BMB
Reports

BMB Rep. 2015; 48(8): 467-472
www.bmbreports.org

Acceleration of heat shock-induced collagen breakdown in human dermal fibroblasts with knockdown of NF-E2-related factor 2

Gunhyuk Park[1] & Myung Sook Oh[1,2,]*

[1]Department of Life and Nanopharmaceutical Science, Graduates School and Kyung Hee East-West Pharmaceutical Research Institute, Kyung Hee University, [2]Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University, Seoul 02447, Korea

Heat shock increases skin temperature during sun exposure and some evidence indicates that it may be involved in skin aging. The antioxidant response mediated by the transcription factor NF-E2-related factor 2 (Nrf2) is a critically important cellular defense mechanism that serves to limit skin aging. We investigated the effects of heat shock on collagenase expression when the antioxidant defense system was downregulated by knockdown of Nrf2. GSH and collagenases were analyzed, and the expression of inducible Nrf2, HO-1, and NQO1 was measured. HS68 cells were transfected with small interfering RNA against Nrf2. Heat shock induced the downregulation of Nrf2 in both the cytosol and nucleus and reduced the expression of HO-1, GSH, and NQO1. In addition, heat-exposed Nrf2-knockdown cells showed significantly increased levels of collagenase protein and decreased levels of procollagen. Our data suggest that Nrf2 plays an important role in protection against heat shock-induced collagen breakdown in skin. [BMB Reports 2015; 48(8): 467-472]

INTRODUCTION

The influence of the environment, especially sunlight, is of considerable importance in skin aging (1, 2). Natural sunlight includes ultraviolet (UV), visible light, and the infrared (IR) spectrum (2). Of solar energy, 6.8% is in the UV range, 38.9% in the visible range, and 54.3% in the IR spectral range (3). Thus, skin is exposed to significant amounts of IR radiation. IR typically induces molecular vibrations and rotations, which cause an increase in temperature (4, 5). Thus, the biological effects induced by IR are usually ascribed to the generation of heat, and heat may also be involved in the developing skin aging. In a previous study, it was shown that heat shock induced the matrix metalloproteinase (MMP)-1 and MMP-3 levels in skin fibroblasts through extracellular signal-regulated kinases (ERK) and c-Jun N terminal kinases (JNK) upregulation and the interleukin-6, in addition to increasing MMP-12 levels in skin (5, 6). Additionally, heat-induced reactive oxygen species (ROS) modulate the signal transduction signaling involved in the upregulating MMP levels in skin keratinocytes (7). Recently, chronic heat shock exposure can cause skin aging including wrinkling in a hairless mouse model. Increased MMP-13, decreased antioxidant levels, and oxidative stress due to chronic heat shock may play important roles in the development of skin aging (8).

"Nuclear factor erythroid-derived 2-related factor 2" (Nrf2) was reported to play a key role in the regulating oxidative response systems (9, 10). In an unstressed state, Nrf2 is anchored in the cytoplasm by specific inhibitors, such as "kelch-like ECH-associated protein 1" (Keap1) (10). In this presence of stimulants, such as oxidants and UV irradiation, Keap1 dissociates from Nrf2, allowing it to accumulate in the nucleus, where it binds to antioxidant response elements (ARE) in the enhancers of target genes (10). Nrf2 regulates many important antioxidant, such as glutathione (GSH) and NAD(P)H dehydrogenase 1 (NQO1) (10). In a previous study, Nrf2 has been shown to confer protection against apoptosis induced by fas, death-inducing signaling complex, and mitochondrial toxins (11). Furthermore, Nrf2 plays a role in the protecting skin cells against UV-induced apoptosis (12). Moreover, we have demonstrated that several Nrf2 regulators promote the survival of keratinocytes and fibroblasts and inhibit oxidative stress-induced mitochondrial-mediated apoptosis and skin aging (12-16). Additionally, studies have indicated that UVB-irradiated Nrf2$^{-/-}$ mice showed significantly increased coarse wrinkle formation, loss of skin flexibility, and epidermal thickening (17). As such, Nrf2 is a key regulator of the cellular stress response, and pharmacological Nrf2 activation is a promising strategy for skin protection and prevention. Thus, in this study, we examined Nrf2 as a putative major component of the protective ma-

*Corresponding author. Tel: +82-2-961-9436; Fax: +82-2-963-9436; E-mail: msohok@khu.ac.kr

http://dx.doi.org/10.5483/BMBRep.2015.48.8.215

Received 17 October 2014, Revised 10 November 2014, Accepted 1 December 2014

Keywords: Collagenase, Glutathione, Heat, NF-E2-related factor 2, Si-Nrf2

ISSN: 1976-670X (electronic edition)
Copyright ⓒ 2015 by the The Korean Society for Biochemistry and Molecular Biology
This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

chinery involved in skin aging induced by heat-shock with knockdown of Nrf2.

