Ultraviolet A-induced Production of Matrix Metalloproteinase-1 Is Mediated by Macrophage Migration Inhibitory Factor (MIF) in Human Dermal Fibroblasts

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Hirokazu Watanabe†, Tadamichi Shimizu†, Jun Nishihira§, Riichiro Abe†,
Toshinori Nakayama¶, Masaru Taniguchi**, Hisataka Sabe‡, Teruo Ishibashi§,
and Hiroshi Shimizu††††

From the Departments of †Dermatology and ‡Molecular Biochemistry, Hokkaido University Graduate School of Medicine, Kita-ku, Sapporo 060-8638, Japan, the †§Department of Medical Immunology and ‡§Department of Molecular Immunology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana Chuo-ku, Chiba 260-8670, Japan, the †‡Laboratory for Immune Regulation, RIKEN Research Center for Allergy and Immunology, Yokohama, 230-0045, Japan, and the ‡‡Department of Molecular Biology, Osaka Bioscience Institute, Osaka 565-0874, Japan

Matrix metalloproteinases (MMPs) are thought to be responsible for dermal photaging in human skin. In the present study, we evaluated the involvement of macrophage migration inhibitory factor (MIF) in MMP-1 expression under ultraviolet A (UVA) irradiation in cultured human dermal fibroblasts. UVA (20 J/cm²) up-regulates MIF production, and UVA-induced MMP-1 mRNA production is inhibited by an anti-MIF antibody. MIF (100 ng/ml) was shown to induce MMP-1 in cultured human dermal fibroblasts. We found that MIF (100 ng/ml) enhanced MMP-1 activity in cultured fibroblasts assessed by zymography. Moreover, we observed that fibroblasts obtained from MIF-deficient mice were much less sensitive to UVA regarding MMP-1 expression than those from wild-type BALB/c mice. Furthermore, after UVA irradiation (10 J/cm²), dermal fibroblasts of MIF-deficient mice produced significantly decreased levels of MMP-13 compared with fibroblasts of wild-type mice. Next we investigated the signal transduction pathway of MIF. The up-regulation of MMP-1 mRNA by MIF stimulation was found to be inhibited by a PKC inhibitor (GF109203X), a Src-family tyrosine kinase inhibitor (herbimycin A), a tyrosine kinase inhibitor (genistein), a PKA inhibitor (H89), a MEK inhibitor (anisomycin), and a JNK inhibitor (SP600125). In contrast, the p38 inhibitor (SB203580) was found to have little effect on expression of MMP-1 mRNA. We found that PKCα, PKCβII, PKCδ (Thr68), PKCδ (Ser643), Raf, and MAPK were phosphorylated by MIF. Moreover, we demonstrated that phosphorylation of PKCαβII and MAPK in response to MIF was suppressed by genistein, and herbimycin A as well as by transfection of the plasmid of C-terminal Src kinase. The DNA binding activity of AP-1 was significantly up-regulated 2 h after MIF stimulation. Taken together, these results suggest that MIF is involved in the up-regulation of UVA-induced MMP-1 in dermal fibroblasts through PKC-, PKA-, Src family tyrosine kinase-, MAPK-, c-Jun-, and AP-1-dependent pathways.

The skin is an important barrier that protects the body from damage due to direct contact with the outside environment, including trauma, bacterial infection, and ultraviolet (UV) irradiation. Regarding the environmental damage to skin, the most common physical injury is that caused by UV irradiation. UV irradiation substantially increases the risk of actinic damage to the skin. Interstitial collagens, the major structural components of the dermis, have been found to be particularly diminished in skin actinically damaged by UV irradiation (1–3). Quantitative and qualitative changes in the dermal extracellular matrix proteins such as elastin, glycosaminoglycans, and interstitial collagens are also associated in dermal photodamage. There are several morphological and biochemical indications that collagen type I is reduced in UV actinically damaged skin (4). Various types of UV-induced matrix-degenerating metalloproteinases present in dermal fibroblasts contribute to the breakdown of dermal interstitial collagen and other connective tissue components.

As for the underlying biological mechanisms of action involved in skin damage, the skin is known to secrete a number of cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)α (5–7). UV irradiation up-regulates the production of these cytokines, and UV-induced collagenases such as matrix metalloproteinase (MMP)-1 from dermal fibroblasts are mediated in part by IL-1α and IL-1β (6). Furthermore, collagenase activity has been shown to be inhibited by a tissue inhibitor of metalloproteinases (TIMP) (8).

