Infrared-A Radiation-Induced Matrix Metalloproteinase 1 Expression is Mediated Through Extracellular Signal-regulated Kinase 1/2 Activation in Human Dermal Fibroblasts

Stefan M. Schieke,*†[1] Helger Stege,†[1] Viola Kürten,† Susanne Grether-Beck,* Helmut Sies,‡§ and Jean Krutmann*§

*Institut für Umweltmedizinische Forschung (IUF) an der Heinrich-Heine-Universität gGmbH, Auf'm Hennekamp 50, D-40225 Duesseldorf, Germany; †Department of Dermatology, Heinrich-Heine-University, Moorenstr. 5, D-40225 Duesseldorf, Germany; ‡Institut für Physiologische Chemie I, Heinrich-Heine-University, Universitätsstr.1, D-40225 Duesseldorf, Germany; §Biologisch-Medizinisches Forschungszentrum, Heinrich-Heine-University, Universitätsstr.1, D-40225 Duesseldorf, Germany

In addition to ultraviolet radiation, human skin is exposed to infrared radiation from natural sunlight as well as artificial ultraviolet and infrared irradiation devices used for therapeutic or cosmetic purposes. The molecular consequences resulting from infrared exposure are virtually unknown. In this study we have investigated whether infrared has the capacity to affect gene expression in human skin cells. Exposure of cultured human dermal fibroblasts to infrared in the range of 760–1400 nm (infrared-A) induced the expression of matrix metalloproteinase 1 at the mRNA and protein level in a time- and concentration-dependent manner. Expression of tissue inhibitor of matrix metalloproteinase 1 remained unaltered. These effects were not mediated by the generation of heat by infrared-A. Furthermore, infrared-A did not induce heat shock protein 70 expression in human dermal fibroblasts under conditions that increased matrix metalloproteinase 1 expression. Here we provide evidence that infrared-A activated mitogen-activated protein kinase pathways. Extracellular signal-regulated kinase 1/2 and p38-mitogen-activated protein kinase were rapidly activated after infrared-A exposure. The mitogen-activated protein kinase/extracellular signal-regulated kinase inhibitor PD 98059, which specifically blocked the extracellular signal-regulated kinase pathway, prevented infrared-A-induced matrix metalloproteinase 1 expression. Upregulation of matrix metalloproteinase 1 expression by infrared-A was thus shown to be dependent on extracellular signal-regulated kinase 1/2 activation. In conclusion, this study demonstrates that infrared-A is capable of inducing matrix metalloproteinase 1 expression in human dermal fibroblasts via activation of the extracellular signal-regulated kinase 1/2 signaling pathway. This previously unrecognized property of infrared-A points to its possible role in the photoaging of human skin.
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The solar radiation that reaches human skin ranges from 290 to 4000 nm and includes ultraviolet (UV) radiation (UVB, 290–320 nm, UVA, 320–400 nm), visible light (400–700 nm), and infrared radiation (IR, 700–4000 nm) (Endres and Breit, 2001). In addition to natural IR, human skin is also exposed to IR from artificial irradiation devices. This includes IR from therapeutic IR devices used in physiotherapy and, as an adjunctive measure, in the treatment of cancer patients, as well as contaminating IR from UVA radiation devices, which are being used at increasing frequency for phototherapeutic or cosmetic purposes (Vaupel and Krüger, 1995; Seegenschmidt *et al*, 1996; Krutmann and Morita, 2003). IR, similar to UV radiation, is likely to exert biologic effects on human skin. Accordingly, as demonstrated by previous studies, IR irradiation is capable of reducing UV-induced cytotoxicity in human dermal fibroblasts and leads to an increased ferritin expression in human skin *in vitro* and *in vivo* (Menezes *et al*, 1998; Applegate *et al*, 2000). In addition, IR was shown to stimulate cutaneous wound repair, which was ascribed to increased expression of transforming growth factor-β1 and matrix metalloproteinase (MMP)-2 (Danno *et al*, 2001). Most interestingly, already 20 y ago it was reported by Kligmann (1982) that chronic IR exposure of epilated albino guinea pigs caused skin changes that were similar to those found in solar UV radiation-induced elastosis.

Exposure of human skin to UV induces the expression of MMP a family of zinc metalloendopeptidases responsible for the

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Reprint requests to: Jean Krutmann, Institut für Umweltmedizinische Forschung (IUF) an der Heinrich-Heine-Universität gGmbH, Auf'm Hennekamp 50, D-40225 Duesseldorf, Germany. Email: krutmann@rz.uni-duesseldorf.de

[1]These authors contributed equally to this work.

