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Iron sensitizes keratinocytes and fibroblasts to UVA-mediated matrix metalloproteinase-1 through TNF- α and ERK activation

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Abstract

Oestrogen deficiency is regarded as the main causative factor in postmenopausal skin ageing and photoageing. While women after menopause experience low levels of oestrogen because of cease of ovarian function, they are also exposed to high levels of iron as a result of cessation of menstruation. In this study, we investigated whether this increase in iron presents a risk to the postmenopausal skin. Because of the lack of appropriate animal models to closely mimic the low oestrogen and high iron conditions, we tested the hypothesis in a high iron and low oestrogen culture model. Here, we showed that primary human dermal fibroblasts exposed to iron did not affect the baseline levels of matrix metalloproteinase-1 (MMP-1) activity. However, the iron-exposed fibroblasts were sensitized to UVA exposure, which resulted in a synergistic increase in MMP-1. UVA activated the three members of MAPK family: ERKs, p38, and JNKs. Additional activation of ERKs by iron contributed to the synergistic increases. Primary normal human epidermal keratinocytes (NHEK) did not respond to iron or UVA exposure as measured by MMP-1, but produced tumor necrosis factor- α (TNF- α) in the media, which then stimulated MMP-1 in fibroblasts. Our results indicate that iron and UVA increase MMP-1 activity in dermal fibroblasts not only directly through ERK activation but also by an indirect paracrine loop through TNF- α released by NHEK. We conclude that in addition to oestrogen deficiency, increased iron as a result of menopause could be a novel risk factor by sensitizing postmenopausal skin to solar irradiation.

Keywords

iron; menopause; MMP-1; photoageing; UV

Introduction

The effects of chronological ageing in human skin result in many physiological and biochemical changes, most noticeably in the appearance of fine lines and wrinkles. A

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Supporting Information Additional Supporting Information may be found in the online version of this article.

Table S1. Primer sequences of MMP-1 and its mediators used for qRT-PCR.

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thinned epidermis in conjunction with the loss of rete ridges and compromised lipid barrier repair contribute to some of the maladies associated with ageing. Although not immediately life threatening, these conditions affect the well-being, quality of life, and psychological state of aged individuals (1,2).

Beyond the intrinsic ageing process, sun-exposed areas such as the face, neck, and dorsum of hands and forearms encounter additional damaging effects, largely because of exposure to UV (3). Photoageing refers to the effects of long-term UV exposure and sun damage superimposed on intrinsically aged skin. Many of the functions of skin that decline with age show an accelerated decline in photoaged skin (4). For example, decreases in type I and III collagen are seen in intrinsically aged skin; however, these decreases are accelerated in sun-exposed regions (5).

In women, the onset of menopause presents a unique gender-specific, age-related factor that affects skin. Most studies suggest that intrinsic skin ageing is more closely related to postmenopausal age than chronological age and, thus, reflect hormonal effects (6). Skin ageing in postmenopausal women is characterized by hair loss, colour change, wrinkle formation, skin atrophy and dryness and loss of collagen. Collagens make up 70–80% of the dry weight of the skin and give the dermis its mechanical and structural integrity (7). Type I and III are the major collagens in skin, and there are also a small portion of collagen IV, V, VI, VII, and XI. Most of the patho-physiological changes of the skin in postmenopausal women are related to a reduction in collagen content (8), either by decreasing collagen synthesis or by increasing collagen degradation. Oestrogen promotes anabolism of collagen, while matrix metalloproteinases (MMPs) degrade collagens. MMP-1 is a collagenase, degrading type I collagen; MMP-2 and MMP-9 are gelatinases, degrading type IV collagen. The postmenopausal years represent a state of oestrogen deficiency, which results in deleterious effects on the skin. Indeed, it has been shown that oestrogen supplementation or hormone replacement therapy (HRT) partially increases skin thickness, collagen content, or both (9,10). The benefits and drawbacks of HRT remain controversial and a substantial number of women have discontinued its use because of concerns about cancer risk (11). Moreover, the partial effects of HRT also suggest that factor(s) other than oestrogen deficiency may contribute to postmenopausal skin ageing and photoageing.