Macrophage migration inhibitory factor (MIF), originally identified as a lymphokine that concentrates macrophages at inflammatory loci, is a potent activator of macrophages in vivo and is considered to play an important role in cell-mediated immunity (9, 10). It has been reported that MIF is expressed primarily by T cells and macrophages; recent studies have however revealed that this protein is ubiquitously expressed by various cells, thus indicating its involvement beyond the immune system in a variety of pathologic states (11, 12). It is of interest that MIF functions as a cytokine, an anterior pituitary hormone, and an anti-tumor protein.
MIF Mediates MMP-1 Expression by UVA

EXPERIMENTAL PROCEDURES

Materials—The following materials were obtained from commercial sources. Genistein, herbimycin A, PP2, GF109203X, H89, PD98059, SB203580, and SP600125 were purchased from Calbiochem (San Diego, CA); Dulbecco’s modified Eagle’s medium (DMEM) from Invitrogen (Groningen, Netherlands); Dig Gel Shift kit and FuGENE 6 was from Roche Applied Science (Mannheim, Germany); the Isogen RNA extraction kit was from Nippon Gene (Toyama, Japan); the Biotrack MMP-1 assay kit, [32P]dCTP, and Hybond N nylon membrane were from Amersham Biosciences (Piscataway, NJ); the consensus AP-1 oligonucleotide (Madison, WI). To examine the signal transduction pathway in the dermal fibroblasts were stimulated with or without MIF and various inhibitors of molecules involved in the signal transduction pathway for 24 h. Total RNA was isolated from monolayered cultures using an Isogen RNA extraction kit according to the manufacturer’s protocols. RNA was quantified by spectrophotometry, and equal amounts of RNA (5–10 μg) from samples were loaded on a formaldehyde-agarose gel. The gel was stained with ethidium bromide to visualize the RNA standards, and the RNA was transferred onto a nylon membrane. Fragments obtained by restriction enzyme treatments for MMP-1, TIMP-1, GAPDH, and MIF were labeled with [32P]dCTP using a DNA random primer labeling kit. Hybridization was carried out at 42 °C for 24 h. The blots were then washed with 0.1× SSC (1× SSC: 0.15 M NaCl, 0.015 M sodium citrate) at 65 °C for 2 h. The radioactive bands were visualized by autoradiography on Kodak X-AR5 film and quantitatively analyzed using the NIH Image system. Multiple autoradiographic data were examined to ensure that the results reflected those produced in the linear range of the film. The results were normalized by GAPDH mRNA levels. Comparison of actin and nidom bromide-stained gels with the corresponding GAPDH mRNA levels showed that GAPDH mRNA levels reflected the total RNA loaded onto the gels.

ELISA for MIF—To examine the concentration of MIF from cultured fibroblasts, supernatants from cultured fibroblasts by UVA were examined. The assay can be adapted for a MIF ELISA CO, an essentially similar (19). For this assay we used recombinant human MIF to obtain the standard curve, in which good linearity was demonstrated between MIF concentrations (1 to 200 ng/ml) and absorbency. ELISA for MMP-1—After reaching confluence, the cells were trypsinized and then plated on a 24-well culture dish at 4 × 10^4 cells per well in 0.5 ml of DMEM containing 10% FCS. After 48 h, the medium was replaced with 0.5 ml of serum-free DMEM containing various doses of MMP (0, 0.1, 1, 10, and 100 ng/ml). After 24 h, the supernatants were collected and subjected to ELISA for MMP-1. The protein level values for MMP-1 were assigned to those of the supernatants. For the time-course study, we used a procedure similar to that used for the dose response and for the MMP-1 assay, except that the supernatants were collected and subjected to ELISA for MMP-1. The minimal sensitivity of the test system was 6.25 ng/ml, and good linearity was observed at amounts up to 100 ng/ml. Using this ELISA system, all forms of MMP, including both the pro-MMP-1, MMP-1, and MMP-bone sialoproteins with TIMP-1, could be measured.

Determination of MMP-1 Activity in Culture Media of Fibroblasts—Culture media of dermal fibroblasts were collected at the indicated intervals in the presence of MIF (100 ng/ml) for up to 48 h, and concentrated (10-fold) using Centriprep YM-30 (Millipore, Bedford, MA), and subjected to Western blot analyses. Then, the cells were cultured in DMEM supplemented with 10% FCS and then further incubated for 24 h. Expression of MMP-1 mRNA and protein levels were assessed by Northern blot and Western blot analyses. The abnormally of MIF-deficient mice or WT mice were carefully shaved, and irradiated with UVA with damaging dose (300J/cm2). After irradiation, cells were cultured in DMEM supplemented with 10% FCS for 24 h, and MMP-1 expression was evaluated by Western blot analysis.

