

UCSF

UC San Francisco Previously Published Works

Title

Ultraviolet A Enhances Cathepsin L Expression and Activity via JNK Pathway in Human Dermal Fibroblasts.

Permalink

<https://escholarship.org/uc/item/70j67639>

Journal

Chinese Medical Journal, 129(23)

Authors

Xu, Qing-Fang
Zheng, Yue
Chen, Jian
et al.

Publication Date

2016-12-05

DOI

10.4103/0366-6999.194654

Peer reviewed

Ultraviolet A Enhances Cathepsin L Expression and Activity via JNK Pathway in Human Dermal Fibroblasts

Qing-Fang Xu¹, Yue Zheng¹, Jian Chen¹, Xin-Ya Xu¹, Zi-Jian Gong¹, Yun-Fen Huang¹, Chun Lu¹, Howard I Maibach², Wei Lai¹

¹Department of Dermato-Venereology, the Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong 510630, China

²Department of Dermatology, School of Medicine, University of California, San Francisco, CA 94143, USA

Abstract

Background: Cathepsin L (CatL) is a cysteine protease with strong matrix degradation activity that contributes to photoaging. Mannose phosphate-independent sorting pathways mediate ultraviolet A (UVA)-induced alternate trafficking of CatL. Little is known about signaling pathways involved in the regulation of UVA-induced CatL expression and activity. This study aims to investigate whether a single UVA irradiation affects CatL expression and activity and whether mitogen-activated protein kinase (MAPK)/activator protein-1 (AP-1) pathway is involved in the regulation of UVA-induced CatL expression and activity in human dermal fibroblasts (HDFs).

Methods: Primary HDFs were exposed to UVA. Cell proliferation was determined by a cell counting kit. UVA-induced CatL production and activity were studied with quantitative real-time reverse transcription polymerase chain reaction (RT-PCR), Western blotting, and fluorimetric assay in cell lysates collected on three consecutive days after irradiation. Time courses of UVA-activated JNK and p38MAPK signaling were examined by Western blotting. Effects of MAPK inhibitors and knockdown of *Jun* and *Fos* on UVA-induced CatL expression and activity were investigated by RT-PCR, Western blotting, and fluorimetric assay. Data were analyzed by one-way analysis of variance.

Results: UVA significantly increased CatL gene expression, protein abundance, and enzymatic activity for three consecutive days after irradiation ($F = 83.11, 56.14, \text{ and } 71.19$, respectively; all $P < 0.05$). Further investigation demonstrated phosphorylation of JNK and p38MAPK activated by UVA. Importantly, inactivation of JNK pathway significantly decreased UVA-induced CatL expression and activity, which were not affected by p38MAPK inhibition. Moreover, knockdown of *Jun* and *Fos* significantly attenuated basal and UVA-induced CatL expression and activity.

Conclusions: UVA enhances CatL production and activity in HDFs, probably by activating JNK and downstream AP-1. These findings provide a new possible molecular approach for anti-photoaging therapy.

Key words: Cathepsin L; JNK Pathway; Photoaging; Ultraviolet A

INTRODUCTION

Photoaging is characterized by structural changes in dermal extracellular matrix, including solar elastosis and collagen degradation.^[1] Proteases capable of degrading extracellular matrix participate in photoaging. Four classes of such proteases are found in mammalian cells: Metalloproteases, aspartic, serine, and cysteine proteases. Metalloproteases and cysteine proteases are believed to be particularly important in extracellular matrix turnover.^[2] Matrix metalloproteinases (MMPs), commonly studied enzymes related to photoaging, degrade most of the dermal extracellular matrices. Their expression and activity are increased by ultraviolet (UV) radiation, resulting in reduced Type I collagen.^[3] However, MMPs are secreted extracellularly and work at neutral pH,^[4] while

cysteine proteases degrade matrix proteins in lysosomes or extracellularly under acidic conditions.^[5] UV increases the production and release of inflammatory factors, leading to local inflammation.^[6] Inflammation tends to cause local changes in pH that may result in acidic environments. Local acidic environments may increase the activity of cathepsins (Cats) rather than that of MMPs. Moreover, cysteine proteases can activate MMPs.^[7] Therefore, the role

Address for correspondence: Prof. Wei Lai,
Department of Dermato-Venereology, the Third Affiliated Hospital
of Sun Yat-Sen University, Guangzhou, Guangdong 510630, China
E-Mail: drlaiwei@126.com

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

© 2016 Chinese Medical Journal | Produced by Wolters Kluwer - Medknow

Received: 25-04-2016 **Edited by:** Li-Min Chen

How to cite this article: Xu QF, Zheng Y, Chen J, Xu XY, Gong ZJ, Huang YF, Lu C, Maibach HI, Lai W. Ultraviolet A Enhances Cathepsin L Expression and Activity via JNK Pathway in Human Dermal Fibroblasts. Chin Med J 2016;129:2853-60.

Access this article online

Quick Response Code:



Website:
www.cmj.org

DOI:
10.4103/0366-6999.194654

of cysteine proteases in photoaging has been investigated recently.^[8]

CatL is a cysteine protease abundant in fibroblasts, which is involved in skin structure and functions, including hair follicle morphogenesis, epidermal differentiation, wound healing, and antigen presentation.^[9,10] The balanced expression of CatL and its inhibitor hurpin is of great importance for normal skin function in mice.^[11] Further, CatL is a prominent protease involved in photoaging because of its potent matrix degrading and protease-activating abilities.^[12] Recent research shows that CatL activity in fibroblasts is significantly suppressed 1 h after four consecutive daily exposures to 9.9 J/cm² UVA, whereas its gene expression level remains unchanged.^[13] However, repetitive UVA irradiation elevates CatL protein synthesis through increased transcript levels in fibroblasts.^[12] Little is known about the molecular mechanisms, whereby UVA induces CatL expression and activity.

