cells were cultured in DMEM plus 10% FBS. Synoviocytes used were third to seventh passage cells. The Saos-2 cell line, a human osteosarcoma cell line containing no endogenous p53, was obtained by enzymatic dispersion of canine synovial tissues. Briefly, the cells were then washed, placed in fresh OPTI-MEM I medium, and cotransfected with varying amounts of p53-expressing plasmid and 1.2 μg of hMMP13CAT reporter plasmid using 5 μl of LipofectAMINE reagent or 4 μl of Superfect transfection reagent (Qiagen, Valencia, CA) following the manufacturer’s instructions. Exponentially growing FLS or Saos-2 were plated at a density of 5 × 10^5 cells/well in 6-well cluster plates (Costar, Cambridge, MA) in 2 ml of DMEM, 10% FBS (FLS) or 2 ml of McCoy’s 10% FBS (Saos-2) and grown until 80% confluent (24–36 h). The cells were then washed, placed in fresh OPTI-MEM I medium, and cotransfected with varying amounts of p53-expressing plasmid and 1.2 μg of hMMP13CAT plasmid together with varying amounts of wt-p53 expression plasmid (pCMV-p53), a 4-fold reduction was observed in CAT activity in a dose-dependent manner (Fig. 1, open bars). Saos-2 cells, a human osteosarcoma cell line lacking endogenous p53 (33), were also used in the transfection assays as recipient cells. We observed up to a 10-fold reduction in CAT activity and up to an 8-fold increase in luciferase activity (p53-luci) when Saos-2 cells were cotransfected with −1599hMMP13CAT, p53-luci, and wt-p53 expression plasmid (p53) (Fig. 1, filled bars).

RESULTS

Suppression of hMMP-13 Transcription by the wt-p53 Protein—When FLS were cotransfected with hMMP13CAT reporter plasmid together with varying amounts of wt-p53 expression plasmid (pCMV-p53), a 4-fold reduction was observed in CAT activity in a dose-dependent manner (Fig. 1, open bars). Saos-2 cells, a human osteosarcoma cell line lacking endogenous p53 (33), were also used in the transfection assays as recipient cells. We observed up to a 10-fold reduction in CAT activity and up to an 8-fold increase in luciferase activity (p53-luci) when Saos-2 cells were cotransfected with −1599hMMP13CAT, p53-luci, and wt-p53 expression plasmid (p53) (Fig. 1, filled bars).
Regulation of hMMP-13 Gene Expression by p53

hMMP13 messenger could be detected in the human osteosarcoma cell line (lane 1), and (ii) the level of hMMP13 messenger RNA was suppressed slightly by transfected wt-p53 (lane 2) but up-modulated substantially by the p53 mutants p53–175H and p53–281G (lanes 3 and 4). The less significant repression of hMMP13 messenger by wt-p53 observed is probably due to low transfection efficiency.

Repression of hMMP13 Gene Transcription by wt-p53 Was Reversed by p53 Mutants—We cotransfected a fixed amount of wt-p53 with increasing amounts of mutant p53 expression plasmids together with hMMP13CAT. The repression of hMMP13CAT reporter activity by wt-p53 could be reversed by p53 mutants p53–143A, p53–248W, p53–273H, and p53–281G, indicating that certain p53 mutants indeed have a dominant effect over wt-p53 on the regulation of hMMP13 gene expression (Fig. 3A). Increasing the ratio of mutant p53 to wt-p53 from 1:1 to 2:1 to 4:1, or more, in FLS or Saos-2 cells, resulted in a significant reversal of the wt-p53 repression of hMMP13CAT reporter activity by p53 mutant p53–281G. Saos-2 cells were transfected with varying amounts of the wt-p53 expression plasmid, pCMV-p53, together with 1.2 μg of −1599hMMP13CAT and 0.4 μg of p53-luci using Lipofetamine in OPTI-MEM I medium for 18 h. The parent vector (pCMV) without the p53 coding sequence was used as control and to maintain the total amount of DNA transfection constant. Cells were then washed and fresh DMEM (for FLS) or McCoy’s 5A (for Saos-2) medium containing 10% serum was added. After 24 h, cells were harvested, and CAT and luciferase activities were assayed. The CAT activity was determined with equivalent amounts of protein extracts by scintillation counting following the CAT assay instructions (Promega, Madison, WI). The p53-luci was used as a positive control, and extracts were assayed for luciferase on an EG & G Berthold Autolumat LB955 Rack Lumimeter. The means ± S.E. were derived from three independent transfections and assays, each run in triplicate. It could be seen that with the increase of p53 expression plasmid transfected, relative reporter activity of p53-luci increased, but promoter activity of −1599hMMP13CAT decreased in a dose-dependent manner.

hMMP13 messenger was repressed by wt-p53 but up-modulated substantially by the p53 mutants p53–175H and p53–281G. Saos-2 cells were transfected with pCMV (the parent vector plasmid) 2nd lane, wt-p53 expressing plasmid-transfected cells; 3rd lane, mutant p53–175H-transfected cells; 4th lane, mutant p53–281G-transfected cells. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. Independent transfections were performed, and similar results were obtained. Fig. 4 shows a representative result.