Using NYU Women's Health Study, we have found that serum iron and ferritin are significantly higher in postmenopausal women than in premenopausal women (12). Subsequent literatures show that concurrent but inverse changes occur between iron and oestrogen levels in healthy women during menopausal transition (13). Whereas oestrogen decreases because of the cessation of ovarian functions, iron increases as a result of decreasing menstrual periods. Therefore, the natural biological system in older women is low oestrogen and high iron.

Considering the significant increase in iron levels from premenopause to postmenopause, we hypothesized that, in addition to oestrogen deficiency, increased iron as a result of menopause could be a risk factor affecting the postmenopausal skin ageing and photoageing. Because of the lack of appropriate animal models that closely mimic the changes in oestrogen and iron levels during menopausal transition, we tested the hypothesis in a cell culture model. By growing primary normal human epidermal keratinocytes (NHEK) and dermal fibroblasts in the postmenopausal condition of high iron and low oestrogen, followed by exposure to UVA, we have found that increased iron sensitizes keratinocytes and fibroblasts to UVA-mediated MMP-1 activities.

Methods

Reagents and cells

Horse liver ferritin, apo-transferrin (Tf without iron), holo-transferrin (two binding sites of Tf are fully saturated with iron), 17 β -estradiol (E2) water soluble, kinase inhibitors PD98059, U0126, SP600126, SB202190, Wortmannin, and anti-tubulin antibody were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Antibodies against phospho-ERK, JNK, and p38, as well as non-phosphorylated counterparts were purchased from Cell Signaling (Danvers, MA, USA).

Primary NHEK and primary human dermal fibroblasts were purchased from Cascade (Portland, OR, USA). NHEK cells were cultured in Epilife® basal medium with a 1% growth supplement (Cascade Biologics, Portland, OR, USA). Fibroblasts were maintained in DMEM with 10% FBS.

Cell cultures and UVA exposure

Normal human epidermal keratinocytes cells and fibroblasts were seeded in 6-well plates and starved in 0.1% supplemented Epilife® medium and 0.1% FBS DMEM, respectively. Two cell culture conditions were developed based on the low E2 and high Fe in postmenopausal women and high E2 and low Fe in premenopausal women. In premenopausal condition (Pre-), E2 (500 ng/ml) and apo-Tf (5 μ g/ml) were added into the cell culture medium. In postmenopausal (Post-) condition, cells were treated with ferritin (20 ng/ml) and holo-Tf (5 μ g/ml). After overnight starving, NHEK and fibroblasts were continued to grow under the control (Ctrl), Pre- (high E2 and low iron), or Post- (low E2 and high iron) conditions before exposure to UVA at 50 kJ/m², a low daily dose (14). Media were collected 24 h later for measurements of MMPs activities.

Media transfer assay between NHEK and fibroblasts

To investigate whether NHEK cells exposed to iron and UVA release factors that further stimulate fibroblasts, a media transfer was carried out. In brief, 24 h after UVA exposure, 1 ml media each from NHEK cells grown under Ctrl, Pre-, and Post-conditions was transferred to the wells of fibroblasts, respectively. These fibroblasts were not exposed to UVA and were grown in 2 ml 0.1% FBS DMEM. The MMP-1 activities in the media of fibroblast (3 ml on total) were measured 24 h later. To illustrate the effects of tumor necrosis factor (TNF)- α from NHEK cells on MMP-1 induction in fibroblasts, NHEK cells were pretreated with control IgG or neutralizing TNF- α antibody at a concentration of 2 μ g/ml for 1 h before the media were transferred to human fibroblasts.

Western blot

Cell extracts from fibroblasts were collected at 15, 30 min, 1, 2 and 4 h post UVA irradiation. Equal amounts of protein were separated in 10% SDS–polyacrylamide gels. After transferring, the polyvinylidene fluoride (PVDF) membranes were probed with the primary antibodies for overnight. After washing, the HRP-labelled secondary antibody was added for 1 h, and the bands were visualized by enhanced chemiluminescence kit (PerkinElmer, Waltham, MA, USA).