Mitogen-activated protein kinase (MAPK) pathway regulates CatL expression in several cultured cell types.^[14-16] In human gingival fibroblasts, interleukin (IL)-6/sIL-6R enhances CatL expression through the caveolin-1-mediated activator protein-1 (AP-1) pathway.^[15] In articular chondrocytes, CatL is enhanced by the N-terminal telopeptide of collagen Type II through the activation of p38MAP kinase.^[16] Since UVA activates MAPK/AP-1 pathway,^[1] we examined whether MAPK/AP-1 pathway regulates UVA-induced CatL expression and activity in human dermal fibroblasts (HDFs).

Here, we evaluated CatL expression and activity following a single UVA irradiation in HDFs. Pharmacologic inhibitors and *Jun* and *Fos* knockdown were utilized to determine the role of MAPK/AP-1 pathway in mediating UVA-induced CatL expression and activity.

METHODS

Ethics statement

Parents signed an informed consent form on behalf of their enrolled children. The parents were informed of our research objectives and their privacy and anonymity were protected. The consent procedure was conducted according to the principles expressed in the *Declaration of Helsinki*. Both the consent procedure and our study were approved by the Clinical Research Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, China (No: [2010]2-22).

Cell culture

HDFs were isolated from circumcised foreskins of children aged 5–9 years. Cells were cultured in Dulbecco's modified Eagle's media (DMEM, Gibco, USA) supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), and 10% fetal bovine serum (FBS, Gibco, USA).

Ultraviolet A irradiation and cell counting kit-8 assay

Cells were seeded in dishes and incubated overnight at 37°C prior to UVA exposure. Dishes with cells were placed

under the irradiation device (SS-03A, Sigma-Aldrich, USA) at a distance of 365 mm from the UVA source. The UVA source was TL10RS lamps (Philips, Netherlands), with an emission spectrum between 320 and 400 nm. Before each irradiation, cells were washed twice with phosphate-buffered saline (PBS). PBS was discarded and fresh culture medium was added after irradiation. Mock-irradiated control cells in PBS were covered with foil and incubated inside a clean bench.

Cell viability was determined using a cell counting kit (CCK-8, Dojindo, Japan). In brief, cells were incubated in 96-well culture plates overnight and then treated with UVA irradiation, chemical inhibitors, or small interfering RNA (siRNA). After treatment, 10 µl CCK-8 solution was added. Cells were incubated at 37°C for 4 h. Absorbance was analyzed at 450 nm using an enzyme-linked immunosorbent assay reader (Bio-Rad, USA).

Mitogen-activated protein kinase inhibitor treatment

SP600125 and SB203580 (Calbiochem, USA) were chosen to block the activation of JNK and p38, respectively.^[17] The inhibitors were dissolved in dimethyl sulfoxide to make 10 mmol/L stock solutions. Working concentrations of 800 nmol/L SP600125 and 10 µmol/L SB203580 were chosen as described previously.^[1] Cells were pretreated with inhibitors for 1 h before UVA irradiation and incubated with inhibitors again after irradiation until harvesting.

Jun and *Fos* siRNA transient transfection

Conditions for the efficient transfection were optimized in preliminary experiments. Fibroblasts at 50–70% confluence were transfected with either 100 nmol/L nontargeting siRNA (Sigma-Aldrich) or 50 nmol/L *Jun* siRNA (SASI_HsO2_00333461, sense strand 5'-GAUGGAAACGACCUUCUAUdTdT-3', anti-sense strand 5'-AUAGAAGGUCGUUCCAUCdTdT-3', Sigma-Aldrich) and 50 nmol/L *Fos* siRNA (SASI_HsO1_00115496, sense strand 5'-CACACAUGAUGUUUGACGAdTdT-3', anti-sense strand 5'-UCGUCAAACAUCAUGUGUGdTdT-3', Sigma-Aldrich) in serum-free Opti-MEM medium (Gibco, USA) using Lipofectamine RNAiMAX transfection reagent (Invitrogen, USA) according to the manufacturer's protocol. The efficiencies of *Jun* and *Fos* gene silencing were determined by reverse transcription polymerase chain reaction (PCR) and Western blotting analysis 24 h after transfection. Cells were then irradiated with 10 J/cm² UVA or mock treated before being transferred into fresh culture medium.

Quantitative real-time reverse transcription polymerase chain reaction

Total RNA was extracted using Trizol (Invitrogen, Germany) and quantified spectrophotometrically. Sequences of primers (Takara Bio Inc., China) for the amplification of each gene were as follows: *CTSL*, 5'-GCATGGGTGGCTACGTAAAG-3' (forward) and 5'-TCCCCAGTCAAGTCCTTCT-3' (reverse)

with a product size of 120 bp; *Jun*, 5'-GAAGTGTCCGAGAACTAAAG-3' (forward) and 5'-AAAAGTCCAACGTTCCGTC-3' (reverse) with a product size of 189 bp; *Fos*, 5'-TCTCCAGTGCCAACTTCATT-3' (forward) and 5'-GTGTATCAGTCAGCTCCCTC-3' (reverse) with a product size of 330 bp; and *GAPDH*, 5'-GCACCGTCAAGGCTGAGAAC-3' (forward) and 5'-TGGTGAAGACGCCAGTGGA-3' (reverse) with a product size of 138 bp. One microgram of RNA was subjected to first-strand cDNA synthesis using a PrimeScript RT reagent kit (Takara Bio Inc.) at 37°C for 15 min and 85°C for 5 s. Real-time PCR amplifications were performed on an ABI PRISM 7500 Sequence Detection System with SYBR Premix Ex Taq™ (Takara Bio Inc.). The PCR program was as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 20 s. Relative expression levels of target genes were calculated using the $2^{-\Delta\Delta C_t}$ method and determined as the fold increase over the expression in controls.^[1]

Immunoblotting analysis

Cells were harvested and total protein concentrations were measured by a BCA protein assay kit (Key-Gen Biotech, China). After heat denaturation, the samples were electrophoresed and transferred to a polyvinylidene difluoride membrane. The membrane was blocked with 5% skim milk in tris-buffered saline with Tween for 1 h at room temperature and then incubated with primary antihuman antibodies such as phospho-JNK (P-JNK), JNK, phospho-p38 (P-p38), p38 and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (Cell Signaling Technology, USA), and CatL antibody (Abcam, USA). After washing, the membrane was incubated with secondary antibody (Cell Signaling Technology) for 1 h at room temperature. Blots were detected by chemiluminescence (Millipore, USA). Band intensity was quantified with Quantity One software (Bio-Rad). All values were normalized to the corresponding GAPDH results.

