Luteolin Prevents Solar Radiation-Induced Matrix Metalloproteinase-1 Activation in Human Fibroblasts: A Role for p38 Mitogen-Activated Protein Kinase and Interleukin-20 Released from Keratinocytes

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Abstract

Human skin is continuously exposed to solar radiation, which can result in photoaging, a process involving both dermal and, to a lesser extent, epidermal structures. Previously, we have shown that the flavonoid luteolin protects the epidermis from ultraviolet (UV)-induced damage by a combination of UV-absorbing, antioxidant, and antiinflammatory properties. The aim of the present study was to determine direct and indirect effects of luteolin on dermal fibroblasts as major targets of photoaging. Stimulation of fibroblasts with UVA light or the proinflammatory cytokine interleukin-20 (IL-20) is associated with wrinkled skin, increased IL-6 secretion, matrix metalloproteinase (MMP-1) expression, and hyaluronidase activity. All of these targets were inhibited by luteolin via interference with the p38 mitogen-activated protein kinase (MAPK) pathway. Next, we assessed the role of conditioned supernatants from keratinocytes irradiated with solar-simulated radiation (SSR) on nonirradiated dermal fibroblasts. In keratinocytes, luteolin inhibited SSR-induced production of IL-20, also via interference with the p38 MAPK pathway. Similarly, keratinocyte supernatant-induced IL-6 and MMP-1 expression in fibroblasts was reduced by pretreatment of keratinocytes with luteolin. Finally, these results were confirmed *ex vivo* on skin explants treated with luteolin before UV irradiation. Our results suggest that SSR-mediated production of soluble factors in keratinocytes is modulated by luteolin and may attenuate photoaging in dermal fibroblasts.

Introduction

SOLAR ULTRAVIOLET (UV) RADIATION leads to various immediate and long-term deleterious effects, including acute erythema (*e.g.*, sunburn) and the degradation of collagen and elastin, leading to wrinkled appearance of the skin if imperfectly repaired (photoaging).¹ Whereas UVB radiation is almost completely absorbed in the epidermis and can directly damage the DNA of epidermal cells (*i.e.*, keratinocytes), UVA radiation penetrates deeper into the skin and acts mainly indirectly on dermal fibroblasts through reactive oxygen species (ROS) and activation of ROS-induced signaling.^{1,2} Furthermore, UV irradiation strongly promotes the expression of proinflammatory cytokines in keratinocytes, such as interleukin-6 (IL-6), IL-1 β , and tumor necrosis factor- α (TNF- α). These cytokines, in turn, induce the recently described p38 mitogen-activated protein kinase (MAPK)-

regulated cytokine IL-20.^{3,4} IL-20 also might play a role in photoaging because the wrinkled skin of IL-20 transgenic mice resembles dermal abnormalities of photoaged skin.⁵ Furthermore, we have shown that the IL-20 receptors IL-20R α and IL-20R β are expressed on human dermal fibroblasts.⁶ Therefore, we hypothesize that solar light-induced IL-20 production in keratinocytes might contribute to aging processes in the dermis.

It has already been demonstrated that conditioned medium from either UV-exposed skin or basal cell carcinomas contains large amounts of active matrix metalloproteinase-1 (MMP-1).^{7,8} MMP-1, the so-called interstitial collagenase, plays a major role in the process of photoaging because it specifically cleaves type 1 collagen, a major constituent of the dermis. MMP-1 also activates other MMPs such as MMP-9, which has the highest substrate specificity for dermal elastin and fibrillin.⁹

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Among the nonfibrous components in dermis and epidermis, the glucosaminoglycan hyaluronic acid (HA) plays an essential role in supporting a tight tissue architecture¹⁰ by its capacity to bind water.¹¹ More than 50% of total body HA is stored within the dermal layer of the skin.¹² HA decreases after UVB exposure,¹³ because UVB irradiation increases hyaluronidase expression in fibroblasts. Additionally, UV-induced ROS production decreases the synthesis of HA,¹⁴ thus contributing to the development of photoaged dry skin.

Recently, we have demonstrated UV-protective properties of the flavonoid luteolin on human keratinocytes.¹⁵ In the present study, we addressed the question of whether luteolin can directly inhibit UVA-induced MMP-1 expression and hyaluronidase activity in dermal fibroblasts. Furthermore, we examined if MMP-1 production in dermal fibroblasts can be reduced indirectly by pretreatment of keratinocytes with luteolin before exposing them to solar irradiation, a setting that is closer to topical treatment *in vivo*. Specifically, we addressed the role of IL-20 released by UV-irradiated keratinocytes and the involvement of p38 MAPK signaling. The *in vitro* findings were then confirmed on UV-irradiated skin explants that were pretreated topically with luteolin.