Abbreviations: AP-1, activator protein 1; ERK, extracellular signal-regulated kinase; Hsp, heat shock protein; IR, infrared radiation; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MMP-1, matrix metalloproteinase 1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; TIMP, tissue inhibitor of matrix metalloproteinases.

turnover and degradation of extracellular matrix components, such as collagen and elastin. As a result, chronic exposure to UV leads to the premature aging of skin, which clinically presents with wrinkle formation, loss of skin tone, and pigmentation abnormalities (Gilchrest and Yaar, 1992). Collagenase 1 (MMP-1) is expressed by human skin cells *in vitro* and *in vivo* in response to UVA and UVB irradiation (Scharffetter *et al*, 1991; Fisher *et al*, 1998). The promoter region of MMP-1 carries binding sites for the activator protein (AP)-1 transcription factors, which lead to an enhanced MMP-1 gene expression (Angel *et al*, 1987; Gutman and Wasylyk, 1990). In response to external stimuli mitogen-activated protein kinase (MAPK) pathways mediate the expression and transcriptional activity of AP-1 leading to the modulation of gene expression (for review see Chang and Karin, 2001; Kyriakis and Avruch, 2001).

Three distinct MAPK pathways have been studied intensively: the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway (Raf → MEK1/2 → ERK1/2), the c-Jun N-terminal kinase (MEKK1/3 → MKK4/7 → JNK1/2/3), and p38 (MEKK → MKK3/6 → p38α-δ) pathways also termed stress-activated protein kinases. The ERK1/2 pathway is primarily induced by mitogens such as growth factors, whereas the stress-activated protein kinase pathways are predominantly induced by inflammatory cytokines as well as environmental stress such as UV, heat, and osmotic shock. Activated MAPK translocate to the nucleus, where they phosphorylate and activate transcription factors such as c-Jun, ATF-2, and ternary complex factors leading to the formation and activation of AP-1 (Hazzalin & Mahadevan, 2002).

In order to understand better the impact of IR on human skin it is important to assess IR-induced biologic effects in human skin cells at a molecular level. Whereas UV radiation-induced molecular effects have been extensively studied within recent years, the effects of IR on gene expression in human skin cells are yet unknown. As IR was shown to contribute to photoaging and as activation of MMP-1 is thought to be of critical importance in this process, we have focused this study on the ability of IR to induce MMP-1 expression in cultured human dermal fibroblasts.

By employing an irradiation device that exclusively emits radiation in the near IR range (760–1400 nm; IR-A) we have observed that IR is indeed capable of inducing MMP-1 expression in these cells. Moreover, IR-A-induced MMP-1 expression was found to be mediated through activation of the MAPK signaling pathway.

MATERIALS AND METHODS

Chemicals and cytokines Recombinant human interleukin (IL)-1α was purchased from Genzyme Inc. (Cambridge, MA). The compounds PD 98059 and SB 203580 were obtained from Calbiochem Bioscience (La Jolla, CA), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was from Roth (Karlsruhe, Germany).

Cell culture and viability measurements Human dermal fibroblasts were isolated from neonatal foreskin obtained from five different donors. Cells were cultured in Eagle's minimum essential medium (Biochrom KG Seromed, Berlin, Germany) supplemented with 10% fetal bovine serum (Gibco, Karlsruhe, Germany), 1% antibiotics/antimycotics (penicillin, streptomycin, amphotericin B), and 1% glutamine (Gibco) and were grown on plastic Petri dishes (Greiner, Solingen, Germany) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C as previously described (Berneburg *et al*, 1999). Cells were used between passages 4 and 16, and grown to 100% confluence before treatment.

Cell viability was measured using the reduction of MTT to the corresponding blue formazan. Skin fibroblasts were treated in 30 mm dishes and incubated with 800 µl per well of MTT (1 mg per ml) in medium for 1–2 h. The reaction was stopped by addition of 200 µl of 10% (w/v) sodium dodecyl sulfate/0.01 M HCl per well, and the formazan released from the cells by incubation at 37°C overnight was measured. Absorbance of the supernatant was measured at 570 nm against a background at 700 nm.