Measurements of MMP-1 activities

Metalloproteinase-1 activities were measured by Förster resonance energy transfer (FRET) assay following the manufacturer's protocol (AnaSpec, San Jose, CA, USA). Briefly, 100 μ l sample or 100 μ l standard was added in the plate pre-coated with anti-MMP-1 antibody for 2 h. After washing, MMP fluorogenic substrate, 5-FAM/QXL™ 520 FRET peptide, was

added and cultured for 16 h at room temperature. The fluorescence is measured at Ex/Em = 490 nm/520 nm upon MMP-1-induced cleavage of the FRET substrate.

Gelatin zymography

The media for measuring MMP-1 were also used to detect MMP-2 and MMP-9 activities by gelatin zymography (15). Media were mixed with 3X non-reducing sample buffer without boiling, and 20 μ l of samples per lane was loaded into a 10% SDS-PAGE containing 2 mg/ml gelatin. After washing with 2.5% triton-100 (20 min each for three time), the gels were incubated in an incubation buffer (50 mM Tris-HCl PH 8.0, 5 mM CaCl_2) on a shaker at 37° C for 18 h. After staining the gels with Coomassie Blue R250 for 2 h and then destaining, clear bands with gelatin digested by MMP-2 and MMP-9 were visualized and photographed.

Real-time PCR

Total RNA was extracted from NHEK cells and reverse-transcribed to cDNA following the manufacturer's instruction (Invitrogen, Carlsbad, CA, USA). The cDNA was mixed with SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) and primers for interleukin-1 β (IL-1 β), IL-6, IL-17, IL-21, macrophage migration inhibitor factor (MIF), tumor necrosis factor- α (TNF- α), transforming growth factor- β (TGF- β), and tissue inhibitors of MMP1 (TIMP-1), respectively. The sequences of the oligonucleotides for the primers were listed in Table S1 (see Supplements). The real-time PCR was run in a 384-well plate (ABI 7900 series; Applied Biosystems, Foster City, CA, USA). The mRNA expression levels of target genes were normalized to the geomean of three house keeping genes, GAPDH, G6PD, and HPRT1 in the control cells without UVA exposure. Data were expressed as fold changes over the control.

Statistical analyses

The experimental differences were determined by two-tailed Student's *t*-test. Graphed data represent the means $\pm$ SD of at least three experiments. A confidence level of $P < 0.05$ was taken to represent a significant difference in all cases.

Results

Iron sensitizes UVA-mediated MMP-1 activity in fibroblasts but not in NHEK cells

Figure 1a shows that UVA at 50 kJ/m² significantly induced MMP-1 activities in primary human dermal fibroblasts but not in primary NHEK cells. Interestingly, the most substantial induction of MMP-1 by UVA was observed in fibroblasts grown under Postcondition, a 44.6-fold increase over the non-UVA-exposed controls ($P < 0.05$, $n = 4$). Fibroblasts grown under the control or Pre-conditions resulted in 17.1-fold and 12.6-fold increases, respectively (Fig. 1a). These results suggest that iron under the Post-condition of high iron in the form of ferritin and holo-Tf could potentiate UVA-mediated MMP-1 expression in human skin fibroblasts. Oestrogen under the Pre-condition of high E2 and apo-Tf provides some protections.

To test whether iron from ferritin and holo-transferrin is indeed responsible for the MMP-1 induction, a new batch of fibroblasts grown under the Post-condition were pretreated with deferoxamine (DFO), an iron chelator, and then followed by UVA irradiation. Figure 1b shows that UVA-mediated MMP-1 activity was strongly inhibited by DFO (614.5 ± 60 ng/ml without DFO *versus* 261.9 ± 27.1 ng/ml with DFO, $P < 0.05$, $n = 3$).