Cathepsin L enzymatic activity measurement

CatL activity was measured using fluorimetric CatL activity assay kit (BioVision, USA) according to the manufacturer's instructions. Cells were lysed in lysis buffer. Cell lysate (50 μ l) was incubated with 50 μ l reaction buffer containing 1 μ mol/L Ca074 (a CatB enzymatic activity-specific inhibitor, Calbiochem, USA) for 15 min to irreversibly inhibit CatB.^[18] CatL substrate (Ac-Phe-Arg- amino-4-trifluoromethylcoumarin [AFC], 200 μ mol/L final concentration) was then added and the mixture was incubated for 1 h at 37°C. Release of free AFC was measured at λ_{ex} 400 nm and λ_{em} 505 nm using a fluorescence plate reader SpectraMax Gemini (Molecular Devices, USA). Fold increases in CatL activity were determined by comparing the relative fluorescence units against the levels of the controls.

Statistical analysis

Results are representative of at least three independent experiments. All values have been expressed as mean \pm standard deviation (SD). Statistical significance

was determined by one-way analysis of variance, followed by the least significant difference test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Effect of ultraviolet A, signaling inhibitors, and siRNA on fibroblast viability and morphology

To clarify the effect of UVA, signaling inhibitors, and *Jun* and *Fos* siRNA on CatL expression and enzymatic activity, we established an appropriate experimental culture system to exclude their cell cytotoxicity. Cell viability was determined using the CCK-8 assay. First, HDFs were irradiated with sham, 5, 10, and 15 J/cm² UVA and harvested at 24 h, 48 h, and 72 h after irradiation. Doses of up to 10 J/cm² UVA did not impair cell viability significantly for 3 days, while 15 J/cm² UVA remarkably reduced cell viability [Figure 1a]. Therefore, 10 J/cm² UVA was selected for the study.

Then, we examined the cytotoxicity of MAPK inhibitors on fibroblasts. Cells were mock irradiated or irradiated with 10 J/cm² UVA after incubation with 800 mmol/L SP600125 or 10 μ mol/L SB203580 for 1 h, and then retreated with or without MAPK inhibitors for 48 h. Viability was measured in control cells (C), SP600125-treated cells (SP), and SB203580-treated cells (SB), without irradiation or with 10 J/cm² UVA irradiation (UVA-C, UVA-SP, and UVA-SB). We demonstrated that SP600125 and SB203580 neither significantly decreased viability nor altered the morphology of control or UVA-treated cells [Figure 1b and 1d].

Finally, the effect of siRNA on cellular viability was detected. Cells were irradiated with sham or 10 J/cm² UVA 24 h after transfection with 50 nM *Jun* siRNA and 50 nmol/L *Fos* siRNA (siRNA group) or with 100 nmol/L nontargeting control siRNA (NC group) and recultured in fresh complete medium for an additional 48 h. No significant differences in cell viability or morphology were observed between control cells and cells treated with *Jun* and *Fos* siRNA, or UVA, or a combination of siRNA and UVA [Figure 1c and 1d].

Ultraviolet A enhances cathepsin L expression and enzymatic activity

To investigate whether single UVA irradiation affects CatL expression and enzymatic activity, HDFs were exposed to a single dose of UVA. Cell lysates were collected for three consecutive days after UVA exposure. As demonstrated in Figure 2a–2c, UVA irradiation significantly enhanced both CatL expression and activity for three days. There was no significant difference of either CatL expression or activity in UVA-irradiated cells among three days after irradiation.

Effect of ultraviolet A on the activation of JNK and p38MAPK pathways

Cells were irradiated with UVA and harvested at 0.75, 1.5, 3, and 6 h after treatment. Phospho-JNK and P-p38 were detected by Western blotting analysis. We demonstrated that JNK