RESULTS

Heat shock reduced the expression or translocation of Nrf2 proteins and expression of HO-1, NQO1, and GSH proteins in HS68 cells

Cell viability of HS68 cells was not affected by treatment at temperatures of 37-44°C, according to a MTT assay 48 h later. However, heat shock at 46°C caused increased cytotoxicity (Supplementary Fig. 1). Additionally, mitogen-activated protein kinase signaling and ROS generation were increased significantly by heat shock at 44°C (Supplementary Fig. 2 and 3). Thus, all the experiments described below were performed at a heat shock temperature of 44°C, similar to the procedures used in previous studies (5). To evaluate whether heat shock impaired Nrf2 regulation in HS68 cells, we measured Nrf2 protein levels. Exposure of HS68 cells to 44°C showed that expression and translocation of Nrf2 protein were increased sig-

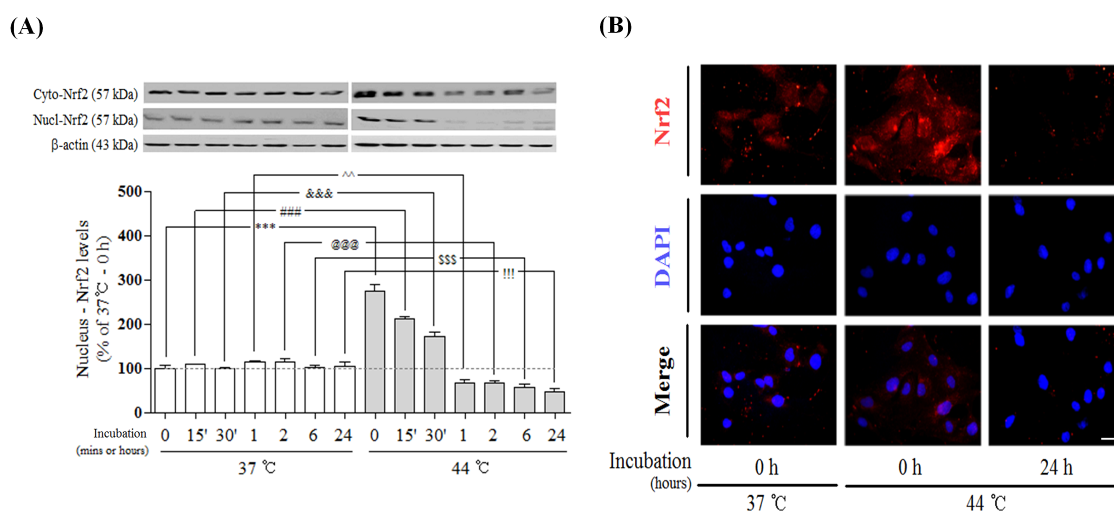

Fig. 1. Effects of heat shock on Nrf2 downregulation in HS68 cells. The levels of nuclear and cytosolic Nrf2 (A) were measured by Western blotting. Additionally, representative photomicrographs are shown (B). ^^P < 0.01 and ***, ###, &&&, @@@ and !!!P < 0.001; one-way analysis of variance followed by *Tukey's post hoc* test, performed using the GraphPad Prism software.