Materials and Methods

Antibodies and reagents

The following antibodies and dilutions were used for immunohistochemical staining or western blotting: Anti-p38 MAPK (Cell Signaling, Frankfurt, Germany), 1:1,000; anti-phospho-p38 MAPK (clone 3D7, Cell Signaling, Frankfurt, Germany); anti-HSC-70 (clone B-6, Santa Cruz Biotechnology, Heidelberg, Germany) 1:5,000; and neutralizing anti-IL-20 (RD Systems, Wiesbaden, Germany) 1:10. The secondary antibody multilink biotin, the streptavidin horseradish peroxidase (HRP)-label, and the 3-amino-9-ethylcarbazole (AEC) substrate were from Dako (Glostrup, Denmark) and were used according to the manufacturer's instructions. Other secondary antibodies were rabbit anti-mouse-HRP (Bio-Rad Laboratories GmbH, München, Germany) and goat anti-rabbit-HRP (Santa Cruz Biotechnology).

Luteolin (purity >80) was provided by NIG (Magdeburg, Germany).¹⁵ Test concentrations were freshly prepared for each cell culture experiment using final nontoxic concentrations of luteolin in cell culture medium. The solvents alone served as negative controls. The following positive controls were used: *N*-acetylcysteine (NAC; Sigma-Aldrich, Seelze, Germany) and the p38 MAPK inhibitor SB203580 (Calbiochem, Darmstadt, Germany).

UV irradiation

A solar simulator (Model 81192, Oriel, Stratford, CT) equipped with a 1,000 W Xenon arc lamp was used. The emission spectrum was between 290 nm and 2500 nm, with a maximal output between 300 nm and 800 nm.¹⁶ UVA-1 radiation between 340 nm and 450 nm was delivered from UVASUN metal halogenid high-pressure lamps (UVASUN 5000, Firma Mutzhas, München) as described.¹⁷

Cell culture

The spontaneously immortalized human keratinocyte cell line HaCaT (from CLS, Heidelberg, Germany) was cultured

in Dulbecco modified essential medium (DMEM; Invitrogen GmbH, Karlsruhe, Germany) as described.¹⁵ Normal human dermal fibroblasts (NHDFs) were isolated from human foreskin as described⁶ and routinely used between passages 3 and 6.

For western blotting and enzyme-linked immunosorbent assay (ELISA), the cells were seeded in 5-cm petri dishes and allowed to attach for 24 hr. Subsequently, the cell culture medium was replaced by phosphate-buffered saline (PBS) with different extract concentrations as indicated and incubated for 30 min. The cells were then irradiated with increasing doses of UVA-1 in a dose kinetic experiment, or with 20 J/cm² UVA-1 or 6 J/cm² solar-simulated radiation (SSR) (corresponding to 24 mJ/cm² UVB) in all other experiments. After 24 hr, the supernatants were snap frozen and stored at -80°C and used for IL-20 ELISA (Pepro Tech, London, Great Britain) according to the manufacturer's protocols. RNA isolation was performed 4 hr after irradiation.

Split-thickness skin organ culture

Skin explants from split-thickness skin were prepared as described.¹⁸ In brief, 6-mm punch biopsies containing epidermis and papillary dermis were prepared and were set in 12-well plates in 400 µL of SFM medium (Promo Cell, Heidelberg, Germany). The test substances were applied to the stratum corneum by a filter paper soaked with the test substances to mimic the *in vivo* situation of a topically applied pre-sun product. After 24 hr, the medium was replaced by PBS, the filter paper was removed, and the skin explants were irradiated on ice with 6 J/cm² using a solar simulator. Subsequently, PBS was replaced by keratinocyte medium (SFM medium; Promo Cell, Heidelberg, Germany), and the cells were incubated for 24 hr before the supernatants were snap frozen in liquid nitrogen and stored at -80°C and used for MMP-1 and IL-6 ELISA (R&D Systems, Wiesbaden, Germany or Pepro Tech, London, Great Britain) according to the manufacturer's protocols.