Whether iron sensitizes a broad spectrum of MMPs was further investigated by measuring MMP-2 and MMP-9 activities. As shown by gelatin zymography, Fig. 1c displays that MMP-2 and MMP-9 activities in NHEK cells were slightly downregulated after UVA

irradiation in all three growing conditions, consistent with previously published studies (16,17). MMP-9 activities in fibro-blasts showed no significant changes after UVA exposure and MMP-2 was undetectable (data not shown).

ERK pathway is responsible for iron- and UVA-mediated MMP-1 induction

To elucidate the mechanism by which iron and UVA induce MMP-1, fibroblasts grown under the Post-condition were pretreated with different kinase inhibitors for 1 h and then followed by UVA exposure. Figure 2a shows that the MMP-1 induction was completely blocked by ERK inhibitor PD98059 and MEK1/2 inhibitor U0126, upstream kinases to activate ERK ($P < 0.05$, $n = 3$). JNK inhibitor SP600126, p38 MAPK inhibitor SB202190, and PI-3K inhibitor Wortmannin enhanced the baseline levels of MMP-1. Pretreatments of these inhibitors further increased UVA-mediated MMP-1 expression.

Figure 2b shows that Post-condition with high iron stimulated baseline levels of ERK and p38 when compared to Pre-condition. ERK, JNK, and p38 signal pathways were all activated by UVA at 15 min post-exposure and were sustained at least up to 4 h. However, the patterns of phosphorylation intensities differed between Pre- and Post-conditions. Activations of ERK1 and ERK2 were more robust and sustained in the fibroblasts grown under Post-than under Pre-condition. JNK activations were comparable between the Pre- and Post-conditions; but p38 MAPK activations were weaker in Post- than in Pre-conditions. As a whole, Figs 1 and 2 indicate that ERK is most likely the pathway responsible for iron- and UVA-induced MMP-1 in fibroblasts.

Iron enhances the ability of NHEK media to stimulate MMP-1 in fibroblasts

Under physiological conditions keratinocytes are the outmost layer to receive UV exposure, whereas fibroblasts are covered by keratinocytes and receive only low doses of UVA irradiation. In this study, we investigated a physiologically relevant scenario that iron may enhance the ability of NHEK cells to release cytokine/growth factors, which further alter MMP-1 activity in fibroblasts. Figure 3a shows that intrinsic MMP-1 activities in NHEK cells were low with or without UVA exposure. When the NHEK media were transferred to fibroblasts, MMP-1 activities in fibroblasts were induced by media from UVA-exposed NHEK cells. The most significant induction was from the cells grown under Post-condition with a 130% increase ($P < 0.05$, $n = 3$). MMP-1 activities in Ctrl and Pre-conditions resulted in less but statistically significant increases at 40% and 30%, respectively ($P < 0.05$, $n = 3$). Because background levels of MMP-1 in the NHEK media were low (Fig. 3a), these results indicated that high levels of MMP-1 in fibroblasts were not directly from the media of NHEK cells but produced by fibroblasts.

TNF- α from the NHEK media stimulates MMP-1 in fibroblasts

Metalloproteinase-1 can be induced by IL-1 β , IL-6, IL-17, IL-21, TNF- α , and MIF or inhibited by TGF- β and TIMP-1 (18–23). To identify which mediator produced by NHEK cells under Post-conditions is responsible for stimulating MMP-1 in fibroblasts, data from qRT-PCR show that mRNA levels of IL-1 α , IL-6 and TNF- α were significantly induced by UVA in NHEK cells grown under Post-condition (Fig. 4). Interestingly, only the patterns of TNF- α induction were similar to those of MMP-1 in fibroblasts grown in NHEK medium (Fig. 3b). TGF- β b and TIMP-1 had no significant changes, while IL-17 and IL-21 were not expressed in NHEK cells. These results suggest that TNF- α may be the factor from the NHEK media that stimulate MMP-1 in fibroblasts.