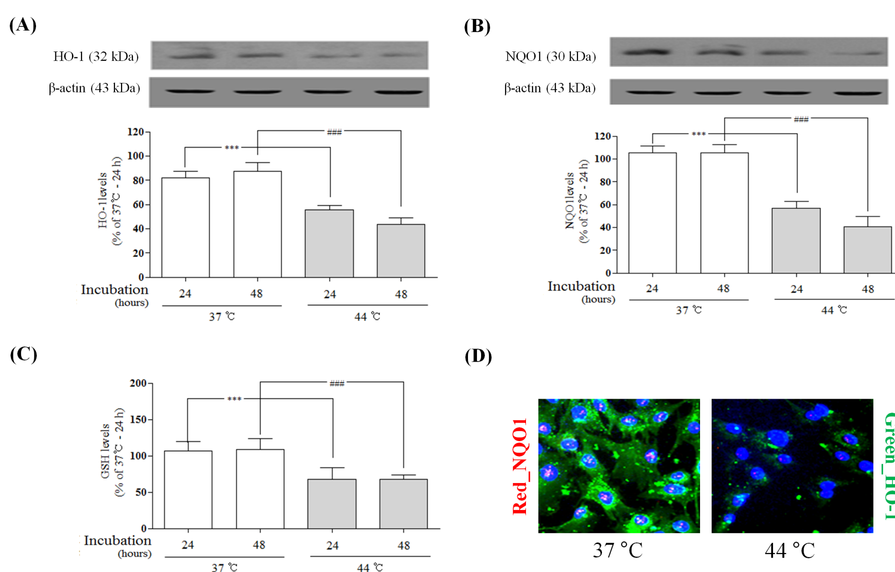

Fig. 2. Effects of heat shock on HO-1, NQO1, and GSH downregulation in HS68 cells. The levels of HO-1 (A), NQO1 (B), and GSH (C) were measured by Western blotting. Representative photomicrographs are shown (D). Scale bar = 50 μm. *** and ###P < 0.001; one way analysis of variance followed by *Tukey's post hoc* test, performed using the GraphPad Prism software.

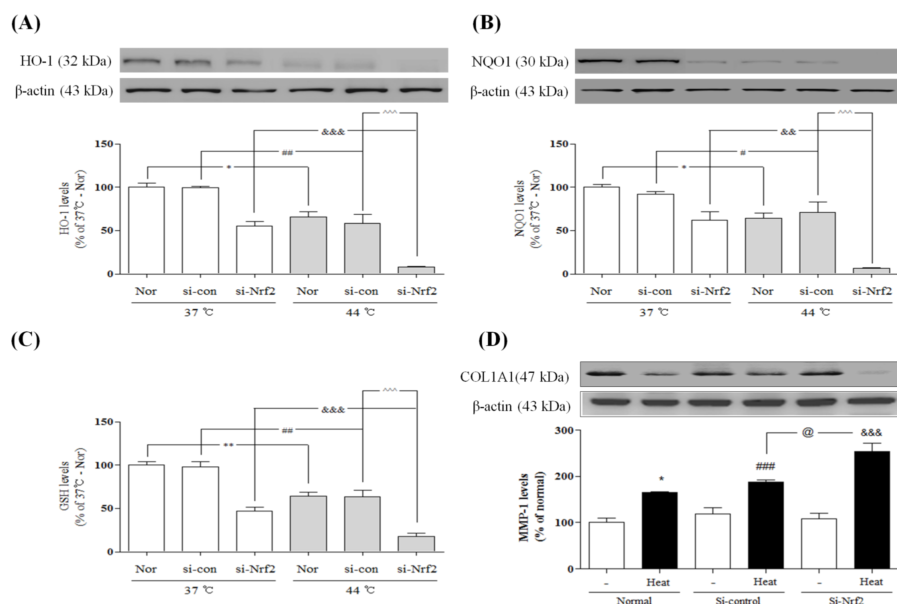

Fig. 3. Effects of heat shock on HO-1, NQO1, and GSH downregulation and accelerated collagen breakdown in Nrf2-siRNA transfected HS68 cells. The levels of HO-1 (A), NQO1 (B), GSH (C), and MMP-1 and COL1A1 (D) were measured by Western blotting or a kit. $^{*,\#,\text{ and }@}P < 0.05$, $^{\#\#\text{ and }\&\&}P < 0.01$ and $^{\&\&\&\text{ and }\wedge\wedge\wedge}P < 0.001$; one-way analysis of variance followed by *Tukey's post hoc test*, performed using the GraphPad Prism software.

nificantly after heat shock for 30 min (274.68 ± 13.57% vs. the 37°C value), and then declined at 24 h (46.67 ± 8.20% vs. the 37°C values) (Fig. 1). Next, to assess whether heat shock impaired Nrf2 target proteins in HS68 cells, we measured HO-1, NQO1, and GSH expression levels. HS68 cells exposed to a temperature of 44°C showed significantly reduced GSH, HO-1, and NQO1 levels at 24-48 h (71.86 ± 15.17 to 66.86 ± 6.51%, 55.66 ± 3.38 to 43.66 ± 5.23%, and 56.66 ± 6.17 to 40.66 ± 8.74% of the corresponding 37°C values, respectively) (Fig. 2).