Zymography

Culture medium containing 20 µg of protein was used without heating or reduction for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) containing HA (Merck, Darmstadt, Germany). The zymography was performed as described.¹⁹ In brief, after electrophoresis, gels were first incubated in Triton X-100 (2.5%), then in incubation buffer (0.1 M sodium formate, 0.15 M NaCl, pH 3.5), and finally with pronase (0.1 mg/mL; Merck, Darmstadt, Germany) before staining with 0.5% Alcian Blue (Carl-Roth, Karlsruhe, Germany). After a destaining procedure, hyaluronidase activity appeared as a white clearing on the blue background.

Western blot and ELISA

NHDFs were grown in 5-cm petri dishes and treated for 30 min with the test compounds. Subsequently, the cells were irradiated with UVA-1 as indicated, washed once with PBS, and supplemented with new DMEM for 30 min for phospho-p38 MAPK, p38 MAPK, and HSC-70 detection. Cell lysates were prepared for western blot analysis, and the lanes were quantified with the ImageJ software.¹⁵

At 24 hr postirradiation, MMP-1 expression as well as the MMP-1 activity, IL-6, and IL-20 concentrations were analyzed in cell supernatants of NHDFs or HaCaT keratinocytes by ELISA (R&D Systems, Wiesbaden, Germany or Pepro Tech, London, Great Britain) according to the manufacturer's protocols. Data were expressed as mean $\pm$ standard deviation (SD) of three independent experiments. Values of stimulated cells were arbitrarily set to 100%; all other samples were indicated as percentage of control, because of variations between the experiments.

Transfer experiment

To evaluate the role of soluble factors released by keratinocytes, NHDFs were treated for 48 hr with keratinocyte-conditioned medium before the supernatants were snap frozen in liquid nitrogen, stored at -80°C , and used for IL-6 and MMP-1 ELISA or HA zymography. Keratinocyte conditioned media were obtained from HaCaT cells pretreated with or without luteolin ($8\text{ }\mu\text{g/mL}$) or p38 MAPK inhibitor SB203580 ($20\text{ }\mu\text{M}$) before irradiation with a solar simulator (6 J/cm^2).

RNA extraction and real-time quantitative PCR

Total RNA was extracted with the RNeasy Mini kit (Quiagen, Hilden, Germany) from subconfluent HaCaT cells. Reverse transcription was performed with the Omniscript RT-kit (Quiagen). Real-time quantitative PCR (RT-qPCR) was carried out as described.⁶ In brief, the reaction was performed in a $20\text{-}\mu\text{L}$ volume containing 750 ng of cDNA, ready-to-use iQ-SYBR Green Supermix (BioRad, München,

Germany), and IL-20 primers (forward primer $5'\text{-ttgcaagacacaaagcctg-3'}$, reverse primer $5'\text{-tggtagaagaaggattggcg-3'}$). The expression levels were calculated relative to those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). For each experiment, three different RNAs were used and each reaction was performed in duplicate.

Statistical analysis

The data were analyzed using the unpaired Student *t*-test (two-tailed), and statistical significance was established at $p \leq 0.05$ (*) and $p \leq 0.01$ (**). Data are expressed as mean $\pm$ standard deviation (SD) of at least three independent experiments.

Results

Luteolin inhibits UVA-1-induced IL-6 and MMP-1 expression as well as hyaluronidase activation in NHDFs via the p38 MAPK pathway

UVA-1 irradiation is known to induce the release of various cytokines in NHDFs.²⁰ Some of these cytokines, including IL-6, enhance MMP-1 production and activation in fibroblasts via a MAPK signaling pathway.²¹ To test if luteolin interferes with this pathway in fibroblasts, we treated NHDFs for 30 min with $4\text{ }\mu\text{g/mL}$ luteolin before the cells were irradiated with 20 J/cm^2 UVA-1. The cells were harvested 20 min following irradiation, and the expression of phosphorylated p38 MAPK was analyzed. UVA-1 irradiation resulted in phosphorylation (activation) of p38 MAPK (Fig. 1), whereas total p38 MAPK expression remained