The media from NHEK cells were then pretreated with control IgG or neutralizing anti-TNF- α antibody for 1 h. After transferring the media from the NHEK cells to fibroblasts under Post-condition, a significant decrease in the MMP-1 activity, at 73.7%, was seen

compared to normal mouse IgG control (Fig.5, $P < 0.05$, $n = 3$). Under Pre-condition, the inhibition was statistically insignificant at 9.4%. These results confirm that TNF- α from irradiated NHEK cells is most likely responsible for the MMP-1 induction in fibroblasts under Post-condition.

Discussion

Oestrogen deficiency has been considered the single most important risk factor in menopause-related skin ageing and photoageing (24,25), which is characterized by a loss of collagen at the histo-logical level (6). Thinning of the dermis, clinically recognized by easy tearing and bruising, is often observed in postmenopausal women. In fact, total dermal collagen content declines at an average rate of 1–2% per year after menopause (9). Collagen is synthesized by fibroblasts from procollagen molecules by the action of neutral endoproteases. It has been shown that E2 exhibits inhibitory effects on proMMP-1, but not type I collagen or TIMP-1 synthesis (26). Although the exact mechanism of oestrogen on collagen integrity is unclear (27), oestrogen deficiency is an undeniable risk factor in postmenopausal skin ageing.

Women after menopause experience not only oestrogen decrease but iron increase as well. Levels of ferritin, an iron storage protein with a molecular capacity of binding up to 4500 atoms of iron, are increased two- to threefold during the menopausal transition (28,29). We have shown that transferrin, an iron transport protein, is more saturated in postmenopausal women than in premenopausal women (12). This iron increase occurs concurrently but inversely with oestrogen decrease during menopausal transition (13). Based on the changes in oestrogen and iron levels, we have varied levels of oestrogen and iron with low oestrogen and high iron to mimic postmenopausal condition and high oestrogen and low iron to simulate premenopausal condition.

By growing primary human NHEK and fibroblasts in these distinct media, baseline levels of MMP-1 did not significantly differ among the fibroblasts grown under Ctrl, Pre-, or Post-conditions. This observation is consistent with reports previously showing that iron overload alone did not induce skin histological changes in iron overload transgenic HFE^{-/-} (hemochromatosis Fe) mice, mice fed with high iron diet, or mice injected with iron compound (30). Interestingly, by exposing human dermal fibroblasts to UVA at a dose of 50 kJ/m², which were significantly lower than previous studies (31,32), UVA-mediated MMP-1 activities in fibroblasts grown under the high iron of Post-condition were increased 2.6- and 3.5-fold over the same cells grown under the low iron of Ctrl and Pre-conditions, respectively (Fig. 1a). These results suggest that increased iron as a result of menopause could sensitize postmenopausal skin to UVA exposure, leading to an enhanced MMP-1 activity.

In the present study, ferritin and holo-transferrin were used to simulate postmenopausal condition. Ferritin is supposed to prevent iron from acting as a catalyst in reactions between oxidants and biomolecules. Dual properties of ferritin as an antioxidant for its capacity to sequester iron and as a pro-oxidant for its ability to release iron under certain environmental stress have been reported (33,34). Exposure of primary skin fibroblasts to UVA has been previously shown to cause an immediate release of 'free' iron in the cells *via* proteolysis of ferritin (35). Holo-transferrin can also release iron and cause brain damage (36). Previous studies have shown that mRNA levels of MMP-1 in human fibroblasts were significantly increased by 2- to 12-fold after exposure to UVA at doses ranging from 100 to 500 kJ/m² (31). Endogenously generated peroxides and iron seem to be involved in the MMP-1 activation by UVA (31). Instead of measuring mRNA levels of MMP-1, we have measured MMP-1 activities. This is significant because collagen degradation is accelerated by

increased MMP-1 activity (37). The inhibitory effects of DFO on MMP-1 indicate that iron released from ferritin and holo-transferrin enhances UVA-mediated MMP-1 activity (Fig. 1b).