Nrf2 knockdown reduced expression of HO-1, NQO1, and GSH and accelerated collagen breakdown induced by heat shock in Nrf2-siRNA transfected HS68 cells

To demonstrate the importance of Nrf2 upregulation, we developed a Nrf2-knockdown model in HS68 cells using siRNA transfection. HO-1, NQO1, and GSH levels were decreased significantly in Nrf2 siRNA-transfected cells by heat shock (14.43 ± 2.17, 10.28 ± 1.28, and 37.09 ± 8.25%), versus control siRNA-transfected cells (Fig. 3A, B, and C). Additionally, MMP-1 levels were increased significantly in Nrf2 siRNA-transfected cells by heat shock (135.23 ± 8.96%), versus control siRNA-transfected cells. Moreover, COL1A1 levels were decreased significantly in Nrf2 siRNA-transfected cells by heat shock (254.30 ± 16.84%), versus control siRNA-transfected cells (Fig. 3D).

Effects of Nrf2 regulators on heat shock-induced expression of GSH proteins and deceleration of MMP-1 in HS68 cells

To confirm that Nrf2 regulation protected cells from heat-shock-induced antioxidative defense proteins and skin aging factor, the Nrf2 regulators CSE, NAC, and quercetin were used. Heat shock exposure significant depleted GSH (by 27.60 ± 8.88%) and elevated MMP-1 (by 231.71 ± 21.72%), while 100 or 500 μg/ml CSE, 2 mM NAC, or 50 μM quercetin increased levels of GSH (by 39.02 ± 5.27 to 72.64 ± 9.97%, 50.67 ± 4.74%, or 58.54 ± 2.98% of the corresponding 37°C values, respectively) and decreased levels of MMP-1 (by 124.21 ± 69.28 to 49.40 ± 6.14%, 58.88 ± 25.56%, or 35.30 ± 11.57% of the corresponding 37°C values, respectively) (Fig. 4 A and C).

Effects of Nrf2 regulators on heat shock-induced expression of HO-1, NQO1, and GSH proteins and deceleration of collagen breakdown in Nrf2-siRNA transfected HS68 cells

To further confirm the involvement of Nrf2, the effects of Nrf2 regulators, CSE, NAC, and quercetin, on heat-shock-induced GSH levels and MMP-1 levels were measured in Nrf2 knockdown HS68 cells. GSH levels were decreased significantly in Nrf2 siRNA-transfected cells by heat shock (36.05 ± 6.84%), versus control siRNA-transfected cells. In contrast, Nrf2 siRNA-transfected cells treated with 500 μg/ml CSE, 2 mM NAC, or 50 μM quercetin before exposure to heat shock were protected, in terms of GSH levels (267.87 ± 26.43%, 241.41 ± 14.08%, and 290.64 ± 19.10%, respectively), as compared to Nrf2 siRNA-transfected cells (Fig. 4B). Additionally, MMP-1 levels were increased significantly in Nrf2 siRNA-transfected cells by heat shock (200.34 ± 14.54%), as compared to control siRNA-transfected cells. In contrast, Nrf2 siRNA-transfected cells treated with 500 μg/mL CSE, 2 mM NAC, or 50 μM quercetin before exposure to heat shock showed decreased MMP-1 levels (57.06 ± 6.38%, 45.51 ± 12.21%, and 41.94 ± 7.13%,