Irradiation For irradiation, medium was replaced by phosphate-buffered saline (37°C), and confluent cells were exposed at a distance of 30 cm to radiation from a water-filtered IR-A irradiation source (Hydrosun Strahler, Hydrosun Medizintechnik, Mülheim, Germany) emitting IR-A in the range of 760–1400 nm. Irradiation times varied from 10 to 60 min, which corresponded to delivered doses ranging from approximately 0.2–1.2 kJ per cm^2. Under these conditions no increase in temperature could be detected in phosphate-buffered saline immediately after exposure to IR-A. For UVA irradiation, phosphate-buffered saline-covered cells were exposed to UVA radiation (30 J per cm^2) using a Sellamed 24,000 A irradiation device (Dr Sellmeier, Sellas GmbH, Gevelsberg, Germany) as previously described (Grether-Beck *et al*, 2000). Control cells were held under similar conditions without irradiation. Following the treatment, cells were incubated with culture medium for the desired time at 37°C. For the detection of MAPK activation and MMP-1 protein cells were kept in serum-free medium after irradiation until harvesting. The treatments applied were nontoxic to the cells as determined by light microscopy and the MTT assay (viability ≥ 90% 0–48 h after exposure).

RNA extraction and reverse transcriptase–polymerase chain reaction Total RNA was isolated using RNeasy Total RNA Kit (Qiagen, Hilden, Germany). Expression of mRNA specific for MMP-1, tissue inhibitor of MMP (TIMP)-1 and heat shock protein (Hsp)70 was assessed by differential reverse transcriptase–polymerase chain reaction as described (Henninger *et al*, 1993; Grether-Beck *et al*, 2000). The following primer pairs were used (5′-3′):
MMP-1: GTA TGC ACA GCT TTC CTC CAC TGC, GAT GTC TGC TTG ACC CTC AGA GAC C
TIMP-1: TTC CGA CCT CGT CAT CAG GG, ATT CAG GCT ATC TGG GAC CGC
Hsp70: ATG CGG CCA AGA ACC, CAA AGA TCA GGA CGT TGC
β-actin: GTG GGG CGC CCC AGG CAC CA, CTC CTT AAT GTC ACG CAC GAT TTC.

Immunodetection of MMP-1 Supernatants from irradiated and sham-irradiated control cells were collected at the indicated time points and concentrated with centrifugal filter units (Centricon, Millipore, Eschborn, Germany). Samples of 18 µl, normalized for protein content (Proteinassay, Bio-Rad Laboratories, Munich, Germany), were subjected to gel electrophoresis on 10% (w/v) polyacrylamide gels, buffered in Tris/glycine, and blotted on to nitrocellulose membranes (Bio-Rad). MMP-1 was detected with a sheep polyclonal anti-human MMP-1 antibody (The Binding Site, Birmingham, U.K.). After incubation with a secondary rabbit anti-sheep IgG antibody coupled to horseradish peroxidase (Dako, Glostrup, Denmark) chemiluminescence was detected (ECL, Amersham, Braunschweig, Germany).

Phosphorylation of MAPK Cell lysis, electrophoresis, and blotting procedures were performed as described before (Schieke *et al*, 1999). Immunodetection of ERK1/2 and p38-MAPK was carried out using polyclonal anti-active-MAPK (Promega, Mannheim, Germany) and polyclonal anti-phospho-p38 MAPK (Cell Signaling Technology, Frankfurt am Main, Germany) antibodies, respectively. Incubation with an anti-rabbit secondary antibody conjugated to horseradish peroxidase (Dako, Glostrup, Denmark) was followed by chemiluminescence detection (ECL, Amersham). After stripping, the membrane was reprobed with polyclonal anti-ERK1/2 (Promega) and polyclonal anti-p38 MAPK (Cell Signaling Technology) antibody that served as gel loading and protein control.

Statistical analysis Data were analyzed with one-sided t-tests. Differences were considered significant when $p < 0.05$.

RESULTS

Effects of IR-A irradiation on MMP-1 expression In previous studies it has been shown that UV irradiation causes dermal photoaging associated with upregulation of MMP, e.g., MMP-1 (Fisher *et al*, 1997). We therefore assessed the capacity of IR-A to affect MMP-1 expression in cultured human dermal fibroblasts. As positive controls, cells were stimulated with recombinant human IL-1 (100 U per ml) and UVA radiation (30 J per cm^2). IR-A radiation exposure increased steady-state levels of mRNA specific for MMP-1 in a concentration- and time-dependent manner (**Fig 1*a***). MMP-1 mRNA expression was maximal after 60 min of IR-A exposure corresponding to a