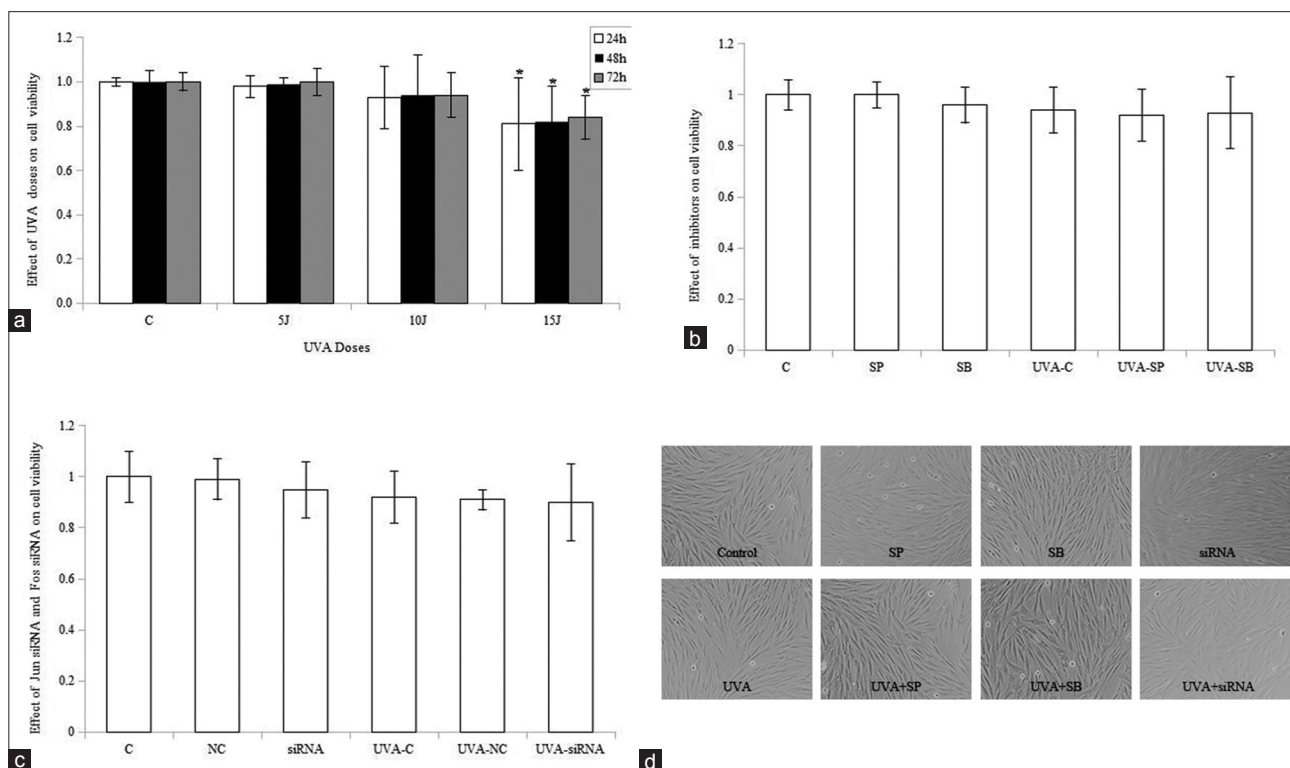


Figure 1: Effect of ultraviolet A, signaling inhibitors, and siRNA on cell viability and morphology. Cellular viability was detected after treatment with ultraviolet A (a), or ultraviolet A and inhibitors (b), or ultraviolet A and siRNA transfection (c), and fibroblasts were photographed (original magnification, $\times 10$) (d). Means \pm standard deviations are from three independent experiments. * $P < 0.05$ versus control. UVA: Ultraviolet A; C: Control; SP: SP600125; SB: SB203580; NC: Nontargeting control siRNA; siRNA: Small interfering RNA.

phosphorylation was significantly induced between 0.75 and 1.5 h, while P-p38 was remarkably enhanced between 0.75 and 3 h [Figure 3]. Total JNK and p38MAPK levels were unaffected.

Inhibition of JNK pathway impairs ultraviolet A-induced cathepsin L expression and enzymatic activity

To determine whether MAPKs play roles in UVA-induced CatL expression and enzymatic activity, fibroblasts were pretreated with 800 nmol/L SP600125 (SP) or 10 μ mol/L SB203580 (SB) for 1 h before 10 J/cm² UVA irradiation and retreated with inhibitors after irradiation for 48 h. We previously confirmed that 800 nmol/L SP600125 and 10 μ mol/L SB203580 specifically inhibited UVA-stimulated activation of JNK and p38MAPK pathways, respectively.^[1] SP600125 treatment significantly attenuated UVA-induced CatL messenger RNA (mRNA) and protein expression compared with UVA-treated controls (UVA-Cs) ($P < 0.05$) [Figure 4a and 4b]. In contrast, inhibition of p38MAPK pathway did not alter UVA-induced CatL mRNA and protein expression. In addition, basal CatL mRNA and protein expression were not affected by either inhibition of JNK pathway or p38MAPK inactivation.

Finally, we examined the effects of MAPK inhibitors on UVA-induced CatL activity. CatL activity in cells cotreated with SP600125 and UVA was remarkably impaired

compared with UVA-C cells ($P < 0.05$), while p38MAPK inhibition did not significantly suppress UVA-induced CatL activity ($P > 0.05$) [Figure 4c].

Jun and *Fos* knockdown reduces ultraviolet A-induced cathepsin L expression and activity

Since AP-1 transactivation is induced by MAPK pathway, we further studied whether UVA-induced CatL expression and activity were also mediated by AP-1. *Jun* and *Fos* siRNAs were cotransfected in fibroblasts to decrease AP-1 expression. First, we confirmed that the expression of *Jun* and *Fos* in transfected cells was significantly reduced compared with control ($P < 0.05$). Cells were then exposed to 10 J/cm² UVA 24 h after transfection and reincubated in fresh medium for 48 h. We observed that cotransfection significantly diminished UVA-stimulated CatL mRNA and protein expression compared with UVA-C ($P < 0.05$) [Figure 5b and 5c].

As protease protein expression is closely associated with activity, we investigated the role of AP-1 on UVA-stimulated CatL activity. *Jun* and *Fos* knockdown significantly decreased UVA-induced CatL enzymatic activity compared with UVA-C [Figure 5d]. In addition, cotransfection remarkably reduced basal CatL expression and activity. In contrast, CatL expression and activity in UVA-treated or non-UVA-treated fibroblasts were not significantly changed by nontargeting siRNA.