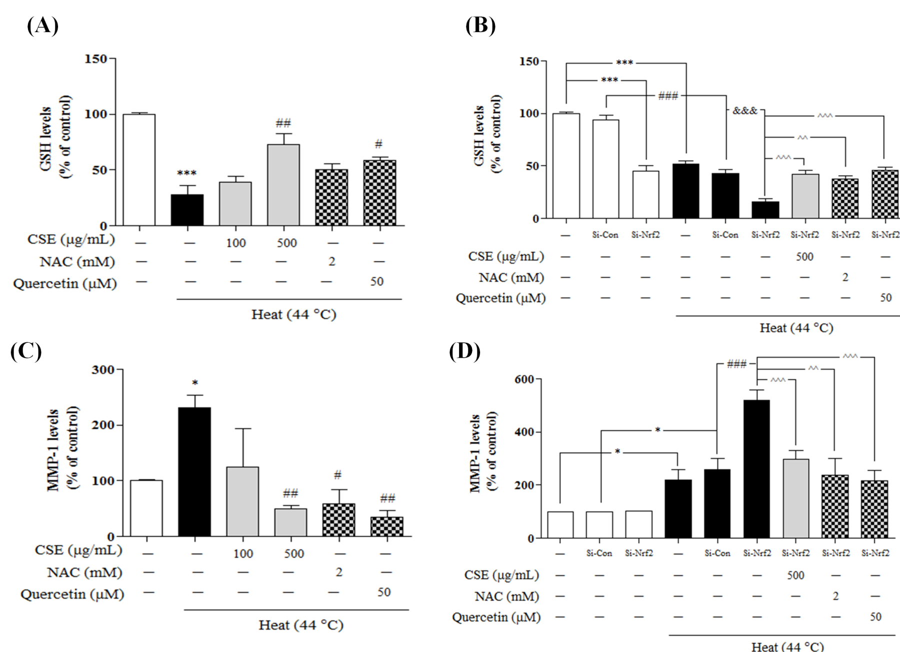

Fig. 4. Effects of Nrf2 regulators on heat shock-induced upregulation of GSH and downregulation of MMP-1 levels in HS68 cells. The levels of GSH (A) and MMP-1 (C) were measured using commercial kits. Then, effects of Nrf2 regulators on heat-shock-induced upregulation of GSH and deceleration of collagen breakdown in Nrf2-siRNA transfected HS68 cells. The levels of GSH (B) and MMP-1 (D) were measured. * and $^{\#}P < 0.05$, $^{\#\#}$ and $^{\wedge\wedge}P < 0.01$, and ***, $^{\#\#\#}$, $^{\wedge\wedge\wedge}$ and $^{\&\&\&}P < 0.001$; one-way analysis of variance followed by *Tukey's post hoc* test, performed using the GraphPad Prism software.

respectively), compared with Nrf2 siRNA-transfected cells (Fig. 4D).

DISCUSSION

Nrf2 can function as a direct oxidative stress sensor, and phase 2 detoxifying enzymes are major executors of cellular defense against oxidative stress. Many studies have shown that the Nrf2 signals plays an important role in protection on UV-induced aging by maintaining antioxidants levels (18). Further, Nrf2/heat-related studies reported that Nrf2 activation is the regulatory pathway of cytoprotective gene and proteins expression against heat shock in dental pulp and liver (19, 20). In fact, Sahin *et al.* reported that Nrf2 activators such as curcumin, epigallocatechin-3-gallate, and resveratrol modulated the Nrf2/HO-1 pathway in quail hepatocytes to counteract the damage caused by heat shock (21). However, no reported study has yet examined the effect of Nrf2 on skin aging induced by heat shock. Therefore, we focused on Nrf2 as a putative major component of the protective involved in skin aging induced by heat shock.

Oxidative stress, including UV, IR, and heat shock, causes depletion of antioxidants such as GSH, NQO1, and HO-1 in skin (22). Moreover, Nrf2 has been shown to be involved in the inducting phase II enzymes or antioxidants (22). In this studies, heat shock induced a certain concentration of ROS and it activates Nrf2 expression and translocation in initial stage, but excessive ROS generation inactivated Nrf2 (Fig. 1). Moreover, time dependently ROS generation and its downstream proteins of ERK, JNK, and P38 MAPKs signaling, demonstrating that Nrf2 activation may be associated with the regulation of heat shock in initial stage (Supplementary Fig. 2 and 3). This result is consistent with the previous report, Shin MH et al.; heat shock induces cellular levels of ROS generation and its downstream proteins of MAPKs signaling in keratinocytes (5). Also, Nrf2 expression in nucleus were increased rapidly after H_2O_2 for 30 min, and then declined at 24 h. In this study, furthermore, Nrf2 siRNA-transfected cells showed a significantly greater decrease in HO-1, NQO1, and GSH levels than siRNA-control cells after heat shock (Fig. 3A, B, and C).