The Dominant Effect of p53 Mutants Is a Promoter and p53 Mutant-specific Effect—The p53 mutants p53–143A, 175H,

FIG. 1. Dose-dependent repression of hMMP13 promoter activity by wild type p53. FLS or Saos-2 cells (triplicate) were plated at a density of 5 × 10⁵ cells/well in 6-well cluster plates and then cotransfected with varying amounts of the wt-p53 expression plasmid, pCMV-p53, together with 1.2 μg of −1599hMMP13CAT and 0.4 μg of p53-luci using Lipofetamine in OPTI-MEM I medium for 18 h. The parent vector (pCMV) without the p53 coding sequence was used as control and to maintain the total amount of DNA transfection constant. Cells were then washed and fresh DMEM (for FLS) or McCoy’s 5A (for Saos-2) medium containing 10% serum was added. After 24 h, cells were harvested, and CAT and luciferase activities were assayed. The CAT activity was determined with equivalent amounts of protein extracts by scintillation counting following the CAT assay instructions (Promega, Madison, WI). The p53-luci was used as a positive control, and extracts were assayed for luciferase on an EG & G Berthold Autolumat LB955 Rack Lumimeter. The means ± S.E. were derived from three independent transfections and assays, each run in triplicate. It could be seen that with the increase of p53 expression plasmid transfected, relative reporter activity of p53-luci increased, but promoter activity of −1599hMMP13CAT decreased in a dose-dependent manner.

FIG. 2. p53 mutants either lose repression activity or up-modulate the gene expression of hMMP13. A, p53 mutants lose wt-p53 repressor activity. One set of the FLS was cotransfected with −1599hMMP13CAT (1.2 μg) together with 100 ng of wt-p53- or p53 mutant-expressing plasmids. The other sets were transfected with −1599hMMP13CAT together with an equal amount of pCMV as control. Relative promoter activity was calculated by arbitrarily setting the activity of the control (cotransfected with parent vector) as 100%. All six of the p53 mutants tested lost wt-p53 repressor activity in FLS (filled bars) and 5 of the p53 mutants lost wt-p53 repressor activity completely in Saos-2 cells (open bars). For Saos-2 cells, 0.3 μg of pCMV-β-galactosidase was included in a parallel transfection experiment, and cells were stained for β-galactosidase as detailed under “Materials and Methods.” The average percentage of cells stained blue was within the range of 4–6% for all transfections. B, dose-dependent stimulation of −1599hMMP13CAT reporter activity by p53 mutant p53–281G. Saos-2 cells were cotransfected with varying amounts of p53–281G plasmid together with 1.2 μg of −1599hMMP13CAT plasmid. The parent vector without the p53 coding sequence was used to maintain the total amount of DNA-transfected constant. The CAT activity was determined approximately 40 h after transfection. Three independent transfections, each run in triplicate, were performed, and the results are expressed as the means ± S.E. C, repression of endogenous hMMP13 messenger by wt-p53. Total RNA was isolated from Saos-2 cells after p53 transfection and subjected to Northern blot analysis. Northern blot showed that hMMP13 messenger was repressed slightly by wt-p53 but up-modulated substantially by the p53 mutants p53–175H and p53–281G. 1st lane, Saos-2 cells were transfected with pCMV (the parent vector plasmid); 2nd lane, wt-p53-expressing plasmid-transfected cells; 3rd lane, mutant p53–175H-transfected cells; 4th lane, mutant p53–281G-transfected cells. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

FIG. 3. Mutant-specific Effect—The p53 mutants p53–143A, 175H,
p53–281G-transfected cells. lane 7, from p53–248W-transfected cells; lane 6, from p53–143A-transfected cells; lane 5, from wt-p53-expressing plasmid-transfected cells; lane 4, Saos-2 cells transfected with pCMV (the parent vector plasmid); lane 3, from p53–175H-transfected cells; lane 2, from p53–280T-transfected cells; lane 1, from Saos-2 cells transfected with p5CMV and p53–281G mutant proteins in FLS (A), and by overexpression of p53–143A, p53–273H and p53–281G mutant proteins in Saos-2 (B). The means ± S.E. were derived from three independent transfections and assays, each run in triplicate. Relative promoter activity was calculated by arbitrarily setting the activity of the control as 100.