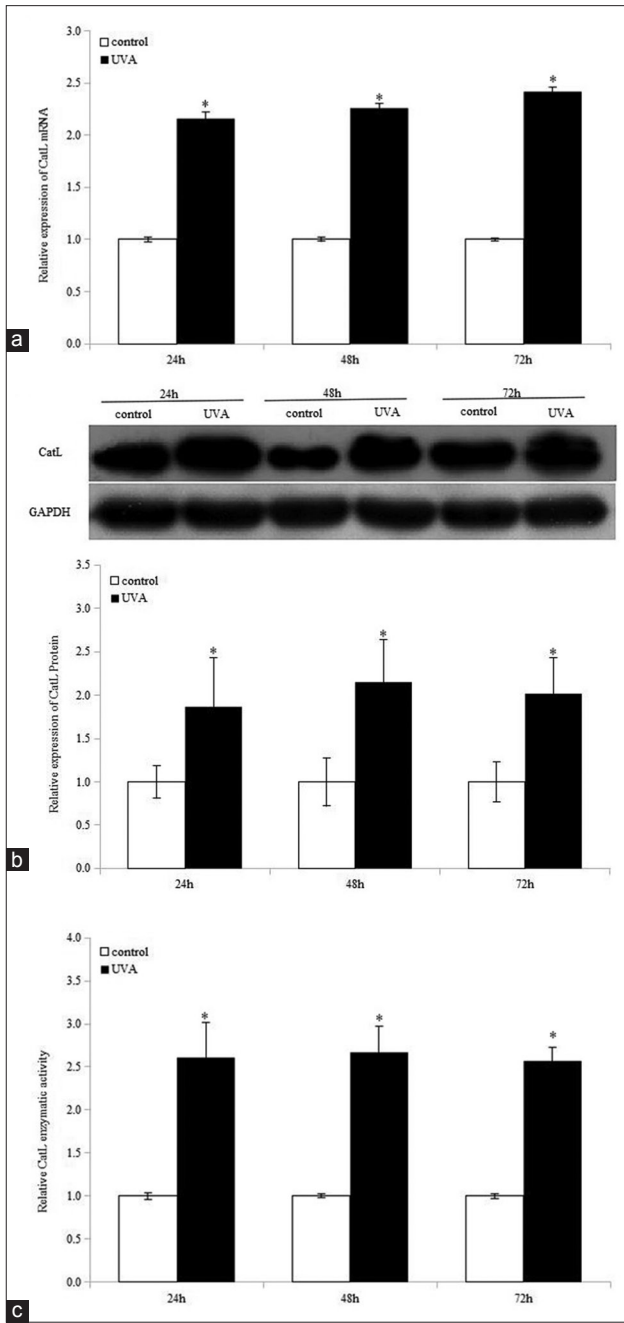


Figure 2: Ultraviolet A improves cathepsin L expression and enzymatic activity for three consecutive days in human dermal fibroblasts. Cathepsin L gene expression (a), protein expression (b), and activity (c) were examined after ultraviolet A irradiation. Means \pm standard deviations are from three independent experiments. * $P < 0.05$ versus control. UVA: Ultraviolet A; CatL: Cathepsin L; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

DISCUSSION

CatL, a member of cysteine Cat family, exhibits strong extracellular matrix degradation activity and participates in photoaging.^[12] We demonstrated that a single UVA irradiation increased CatL expression and activity in fibroblasts for three consecutive days. Similar to our results, Klose *et al.*^[12] reported that consecutive daily UVA irradiation (each 20 J/cm²) increased CatL expression and

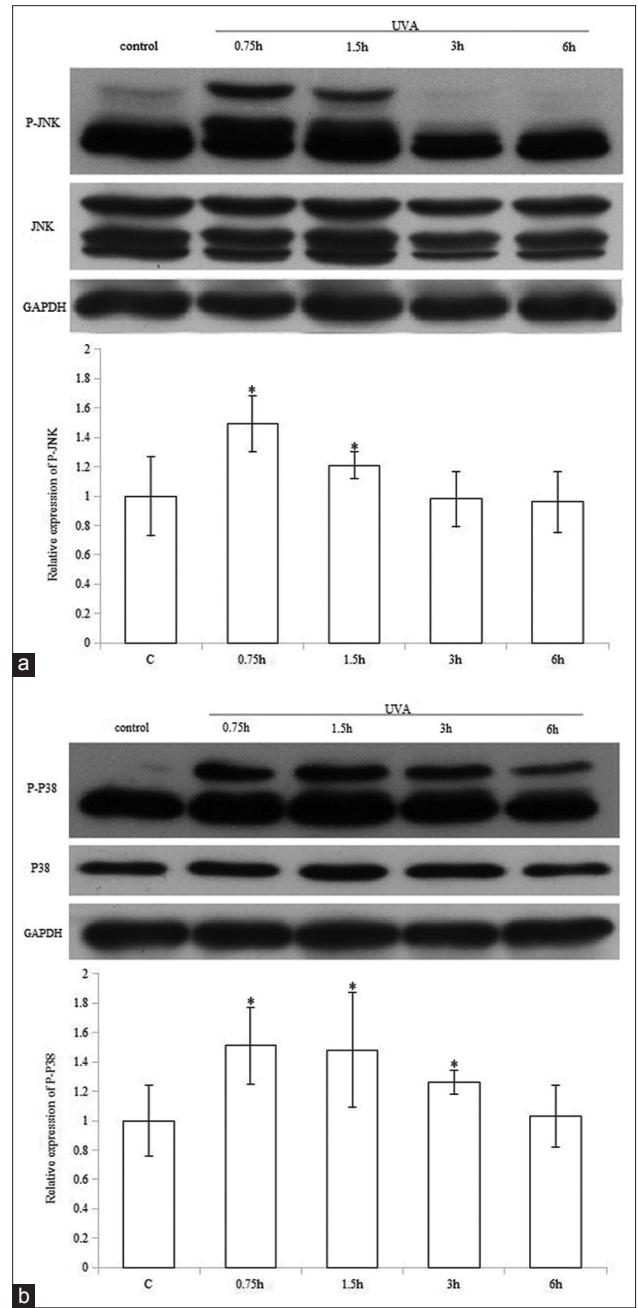


Figure 3: Ultraviolet A activates JNK and p38MAPK pathways. Western blotting analysis was performed to detect the activation of JNK (a) and p38 (b). The representative data of three independent experiments are shown. * $P < 0.05$ versus control. UVA: Ultraviolet A; P-: Phospho-; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; MAPK: Mitogen-activated protein kinase.

enhanced the extracellular release of gelatinolytic active CatL in fibroblasts. In contrast, CatL activity in fibroblasts was significantly suppressed 1 h after four consecutive daily exposures to 9.9 J/cm² UVA, whereas its mRNA level was unchanged.^[13] The discrepancy between their results and our research may be a consequence of different UVA treatment regimens. Furthermore, covalent adduction and modification of amino acid residues have been shown to be involved in the inactivation of cysteine Cats in response to electrophilic stressors (including singlet oxygen, nitroxyl, and reactive