Additionally, to determine whether mechanisms of heat shock in collagen breakdown were regulated by Nrf2, we measured collagenase levels following Nrf2 knockdown. The family of COL gene products is composed of various chain types, such as collagens types I, II, III, IV, and V. Specially, COL1A1 is important for the skin development and for maintain physiological functions (23-25). MMP-1, a fibroblast-type or interstitial collagenase, is secreted by fibroblasts and macrophages (24). It degrades collagen and is thought to play a role in skin aging (24). Sahin *et al.* reported that heat shock induced increasing MMP-1 by production of ROS in skin cells (Fig. 3D and supplementary Fig. 2) (5). Further, as mentioned previously, Nrf2 is the sensory mediator for protection on ROS by induction of antioxidants, even at low levels of oxidative damage. Due to its central role in ROS detoxification, Nrf2 is an attractive target for pharmacological protection of the skin aging (26). However, no reported study has yet examined the effect of Nrf2 on collagen-relationships induced by heat shock. In our study, MMP-1 levels were significantly increased in Nrf2 siRNA-transfected cells by heat shock, compared with

siRNA-control cells (Fig. 3D). Also, COL1A1 was decreased significantly in Nrf2 siRNA-transfected cells by heat shock (Fig. 3D). No difference in MMP-1 and COL1A1 levels was observed between Nrf2 siRNA-transfected cells and siRNA-control cells. As the constitutively lower Nrf2 levels dependent antioxidant enzymes in Nrf2-knockdown cells were shown here to accelerate skin aging, heat shock-induced Nrf2 inactivation was not directly related to the results of the present study.

Furthermore, we measured the effects of Nrf2 regulators against heat-shock-induced GSH and MMP-1 levels in normal and Nrf2 knockdown HS68 cells. In our previous study, we demonstrated that Nrf2 activators (coriander, walnut, and green tea extract, sauchinone, and NAC) inhibited oxidative-stress-induced apoptosis and skin aging (14-16). In the present study, CSE, NAC, and quercetin significantly protected skin cells against heat shock-induced damage without inducing toxicity (data not shown). Additionally, heat shock caused significant depletion of GSH and elevation of MMP-1, whereas CSE, NAC, and quercetin induced GSH and reduced MMP-1 levels. Also, in Nrf2 siRNA-transfected cells treated with CSE, NAC, or quercetin showed significantly increased GSH expression levels and decreased MMP-1 expression levels (Fig. 4). These results indicate that Nrf2 plays an important role in preventing skin aging via upregulating antioxidants through Nrf2 activators.

Our findings show that Nrf2 plays a crucial role in protection against heat shock-induced skin aging. Based on this concept of thermal aging through the Nrf2 pathway, development of Nrf2-activator may provide effective therapeutic strategies for thermal skin aging.

MATERIALS AND METHODS

Cell culture and treatments

The cell culture system was established according to previously published methods (13). The HS68 cell line, human skin fibroblast cell, was obtained from the American Type Culture Collection (ATCC; Rockville, USA). Cells were maintained in DMEM supplemented with 10% heat inactivated FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin in condition of 95% air and 5% CO_2 at 37°C. Then, for heat shock treatment, cells were cultured to 80% confluence and then maintained in culture medium without FBS for 24 h; the culture dishes were sealed with Parafilm (Pechiney Plastic; USA) and immersed for 30 min in a circulating water bath thermo-regulated at 37°C (control) or 44°C (heat shock treatment). In addition, for Nrf2 regulator treatment, HS68 cells were seeded on 12-well plates and pretreated with coriander extract (CSE) at dose of 100 or 500 μg/ml, N-acetyl-cysteine (NAC) at dose of 2 mM, and quercetin at dose of 50 μM for 1 h, and then stimulated with heat stress for an additional 23 h.

siRNA transfection

HS68 cells were used at a confluence of 80-85% in 100-mm dish or 12-well plates. Cells were transfected with Stealth small interfering RNA (siRNA) using Lipofectamine 2000 (Invitrogen; CA). Lipofectamine 2000 (25 μl) was mixed with 50 μM siRNA solution (an equimolar mix of both Nrf2 siRNA and scramble siRNA) and 2.5 ml of Opti-MEM medium (Gibco-BRL; CA). After 30 min at room temperature, 300 μl of the mix was added to 300 μl of serum-free DMEM in each dish and incubated for 24 h (Supplementary Fig. 4).