FIG. 1. Luteolin reduces ultraviolet A-1 (UVA-1)-induced p38 mitogen-activated protein kinase (MAPK) phosphorylation in normal human dermal fibroblasts (NHDFs). NHDFs were treated for 30 min with different substances ($4\text{ }\mu\text{g/mL}$ luteolin, $20\text{ }\mu\text{M}$ SB203580, $816\text{ }\mu\text{g/mL}$ NAC). Subsequently, the cells were irradiated with 20 J/cm^2 UVA-1. P38 MAPK, and phosphorylated p38 MAPK protein expression was assessed 30 min after irradiation by western blotting. The histogram depicting relative protein expression levels of phosphorylated p38 MAPK was normalized to the expression level of p38 MAPK. Values of irradiated NHDFs were arbitrarily set to 100%; all other samples were indicated as percentage of control. The values are means $\pm$ (SD) of three independent experiments (**) $p < 0.01$.

unchanged. Similarly, the antioxidant NAC also inhibited the activation of p38 MAPK, but higher concentrations (5 mM or 816 $\mu\text{g}/\text{mL}$) were necessary. As positive control, we used the p38 MAPK inhibitor SB203580 that also specifically blocked the phosphorylation of p38 MAPK.

Next, we measured the IL-6 and MMP-1 content in cell culture supernatants of NHDFs 24 hr after UVA-1 irradiation. UVA-1-induced IL-6 and MMP-1 production in NHDFs was measured by ELISA (Fig. 2A,B), whereas the MMP-1 expression correlated with the MMP-1 activity (Fig. 2B). This effect was inhibited by both luteolin and SB203580 (Fig. 2A,B). This indicates that the MAPK pathway is required for UVA-1-induced IL-6 and MMP-1 production in NHDFs. Hyaluronidase activity was also inhibited by luteolin and SB203580 as determined with HA zymography (Fig. 2C).

Luteolin inhibits SSR-induced IL-20 upregulation in keratinocytes and IL-20-induced MMP-1 expression and hyaluronidase activity in NDHFs

Fibroblasts are directly activated not only by UVA irradiation but also by soluble factors released from irradiated keratinocytes, *e.g.*, the proinflammatory cytokine IL-6. Recently, we have shown that IL-20, an early proinflammatory cytokine, is also released by keratinocytes in response to UVB irradiation,⁶ whereas UV irradiation did not induce IL-20 expression in human fibroblasts (data not shown). When HaCaT keratinocytes were treated with 8 $\mu\text{g}/\text{mL}$ luteolin before irradiation with a solar simulator, IL-6 expression (Fig. 3A) as well as IL-20 expression was inhibited both at the RNA and protein levels (Fig. 3B,C).

FIG. 2. Luteolin decreases ultraviolet A-1 (UVA-1)-induced interleukin-6 (IL-6), matrix metalloproteinase-1 (MMP-1) expression, and hyaluronidase activity in normal human dermal fibroblasts (NHDFs). NHDFs were incubated for 1 hr with luteolin (4 $\mu\text{g}/\text{mL}$) or p38 MAPK inhibitor SB203580 (20 μM) and exposed to 20 J/cm² UVA-1 radiation. At 24 hr after irradiation, the supernatant was analyzed. (A) Measurement of IL-6 with an enzyme-linked immunosorbent assay (ELISA) in the supernatant. (B) Measurement of MMP-1 expression and activity with an ELISA in the supernatant. Values of irradiated NHDFs were arbitrarily set to 100%; all other samples were indicated as percentage of control. (C) Detection of hyaluronidase activity by hyaluronic acid (HA) zymography. The histograms depict relative protein activity levels to the irradiated sample. The values are means $\pm$ standard deviation (SD) of three independent experiments (**) $p < 0.01$.

FIG. 3. Luteolin reduces solar simulator-induced interleukin-6 (IL-6) and IL-20 expression in keratinocytes and influences IL-20-induced matrix metalloproteinase-1 (MMP-1) expression and hyaluronidase activity in normal human dermal fibroblasts (NHDFs). HaCaT cells were treated for 1 hr with luteolin (8 μ g/mL) or p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580 (20 μ M) and exposed to 6 J/cm² solar simulator (SS) irradiation on ice (A–C), and NHDFs were treated with IL-20 (10 ng/mL) with or without luteolin (4 μ g/mL) for 30 min (D–E). After 24 hr, the culture supernatant was collected. (A) Amount of IL-6 protein in the culture supernatant of HaCaT cells 24 hr after irradiation was measured by enzyme-linked immunoassay (ELISA). Values of irradiated HaCaT cells were arbitrarily set to 100%; all other samples were indicated as percentage of control. (B) IL-20 gene expression was quantified by real-time quantitative PCR (RT-qPCR) in HaCaT cells. For each condition three RNAs isolated and transcribed independently were submitted in duplicate to the RT-qPCR reaction. Values of untreated HaCaT cells were arbitrarily set to 1; all other samples were indicated as fold change compared to control. (C) Amount of IL-20 protein in the culture supernatant of HaCaT cells 24 hr after irradiation as measured by ELISA. Values of irradiated HaCaT cells were arbitrarily set to 100%; all other samples were indicated as percentage of control. (D) Measurement of MMP-1 with an ELISA in the supernatant of NHDFs. (E) Detection of hyaluronidase activity by hyaluronic acid (HA) zymography in the supernatant of NHDFs. The histograms depict relative protein expression levels to the irradiated sample. Values of stimulated NHDFs were arbitrarily set to 100%; all other samples were indicated as percentage of control. The values are means $\pm$ standard deviation (SD) of three independent experiments.