Northern Blot Analysis—Complete coding cDNA for human MMP-1 in a pSP64 vector was obtained from the American Type Culture Collection. Templates of human TIMP-1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA for Northern blot analyses were obtained by reverse transcription-polymerase chain reaction (RT-PCR) from human RNA library of human primary dermal fibroblasts. Preparation of each template proceeded using the following primers: TIMP-1 (535 bp), forward primer 5’-TCTCTGTTGTTGCTTAGTGAT-3’ and reverse primer 5’-CAGCAAGGTGACGGGACTGAAGG-3’, GAPDH (306 bp), forward primer 5’-CGGAGTCACAGGATTGGTGTGGT-3’ and reverse primer 5’-AGCCCTTCTCATGGTGTGGTGAACGC-3’. Preincubation of the RNA samples with the DNA (5 ng) was performed in 100 ng anti-MMP-1 antibody, and were incubated at room temperature and electrophoresed until the dye-stained gels with the corresponding GAPDH mRNA levels. The results were normalized by GAPDH mRNA levels showed that GAPDH mRNA levels reflected the total RNA loaded onto the gels.

UVA Irradiation—Dermal fibroblasts were obtained from MIF-deficient mice and control WT mice. Newborn mouse skin was carefully shaved, a segment of skin excised, and fibroblasts were obtained using the standard explant technique. Briefly, the skin was cut into 3 × 5-mm pieces and placed onto large Petri dishes with the subcutaneous side down. Once a sufficient number of fibroblasts had migrated out from the skin sections, pieces of the skin were removed and the cells were passaged by the use of a plastic lid covered with aluminum foil onto a flat-bottomed 6-well plate. After irradiation, the cells were cultured in DMEM supplemented with 10% FCS at 37 °C. Control samples were mock-irradiated and maintained under the same culture conditions as those used for the UVA-irradiated specimens. To examine the effects of anti-MIF antibody on the UVA-induced MMP-1 mRNA, fibroblasts were UVA-irradiated (20 J/cm2) in the presence of an anti-MIF antibody (1 and 10 μg/ml) in DMEM supplemented with 10% FCS and then further incubated for 24 h. Expression of MMP-1 mRNA and protein levels were assessed by Northern blot and Western blot analyses. The abnormally of MIF-deficient mice or WT mice were carefully shaved, and irradiated with UVA (0–30 J/cm2). After UVA irradiation for 24 h, skin was surgically obtained and was assessed by Western blot analysis. Dermal fibroblasts from MIF-deficient mice or WT mice were harvested after achieving 70% confluence. The cells were washed twice with PBS and exposed to a UVA fluorescent lamp for the indicated doses (0–10 J/cm2). After irradiation, cells were cultured in DMEM supplemented with 10% FCS for 24 h, and MMP-1 expression was evaluated by Western blot analysis.

It is of note that UVA irradiation reaches the reticular dermis, rendering fibroblasts accessible targets (14). In the skin, MIF is expressed in the epidermis, particularly in the basal layer (15). However, the precise role of MIF in the dermis and the effects of UVA on MIF expression of dermal fibroblasts remain to be elucidated. In the present study, we attempted to determine whether MIF mediates the up-regulation of MMP-1 expression in response to the stimulation of UVA irradiation. We also investigated the signal transduction pathway of MIF in human dermal fibroblasts.

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front was near the bottom of the gel. To produce the enhancing effects, 10 ml heparin (0.3 mg/ml in 1× sample buffer without SDS) was added to the lanes 20–30 min after electrophoresis began. Each gel was washed twice with 2.5% Triton X-100, 50 mM Tris, pH 7.5, 4 °C, 20 min each, to remove SDS and then two times with buffer plus 5 mM CaCl₂. The gel was washed three times with incubation buffer (50 mM Tris, pH 7.5, 5 mM CaCl₂) and then incubated in this buffer with added protease inhibitors (50 μM each of z-phe-chloromethylketone and tosyl-phenylchloromethylketone) for 18 h at 37 °C with gentle shaking. Gels were stained with 0.1% Coomassie Blue in 40% MeOH, 10% acetic acid, for 45 min, and destained with 7% acetic acid. A positive control for recombinant human MMP-1 was used to detect the molecules of MMP-1.

