Original Article

UVB-Induced Senescence of Human Dermal Fibroblasts Involves Impairment of Proteasome and Enhanced Autophagic Activity

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Abstract

In the current study, we have extended previous findings aiming at a better understanding of molecular mechanisms underlying UVB-induced senescence of diploid human dermal fibroblasts (HDFs), an experimental model to study the process of photoaging in the skin. We provide evidence that the inhibition of proteasomal degradation of damaged proteins and the activation of autophagosome formation are early events in UVB-induced senescence of HDFs, dependent on UVB-induced accumulation of reactive oxygen species. Our data suggest that autophagy is required for the establishment of the senescent phenotype in UVB-treated HDFs and that inhibition of autophagy is sufficient to change the cell fate from senescence to cell death by apoptosis. Studies in reconstructed skin equivalents revealed that UVB irradiation triggers hallmarks of autophagy induction in the dermal layer. These findings have potential implications for fundamental as well as translational research into skin aging, in particular photoaging.

Keywords: Senescence—Skin aging—UVB—Proteasome—Autophagy

Cellular senescence is increasingly recognized as a physiological process involved in tumor suppression, age-associated dysfunction in various tissues (1,2), and as a regulatory switch in mammalian development (3). Senescent cells express a number of nonexclusive markers, including the cell cycle inhibitor p16INK4A and elevated levels of senescence-associated β-galactosidase (SA-β-Gal) (1). Many senescent cells also secrete several cytokines, growth factors, and matrix metalloproteinases, collectively referred to as the senescence-associated secretory phenotype (4). In human skin, cells positive for the senescence markers SA-β-Gal (5) and p16INK4A (6,7) accumulate with age, and there is increasing evidence that the persistence of senescent fibroblasts in aged skin contributes to functional and morphological alterations associated with skin aging (8).

Photoaging of the skin occurs due to UV irradiation, in particular UVA and UVB, which, based on their distinct physical properties, induce distinct albeit overlapping biological responses in both the epidermal and dermal layers of the skin (9). In the case of human dermal fibroblasts (HDFs), repeated mild treatment with UVB has been established as an experimental model to study some aspects of extrinsic skin aging (10), which depends on the accumulation of senescent cells, in particular fibroblasts in the dermis (11). Transcriptional profiling of HDFs undergoing UVB-induced senescence has revealed differentially regulated miRNAs and mRNAs and suggested a role for several regulatory pathways in this process. In this study, we also noted upregulation of both autophagy-related and pro-apoptotic genes; however, apoptotic cell death was not observed in UVB-induced senescence (12).
Macroautophagy, referred to as autophagy below, is a universal quality control mechanism acting in most if not all eukaryotic systems to ensure cellular homeostasis, and in particular proteostasis (13). Microtubule-associated protein 1 light chain 3 (LC3) is a key factor in the process of autophagosome formation. In untreated cells, LC3 is present mainly in its unlipidated form. Upon autophagy activation, LC3 is covalently linked to the lipid PE and lipidated LC3 is then recruited to the membrane of the autophagosomes (14). Autophagy is an essential metabolic process that is upregulated by nutrient depletion, mTOR inhibition, and activation of AMPK (5’ adenosine monophosphate-activated protein kinase) (15). In young cells, autophagy is maintained at basal levels under nonstressed conditions and further activated by different stressors, such as excess production of reactive oxygen species (ROS) (16). In the context of skin aging, basal levels of autophagy are increased during replicative senescence of human facial skin fibroblasts (17). Furthermore, autophagy is induced in HDFs by UVA (18) and in human keratinocytes by UVB (19); however, it seems that autophagy delays but does not abrogate the senescence response of these cell types (20,21). Specifically in the dermis, the increased number of autophagosomes found in fibroblasts of aged donors is mainly caused by impaired autophagic flux, which triggers alterations in the content of extracellular matrix proteins and, consequently leads to deterioration of dermal integrity and increased skin fragility (21). Recent work has established a complex interplay between autophagy and senescence that strongly depends on cellular context. Briefly, autophagy was established as an essential mediator of oncogene-induced senescence in several human cell types (22,23), whereas it seems to play no role in senescence due to telomere shortening (24). In certain cell types, and in response to certain types of senescence-inducing stimuli, autophagy can inhibit senescence (25–28). The role of autophagy in UVB-induced senescence of HDFs is currently unknown.