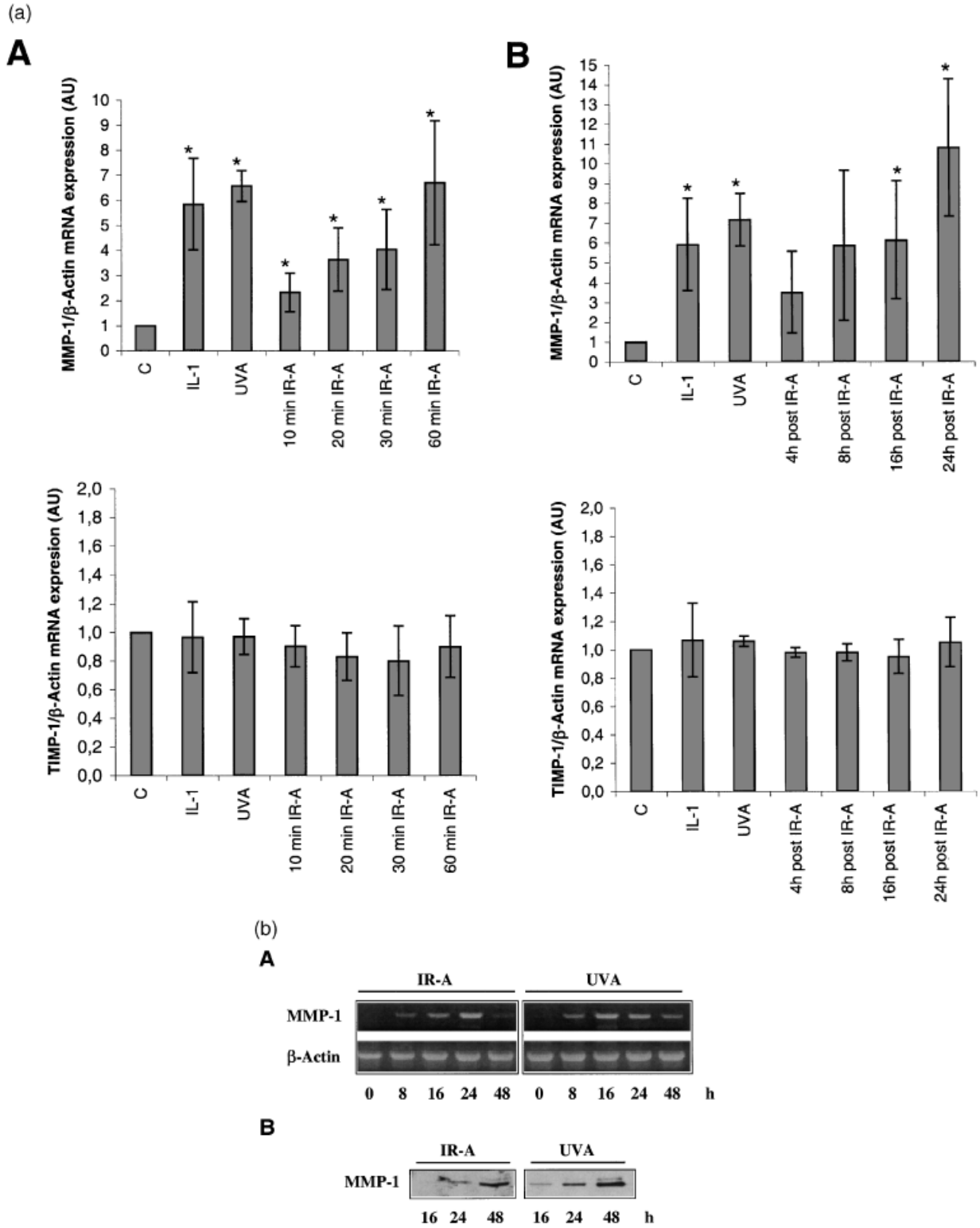

Figure 1. IR-A irradiation-induced MMP-1 mRNA and protein expression in human dermal fibroblasts. (*a*) Concentration- and time-dependent MMP-1 and TIMP-1 mRNA expression in human dermal fibroblasts following IR-A irradiation. Human dermal fibroblasts were exposed to recombinant human IL-1α (100 U per ml; added to culture medium for 24 h), UVA (30 J per cm^2), or IR-A and subsequently analyzed for MMP-1 and TIMP-1 mRNA expression by differential reverse transcriptase–polymerase chain reaction as described in *Materials and Methods*. (*A*) Cells were exposed to IR-A for varying periods of time (10–60 min), corresponding to increasing doses in the range of 0.2–1.2 kJ per cm^2. Cells were harvested 24 h after irradiation to assess MMP-1 mRNA expression. As a positive control fibroblasts were treated with IL-1α and UVA and harvested 24 h after treatment to determine MMP-1 mRNA. (*B*) Cells were exposed to IR-A for 60 min (1.2 kJ per cm^2) and harvested after 4–24 h after irradiation. Control cells treated with IL-1 and UVA were harvested 24 h after treatment. Data are given as relative MMP-1 and TIMP-1 mRNA expression (normalized for β-actin expression). Results are mean ± SD; $^*p < 0.05$ vs. untreated control. (*b*) Time-dependent expression of MMP-1 mRNA and protein in IR-A- or UVA-exposed human dermal fibroblasts. Cells were exposed to IR-A (1.2 kJ per cm^2) or UVA (30 J per cm^2). (*A*) After irradiation cells were harvested immediately (0 h) or after 8, 16, 24, and 48 h. Expression of MMP-1 mRNA was assessed as in (*a*). (*B*) In addition to harvesting of cells, at 16, 24, and 48 h after irradiation supernatants were collected for determination of MMP-1 protein expression by western blot analysis as described in *Materials and Methods*. Data represent one of three experiments.