Western Blot Analysis—Cells (1 × 10⁶ cells) were disrupted with a Polytron homogenizer (Kinematica, Lucerne, Switzerland). The protein concentrations of the cell homogenates were quantified using a Micro BCA protein assay reagent kit. Equal amounts of homogenates were homogenized (Kinematica, Lucerne, Switzerland). The protein concentrations of the cell homogenates were quantified using a Micro BCA protein assay reagent kit. Equal amounts of homogenates were dissolved in 20 μl of 1× sample buffer (50 mM Tris, pH 6.8, containing 2-mercaptoethanol (1%), sodium dodecyl sulfate (SDS) (2%), glycerol (20%), and bromphenol blue (0.04%), and the samples were heated to 100 °C for 5 min. The samples were then subjected to SDS-PAGE and transferred electrochemically onto a nitrocellulose membrane. The membranes were blocked with 1% nonfat dry milk in phosphate-buffered saline, probed with anti-phospho-Raf, or anti-phospho-MAPK antibody, then allowed to react with goat anti-rabbit IgG Ab coupled with horseradish peroxidase. The resultant complexes were processed for the detection system according to the manufacturer’s protocol. To investigate the involvement of tyrosine kinase in PKC phosphorylation, cells were serum-starved for 24 h and challenged with MIF (100 ng/ml) 30 min after the addition of 50 mM sodium pyrophosphate (50 μM) in serum-free medium. After 60 min, the cells were harvested and subjected to Western blot for phosphorylation of phospho-PKCα/βII. To investigate the involvement of tyrosine kinase and PKC in the phosphorylation of MAPK by MIF, fibroblasts pretreated with inhibitors against tyrosine kinase and PKC for 30 min were stimulated with MIF for 60 min. Then cell lysates were prepared and subjected to Western blot analysis. For loading controls, we carried out Western blot analysis on β-actin using an anti-β-actin antibody.

Transfection of CSK and CSK—At 24 h after plating the fibroblasts on 6-well dishes, plasmid DNAs of CSK and CSK were transfected using FuGENE 6 according to the manufacturer’s protocol. For each dish, CSK or CSK plasmid DNA (1 μg) was transfected to cultured cells in serum-free medium for 24 h. Following this, cells were stimulated with MIF (100 ng/ml) for 60 min. The cell lysates were prepared and subjected to Western blot analysis for phosphorylation of PKC and MAPK.

Electrophoretic Gel Mobility Shift Assay (EMSAs)—Human dermal fibroblasts were incubated with 500 ng/ml MIF for the indicated times, and the nuclear proteins were extracted. The consensus AP-1 oligonucleotide was annealed and labeled with digoxigenin-11-deUTP. To prevent non-specific binding, 0.1 μg of poly(dI-C)) was added to the binding reaction. The mixture was transferred to a 6% polyacrylamide gel and submitted to gel electrophoresis. Following electrophoresis, the oligonucleotide-protein complexes were electroblotted to a Nylon membrane. The digoxigenin-labeled DNA oligonucleotides were visualized by an enzyme immunoassay using anti-digoxigenin-AP, Fab-fragments, and the chemiluminescent substrate CSPD, as described by the manufacturer (Roche Applied Science). The generated chemiluminescence was visualized on x-ray film.

Statistics—Values are expressed as means ± S.E. of the respective test or control group. Statistical significances between the control group and test groups were evaluated by the Student’s t test. Data are representative of at least three experiments.

RESULTS

MIF Production in Response to UVA Irradiation in Human Dermal Fibroblasts—We first examined whether UVA is able to stimulate production of MIF in dermal fibroblasts. Fibroblasts were irradiated with UVA, and it was found that UVA up-regulated MIF production in a dose-dependent manner (Fig. 1). After 24-hr UVA stimulation at an intensity of 20 J/cm², the MIF content was remarkably elevated, showing a more than 8-fold increase compared with levels in the absence of UVA stimulation.

Effects of MIF on MMP-1 and TIMP-1 mRNA Expression—In human dermal fibroblasts, MMP-1 mRNA was up-regulated in a dose-dependent manner in response to MIF, ranging from 1 ng/ml to 100 ng/ml for per 24-hr treatment (Fig. 2a). A time-course study of MMP-1 and TIMP-1 in dermal fibroblasts was then performed. MMP-1 mRNA expression increased in response to MIF (100 ng/ml) at 1 h post stimulation and reached a maximum at 24 h (Fig. 2b). MMP mRNA levels were slightly down-regulated at 48 h. TIMP-1 mRNA was also elevated, but the increase was less significant than MMP-1 mRNA, although the levels were sustained for at least 48 h.