Besides autophagy, the 20S proteasome is the second essential quality control system to maintain proteostasis. Consistent with the observed accumulation of oxidized and damaged proteins in senescent HDFs, a general reduction of proteasome activity has been observed in HDFs undergoing replicative senescence (29). The activity of proteasomes and autophagosomes is regulated by overlapping signals, and regulatory cross-talk between both quality control systems has been described. Thus, it has been shown that proteasome inhibition induces autophagy, implying the role of autophagy as a compensatory mechanism upon impairment of proteasomal degradation (30,31). Others reported that the proteasome was activated in response to autophagy inhibition (32). These observations suggest a complex regulatory module linking the two main protein quality control systems. However, the regulation of this module in UVB-induced senescence of HDFs has remained elusive. In the current communication, we address the role of protein quality control systems and their functional interaction in mediating the senescence response in UVB-treated HDFs.

**Methods**

**Chemicals**

All chemicals were purchased from Sigma (Steinheim, Germany) unless stated otherwise.

**Cell Culture and UVB Irradiation**

HDFs derived from newborn foreskin, purchased from ATCC (Manassas, VA, SCRC-1042) at Passage 0, were expanded until Passage 4 and cultivated in normal DMEM or in DMEM containing 4-mM N-acetyl-cysteine (NAC). Cumulative population doublings (cPDLs) were calculated as described (33). Under our experimental conditions, HFF-2 cultivated from Passage 4 were able to perform up to 45 cPDLs (34). Human keratinocytes were cultivated in Keratinocyte Growth Medium-2 (KGM-2; Lonza, Walkersville, MD) without calcium, to avoid differentiation, and splitted under less than 85% of confluence. UVB treatment of HDFs was performed as described (12), using eight irradiations of 3,250 J/m² each in 4 days.

**Detection of ROS and Tracking of Acidic Organelles**

Production of intracellular ROS in HDFs submitted to UVB was measured by flow cytometry with the use of dihydroethidium (DHE; Molecular Probes, Vienna, Austria) staining. Abundance of acidic organelles in the cytoplasm of living cells was also measured by flow cytometry with the use of the fluorescent probe LysoTracker Red (LTR, Life Technologies, Vienna, Austria). For this purpose, cells were washed, trypsinized, and incubated in media containing 20-pM DHE for 30 minutes at 37°C or 50-nM LTR for 1 hour also at 37°C. After incubation, cells were washed and resuspended in phosphate-buffered saline. Fluorescence was measured using the fluorescence-activated cell sorting (FACS) Canto II flow cytometer (BD Biosciences, Franklin Lakes, NJ). A positive control for DHE, 0.5 µM of the mitochondrial complex I inhibitor rotenone was used along with the DHE staining. The positive control for LTR consisted of cells incubated with 30-mM ammonium chloride for 4 hours prior to LTR staining (35).

**RNA Isolation and Quantitative Real-Time PCR**

Total RNA was extracted from cells using the RNeasy Mini Kit (Qiagen, Hilden, Germany; 74106), and cDNA synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit.
Stable Knockdown and Overexpression
Degron-destabilized green fluorescent protein (GFP-degron) expressing HDFs were produced as described (37). Lentiviral vectors carrying ATG7-targeting shRNA sequence and expression vectors containing the human LC3B gene fused at its 5’ end to the GFP gene were produced as described (38) and used for the production of viral particles (38). For stable knockdown, HDFs (Passage 4) were seeded at 80% confluence in six-well plates and incubated overnight. On the next day, medium was replaced by DMEM containing 8 multiplicity of infection (MOI) of virus plus 8 µg/mL of hexodimethine bromide. Lentivirus containing empty vectors were used as controls. The media was exchanged 24 hours after infection. Selection with 500 ng/mL of puromycin started on Day 3 after infection. For stable overexpression, cells were infected with 6 MOI of viral particles and selected with 10 µg/mL of blasticidin for 12 days.

Monitoring of Proteasome Activity
To monitor proteasome activity, fibroblasts carrying the GFP-degron fusion protein were analyzed by flow cytometry as described (37).

Detection of Apoptotic and Necrotic Cells
Cells were stained with FITC-conjugated Annexin V (BD PharMingen, Vienna, Austria) and propidium iodide according to the manufacturer’s protocol (BD PharMingen). Cells were analyzed using the FACS Canto II system (BD Biosciences).