Measuring Nrf2, HO-1, NQO1, and COL1A1 levels by Western blotting

Western blotting was performed according to previously published methods (16). For the detection of Nrf2, HO-1, NQO1, and COL1A1 proteins, the cells were lysed. The lysates were separated by 15% SDS-PAGE, and were then transferred to a membrane. The membranes were incubated with 5% skim milk in TBST for 1 h. Then they were incubated with primary antibody (1:1,000 dilutions) overnight at 4°C, followed by incubated with HRP-conjugated secondary antibody for 1 h. Immunoreactive bands were detected using an ECL detection kit and a LAS-4000 mini system (Fujifilm Corporation, Tokyo, Japan) was used for visualization.

Measuring Nrf2, HO-1, and NQO1 levels by immunofluorescence

Immunofluorescence was performed according to previously published methods (16). Cells were fixed through incubation with 4% PFA at room temperature for 30 min. Fixed cells were rinsed in PBS and then incubated overnight at 4°C with primary antiantibody (dilution 1:500). They were then incubated for 2 h with an Alexa Fluor conjugated secondary antibody (dilution 1:250). Cells were finally washed in PBS and mounted using Vectashield Mounting Medium containing DAPI. Confocal immunofluorescent images were captured using an LSM 700 confocal microscope (Carl Zeiss, Thornwood, USA) and the fluorescence intensity was measured using Axio Vision 4.4 (Carl Zeiss, Oberkochen, Germany). Data are presented as percentages of control values. For the assessment of intra-experimental variability, three independent experiments were carried out in triplicate.

Measuring MMP-1 and GSH levels

MMP-1 and total GSH levels were measured using a Human MMP-1 ELISA Kit (RayBiotech; USA) and Total Glutathione Quantification Kit (Dojindo; Japan), respectively, according to the instruction manuals.

ACKNOWLEDGEMENTS

This study was supported by the grant of the Korea Healthcare technology R&D Project, Ministry of Health & Welfare, Republic of Korea (Grant No.: A103017) and this research was supported by a grant from the Korea Institute of Oriental Medicine (KIOM) [K14301].