MMP-1 Production and Activation in Response to MIF—MMP-1 protein was detected in the culture supernatant of human dermal fibroblasts. In a dose-response study, the MMP-1 protein levels were significantly up-regulated at doses of 100 ng/ml (Fig. 3a). For the time-course study, MMP-1 in the supernatant was elevated at 12 h after MIF stimulation (100 ng/ml), reached a maximum at 24 h, and was sustained for at least 48 h (Fig. 3b). To investigate MIF induced MMP-1 activity, zymography was performed. We used heparin to enhance the signal, because MMP-1 is difficult to detect at low levels in

FIG. 1. Induction of MIF in human fibroblasts by UVA radiation. Fibroblasts were treated with UVA radiation and cultured for 24 h. MIF in the culture media was measured by ELISA, as described under “Experimental Procedures.” The values are the mean ± S.E. of three different experiments. *p < 0.01 and **, p < 0.05 for 20 J/cm² versus 0 J/cm².

FIG. 2. Effects of MIF on MMP-1 mRNA expression in dermal fibroblasts. Total RNAs extracted from dermal fibroblasts were treated with various concentrations of MIF in serum-free medium at the indicated intervals. Northern blot analysis was carried out as described under “Experimental Procedures.” The membranes were hybridized with radiolabeled cDNA probes of MMP-1 and GAPDH and then visualized by autoradiography. a, the dose-dependent expression of MMP-1 mRNA expression in response to MIF ranging from 0 to 100 ng/ml after 24 h-MIF stimulation. b, time-dependent expression of MMP-1 mRNA in response to MIF (100 ng/ml).
MIF Mediates MMP-1 Expression by UVA

Fig. 3. Effects of MIF on the production and activation of MMP-1 in dermal fibroblasts. a, aliquots of the culture supernatants of dermal fibroblasts in serum-free medium were collected after treatment with various concentrations of MIF for 24 h, then subjected to ELISA for MMP-1 (n = 5). **, p < 0.01 and *, p < 0.05 versus control (0 ng/ml). b, culture supernatants of dermal fibroblasts were collected at the indicated times in the presence of 100 ng/ml MIF for up to 48 h and then subjected to ELISA on MMP-1 (n = 5). *, p < 0.05 versus control (0 h). c, culture supernatants of dermal fibroblasts were collected at the indicated times in the presence of 100 ng/ml MIF for up to 48 h. The supernatants were concentrated 10-fold and subjected to heparin-enhanced zymography. Molecular weight markers at 56 and 45 kDa show latent and active forms of MMP-1, respectively. Cont, stimulation with recombinant MMP-1 for 48 h.

conventional gelatin zymography (20). MMP-1 in the active form (45 kDa) in fibroblasts was enhanced by 100 ng/ml MIF stimulation, reached a maximum at 24 h, and slightly decreased at 48 h (Fig. 3c).

Inhibition of MMP-1 Production of Dermal Fibroblasts by a Neutralizing anti-MIF Antibody—We attempted to determine whether neutralizing anti-MIF antibody influences UVA-induced MMP-1 expression in human dermal fibroblasts. By Northern blot analysis, we found that the anti-MIF antibody (1 and 10 μg/ml) significantly down-regulated the expression of MMP-1 mRNA induced by UVA stimulation (20 J/cm²) (Fig. 4a). Based on the results of the Western blot analysis, we confirmed that MMP-1 production is inhibited by the anti-MIF antibody (Fig. 4b).

UVA-induced MMP-13 Production in Cultured Dermal Fibroblasts and Skin Tissue in Vivo from MIF-deficient Mice—To clarify whether synthesis of MIF is required for the UVA-induced collagenase, we used dermal fibroblasts from MIF-deficient mice in the production of mouse collagenase MMP-13. Although MMP-13 plays a restricted role in human tissues, it is the predominant tissue collagenase in rodents. Twenty-four hours after UVA irradiation, a significant decrease in viability was observed only after more than 15 J/cm² UVA irradiation in fibroblasts from MIF-deficient mouse (data not shown); we therefore used up to 10 J/cm² UVA irradiation for experiment.