MAPK signaling pathway consists of three major pathways of ERK, p38, and JNK. Our results have shown that ERK signal transduction pathway is most likely responsible for UVA-mediated MMP-1 activation under the Post-conditions of high iron (Fig. 2a, b). Iron alone appears to activate ERK and p38 but not JNK pathways in human dermal fibroblasts (Pre- versus Post- without UVA), confirming our previous findings with iron on lung epithelial A549 cells and liver HepG2 cells (38). Both p38 and ERK activation had been reported to induce MMP-1 expression in cardiac fibroblasts (22) and monocyte (39) by cytokines and LPS. When compared to the selective effects of iron on ERK and p38, UVA significantly activated all three major pathways in the MAPK family regardless of the cell culture conditions (Fig. 2b). Interestingly, ERK inhibitor PD98059 and MEK1 inhibitor U0126 completely blocked the MMP-1 activity. Inhibitors for JNK, p38, and PI-3 kinase altered the baseline and did not attenuate its increases (Fig. 2a). These results strengthen our argument that ERK coactivation by iron and UVA contributes to the synergistic increase in MMP-1 activities in human dermal fibroblasts.

Normal human epidermal keratinocytes is the outmost layer to be exposed to UVA. Although UVA did not induce MMP-1 in the cells, this led us to investigate whether NHEK cells exposed to UVA release certain mediators to stimulate MMP-1 in dermal fibroblasts. Previous studies have shown that the induction of MMP-1 by UVA seems to be at least in part mediated by the proinflammatory cytokines IL-1 and IL-6 (40,41). By transferring media from NHEK cells into dermal fibroblasts, the media from all UVA-exposed NHEK cells indeed induced MMP-1 when compared to the media without UVA (Fig. 3). Most interestingly, media from NHEK cells grown under the Post-condition produced the highest MMP-1 activity (Fig. 3b).

This observation is physiologically relevant to postmenopausal skin photoageing. Thus, we attempted to identify the potential mediators responsible for this phenomenon. Among all known MMP-1 stimulators and inhibitors (IL-1, IL-6, MIF, TIMP-1, etc.), changes in TNF- α in NHEK cells following UVA exposure (Fig. 4) showed the similar pattern as MMP-1 induction in fibro-blasts with the media transferred from NHEK cells (Fig. 3). Using TNF- α neutralizing antibody, the UVA-mediated increase in MMP-1 is significantly attenuated (Fig. 5). Our results suggest that TNF- α is probably the mediator released from UVA-exposed NHEK cells, which can induce MMP-1 in fibroblasts.

Taken together, we have shown that iron in the form of ferritin and holo-transferrin sensitizes both keratinocytes and fibroblasts to UVA-mediated MMP-1 activities. Since postmenopausal women experience oestrogen decrease as well as iron increase, our present study suggests that increased iron as a result of menopause could be of equal important determinant as oestrogen deficiency in postmenopausal skin ageing and photoageing. As illustrated in Fig. 6, two possible mechanisms of iron sensitization exist to induce MMP-1 when skin is exposed to UVA. Either, fibroblasts are directly exposed to UVA and then secrete MMP-1 through the ERK pathway. Or, following UVA exposure, keratinocytes release different mediators, such as TNF- α and IL-6, which subsequently stimulate MMP-1 induction in fibroblasts. Therefore, NHEK cells indirectly contribute to the induction of MMP-1 in fibroblasts (42–44). Increased iron levels could synergize the UVA-mediated MMP-1 by producing reactive oxygen species through Fenton, Haber-Weiss, and autoxidation reactions (31,45). Further studies should validate these findings with human subjects from before and after menopause.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

DFO	deferoxamine
ECM	extracellular matrix
HRT	hormone replacement therapies
IL-6	interleukin-6
MIF	macrophage migration inhibitory factor
MMP	matrix metalloproteinases
NHEK	normal human epidermal keratinocytes
ROS	reactive oxygen species
Tf	transferrin
TGF-β	transformation growth factor- β
TIMP-1	tissue inhibitor of metalloproteinases-1
TNF-α	tumor necrosis factor- α

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Figure 1.