REFERENCES

1. Zastrow L, Groth N, Klein F et al (2008) The missing link-light-induced (280-1,600 nm) free radical formation in human skin. Skin Pharmacol Physiol 22, 31-44
2. Schröder P and Krutmann J (2005) Environmental oxidative stress-Environmental sources of ROS. The handbook of Environmental Chemistry 2, 19-31.
3. Schieke SM, Schroeder P and Krutmann J (2003) Cutaneous effects of infrared radiation: from clinical observations to molecular response mechanisms. Photodermatol Photoimmunol Photomed 19, 228-234
4. Cho S, Lee MJ, Kim MS et al (2008) Infrared plus visible light and heat from natural sunlight participate in the expression of MMPs and type I procollagen as well as infiltration of inflammatory cell in human skin in vivo. J Dermatol Sci 50, 123-133
5. Shin MH, Moon YJ, Seo J-E, Lee Y, Kim KH and Chung JH (2008) Reactive oxygen species produced by NADPH oxidase, xanthine oxidase, and mitochondrial electron transport system mediate heat shock-induced MMP-1 and MMP-9 expression. Free Radic Biol Med 44, 635-645
6. Park C-H, Lee MJ, Ahn J et al (2004) Heat shock-induced matrix metalloproteinase (MMP)-1 and MMP-3 are mediated through ERK and JNK activation and via an autocrine interleukin-6 loop. J Invest Dermatol 123, 1012-1019
7. Lee YM, Li WH, Kim YK, Kim KH and Chung JH (2008) Heat-induced MMP-1 expression is mediated by TRPV1 through PKCα signaling in HaCaT cells. Exp Dermatol 17, 864-870
8. Shin MH, Seo JE, Kim YK et al (2012) Chronic heat treatment causes skin wrinkle formation and oxidative damage in hairless mice. Mech Ageing Dev 133, 92-98
9. Braun S, Hanselmann C, Gassmann MG et al (2002) Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in the healing skin wound. Mol Cell Biol 22, 5492-5505
10. Osburn WO and Kensler TW (2008) Nrf2 signaling: an adaptive response pathway for protection against environmental toxic insults. Mutat Res 659, 31-39
11. Aleksunes LM and Manautou JE (2007) Emerging role of Nrf2 in protecting against hepatic and gastrointestinal disease. Toxicol Pathol 35, 459-473
12. Zhong JL, Edwards GP, Raval C, Li H and Tyrrell RM (2010) The role of Nrf2 in ultraviolet A mediated heme oxygenase 1 induction in human skin fibroblasts. Photochem Photobiol Sci 9, 18-24
13. Park G, Jang DS and Oh MS (2012) *Juglans mandshurica* leaf extract protects skin fibroblasts from damage by regulating the oxidative defense system. Biochem Biophys Res Commun 421, 343-348
14. Park G, Kim H, Kim Y, Park S, Kim S and Oh M (2012) *Coriandrum sativum* L. protects human keratinocytes from oxidative stress by regulating oxidative defense systems. Skin Pharmacol Physiol 25, 93-99
15. Park G, Kim HG, Sim Y, Sung SH and Oh MS (2012) Sauchinone, a Lignan from *Saururus chinensis*, Protects Human Skin Keratinocytes against Ultraviolet B-Induced Photoaging by Regulating the Oxidative Defense System. Biol Pharm Bull 36, 1134-1139
16. Park G, Kim H, Hong S-P, Kim S and Oh M (2014) Walnuts (Seeds of *Juglandis sinensis* L.) Protect Human Epidermal Keratinocytes against UVB-Induced Mitochondria-Mediated Apoptosis through Upregulation of ROS Elimination Pathways. Skin Pharmacol Physiol 27, 132-140
17. Hirota A, Kawachi Y, Yamamoto M, Koga T, Hamada K and Otsuka F (2011) Acceleration of UVB-induced photoageing in Nrf2 gene-deficient mice. Exp Dermatol 20, 664-668
18. Hirota A, Kawachi Y, Itoh K et al (2005) Ultraviolet A irradiation induces NF-E2-related factor 2 activation in dermal fibroblasts: protective role in UVA-induced apoptosis. J Invest Dermatol 124, 825-832
19. Chang SW, Lee SI, Bae WJ et al (2009) Heat stress activates interleukin-8 and the antioxidant system via Nrf2 pathways in human dental pulp cells. J Endod 35, 1222-1228
20. Sahin K, Orhan C, Akdemir F, Tuzcu M, Iben C and Sahin N (2012) Resveratrol protects quail hepatocytes against heat stress: modulation of the Nrf2 transcription factor and heat shock proteins. J Anim Physiol Anim Nutr 96, 66-74
21. Sahin K, Orhan C, Tuzcu Z, Tuzcu M and Sahin N (2012) Curcumin ameloriates heat stress via inhibition of oxidative stress and modulation of Nrf2/HO-1 pathway in quail. Food Chem Toxicol 50, 4035-4041
22. Chen X-L and Kunsch C (2004) Induction of cytoprotective genes through Nrf2/antioxidant response element pathway: a new therapeutic approach for the treatment of inflammatory diseases. Curr Pharm Des 10, 879-891
23. van der Rest M, Garrone R (1991) Collagen family of proteins. FASEB J 5, 2814-2823
24. Chung JH, Seo JY, Choi HR et al (2001) Modulation of skin collagen metabolism in aged and photoaged human skin in vivo. J Invest Dermatol 117, 1218-1224
25. Yoon BH, Jeon YH, Hwang B et al (2013) Anti-wrinkle effect of bone morphogenetic protein receptor 1a-extracellular domain (BMPR1a-ECD). BMB Rep 46, 465-470
26. Lee EK, Kim JA, Park SJ et al (2013) Low-dose radiation activates Nrf1/2 through reactive species and the Ca^{2+}/ERK1/2 signaling pathway in human skin fibroblast cells. BMB Rep 46, 258-263