Skin Equivalents
Foreskin fibroblasts were mixed with collagen matrix (Biochrom, Berlin, Germany) and seeded in deep wells (BD Biosciences). Human keratinocytes were placed over the dermal equivalent and grown submerged in KGM-2 Bullet Kit (Lonza) for 48 hours. Skin equivalents were subjected to air–liquid interface (ALI) in differentiation media (KGM Bullet Kit, Lonza, with 25 mg of ascorbic acid, 10 µg/mL of transferrin, and 1 M of CaCl2) for 7 days (39). Starting on Day 8 after the establishment of ALI, skin equivalents were subjected to up to six consecutive UVB irradiations, as described earlier for HDF monolayers.

Histology and Immunostaining of Reconstructed Skin
Reconstructed skin samples were fixed in 4% paraformaldehyde and processed for histology. 5-µm serial paraffin sections of skin equivalents were either stained with hematoxylin–eosin and observed under normal light microscope or further processed for routine indirect immunofluorescence. Sections were incubated with the following primary antibodies: Atg7 (Promokine, Bethesda, MD) and Lamp-1 (Sigma) overnight at 4°C. Alexa Fluor 594 goat anti-rabbit was used as secondary antibody. The nuclei were counterstained with 7-aminoactinomycin D (7-AAD), and the samples were observed under confocal laser-scanning system MicroRadiance (Bio-Rad, Hemel Hempstead, Hertfordshire, UK).

Statistics
All experiments were repeated at least three times unless otherwise specified. Results are displayed as mean values of independent experiments ± SD. Differences were compared using Student’s t test. In all graphics *p < .5; **p < .1; ***p < .01

Ethical Statement
For work with skin equivalents, the Declaration of Helsinki protocols were followed, and patients gave their written, informed consent.

Results
Autophagy Is Activated in the Early Phase of UVB-Induced Senescence
HDFs, derived from newborn foreskin, were submitted to mild and repeated doses of UVB, as described (12). Cytoplasmic ROS production and the content of acidic organelles were assessed in living cells by DHE staining (Supplementary Figure 1A) and LTR staining (Supplementary Figure 1B), respectively. Upon staining of living cells with the redox-sensitive dye DHE, we observed increased levels of cytoplasmic ROS, starting from Day 2 after the first UVB irradiation (Supplementary Figure 1A). This process was accompanied by a significant increase in the number and size of acidic organelles, as shown by LTR staining (Supplementary Figure 1B), suggesting a potential involvement of autophagy. A reporter protein (referred to as LC3-GFP fusion protein), consisting of microtubule-associated protein 1 light chain 3 (LC3) fused to a GFP, provides a useful tool to track autophagy activation through the formation of green punctuated structures in the cytoplasm of the cells (35). In order to verify the activation of autophagy upon UVB, we assessed the generation of LC3-GFP–positive vesicles (autophagosomes) in control nonirradiated and UVB-treated LC3-GFP–expressing fibroblasts by confocal microscopy. A basal level of autophagosomes was observed in control nonirradiated cells (Figure 1A, Supplementary Figure 2A). In contrast, cells treated with UVB showed a boost of autophagosome formation, with special emphasis on Day 2 where the average number of autophagosomes per cell was eight times higher compared with their respective controls (Figure 1A, Supplementary Figure 2A). Autophagic flux remained high until Day 7 after the first stress and returned to values of control nonirradiated cells on Day 9 (Supplementary Figure 2B). Treatment with UVB did not increase apoptosis in these conditions (Supplementary Figure 2C). To further analyze the process of UVB-induced LC3 lipidation, proteins isolated from control and irradiated cells were analyzed by Western blotting using anti-LC3 antibodies (Figure 1B, Supplementary Figure 2D). We observed an increased conversion of LC3-I to its lipidated form LC3-II upon UVB treatment, particularly visible at Day 3. UVB-induced formation of autophagosomes and autophagic vacuoles was confirmed by ultrastructural analysis of UVB-treated and control cells at Day 2 after starting the UVB treatment (Figure 1C, Supplementary Figures 3 and 4).