FIG. 3. Dominant effect of mutant p53 over wt-p53. FLS (A) or Saos-2 (B) cells were cotransfected with varying amounts of p53 mutant expression plasmids and a fixed amount of wt-p53 expression plasmid together with 1.2 μg of −1599hMMP13CAT. The parent vector without the p53 coding sequence was used to maintain the total amount of DNA transfected constant. The CAT activities were determined approximately 40 h after transfection. The repression of hMMP13CAT reporter activity by wt-p53 protein could be reversed by overexpression of p53–143A, p53–248W, p53–273H, and p53–281G mutant proteins in FLS (A), and by overexpression of p53–143A, p53–273H and p53–281G mutant proteins in Saos-2 (B). The means ± S.E. were derived from three independent transfections and assays, each run in triplicate. Relative promoter activity was calculated by arbitrarily setting the activity of the control as 100.

FIG. 4. Approximately equivalent amounts of mutant and wt-p53 protein were expressed. Saos-2 cells were transfected with empty vector, wt-p53, or mutant p53-expressing plasmids. Total p53 protein was determined in cell lysates by Western blot analysis. No p53 protein was detected in the empty vector-transfected cells (lane 1), and transfection with wt-p53 and mutant p53 expression plasmids increased the immunoreactive p53 protein levels. Approximately equal amounts of p53 were detected in wt-p53 and mutant p53-transfected cells. Three independent transfections were performed, and similar results were obtained. Figure shows a representative result. Protein samples are as follows: Lane M, molecular weight marker; lane 1, from Saos-2 cells transfected with pCMV and p53–281G mutant proteins; lane 2, from wt-p53-transfected cells; lane 3, from mutant p53–143A-transfected cells; lane 4, from p53–175H-transfected cells; lane 5, from p53–248W-transfected cells; lane 6, from p53–273H-transfected cells; lane 7, from p53–280T-transfected cells; and lane 8, from p53–281G-transfected cells.

280T, and 281G, which have been shown to lose wt-p53 activity to repress the promoter activity of hMMP1 (1), were tested to see whether they were dominant over wt-p53 in the regulation of the promoter activity of hMMP1 in Saos-2 cells. Results revealed that among the four p53 mutants only p53–281G was dominant over wt-p53 (Fig. 5A). Complete dominance of wt-p53 over mutant was observed for p53 mutant p53–143A, p53–143A, p53–248W, p53–273H, and p53–281G mutant proteins in FLS (A), and by overexpression of p53–143A, p53–273H and p53–281G mutant proteins in Saos-2 (B). The means ± S.E. were derived from three independent transfections and assays, each run in triplicate. Average promoter activity was calculated by arbitrarily setting the activity of the control as 100.

FIG. 5. The dominant effect of p53 mutants is a promoter and p53 mutant-specific effect. A, Saos-2 cells were cotransfected with increasing amounts of p53 mutant expression plasmids and fixed amounts of wt-p53 expression plasmid together with 1.2 μg of −4372hMMP1luci. The parent vector without the p53 coding sequence was used to maintain the total amount of DNA transfected constant. The luciferase activities were determined approximately 40 h after transfection. Results revealed that among the four p53 mutants only p53–281G was dominant over wt-p53. The mutant p53–143A, which was dominant over wt-p53 in the regulation of the promoter activity of hMMP13, lost its dominant effect over wt-p53 completely in the regulation of the promoter activity of hMMP1. The means ± S.E. were derived from three independent transfections and assays, each run in triplicate. Relative promoter activity was calculated by arbitrarily setting the activity of the control as 100. B, Saos-2 cells were cotransfected with increasing amounts of p53 mutant expression plasmids and fixed amounts of wt-p53 expression plasmid together with 1 μg of p53-luci. The parent vector without the p53 coding sequence was used to maintain the total amount of DNA transfected constant. The luciferase activities were determined approximately 40 h after transfection. Results revealed that reversions of the reporter activity of p53-luci were observed for p53 mutants p53–143A (circles), p53–273H (reverse triangles), p53–280T (diamonds), and p53–281G (hexagons). However, wt-p53 was completely dominant over p53–175H (squares) and p53–248W (triangles). Three independent transfections and assays, each run in triplicate, were performed, and 20–40% of variation in the luciferase activity of p53-luci was observed from experiment to experiment. Relative promoter activity was calculated by arbitrarily setting the activity of the control as 1 (cotransfected with pCMV). 175H, and p53–280T, despite a 9-fold lower quantity of wt-p53 expression plasmid than mutated p53 expression plasmid used in cotransfection experiments (data not shown). Increasing the ratio of mutant p53–281G to wt-p53 to 3-fold produced a reversal of up to 100%, and the reversal was dose-dependent (Fig. 5A). Further increasing the ratio of p53–281G to wt-p53 led to an induction of the promoter activity of hMMP1. However, the mutant p53–143A, which was dominant over wt-p53 in the regulation of the promoter activity of hMMP13 (Fig. 3), lost its dominant effect completely in the regulation of the promoter activity of hMMP1 (Fig. 5A). These results indicate that the dominant effect of p53 mutants over wt-p53 and vice versa is a promoter- and p53 mutant-specific effect. To exclude the possibility that our observation is an artifactual effect, we cotransfected the cells with hMMP13CAT and hMMP1luci together with wt-p53 and mutant p53–143A expression plasmids. With
the increase of the amount of p53–143A expression plasmid, the repression of hMMP13CAT by wt-p53 was gradually reversed, but the repression of hMMP11luci by wt-p53 was maintained (data not shown).