delivered dose of 1.2 kJ per cm^2 (**Fig 1*aA***). Time-course experiments revealed that a maximum of MMP-1 mRNA was reached 24 h after irradiation (**Fig 1*aB,bA***). Upregulation of MMP-1 mRNA expression in IR-A-exposed cells was associated with an increased expression of MMP-1 protein, as was demonstrated by western blot analysis of supernatants prepared from IR-A-treated cells (**Fig 1*b***). Maximal upregulation of MMP-1 protein was detected 48 h after irradiation with IR-A, which was similar after UVA exposure of human fibroblasts.

Furthermore, we investigated the expression of TIMP-1 mRNA. In contrast to MMP-1, TIMP-1 mRNA expression remained essentially unaltered in response to IR-A as well as IL-1α and UVA.

IR-A irradiation does not upregulate Hsp70 IR induces molecular vibrations that manifest as an increase in cell temperature and might cause a heat shock response and thereby modulate gene expression (Pirkkala *et al*, 2001). In order to assess whether IR-A-induced MMP-1 expression was associated with upregulation of Hsp70, human dermal fibroblasts were exposed to either UVA, IR-A, or treated with a heat shock, which was induced through a 4 h incubation at 42°C. We determined the expression of Hsp70 mRNA in human skin fibroblasts after treatment.

As is shown in **Fig 2**, UVA and IR-A failed to induce the expression of Hsp70 mRNA in contrast to the heat shock treatment, which led to a significant increase in Hsp70 mRNA 1 h after treatment. Moreover, we assessed MMP-1 mRNA after UVA, IR-A, and heat shock treatment. Whereas UVA and IR-A induced a marked increase in MMP-1 mRNA level, heat shock treatment failed to induce the expression of MMP-1 mRNA.

Activation of MAP kinase pathways by IR-A Activation of the MAP kinase signaling pathways has previously been shown to occur after UV radiation exposure. In order to examine whether MAPK pathways could also be activated by IR-A irradiation, cultured human dermal fibroblasts were exposed to IR-A at dose levels that were known to cause maximal MMP-1 induction. Irradiation of fibroblasts with IR-A (1.2 kJ per cm^2) resulted in a rapid dual phosphorylation of p38-MAPK and ERK1/2, which was maximal immediately and 15 min after exposure, respectively (**Fig 3**). In contrast to p38 activation, which was markedly decreased 15 min after irradiation, activation of ERK1/2 sustained for up to 120 min.