Blockage of ROS Accumulation Prevents UVB-Induced Autophagy
In order to understand the role of ROS in the development of UVB-induced senescence and autophagy induction during this process, HDFs were cultivated in the presence of the antioxidant NAC and submitted to mild UVB stress, as described earlier. The cells were monitored for cell proliferation (Figure 2A), number of autophagosomes (Supplementary Figure 2A), and number and size of acidic organelles in the cytoplasm (Supplementary Figure 5). In comparison with control cells cultivated in normal media, cells grown in the presence of NAC achieved a slightly lower number of PDLs (Figure 2A). UVB-irradiated cells cultivated in normal media performed around two PDLs during the 15 days of the experiment, in accordance with previous findings (12) whereas, in cells cultivated in the presence of
NAC, UVB led to cell loss, reflected as negative values in the proliferation curve (Figure 2A). When autophagy initiation was monitored through the formation of LC3-GFP puncta in the cytoplasm of UVB-treated HDFs, we found that NAC treatment reduced the number of autophagosomes per cell, suggesting that the increase in ROS levels is an important event in autophagy activation upon UVB (Supplementary Figure 2A). When the abundance of acidic organelles upon UVB treatment was determined by FACS analysis using the fluorescent probe LTR, we observed a significant decrease in LTR fluorescence in UVB-treated cells cultivated in media containing NAC on Days 1, 2, 7, and 9, when compared with UVB-treated cells grown in control media (Supplementary Figure 5). No statistical difference in LTR fluorescence was observed on Days 3 and 4.

Interplay Between Proteasome and Autophagy Activity During UVB-Induced Senescence

To monitor proteasome activity upon UVB irradiation of HDFs, we generated a reporter cell strain consisting of HDFs carrying a GFP-degron. In control/untreated GFP-degron expressing fibroblasts, the destabilized protein is efficiently ubiquitinated and hence targeted to proteasomal degradation, leading to low GFP fluorescence (37). When proteasome activity is blocked either by pharmacological inhibitors or by oxidative stress, GFP-degron fusion protein is stabilized and accumulates in the cytoplasm, providing a convenient tool to study proteasome activity in living cells (37). When HDFs expressing GFP-degron

Figure 1. UVB induces autophagy. (A) LC3-GFP expressing HDFs were submitted to UVB and processed for confocal microscopy. Wild type HDFs were subjected to the same treatment and analyzed by (B) Western blot or (C) electron microscopy. Representative images of three independent experiments showing the punctuation of LC3 upon 1, 2, 3, and 4 days of UVB treatment (D1, D2, D3 and D4, respectively) and 3 days after the last UVB stress (D7). (B) Representative Western blot showing the lipiddation of LC3 upon UVB, where LC3-I corresponds to unlipiddated and LC3-II to lipidated form of the protein. (C) Electron micrograph of cells submitted for 2 days to UVB irradiation compared with nonirradiated control cells. Arrows indicate autophagosomes.

Figure 2. Autophagy activation in UVB-treated fibroblasts occurs subsequently to proteasome impairment and is decreased upon reactive oxygen species scavenging. (A) Wild type fibroblasts were cultivated in normal media or in media containing 4 mM of the antioxidant N-acetyl cysteine (Nac) submitted to UVB and monitored for cell proliferation. Proliferative capacity was compared with nonirradiated cells grown in the same conditions. (B) Fibroblasts expressing the degron-destabilized green fluorescent protein reporter were UVB irradiated, and the green fluorescence resulting from proteasome inactivation was analyzed by flow cytometry. For FACS analysis, untransfected cells were used as negative controls to determine the correct gating. (C) LC3-GFP expressing fibroblasts were incubated with 100 µM of the proteasome inhibitor LLnL for 3 hours, and the number of LC3 puncta per cell was counted and compared with the punctuation of cells cultivated in the absence of LLnL. Data present mean values ± SD. *p < .5; **p < .1; ***p < .01.
protein were subjected to UVB treatment, a significant decrease of proteasome activity, relative to nonirradiated controls, was observed by flow cytometry (Figure 2B) and confirmed by confocal microscopy (Supplementary Figure 6A). Addition of the proteasome inhibitor N-Acetyl-Leu-Leu-Norleu-al (LLnL), used as a positive control, increased the percentage of cells positive for GFP fluorescence from 6% to 70% (Supplementary Figure 6, and data not shown).

Of interest, a significant increase of GFP fluorescence could already be detected after the first day of UVB treatment, indicating that inhibition of proteasome activity might actually trigger autophagy activation. To investigate this hypothesis, LC3-GFP expressing HDFs were treated with the proteasome inhibitor LLnL and autophagosome formation was analyzed by confocal microscopy (Supplementary Figure 6B). The number of puncta was quantified and compared with untreated cells (Figure 2C). We observed around 50% increase in cytoplasmic LC3-GFP punctuation upon suppression of proteasome activity. Conversely, when autophagy was inhibited by 3-methyl adenosine (3-MA) in GFP-degron expressing HDFs, no change of proteasome activity was observed (Supplementary Figure 6A).