irradiation was measured by enzyme-linked immunoassay (ELISA). Values of irradiated HaCaT cells were arbitrarily set to 100%; all other samples were indicated as percentage of control. (B) IL-20 gene expression was quantified by real-time quantitative PCR (RT-qPCR) in HaCaT cells. For each condition three RNAs isolated and transcribed independently were submitted in duplicate to the RT-qPCR reaction. Values of untreated HaCaT cells were arbitrarily set to 1; all other samples were indicated as fold change compared to control. (C) Amount of IL-20 protein in the culture supernatant of HaCaT cells 24 hr after irradiation as measured by ELISA. Values of irradiated HaCaT cells were arbitrarily set to 100%; all other samples were indicated as percentage of control. (D) Measurement of MMP-1 with an ELISA in the supernatant of NHDFs. (E) Detection of hyaluronidase activity by hyaluronic acid (HA) zymography in the supernatant of NHDFs. The histograms depict relative protein expression levels to the irradiated sample. Values of stimulated NHDFs were arbitrarily set to 100%; all other samples were indicated as percentage of control. The values are means $\pm$ standard deviation (SD) of three independent experiments.

Similarly, inhibition of the p38 MAPK signaling pathway decreased IL-20 RNA and protein expression to the level of the untreated control (Fig. 3B,C), demonstrating the importance of this reaction pathway.

We then tested if IL-20 can induce MMP-1 expression and hyaluronidase activity in NHDFs and if pretreatment of NHDFs with luteolin modulates the effect of IL-20. MMP-1 expression as well as hyaluronidase activity was increased by IL-20, as determined by ELISA or zymography. Luteolin blocked MMP-1 expression (Fig. 3D) and reduced hyaluronidase activity (Fig. 3E).

Luteolin inhibits p38 MAPK-mediated, keratinocyte-induced IL-6, and MMP-1 expression and hyaluronidase activity in NHDFs in vitro

To analyze the interaction of SSR-activated keratinocytes with NHDFs, HaCaT cells were irradiated with 6 J/cm² SSR.

Afterward, the culture medium from irradiated HaCaT cells was transferred to NHDFs, leading to an increase in IL-6 and MMP-1 production and hyaluronidase activity compared to NHDFs incubated with culture medium from nonirradiated keratinocytes (Fig. 4A–C). Figure 4, A and B, shows that conditioned medium from SS-irradiated keratinocytes was able to upregulate IL-6 and MMP-1 production in NHDFs, whereas the MMP-1 expression correlated with the MMP-1 activity (data not shown). This effect was inhibited by pretreatment of the keratinocytes with luteolin, the p38 MAPK inhibitor SB203580, neutralizing antibodies against IL-20, and NAC. In contrast to SB203580, anti-IL-20 antibody did not reduce IL-6 and MMP-1 expression to background, which indicates that a combination of soluble factors might be required for this effect and that p38 MAPK is a key molecule in this setting. HA zymography of NHDFs supernatants showed that pretreatment of keratinocytes