The effect of p53 mutants was also tested with the p53-luci reporter construct. As expected, all six of the p53 mutants tested lost the ability to activate p53-luci almost completely (data not shown). When cotransfected with wt-p53 expression plasmid, a 35–65-fold activation of the reporter activity of p53luci was observed, and when cotransfected with the p53 mutants, a range from a 2-fold repression to a 2-fold activation was observed (data not shown). In addition, results revealed that reversion of the reporter activity of p53-luci activated by wt-p53 was observed for p53 mutants p53–143A (Fig. 5 B, circles), p53–273H (Fig. 5B, reverse triangles), p53–280T (Fig. 5B, diamonds), and p53–281G (Fig. 5B, hexagons) indicating that the four mutants above have some dominant effect over wt-p53 protein in the transactivation of p53-luci. However, wt-p53 was completely dominant over p53–175H (Fig. 5B, squares) and p53–248W (Fig. 5B, triangles). Next, we cotransfected the Saos-2 cells with hMMP13CAT and p53-luci together with wt-p53 and mutant p53–280T expression plasmids. With the increase of the amount of p53–280T expression plasmid, the repression of hMMP13CAT by wt-p53 was maintained, but the activation of p53-luci by wt-p53 was gradually reversed (data not shown). These results further confirm that the dominant effect of p53 mutants over wt-p53 and vice versa is a promoter- and p53 mutant-specific effect.

**DISCUSSION**

Numerous studies have indicated that changes in the synovial membrane in RA share common features with the process of tumor invasion (34–38). Synovial hyperplasia is accompanied by the occurrence of cells with a transformed appearing phenotype (39) and the expression of oncopogenes (40–44). The discovery that p53 is overexpressed in RA synovium and that the p53 gene in RA is abnormal further highlights the common features shared between RA synovial cells and cancer cells. Several recent studies have implied that p53 inactivation in cancer cells might contribute to elevated MMP expression (45, 46). Our data here show directly for the first time that hMMP13 is indeed a p53 target gene subject to repression and that mutant p53 either loses this repression activity or up-modulates the promoter activity of the hMMP13 gene. It has been reported that wt-p53 represses, whereas mutant p53 activates, transcription driven by the multidrug resistance gene, the human interleukin-6 gene, the human basic fibroblast growth factor gene, and c-MYC (27–30). Here we report another case where mutant p53 gains a function as it transactivates transcription, whereas wt-p53 represses transcription driven by the hMMP13 gene promoter. The activation of hMMP13 promoter by p53–281G suggests a possible direct interaction between p53–281G and the promoter that is currently under examination.

hMMP13 was originally isolated from breast carcinoma (8), and it has recently been found in some other tissues related to malignancy (47, 48). It has also been found in the synovium of RA and osteoarthritis patients, but the overall expression of MMP13 was higher in RA synovium compared with osteoarthritis synovium (6). Our findings that hMMP13 is a p53 target gene raises the possibility that the regulation of hMMP13 gene transcription by p53 might be an important regulatory mechanism of hMMP13. The higher hMMP13 protein expression and accumulation in RA and in certain tumors could be attributed to the combined effects of the activation by cytokines/growth factors and the inactivation of negative regulator(s) of hMMP13 gene transcription, such as the p53 tumor repressor protein.