Blocking Autophagy Changes the Fate of UVB-Irradiated HDFs From Senescence to Apoptosis

Based on our findings on autophagy activation upon UVB treatment, HDFs were depleted for the autophagy-related gene Atg7 using a lentiviral vector carrying Atg7-specific shRNA. Nontargeting shRNA was used as a control. Atg7 is essential for the conjugation between Atg5 and Atg12 as well as the lipidation of LC3, two fundamental mechanisms during autophagosome formation (40). Thus, silencing of this gene prevents autophagy initiation (41), providing a useful tool to study the relevance of autophagosome formation during UVB-induced senescence. Infection of HDFs with the Atg7 shRNA vector led to roughly 50% reduction of Atg7 mRNA and protein levels (Figure 3A). Atg7-silenced and control HDFs were submitted to UVB irradiation, and their senescence response was monitored (Figure 3B). In the absence of UVB irradiation, growth curves obtained for Atg7 knockdown and control HDFs during 15 days were not significantly different and matched the growth curves obtained with uninfected HDFs (12). However, upon UVB irradiation, clear differences emerged. The cells treated with nontargeting shRNA went through less than 2 PDLs during 15 days as described for UVB-treated HDFs before (12), with no detectable cell death. In contrast, UVB treatment of Atg7-depleted HDFs triggered a significant loss of cell numbers in the same time frame. When the frequency of apoptotic and necrotic cell death was assessed by Annexin V-PI staining followed by flow cytometry, no necrosis was detected in any of the groups (data not shown); however, UVB treatment induced a significant increase in apoptotic cell death in Atg7-depleted cells (Figure 3C). This effect was especially visible at Day 4, that is, the last day of irradiation. When cells were stained for senescence-associated beta galactosidase (SA-β-Gal) activity, we observed that roughly 70% of the cells treated with nontargeting shRNA reached the senescent phenotype by Day 9, as described before (12). In contrast, UVB treatment of Atg7-depleted cells resulted mainly in apoptotic cell death (almost 57%) with a relatively small proportion of cells (27%) undergoing senescence under these conditions (Figure 3D).

Studies With Skin Equivalent Models Submitted to UVB Irradiation

Reconstructed human skin was prepared as described (39,42) and subjected to up to 3 days of repeated UVB irradiation (3,250 J/m²), as described earlier for HDF monolayers. The tissues were then fixed and processed for routine histology. For morphological analyses, 5-µm paraffin sections were stained with hematoxylin–eosin (Figure 4A). Nonirradiated (control) skin equivalents exhibited a normal morphology, similar to healthy skin. After 2 days of UVB treatment, we observed disorganization of the epidermal layers and loss of basal layer polarization (Figure 4A central panel). Moreover, we observed the appearance of the so called “sunburn
The keratinocytes (merge) and in fibroblasts (detail), where the signal was observed in granular structures (Figure 4B). Lamp-1, on the other hand, was poorly expressed in the cytoplasm of epidermal cells from the control group (Figure 4C, upper panel, detail), and no fibroblasts positive for Lamp-1 were observed in this group (detail). Exposure of skin equivalents to UVB increased the expression of Lamp-1 both in the epidermis and in the dermis especially after 3 days of irradiation. We observed that in the dermal fibroblasts, this protein appeared organized in cytoplasmic granular structures after 2 and 3 days of irradiation (Figure 4C, middle and lower panels, detail).

**Discussion**

The concept of proteostasis or protein homeostasis implies that integrated biological pathways control the synthesis, modification, transport, and degradation of proteins in a cell (44). During aging, the ability of the organisms to maintain the quality of these processes is compromised, and disruption of protein quality control mechanisms is associated with aberrant accumulation of misfolded proteins or protein aggregates (20,45). The ubiquitin–proteasome pathway and autophagy are the major cellular proteolytic machineries, and chronic imbalance of these systems, and consequently loss of proteostasis, has a negative impact on both cellular and organismal life span (17,37,46,47). The functional relationship between autophagy and cellular senescence is complex. Thus, autophagy was described as an essential process that promotes the senescence transition in response to oncogenic HRASG12V (23). Similarly, senescence triggered by chronic expression of a proteolytic Cyclin E fragment in human epithelial cells exposed to DNA damage is prevented by depletion of Atg7 (22). Autophagy is also enhanced during replicative senescence of human skin fibroblasts (17). It was shown that telomere dysfunction rapidly induces autophagic flux when human fibroblasts approach replicative senescence (24). In contrast to our observations with UVB-induced senescence, inhibition of autophagy did not have a significant impact upon the transition to replicative senescence caused by telomere attrition (24). Finally, in several instances, autophagy was found to inhibit senescence (26,27,40,48), highlighting the complex relationship between autophagy and senescence (20). Together these results suggest that functional links between autophagy and senescence are strongly context dependent and vary with cell type as well as with the senescence-inducing stimuli (25).