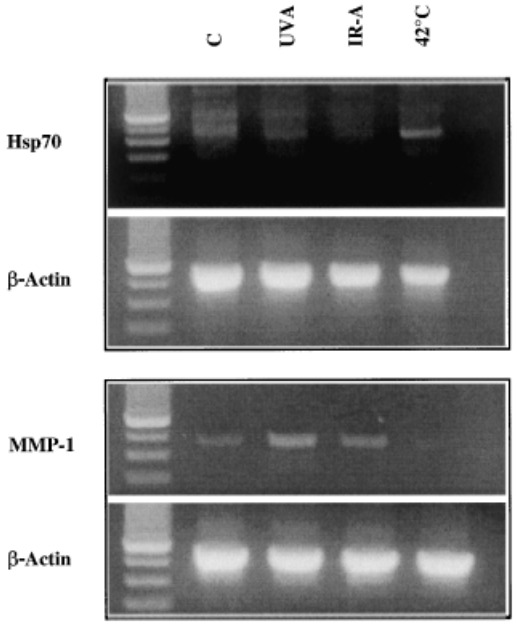

Figure 2. IR-A-induced expression of MMP-1 mRNA in dermal fibroblasts is not mediated by a heat shock response. Confluent fibroblasts were exposed to either UVA (30 J per cm^2), IR-A (1.2 kJ per cm^2), or a heat shock treatment (incubation in culture medium at 42°C for 4 h). Treatment of cells was followed by incubation for 1 or 24 h in culture medium after which cells were harvested to analyze Hsp70 and β-actin mRNA expression or MMP-1 and β-actin mRNA expression, respectively. Data represent one of two experiments.

Role of the ERK1/2 pathway in IR-A-induced MMP-1 expression Previous studies showed that MMP-1 expression induced by various stimuli is mediated by activation of MAPK pathways (for review see Westermarck and Kähäri, 1999). Therefore, we examined the functional relevance of MAPK activation for IR-A-induced MMP-1 expression in human skin fibroblasts.

First, we examined the effects of pharmacologic MAPK cascade inhibitors on IR-A-induced MAPK activation. In order to study the role of ERK1/2 activation, the inhibitor PD 98059 preventing the activation of MAPK/ERK kinase (MEK1) (Alessi *et al*, 1995) was employed. MEK lying immediately upstream of ERK in the signaling cascade is responsible for its phosphorylation and activation. As shown in **Fig 4(*A*)**, treatment of confluent fibroblasts with 20 μM PD 98059 resulted in a significant inhibition of ERK1/2 activation by IR-A. The level of p38 activation was not affected, thus confirming the specificity of PD 98059 for the ERK cascade. The specific inhibitor for p38-MAPK, SB 203580 (Cuenda *et al*, 1995), was employed to examine the role of the p38 pathway. After treatment of cells with 10 μM SB 203580 the activation of p38 by IR-A irradiation was completely abolished, whereas activation of ERK was not altered. Inhibition of phosphorylation was not due to an effect of dimethyl sulfoxide used as a solvent for these agents.

Next, we investigated the effects of the pharmacologic inhibitors on IR-A-induced MMP-1 expression (**Fig 4*B***). Treatment of fibroblasts with 20 μM PD 98059, which efficiently blocked the activation of ERK1/2, resulted in a complete inhibition of MMP-1 induction by IR-A. This effect was not caused by dimethyl sulfoxide. In contrast, incubation of fibroblasts with SB 203580 (10 μM) leading to an inhibition of p38 activation did not prevent upregulation of MMP-1 expression by IR-A.

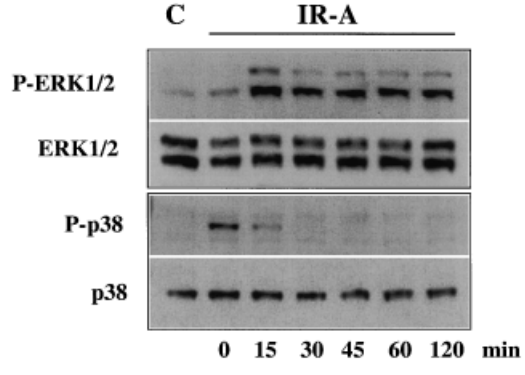

Figure 3. Time course of the activation of ERK1/2 and p38-MAPK in human dermal fibroblasts after IR-A irradiation. Confluent human skin fibroblasts were irradiated with IR-A (1.2 kJ per cm^2) or left unstimulated (sham-irradiated control, C). The cells were lyzed after treatment at the times indicated. Activation of MAPK was assessed as the dual phosphorylation of ERK1/2 and p38 in western blots employing polyclonal phospho-specific antibodies. Equal protein loading was assured by stripping and reprobing with anti-ERK1/2 and anti-p38 MAPK antibodies. Data represent one of four experiments.