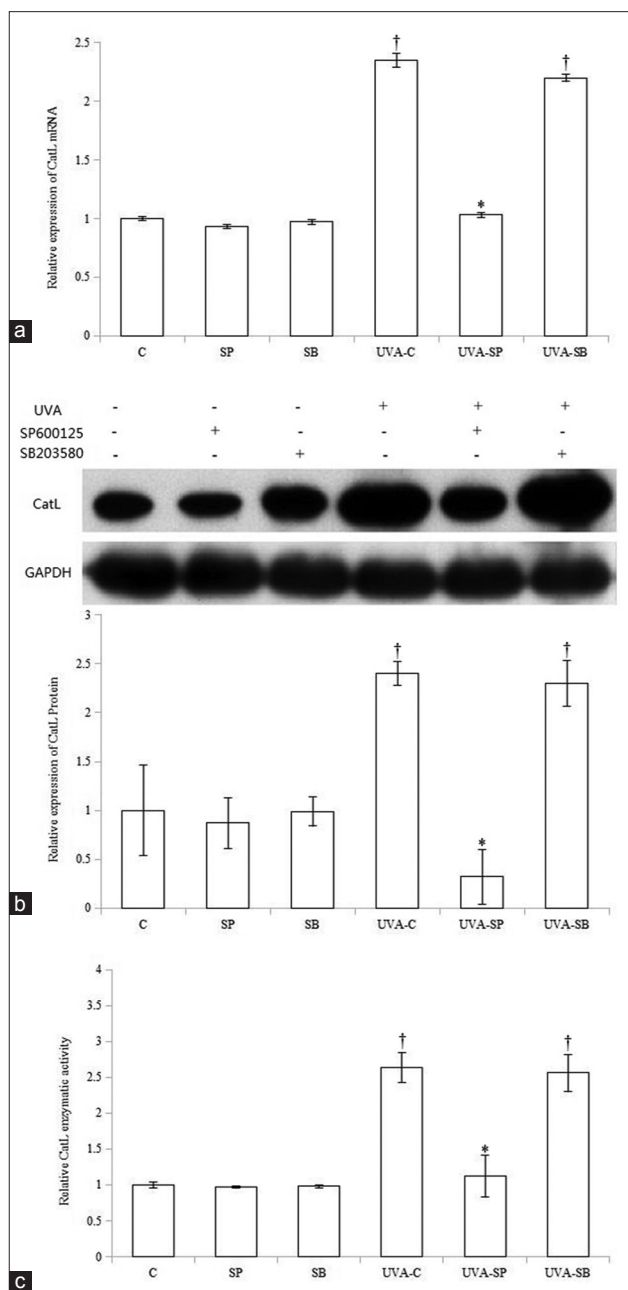


Figure 4: Effect of SP600125 and SB203580 on ultraviolet A-induced cathepsin L expression and enzymatic activity. SP600125 decreased ultraviolet A-induced cathepsin L messenger RNA (a), protein (b), and activity (c). All bar graphs represent data as means \pm standard deviation ($n = 3$). * $P < 0.05$ versus ultraviolet A-treated control or ultraviolet A-SB; † $P < 0.05$ versus control. UVA: Ultraviolet A; CatL: Cathepsin L; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; C: Control; SP: SP600125; SB: SB203580.

carbonyl species).^[19] CatL activity is also inhibited by singlet oxygen.^[20] Moreover, CatL inactivation can be antagonized by the thiol antioxidant *N*-acetyl-L-cysteine.^[13] Based on these observations, it is possible that photooxidative stress increased by a single UVA irradiation could damage CatL activity. However, enhancement of CatL production by UVA may counteract this photooxidative inhibition and lead to enhanced enzymatic activity for three consecutive days. Enhanced CatL expression and activity contribute

to extracellular matrix degradation and play roles in photodamage.

The mechanisms underlying UVA-induced CatL expression and activity are not well understood. Earlier research illustrated that mannose phosphate-independent sorting pathways were involved in mediating UVA-induced alternate trafficking of CatL.^[12] Less is known about signaling pathways involved in the regulation of UVA-induced CatL expression and activity. As UVA activates MAPK pathways,^[1] we further studied whether this signaling pathway plays a role in regulating UVA-induced CatL expression and activity. As shown in Figure 3a and 3b, we demonstrated that 10 J/cm² UVA could activate JNK and p38MAPK pathways. We had previously confirmed that 800 nmol/L SP600125 and 10 μ mol/L SB203580 specifically inhibited UVA-stimulated activation of JNK and p38MAPK pathways, respectively.^[1] We then clarified the effect of inhibition of JNK and p38MAPK pathways on UVA-induced CatL expression and activity. Interestingly, we found that UVA-induced CatL expression and activity were significantly decreased by the inactivation of JNK pathway, but were not affected by p38MAPK inhibition [Figure 4a-4c]. These results indicate that UVA-induced CatL expression and activity are regulated by JNK signaling, but not by the p38MAPK pathway. Consistent with our results, CatL expression and activity were elevated by IL-6/sIL-6R stimulation through the caveolin-1-mediated JNK pathway, but not by the p44/42 MAPK pathway in human gingival fibroblasts.^[14] However, CatL expression appears to be modulated by a complex interplay between p38 and p44/42 MAPK pathways in human fibroblasts.^[15] In addition, Ruetger *et al.*^[16] observed that both CatL and CatK expression in articular chondrocytes was enhanced by the N-terminal telopeptide of collagen Type II through p38MAPK pathway, but not through JNK pathway. Moreover, our earlier research showed that UVA-induced CatK expression correlated more with JNK activation than with p38MAPK activation.^[1] Taken together, we hypothesize that UVA-induced CatL and CatK expression might be mainly regulated by JNK pathway.

Since UVA enhances AP-1 transactivation through activating MAPK pathways,^[21] we hypothesize that AP-1 may play a role in the mediation of UVA-induced CatL expression and activity. As shown in Figure 5b-5d, *Jun* and *Fos* knockdown significantly decreased both basal and UVA-induced CatL expression and activity, suggesting that AP-1 is essential for UVA-induced CatL expression and activity. Similar to our findings, AP-1 has been found to regulate IL-6/sIL-6R-induced CatL expression and activity in human gingival fibroblasts.^[14] Moreover, the promoter of *CatL* gene contains a binding site for AP-1.^[14] These results support our finding that UVA activates JNK and its downstream transcript factor AP-1, leading to elevated CatL expression and activity.