In UVB-treated HDFs, we found clear evidence for autophagy initiation. We postulate that autophagy is essential for the establishment of the senescence phenotype in HDFs upon UVB treatment and that cells deficient for this mechanism are unable to recover from the cumulative damage caused by irradiation and instead undergo apoptosis. Compared with monolayer cultures of HDFs, reconstructed human skin is an innovative and efficient model reflecting more precisely the skin’s physiological state (39). When reconstructed skin was subjected to the same UVB irradiation protocol applied for the experimental HDF monoculture cultures, we observed that UVB caused abnormal morphology of the epidermis of skin equivalents and changed the expression and subcellular organization of autophagy markers, especially in the dermal layer. Whereas these observations indicate that autophagy may be activated by UVB also in skin equivalents, technical difficulties precluded a more detailed analysis of autophagy in this experimental model. Thus, when skin equivalents containing LC3-GFP

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**Figure 4.** UVB acts on the morphology of reconstructed skin and on the expression and subcellular localization of autophagy-related proteins. Skin equivalents were (A) submitted to UVB and stained by hematoxylin–eosin for morphological analysis or (B) subjected to immunofluorescence to label the autophagy initiator Atg7 or (C) the lysosome marker Lamp-1. (A) Morphological comparison of control and skin equivalents submitted to 2 and 3 days of UVB irradiation. Arrows indicate “sunburn cells.” Scale bar 50 μm. (B) Immunofluorescence for Atg7. (C) Immunofluorescence for Lamp-1. In (B) and (C), dotted lines represent the separation between dermis “D” and epidermis “E.” Scale bar 20 μm.
expressing fibroblasts were prepared and analyzed, the histological processing quenched the fluorescence of the LC3-GFP reporter protein (data not shown). Moreover, our attempts to detect GFP and LC3 proteins by immunohistochemistry with available antibodies failed (data not shown).

Activation of autophagy upon oxidative stress has been well demonstrated in various biological models (49). In turn, autophagy is known to be activated in order to reduce oxidative damage (50). Nonetheless, ROS can also function as signaling molecules, and here we demonstrate that UVB increases cytoplasmic ROS production followed by autophagy activation. Blocking of ROS accumulation by treatment with the antioxidant NAC not just prevents autophagy activation upon UVB but also leads to cell loss, suggesting that ROS-mediated signaling might have an important role in cell survival and autophagy initiation during the process of UVB-induced senescence of fibroblasts, reinforcing the complex relationship between ROS, autophagy, and longevity.

Proteasome activity is decreased in skin fibroblasts derived from middle-aged and aged donors (37). Furthermore, decrease of proteasome peptidase activities and accumulation of oxidized ubiquitinated proteins was shown to trigger autophagy in UVB-treated human keratinocytes (29). Accordingly, previous studies highlighted functional links between autophagy and proteasome-mediated degradation of damaged proteins, suggesting a functional interplay between these major protein quality assurance pathways. Still, proteasome inhibition is able to induce autophagy in flies (30), and it was shown that inhibition of autophagy compromises the degradation of ubiquitin–proteasome pathway substrates in HeLa cells (51). Our studies with GFP-LC3 and GFP-degron expressing HDFs suggest that the inactivation of proteasome subsequent to UVB irradiation triggers autophagy upregulation, and these two pathways are important in the establishment of UVB-induced senescence.

In conclusion, our findings suggest that increased ROS production and inhibition of proteasome followed by autophagy activation are early events in the process of UVB-induced senescence of HDFs. Autophagy is required for the establishment of the senescent phenotype in UVB-treated HDFs, and inhibition of autophagy is sufficient to change the cell fate from senescence to cell death by apoptosis. Our work demonstrates that reduced proteasome activity and increased autophagy contribute to the development of UVB-induced senescence in HDFs and suggests mechanisms of protein quality control as novel targets for intervention in photoaging of the skin.

Supplementary Material

Supplementary material can be found at: http://biomedgerontology.oxfordjournals.org/

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Conflict of Interest

The authors declare no conflict of interest.

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