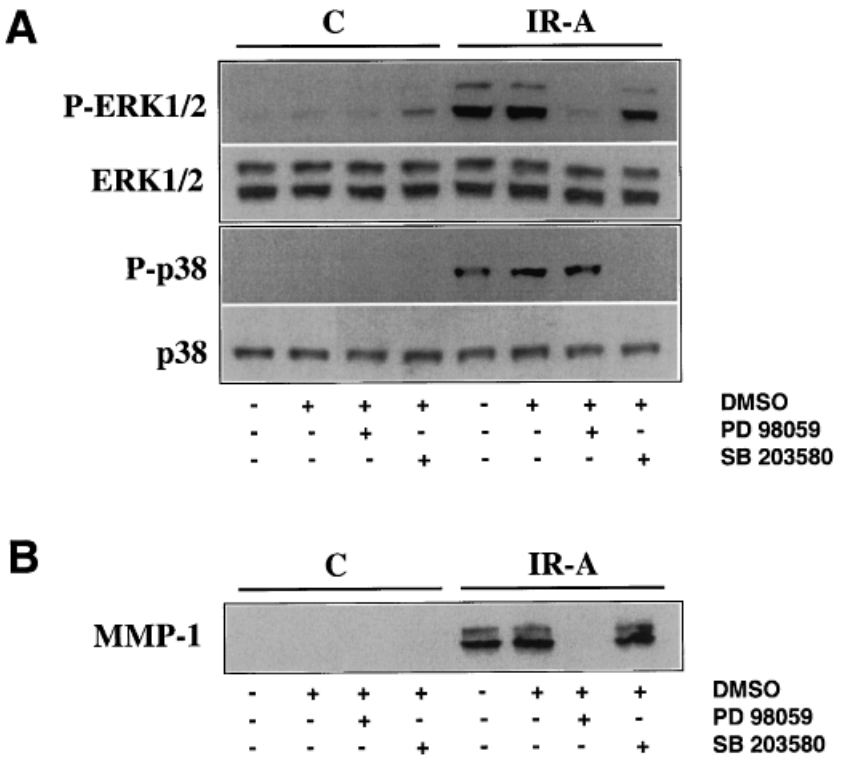

Figure 4. MMP-1 induction by IR-A is dependent on activation of the ERK1/2 pathway. Confluent human skin fibroblasts were pretreated with PD 98059 (20 μM), SB 203580 (10 μM), or dimethyl sulfoxide (DMSO) added to medium for 1 h. Following exposure to IR-A (1.2 kJ per cm^2), cells were post-treated with PD 98059 (20 μM), SB 203580 (10 μM), or dimethyl sulfoxide added to the medium until harvesting. MAPK activation and MMP-1 expression were determined in cultures run at the same time. (*A*) Cells were lyzed immediately and 15 min after exposure to assess the phosphorylation status of p38 and ERK1/2, respectively. (*B*) MMP-1 protein was detected in supernatants by western blot analysis using polyclonal anti-human MMP-1 antibodies 48 h after irradiation. Data represent one of three experiments for ERK and p38 and one of two for MMP-1.

DISCUSSION

The ability of UVA (Scharffetter *et al*, 1991; Wlaschek *et al*, 1995), UVB (Fisher *et al*, 1997; Brenneisen *et al*, 1998), and UVC (Stein *et al*, 1989; Schreiber *et al*, 1995) irradiation to induce MMP-1 synthesis has been well described. The results of our study demonstrate for the first time that IR, in particular IR-A, is also capable of activating cellular signaling pathways inducing synthesis of MMP-1 by human dermal fibroblasts. Induction of MMP-1 expression by IR-A was detected in all fibroblast strains examined. The magnitude of MMP-1 expression, however, showed interindividual variability but no correlates such as skin type or age of the respective donor could be identified because of the small number of individual strains examined.

Similar to UVB and UVA, exposure of dermal fibroblasts to IR-A activates the MAPK signaling cascades. We have found that IR-A leads to a rapid activation of ERK1/2 and p38. The observed activation pattern is distinct from that reported for UVB and UVA. Exposure to UVA and singlet oxygen shows no phosphorylation and activation of ERK1/2 in human skin fibroblasts (Klotz *et al*, 1999). Exposure of human keratinocytes to UVB leads to a more prolonged activation of p38, which remains activated for 2 h, and a rapid activation of ERK1/2, which markedly decreases after 15 min (Peus *et al*, 1999). In addition, MAPK were also shown to be activated by UVC (Dèrijard *et al*, 1994; Raingeaud *et al*, 1995), but it should be noted that this type of radiation does not reach the earth's surface.