Effects of UVA and iron on metalloproteinase (MMP)-1, 2, 9 activities in primary normal human epidermal keratinocytes (NHEK) cells and human dermal fibroblasts. (a) Primary human NHEK cells and dermal fibroblasts were grown in Ctrl (base media), Pre- (base media plus 500 ng/ml E2 and 5 μ g/ml Apo-Tf) or Post- (base media plus 20 ng/ml ferritin and 5 μ g/ml holo-Tf) conditions for overnight and then exposed to UVA at 50 kJ/m². After 24 h cultures, the media were collected for MMP-1 activities. (b) Fibroblasts grown under Post-condition were pretreated with deferoxamine at 50 μ g/ml for 1 h before UVA exposure. (c) Twenty microlitres of cell culture media from NHEK cells and fibroblasts was loaded into a 10% non-reducing SDS-PAGE containing 2 mg/ml gelatin. After electrophoresis, the gels were washed to remove SDS and incubated overnight in an incubation buffer. Clear bands show digestion of gelatin by MMP-2 and MMP-9. * Significantly different from their respective controls ($P < 0.05$).

Figure 2.

Participation of ERK pathway in iron- and UVA-mediated metalloproteinase-1 activities. (a) Fibroblasts grown under Post-condition were pretreated with different kinase inhibitors for 1 h before UVA exposure. DMSO was used as control, PD98059 (final concentration 10 μ M) as ERK inhibitor, U0126 (20 μ M) as MEK1 inhibitor, SP600126 (10 μ M) as JNK inhibitor, SB202190 (10 μ M) as P38 inhibitor, Wortmannin (0.5 μ M) as PI-3K inhibitor. (b) Phosphorylations of ERK, P38 and JNK were measured in fibroblasts grown under Pre- and Post-conditions and post UVA exposure. *Significantly different from their respective controls ($P < 0.05$).

Figure 3.

Increases of metalloproteinase (MMP)-1 activities in fibroblasts by media transferred from UVA-exposed normal human epidermal keratinocytes (NHEK) cells. (a) Background levels of MMP-1 were low in NHEK cells and were slightly decreased after UVA exposure. (b) Levels of MMP-1 in fibroblasts were significantly induced by media transferred from UVA-exposed NHEK cells grown under Post-condition. One millilitre of media from NHEK cells (top) were added into 2 ml of fibroblasts and 24 h later the media were collected for MMP-1 measurements. *Significantly different from the controls without UVA exposure ($P < 0.05$).

Figure 4.

Levels of mRNA expressions of potential metalloproteinase (MMP)-1 mediators in normal human epidermal keratinocytes (NHEK) cells. NHEK cells grown under Ctrl, Pre- and Post-conditions were exposed to UVA at 50 kJ/m². After RNA extraction and reverse-transcription, the mRNA expression levels of different MMP-1 mediators were measured by real-time-PCR. *Significantly different from the controls without UVA exposure ($P < 0.05$).

Figure 5.

Neutralizing effects of anti-tumor necrosis factor (TNF)- α antibody on normal human epidermal keratinocytes (NHEK) media-induced metalloproteinase (MMP)-1 activities in fibroblasts. Media from NHEK cells grown under Ctrl, Pre- and Post-conditions $\pm$ UVA exposure were pretreated with control IgG or TNF- α neutralizing antibody at 2 μ g/ml for 1 h. After transferring the media to fibroblasts for an additional 24 h, MMP-1 activities were measured. *Significantly different from the mIg controls ($P < 0.05$).

Figure 6.

Proposed mechanisms of iron sensitization in UVA-mediated metalloproteinase (MMP)-1 activities. Left: Iron sensitizes normal human epidermal keratinocytes cells to release tumor necrosis factor- α (TNF- α) after UVA exposure. The secreted TNF- α subsequently stimulates MMP-1 in fibroblasts, indirect effects of iron and UVA on MMP-1 of fibroblasts. Right: Iron in the epidermis sensitizes fibroblasts to UVA-mediated ERK activation and MMP-1 induction. Because iron is in place and UVA penetrates the epidermis, this pathway is considered direct effects of iron and UVA on MMP-1 of fibroblasts.