FIG. 4. Conditioned medium of irradiated HaCaT cells influences interleukin-6 (IL-6) and matrix metalloproteinase-1 (MMP-1) expression in normal human dermal fibroblasts (NHDFs). In luteolin-pretreated HaCaT cells, this effect could be inhibited. HaCaT cells were treated as indicated under the graphic before irradiation (8 μ g/mL luteolin, 20 μ M p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580, 816 μ g/mL *N*-acetylcysteine [NAC]). At 24 hr after irradiation at 6 J/cm² with a solar simulator, the conditioned medium was transferred to NHDF. The neutralizing IL-20 antibody was added after irradiation, as recommended by the manufacturer. (A) Measurement of IL-6 with an enzyme-linked immunosorbent assay (ELISA) in the NHDFs supernatant. (B) Measurement of MMP-1 with an ELISA in the NHDFs supernatant. (C) Detection of hyaluronidase activity by hyaluronic acid (HA) zymography. The active enzymes were detected as clear bands on a dark background after staining. The photo shows an inversion of the original gel to facilitate the detection of the bands. Values of irradiated NHDFs were arbitrarily set to 100%; all other samples were indicated as percentage of control. The values are means $\pm$ standard deviation (SD) of three independent experiments.

with luteolin, NAC, and SB203580 before irradiation also reduced hyaluronidase activity (Fig. 4C).

Luteolin inhibits SSR-induced IL-6 and MMP-1 expression *ex vivo*

To test if this is also true in viable skin, skin explants were treated with luteolin-soaked filter paper before irradiation, and the supernatant was examined for IL-6 and MMP-1 expression. In accordance with the *in vitro* results, irradiation with a solar simulator induced the release of IL-6 and MMP-1 into the supernatant. Again, this effect could be inhibited by luteolin, NAC, and the p38 MAPK inhibitor SB203580 (Fig. 5A,B).

Discussion

Photodamaged skin shows major modifications in the dermis, including loss of mature collagen and other alterations of the extracellular matrix (ECM), which can lead to wrinkles, pigmented spots, dryness, and tumors.²² One approach to prevent photoaging is the prevention of UV penetration into the skin by sunscreens. Recently, we have shown that the flavonoid luteolin absorbs UV radiation and reduces UV transmission in a first line of defense¹⁵ and that luteolin can neutralize ROS caused by UV radiation. ROS formation in the dermis can directly degrade the collagen matrix or cause MMP-1 activation through the formation of peroxynitrite, a reaction product of UV-induced nitric oxide and superoxide anions.^{23–27} In 1996, Fisher and colleagues

FIG. 5. Effect of luteolin on irradiation-induced interleukin-6 (IL-6) and matrix metalloproteinase-1 (MMP-1) expression *ex vivo*. Human skin explants were treated with luteolin (8 $\mu\text{g}/\text{mL}$), p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580 (20 μM), or *N*-acetylcysteine (NAC; 816 $\mu\text{g}/\text{mL}$) before irradiation with a solar simulator (SS) at 6 J/cm². At 24 hr after irradiation, the supernatant was collected. **(A)** Measurement with an enzyme-linked immunosorbent assay (ELISA) of IL-6 in the supernatant. **(B)** Measurement with an ELISA of MMP-1 in the supernatant. Values of irradiated skin explants were arbitrarily set to 100%; all other samples were indicated as percentage of control. The values are means $\pm$ standard deviation (SD) of three independent experiments.

FIG. 6. Schematic summary of the complex fibroblast-protecting properties of luteolin. Luteolin protects fibroblasts directly from deleterious effects of ultraviolet A (UVA) (applicable via mesotherapy) or indirectly by influencing the cytokine release from irradiated keratinocytes in the epidermis (applicable topically). UVA, Ultraviolet A light; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; IL-6, interleukin-6; MMP-1, matrix metalloproteinase-1; HA, hyaluronic acid.

showed for the first time the *in vivo* induction of MMP expression and activity after exposure of human skin to low-dose UVB irradiation via the upregulation of the transcription factors activator protein-1 (AP-1) and nuclear factor- κ B (NF- κ B) in keratinocytes and fibroblasts.^{28,29} Luteolin prevents UVA-induced ROS formation in NHDFs (data not shown) and in this way prevents collagen fragmentation. This is in line with the findings of Kang and colleagues, who have shown that UV-induced collagenase expression in human skin can be inhibited by topical treatment with the antioxidants genistein (an isoflavone that was first isolated from soybeans with potent inhibitory effects for tyrosine kinase activity) and NAC.³⁰