MAPK are activated by phosphorylation of Tyr and Thr within a conserved Thr-X-Tyr motif. The phosphorylation and activation of MAPK are catalyzed by a family of dual specificity kinases referred to as MAPK/ERK kinases (MEK or MKK). Phosphorylation and activation of ERK1/2 is mediated by the upstream kinase MEK1/2, which is specifically inhibited by the pharmacologic compound PD 98059. By using this inhibitor we investigated whether IR-A-induced MMP-1 expression is dependent on ERK1/2 signaling. Our results clearly show that inactivation of the ERK1/2 pathway by PD 98059 abolishes the IR-A-induced MMP-1 expression. In contrast, inhibition of the p38-MAPK activation by IR-A has no effect on the level of MMP-1 synthesis. This suggests that the observed MMP-1 synthesis in response to IR is mediated by activation of the ERK1/2 signaling cascade.

This is consistent with results of previous studies, showing that MMP-1 expression by various stimuli is regulated by activation of MAPK. The promoter region of the MMP-1 gene contains binding sites for the transcription factors AP-1 and ETS (Gutman and Wasylyk, 1990; Westermarck *et al*, 1997), activation of which is mediated by MAPK (for review see Hazzalin & Mahadevan, 2002). Induction of MMP-1 gene expression by phorbol ester and basic calcium phosphate crystals in osteoarthritis is mediated by activation of ERK1/2 (Westermarck *et al*, 2001; Sun *et al*, 2002), whereas induction by ceramides and cytokines requires activation of p38-MAPK (Ridley *et al*, 1997; Reunanen *et al*, 1998). In a recent publication by Westermarck *et al* (2001) it is shown that activation of p38 inhibits the phorbol ester-induced MMP-1 expression by blocking the ERK1/2 cascade. From these studies it becomes obvious that MMP-1 expression is tightly regulated by MAPK cascades. In addition, it is shown that induction of MMP-1 expression by UVB (Fisher *et al*, 1998; Brenneisen *et al*, 1998) and by UVA (Wenk *et al*, 1999) is dependent on AP-1 transcriptional activity. It is thus tempting to speculate that the IR-A-induced MMP-1 synthesis observed in our study is mediated by AP-1 activation through the ERK1/2 pathway.

Biologic effects induced by IR are usually ascribed to the generation of heat. In this study expression of MMP-1 and MAPK activation by IR-A were not mediated by generation of heat in irradiated cultures. Furthermore, IR-A did not induce Hsp70 expression in human dermal fibroblasts under conditions that increased MMP-1 expression. It is important to note, however, that this study does not exclude upregulation and phosphorylation of other Hsp in the cellular response to IR-A. Furthermore, Hsp70 expression in thermally stressed fibroblasts was not associated with increased MMP-1 expression. This is in accordance with other studies, which showed heat shock independent effects of IR irradiation. The protective effect of IR against solar UV radiation-induced cytotoxicity in dermal fibroblasts (Menezes *et*

al, 1998) as well as the IR-A-induced increase in ferritin protein expression in human skin (Applegate *et al*, 2000) were not associated with increased expression of Hsp72 and Hsp70, respectively. In addition, IR-stimulated wound repair was shown to be independent of generated heat (Danno *et al*, 2001). Therefore, the question arises whether IR-A triggers a "photochemical" reaction, such as the generation of reactive oxygen species, which was shown to be the case for UVA and UVB. Oxidative stress was previously found to mediate UVA and UVB radiation induced MAPK activation and MMP-1 expression (Wlaschek *et al*, 1995, 1997; Brenneisen *et al*, 1998; Klotz *et al*, 1999; Peus *et al*, 1999). Analysis of the precise photobiologic mechanism by which IR-A is capable of upregulating MMP-1 expression, however, is beyond the scope of this study and remains to be evaluated.

In contrast to MMP-1, the expression of its endogenous inhibitor, TIMP-1, was not increased by IR-A in dermal fibroblasts. This unbalanced upregulation of MMP-1 indicates the possibility that IR-A-irradiated fibroblasts have an increased capacity to proteolytically degrade dermal collagen fibers. Collagen degradation is a key feature of photoaged human skin (Gilchrest and Yaar, 1992; Fisher *et al*, 1997) and these findings therefore offer a possible mechanism to explain previous observations indicating that IR exposure of guinea pigs caused skin changes similar to those found in solar elastosis and enhanced UV-induced dermal damage (Kligmann, 1982). The IR-A device used in this study does not emit any contaminating UV radiation. It is thus conceivable that IR-A itself by virtue of its capacity to induce MMP-1 expression may contribute to photoaging and that sun protection of human skin has to include not only protection from UV, but from IR-A as well. Further studies, however, are required to evaluate the *in vivo* relevance of IR for the photoaging of human skin.

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