It is well known that MAPK/AP-1 pathway is essential for UV-increased MMP expression.^[22] We hypothesize that UV-induced expression of MMPs and CatL might be

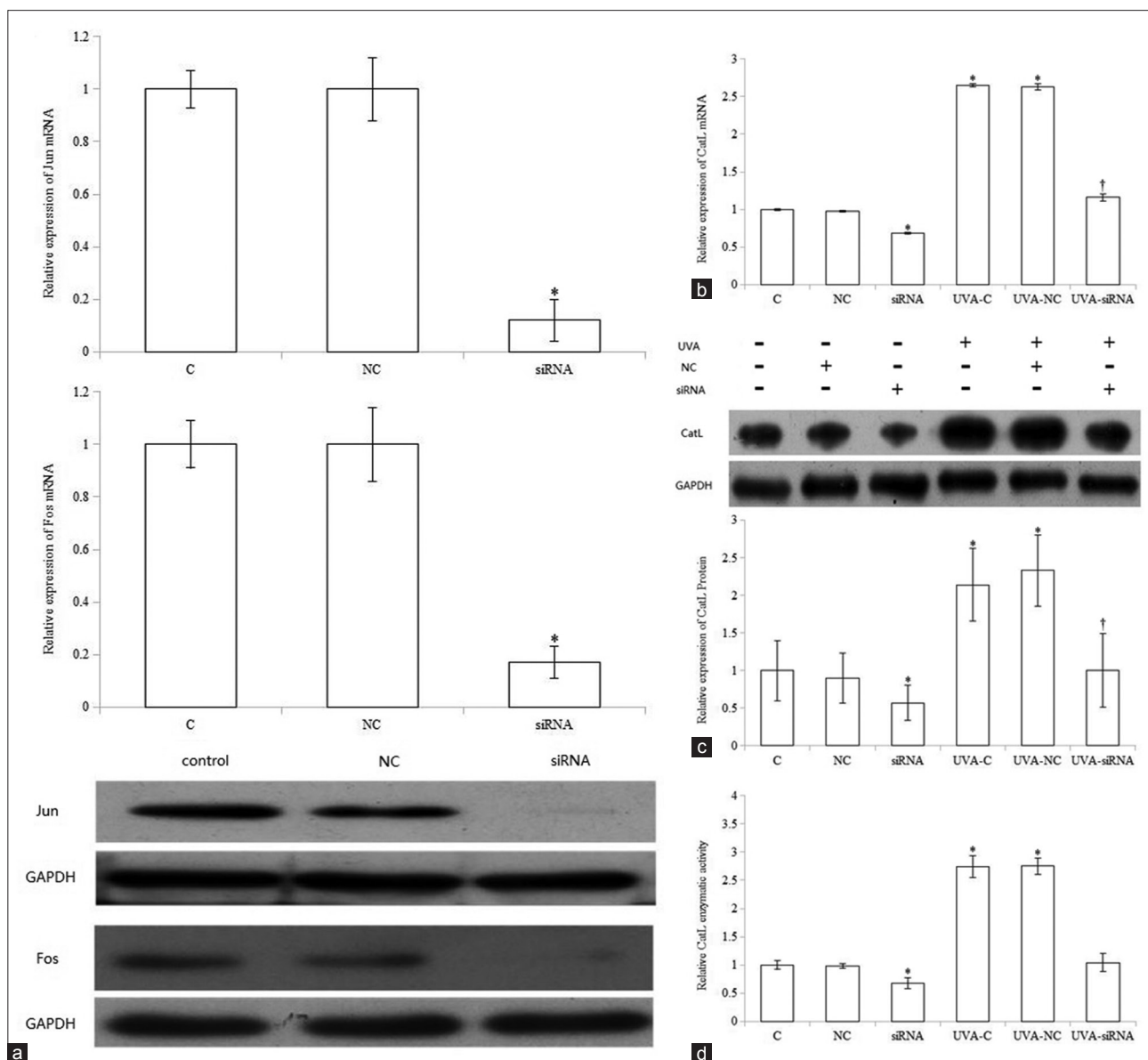


Figure 5: Effect of siRNA-mediated knockdown of activator protein-1 on ultraviolet A-induced cathepsin L expression and enzymatic activity. siRNA transfection knocked down activator protein-1 expression (a), which reduced ultraviolet A-induced cathepsin L messenger RNA (b), protein (c), and activity (d). Data are from three independent experiments. * $P < 0.05$ versus control; † $P < 0.05$ versus ultraviolet A-treated control or ultraviolet A-nontargeting control. UVA: Ultraviolet A; CatL: Cathepsin L; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; C: Control; SP: SP600125; SB: SB203580; NC: Nontargeting control siRNA; siRNA: Small interfering RNA.

modulated by some common molecular mechanisms. The exact mechanisms need further study. CatL not only directly degrades Type I collagen, but also converts pro-urokinase into urokinase.^[23] Urokinase activates plasminogen to plasmin and plasmin activates MMPs.^[7] We thus postulate that MMPs and CatL may enhance each other's expression and activity during photoaging, which then increases dermis matrix degradation and photoaging. The limitation of the present study is that we did not investigate CatL expression and activity in photoaged dermal fibroblasts or skin and whether they were also regulated by JNK pathway.

In conclusion, we demonstrated that UVA enhances CatL production and activity through JNK pathway in HDFs. Knowledge regarding these molecular mechanisms will be

useful in investigating the decrease in dermal collagen in photoaging. Our finding provides a new possible molecular basis for antiphotaging therapy.

Financial support and sponsorship

This work was supported by grants from National Natural Science Foundation of China (No. 81171523 and No. 81201241), Provincial Natural Science Foundation of Guangdong (No. 2016A030313236).