Because up to 20–40% of p53 cDNA isolated from RA synovium has been found to harbor mutation (13), we asked whether wt-p53 or mutant p53 was clearly dominant. The configuration of one wild type and one mutated allele is a necessary intermediate step on the pathway toward complete loss of wt-p53 and possibly relevant to RA disease progression. The repression of hMMP13CAT reporter activity by wt-p53 could be reversed by most of the p53 mutants examined indicating that p53 mutants indeed have some dominant effect over wt-p53 on the regulation of hMMP13 gene expression. However, the complete dominance of wt-p53 over certain p53 mutants suggests that in certain cells both wt-p53 alleles must be lost for a complete loss of wt-p53 activity in the regulation of hMMP13 gene expression.

Our results have shown that that the dominant effect of p53 mutants over wt-p53 and vice versa is a promoter- and mutant-specific effect. This promoter- and mutant-specific effect was further confirmed by cotransfection assays. We have cotransfected the cells with hMMP13CAT and hMMP11luci together with wt-p53 and mutant p53–143A expression plasmids or hMMP13CAT and p53luci together with wt-p53 and mutant p53–280T expression plasmids. With the increase of the amount of mutant p53 expression plasmid, the regulation of one of the two cotransfected promoter by wt-p53 was gradually reversed, whereas the regulation of the other promoter was maintained (data not shown). Therefore, only certain genes will be dysregulated if one wt-p53 allele is lost or mutated. These results indicated that the regulation of gene promoters by p53 and p53 mutants is much more complex than previously thought. The promoter-specific and mutant-specific effects suggest a direct regulation of the hMMP-13 and MMP-1 promoters by p53 and its mutants. It also suggests that p53 mutants not only act to inactivate the wt-p53 and that a direct interaction between p53 mutant and the promoter may exist.

Another interesting finding was that the reporter activity of p53-luci was increased further when low amounts of mutant p53 were cotransfected with wt-p53 (Fig. 5B). This phenomenon suggests that the transcription complex formed by wt-p53 and mutant p53 at a ratio 1:1 has a higher transactivation activity than the transcription complex formed by wt-p53. One explanation for this observation is that p53 mutants inactivate the wt-p53 repressor activity more effectively than they inactivate the wt-p53 transactivation activity. Since the minimal promoter of p53-luci contains the luciferase reporter gene driven by a basic promoter element (TATA box) plus tandem repeats of the consensus binding sequence for p53, wt-p53 may transcriptionally regulate the minimal promoter of p53-luci through two mechanisms as follows: transactivation through binding to the consensus p53-binding sites, and repression through interfering with the binding basal transcription factors to the TATA motif within the minimal promoter of p53-luci (49, 50). The transcription complex formed by wt-p53 and mutant p53 at a ratio 1:1 may have lost the wt-p53 repressor activity but still maintains most of the wt-p53 transactivation activity; therefore, the net effect is the further increase of the reporter activity of p53-luci. With the increase of the ratio of mutant p53 to wt-p53, the transactivation activity of wt-p53 was gradually reversed.

Unlike the activation of gene expression, the mechanism of repression of gene expression by wt-p53 is not well defined. p53 can bind to the TATA-binding protein and repress its promoter activity (51, 52). p53 has also been demonstrated to interact with many other transcription factors as follows: Sp1 (53), CCAAT-binding factor (54), cAMP-response element-binding protein (55), and glucocorticoid receptors (56). A cis-acting el-
Regulation of hMMP-13 Gene Expression by p53

...ement, G(G/C)AA(G/C)TG(T/GA) capable of mediating the down-regulation by p53 was mapped within the Rb promoter (57). Examination of the hMMP-13 promoter indicates that there are two sites (−378GGAAGTAA and −82GGAAGTG) that closely match the sequence G(G/C)AA(G/C)TG(T/GA) identified in the Rb promoter. In addition there is a p53 response element located in the −123 to −97 region of the hMMP13 promoter, a CCAAT box in −500 and AP-1 in −56. The potential role of these sites in the regulation of hMMP13 by wt-p53 and mutant p53, especially p53−/−G1, is currently under examination in our laboratory.

In recent years many genes that are believed to play an important role in RA pathogenesis have been shown to be down-regulated by p53 (1, 23, 28–30). Interestingly, within the hMMP13 gene. Although the significance that p53 mutations cause the dysregulation of gene expression of hMMP13, hMMP-1, and other genes in the process of joint degeneration in RA is currently not clear, an association of overexpression of MMPs and synovial hyperplasia with the inactivation of p53 may exist and warrants further investigation.

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