After 24 h of UVA irradiation (0–10 J/cm²), elevated MMP-13 production was observed in cell lysates of dermal fibroblasts in control WT mice in a dose-dependent manner (Fig. 5a). On the other hand, UVA irradiation appeared to have no effect on MMP-13 production in dermal fibroblasts of MIF-deficient mice. Consistent with these results in vitro, elevated MMP-13 production was also observed in the skin of control WT mice in
Effects of reagents on MIF-induced MMP-1 mRNA expression. Dermal fibroblasts were preincubated for 30 min with various concentrations of inhibitors prior to challenge with MIF. The cells were then incubated for 24 h in the presence or absence of inhibitors and visualized by autoradiography. a, tyrosine kinase inhibitor genistein and Src family tyrosine kinase inhibitor herbimycin A; lane 1, no stimulation; lane 2, genistein 100 μM; lane 3, herbimycin A 10 μM; lane 4, MIF 100 ng/ml; lane 5, MIF 100 ng/ml + genistein 10 μM; lane 6, MIF 100 ng/ml + genistein 100 μM; lane 7, MIF 100 ng/ml + herbimycin A 1 μM; lane 8, MIF 100 ng/ml + herbimycin A 10 μM b, MEK inhibitor PD98089 and p38 inhibitor SB203580; lane 1, no stimulation; lane 2, PD98059 40 μM; lane 3, SB203580 10 μM; lane 4, MIF 100 ng/ml; lane 5, MIF 100 ng/ml + PD98059 10 μM; lane 6, MIF 100 ng/ml + PD98059 40 μM; lane 7, MIF 100 ng/ml + SB203580 5 μM; lane 8, MIF 100 ng/ml + SB203580 10 μM c, PKA inhibitor H89 and PKC inhibitor GF109203X. Lane 1, no stimulation; lane 2, H89 10 μM; lane 3, GF109203X 10 μM; lane 4, MIF 100 ng/ml; lane 5, MIF 100 ng/ml + H89 1 μM; lane 6, MIF 100 ng/ml + H89 10 μM; lane 7, MIF 100 ng/ml + GF109203X 1 μM; lane 8, MIF 100 ng/ml + GF109203X 10 μM.

Phosphorylation of PKC, Raf, and MAPK—an important step for downstream signaling. Dermal fibroblasts were stimulated with MIF (100 ng/ml). Western blot analysis was performed on whole cell lysates (40 μg) and antibodies against phospho-PKCα/β, PKCδ (Thr505), PKCδ (Ser645), and PKCα/β (Thr410/403) were used. After removal of the original signals, we carried out Western blot analysis of β-actin on the same membranes as loading controls for each PKC isoform as described under “Experimental Procedures.” Since the patterns of protein bands of β-actin for all 5 isoforms were similarly detected, we present the results of β-actin on phospho-PKCα/β as a representative at the bottom of the lanes.

Phosphorylation of Raf and MAPK in response to MIF. Dermal fibroblasts were preincubated for 30 min with various concentrations of inhibitors prior to challenge with MIF similar to the procedure in Fig. 6. The cells were then incubated for 24 h in the presence or absence of JNK inhibitor SP600125. Lane 1, no stimulation; lane 2, SP600125 30 μM; lane 3, MIF 100 ng/ml; lane 4, MIF 100 ng/ml + SP600125 3 μM; lane 5, MIF 100 ng/ml + SP600125 30 μM. 

Effects of JNK inhibitor on MIF-induced MMP-1 mRNA expression. Dermal fibroblasts were stimulated for the indicated time intervals (0–120 min) by MIF (100 ng/ml). Western blot analysis was performed on whole cell lysates (40 μg) and antibodies against phospho-PKCα/β, and anti-phospho-p44/p42-MAPK were used. Western blot analysis for β-actin is shown as a control.
Fig. 10. Effects of inhibitors on MIF-induced phosphorylation of PKC and MAPK. Phosphorylation of PKCα/βII and MAPK in dermal fibroblasts induced by MIF was examined in the presence of various inhibitors against PKC and tyrosine kinases. a, MIF-induced PKC phosphorylation was measured at 60 min in the presence or absence of tyrosine kinase inhibitors, PP2, genistein, and herbimycin A. Western blot analysis of the cell lysates (40 µg) was carried out using a phospho-PKCα/βII antibody. Lane 1, control; lane 2, MIF 100 ng/ml; lane 3, MIF 100 ng/ml + PP2 10 µM; lane 4, MIF 100 ng/ml + genistein 100 µM; lane 5, MIF 100 ng/ml + herbimycin A 10 µM. Western blot analysis for β-actin is shown as a control. b, MIF-induced MAPK phosphorylation was evaluated at 60 min in the presence or absence of tyrosine kinase inhibitors (genistein, herbimycin A), and PKC inhibitor GF109203X. Lane 1, control; lane 2, MIF 100 ng/ml; lane 3, MIF 100 ng/ml + genistein 100 µM; lane 4, MIF 100 ng/ml + herbimycin A 10 µM; lane 5, MIF 100 ng/ml + GF109203X 10 µM. Western blot analysis for β-actin is shown as a control.