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Xu Q, Hou W, Zheng Y, Liu C, Gong Z, Lu C, *et al.* Ultraviolet

- A-induced cathepsin K expression is mediated via MAPK/AP-1 pathway in human dermal fibroblasts. *PLoS One* 2014;9:e102732. doi: 10.1371/journal.pone.0102732.
2. Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: An overview. *Mol Cell Biochem* 2003;253:269-85.
 3. Quan T, Qin Z, Xia W, Shao Y, Voorhees JJ, Fisher GJ. Matrix-degrading metalloproteinases in photoaging. *J Invest Dermatol Symp Proc* 2009;14:20-4. doi: 10.1038/jidsymp.2009.8.
 4. Malemud CJ. Matrix metalloproteinases (MMPs) in health and disease: An overview. *Front Biosci* 2006;11:1696-701.
 5. Chapman HA, Riese RJ, Shi GP. Emerging roles for cysteine proteases in human biology. *Annu Rev Physiol* 1997;59:63-88. doi: 10.1146/annurev.physiol.59.1.63.
 6. Park HS, Jin SP, Lee Y, Oh IG, Lee S, Kim JH, *et al.* Toll-like receptor 2 mediates a cutaneous reaction induced by repetitive ultraviolet B irradiation in C57/BL6 mice *in vivo*. *Exp Dermatol* 2014;23:591-5. doi: 10.1111/exd.12477.
 7. Fleetwood AJ, Achuthan A, Schultz H, Nansen A, Almholt K, Usher P, *et al.* Urokinase plasminogen activator is a central regulator of macrophage three-dimensional invasion, matrix degradation, and adhesion. *J Immunol* 2014;192:3540-7. doi: 10.4049/jimmunol.1302864.
 8. Lai W, Zheng Y, Ye ZZ, Su XY, Wan MJ, Gong ZJ, *et al.* Changes of cathepsin B in human photoaging skin both *in vivo* and *in vitro*. *Chin Med J* 2010;123:527-31.
 9. Vasiljeva O, Reinheckel T, Peters C, Turk D, Turk V, Turk B. Emerging roles of cysteine cathepsins in disease and their potential as drug targets. *Curr Pharm Des* 2007;13:387-403.
 10. Bylaite M, Moussali H, Marciukaitiene I, Ruzicka T, Walz M. Expression of cathepsin L and its inhibitor hurpin in inflammatory and neoplastic skin diseases. *Exp Dermatol* 2006;15:110-8. doi: 10.1111/j.1600-0625.2005.00389.x.
 11. Walz M, Kellermann S, Bylaite M, Andrée B, Rütter U, Paus R, *et al.* Expression of the human cathepsin L inhibitor hurpin in mice: Skin alterations and increased carcinogenesis. *Exp Dermatol* 2007;16:715-23. doi: 10.1111/j.1600-0625.2007.00579.x.
 12. Klose A, Wilbrand-Hennes A, Brinckmann J, Hunzelmann N. Alternate trafficking of cathepsin L in dermal fibroblasts induced by UVA radiation. *Exp Dermatol* 2010;19:e117-23. doi: 10.1111/j.1600-0625.2009.01014.x.
 13. Lamore SD, Wondrak GT. UVA causes dual inactivation of cathepsin B and L underlying lysosomal dysfunction in human dermal fibroblasts. *J Photochem Photobiol B* 2013;123:1-12. doi: 10.1016/j.jphotobiol.2013.03.007.
 14. Yamaguchi T, Naruishi K, Arai H, Nishimura F, Takashiba S. IL-6/sIL-6R enhances cathepsin B and L production via caveolin-1-mediated JNK-AP-1 pathway in human gingival fibroblasts. *J Cell Physiol* 2008;217:423-32. doi: 10.1002/jcp.21517.
 15. Urbanelli L, Trivelli F, Ercolani L, Sementino E, Magini A, Tancini B, *et al.* Cathepsin L increased level upon Ras mutants expression: The role of p38 and p44/42 MAPK signaling pathways. *Mol Cell Biochem* 2010;343:49-57. doi: 10.1007/s11010-010-0497-3.
 16. Ruettger A, Schueler S, Mollenhauer JA, Wiederanders B. Cathepsins B, K, and L are regulated by a defined collagen type II peptide via activation of classical protein kinase C and p38 MAP kinase in articular chondrocytes. *J Biol Chem* 2008;283:1043-51. doi: 10.1074/jbc.M704915200.
 17. Silvers AL, Bachelor MA, Bowden GT. The role of JNK and p38 MAPK activities in UVA-induced signaling pathways leading to AP-1 activation and c-Fos expression. *Neoplasia* 2003;5:319-29.
 18. Levicar N, Dewey RA, Daley E, Bates TE, Davies D, Kos J, *et al.* Selective suppression of cathepsin L by antisense cDNA impairs human brain tumor cell invasion *in vitro* and promotes apoptosis. *Cancer Gene Ther* 2003;10:141-51. doi: 10.1038/sj.cgt.7700546.
 19. Krohne TU, Kaemmerer E, Holz FG, Kopitz J. Lipid peroxidation products reduce lysosomal protease activities in human retinal pigment epithelial cells via two different mechanisms of action. *Exp Eye Res* 2010;90:261-6. doi: 10.1016/j.exer.2009.10.014.
 20. Nagaoka Y, Otsu K, Okada F, Sato K, Ohba Y, Kotani N, *et al.* Specific inactivation of cysteine protease-type cathepsin by singlet oxygen generated from naphthalene endoperoxides. *Biochem Biophys Res Commun* 2005;331:215-23. doi: 10.1016/j.bbrc.2005.03.146.
 21. Fisher GJ, Voorhees JJ. Molecular mechanisms of photoaging and its prevention by retinoic acid: Ultraviolet irradiation induces MAP kinase signal transduction cascades that induce Ap-1-regulated matrix metalloproteinases that degrade human skin *in vivo*. *J Invest Dermatol Symp Proc* 1998;3:61-8.
 22. Kim SR, Jung YR, An HJ, Kim DH, Jang EJ, Choi YJ, *et al.* Anti-wrinkle and anti-inflammatory effects of active garlic components and the inhibition of MMPs via NF- κ B signaling. *PLoS One* 2013;8:e73877. doi: 10.1371/journal.pone.0073877.
 23. Ikeda Y, Ikata T, Mishiro T, Nakano S, Ikebe M, Yasuoka S. Cathepsins B and L in synovial fluids from patients with rheumatoid arthritis and the effect of cathepsin B on the activation of pro-urokinase. *J Med Invest* 2000;47:61-75.