In recent years, various polyphenols isolated from green tea, grape seed, *Polypodium leucotomos*, *Reseda luteola*, and other plants have been analyzed for their effectiveness in reducing radiation-induced MMP expression to prevent cellular photodamage.^{15, 31–33} Epigallocatechin-3-gallate from green tea has been shown to possess the potential to reduce photoaging and cancer. However, direct interaction of such polyphenols with fibroblasts is not achieved with topical application, because the epidermis prevents their penetration into the dermis. Although it has been shown that topically applied luteolin can interact with superficial epidermal layers,³⁴ it is unlikely to penetrate into the dermis. However, keratinocytes are a major source of cytokines²⁰ that indirectly trigger the MMP-1 production in dermal fibroblasts.^{2,7}

Accordingly, removal of the epidermal layer from an *in vitro* skin equivalent immediately after SSR exposure abolished MMP-1 production in the remaining fibroblasts.² This is consistent with our results showing that luteolin can reduce SSR-induced cytokine release from keratinocytes, eventually preventing MMP-1 expression in fibroblasts. This reduced IL-6 expression after SSR induction is caused by antioxidant and radical scavenging activities of luteolin as already described, leading to reduced amounts of ROS (*e.g.*, hydrogen peroxide) in the supernatant or to a reduced release of proinflammatory cytokines from ROS-activated keratinocytes. Similarly, it has been shown that the antioxidant ubiquinone suppresses UV-induced MMP-1 production of fibroblasts by inhibiting the production of inflammatory cytokines, *e.g.*, IL-6, in UV-irradiated keratinocytes.³⁵ As neutralizing IL-6 antibodies alone are not able to fully block SSR-induced MMP-1 production in fibroblasts³⁶ and IL-6 transgenic mice show a growth defective phenotype but no pathological signs of photoaging,³⁷ further soluble factors seem to be involved in this process, *e.g.*, the IL-10 family member IL-20. IL-20 transgenic mice exhibit wrinkled skin with epidermal hyperplasia and increased cell proliferation with abnormal expression of differentiation markers (*e.g.*, keratin-5, keratin-6, and keratin-14).³⁸ Intriguingly, many of these changes have also been described for UV-irradiated mouse and human skin.⁵

Recently, we have shown that UVB irradiation enhances IL-20 expression in human keratinocytes, and the IL-20 heterodimeric receptor complex consisting of the subunit IL20R1 and IL20R2 is expressed on NHDFs.⁶ These findings suggest a role of this novel ligand in dermal photoaging. In several pathological conditions, including cutaneous inflammation, psoriasis, and eczema, the expression of IL-20 is dramatically increased, so that the IL-20 system may be a useful target for therapeutic intervention.³⁹ As we have

shown here, luteolin may be such a candidate because it reduces the SSR-induced release of IL-20 in keratinocytes, eventually reducing MMP-1 production in fibroblasts that are treated with such a conditioned keratinocyte medium. Neutralizing IL-20 antibodies also reduced MMP-1 production, but the effect was less pronounced compared to the p38 MAPK inhibitor, indicating that several p38 MAPK-dependent cytokines are involved in MMP-1 upregulation in fibroblasts, as suggested by Dong and colleagues.⁴⁰

Besides collagen, HA as the major nonfibrous component in dermis and epidermis plays an essential role in protecting the skin from dryness by its capacity to bind water.¹¹ The HA content of epidermis and dermis decreases after UVB irradiation due to ROS-mediated decreased synthesis and increased degradation of HA.¹⁴ As we have shown here, luteolin can directly and indirectly inhibit hyaluronidase activation and thus prevent the degradation of HA. A direct link between UVB-induced collagen cleavage and the loss of HA synthesis seems to occur via collagen fragment-induced inhibition of Rho kinases during skin aging.¹³

A third way to reduce photoaging is the use of hyaluronidase and collagenase inhibitors that block already active enzymes. Luteolin is also a potent hyaluronidase inhibitor. This activity of luteolin was originally discovered by its ability to dose-dependently inhibit five different venoms from honey bees, snakes, and scorpions.⁴¹ Lim and Kim demonstrated that the flavonoids quercetin and apigenin directly inhibit MMP-1 activity.⁴² Although luteolin was not tested in this study, it may also possess direct MMP-1 inhibitory activity because its chemical structure is closely related to that of quercetin and apigenin.

In conclusion, luteolin inhibits UV-induced IL-20 expression in keratinocytes and consequently reduces MMP-1 expression in NHDFs. Topical application of luteolin may reduce solar radiation-induced collagen and HA degradation and thus may prevent photoaging of the skin (Fig. 6).

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Author Disclosure Statement

The Competence Center skintegral holds a patent on the production of luteolin-rich extracts from *Reseda luteola* and their use.

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