DNA Binding Activity of AP-1 in Response to MIF—By using the AP-1 consensus oligonucleotide, the DNA binding activities of AP-1 were examined after MIF stimulation (100 ng/ml). The DNA binding activities of AP-1 were significantly up-regulated for up to 120 min. The binding activity was significantly down-regulated with the addition of an excessive amount of non-labeled AP-1 oligonucleotide (100-fold) (Fig. 12).

DISCUSSION

The effects of sunlight have fascinated researchers for decades because nearly every living organism on earth is likely to be exposed to sunlight, including its ultraviolet (UV) fraction. Among sunlight’s detrimental long term effects is skin photaging, which is a well-documented consequence of exposure to UVA and UVB radiation. Photaged skin is biochemically characterized by a predominance of abnormal elastic fibers in the dermis and by a dramatic decrease in distinct collagen types. MMPs are crucial factors involved in the connective tissue remodeling accompanying ultraviolet radiation-induced skin damage.

MIF functions as a pleiotropic cytokine by participating in inflammation and immune responses. MIF was originally discovered as a lymphokine involved in delayed hypersensitivity and various macrophage functions, including phagocytosis, spreading, and cell growth activity (22–24). MIF was recently reevaluated as a proinflammatory cytokine and putative-derived hormone that potentiates endotoxemia (25). This protein is ubiquitously expressed in various organs, including the skin, brain, and kidney (27–33). In the skin, MIF is expressed in the epidermis, particularly in the basal layer (15).

Premature aging of the skin secondary to chronic exposure to UV radiation is primarily due to qualitative and quantitative changes in the dermal extracellular matrix, resulting in increased fragility, reduced recoil capacity, blister formation, and impaired wound healing. Interstitial collagens, the major structural components of the dermis, have been found to be particularly diminished in actinically damaged skin. Although there is a direct role for human dermal fibroblasts and an indirect participation of epidermal keratinocytes in MMP-1 production after UVB irradiation, UVA irradiation is known to reach the reticular dermis, rendering fibroblasts possible targets. Recent studies have shown that UV irradiation significantly affects the coordinated regulation of various MMPs and TIMPs (2). The expression of MMPs is regulated at a transcriptional level by various cytokines and other mediators in both a positive and negative manner under certain physiological conditions. Moreover, the enzyme activities of MMPs are post-translationally controlled by activation of latent proenzymes as well as by interactions with their specific inhibitors, referred to as TIMPs.

It has been reported that the biosynthesis of MMP-1 is up-regulated by 12-O-tetradecanoylphorbol-13-acetate (TPA), cytokines, and growth factors such as IL-1, TNF-α, IL-6, epidermal growth factor, and platelet-derived growth factor, in a variety of cells, including fibroblasts. In contrast, transforming growth factor-β, retinoic acid, and dexamethasone down-regulate MMP-1 (34). We have recently demonstrated that MIF expression is significantly up-regulated by growth factors such as TGF-β and PDGF (35). These findings indicate that the mRNA of metalloproteinases may be precisely regulated through a complex mechanism that includes both growth factors and cytokines. UVA irradiation of human dermal fibroblasts was found to elicit an increase in specific quantities of mRNA and the bioactivities of the cytokines IL-1α and IL-1β; it
then induced interrelated IL-1 autocrine feedback loops, ultimately leading to tissue degradation in photoaging. Singlet oxygen is an early intermediate in the signaling pathway of IL-1-mediating UVA induction of interstitial collagenase. IL-1α and IL-1β may, at least in part, cause the imbalance between MMPs and TIMPs. For example, there have been reports that the generation of singlet oxygen and other reactive oxygen species precedes the induction of IL-1 (36).

We have also demonstrated that UVA stimulation leads to a significant increase in specific MIF mRNA and protein levels in human dermal fibroblasts. This remarkable increase in MIF occurred by UVA irradiation at intensities above 2 J/cm². We also found that IL-1α and IL-1β up-regulate MIF (data not shown). Furthermore, it was found that MIF has the potential to stimulate IL-1β production. Constitutive collagenase synthesis has been reported to be regulated by an IL-1β autocrine mechanism (36). In this study, we demonstrated that anti-MIF neutralizing antibody suppresses the expression of MMP-1 induced by UVA. It is therefore possible that UVA irradiation may stimulate MIF production by an autocrine loop of both MIF and IL-1. MIF up-regulates MMP-1 mRNA as well as protein levels and MMP-1 activity by zymography in dermal fibroblasts. In contrast, TIMP-1 is slightly up-regulated by MIF.

In the present study, we also demonstrated that protein expression of MMP-13 is significantly decreased compared with the levels in control WT-mice after UVA irradiation (10 J/cm²). In the rodent, regulation of MMP-13 most likely plays an important role in extracellular matrix degradation. These results indicate that MIF-deficient skin especially fibroblasts produce less MMP-13 after UVA irradiation. We therefore hypothesize that MIF participates in the production of MMP-13 and is a significant factor in the degradation of the extracellular matrix in the dermis.

The molecular mechanisms of UV-induced MMPs have yet to be defined. UV-induced expression of pro-inflammatory cytokines such as IL-1β and TNF-α may also in part account for the expression of MMPs. IL-1β-induced expression of MMP-1 is mediated by transactivation of the EGF receptor and through an ERK pathway in human keratinocytes. Collagenase synthesis by fibroblasts and keratinocytes involves the PKC second-messenger system, and corticosteroids have been shown to suppress its synthesis at the level of gene transcription. Long-wavelength UV light (UVA, 320–400 nm) stimulates the synthesis of interstitial collagenase and increases PKC activity in wavelengths UV light (UVA, 320–400 nm) stimulates the synthesis of interstitial collagenase and increases PKC activity in human skin fibroblasts in vitro. Ultraviolet irradiation activates growth factor and cytokine receptors on keratinocytes and dermal cells, resulting in downstream signal transduction through an activation of MAP kinase pathways. These signaling pathways converge in the nucleus of cells to induce c-Jun, which heterodimerizes with the constitutively expressed c-Fos to form activated complexes of the transcription factor AP-1. In the dermis and epidermis, AP-1 induces the expression of the matrix metalloproteinases, such as collagenase, 92-kDa gelatinase, and stromelysin, which degrade collagen and other proteins that comprise the dermal extracellular matrix. It has been reported that MIF induces MMP-1 via tyrosine kinase, PKC-, and AP-1-dependent pathways in synovial fibroblasts in patients with rheumatoid arthritis (34). Consistent with this finding, we showed that the DNA binding activity of AP-1 was up-regulated by MIF stimulation. Furthermore, a JNK inhibitor blocks the activation of c-Jun and has no effect on p38 and MAPK activities (37, 38). In the present study, we observed a reduction in the MMP-1 mRNA level using a specific JNK inhibitor. Therefore, it is conceivable that activation of c-Jun plays an important role in the signal transduction pathway of MIF-induced MMP-1 expression.

Furthermore, we demonstrated that protein kinase C, Raf, and MAPK were phosphorylated, but p38 was not phosphorylated in the same manner. Activation of PKC is one of the earliest events in the cascade leading to a variety of cellular responses. There are multiple PKC isoforms, including classical PKCs (α, β1, βII, and γ), which bind calcium, diacylglycerol (DAG) and phospholipids; novel PKCs (δ, ε, η, and θ), which are regulated by DAG and phospholipids; and atypical PKCs: ε and λ, which lack calcium- and DAG-binding domains. Human dermal fibroblasts are known to express α, δ, ε, and ϵ isoforms of PKC. Among them, PKC-δ is thought to be the dominant isoform in the fibroblasts (26). By MIF stimulation, we showed the phosphorylation of PKCα/βII and δ occurred, but not that of PKCζ/λ, suggesting that activation of PKCα or δ can play an important role in MIF signal transduction. CSK has been reported to phosphorylate the carboxyl tyrosine residues of Src family tyrosine kinases and inhibit their functions (17). Using CSK and a kinase-negative mutant of CSK (CSK-) in addition to chemical inhibitors, phosphorylation of PKC and MAPK by MIF stimulation was suppressed by CSK, genistein, and herbimycin A. These facts strongly suggest that PKC and MAPK activation depends on activation of Src family tyrosine kinases.

In conclusion, MIF was found to be up-regulated by UVA irradiation in association with IL-1 in human dermal fibroblasts. Upon MIF stimulation, PKC, Raf, and MAPK can be activated in dermal fibroblasts, and up-regulation of the DNA-binding activity of AP-1 might also take place. Clinically, it has been reported that MIF is closely related to the exacerbation of a variety of diseases, including autoimmune diseases, allergic disease, and carcinogenesis. Hence, this newly identified mechanism may contribute to our understanding of photo-induced dermal connective tissue damage, which results in photoaging. These findings are promising for the potential of MIF inhibitors for therapeutic use in patients with severe photodamage related disorders.

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