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Inhibitory effect of naringenin on LPS-induced skin senescence by SIRT1 regulation in HDFs

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Abstract

Background: This study aims to investigate the ability of naringenin to regulate the expression of nuclear factor-κB, the upper gene of lipopolysaccharide-induced SIRT1; regulate signal transduction in the extracellular matrix, which plays an important role in the dermis; and alter the matrix metalloproteinase 1 and matrix metalloproteinase 3 gene expression, therefore having suppressing effect on skin cell senescence and deoxyribonucleic acid protection and cell protection effects and confirm naringenin's potential as an important cosmetic ingredient.

Methods: The efficacy of naringenin was assessed through reactive oxygen species assay, water-soluble tetrazolium salt assay, nuclear factor-κB luciferase assay, enzyme-linked immunosorbent assay, nicotinamide adenine dinucleotide phosphate oxidase activity assay, and quantitative real-time polymerase chain reaction.

Results: Sirt1, which regulates naringenin upstream of the nuclear factor-κB pathway, inhibits nuclear factor-κB activity and decreases matrix metalloproteinase expression level and SIRT1 gene dose-dependently. The results confirmed naringenin had an anti-aging effect on lipopolysaccharide-induced skin senescence. Various age-related biomarkers were used to analyze the inhibitory effect naringenin had on cell senescence progress and oxidative activity. Further experiments also showed naringenin also acted on NADPH oxidase, which produces superoxide (O2-), resulting in inhibiting NADPH oxidase activity. Experimental results reported that naringenin regulated the activity of SIRT1, which is the cause of modern skin cell aging, and in turn had a regenerative effect on reactive oxygen species-induced skin cellular senescence which affects skin elasticity and wrinkles.

Conclusions: This study confirmed that naringenin inhibits cellular senescence and regenerates human dermal fibroblasts damaged by lipopolysaccharide, and suggests that naringenin will be a cosmetic ingredient that has cell regenerative effects and anti-aging effects.

Keywords: Naringenin, Lipopolysaccharide, SIRT1, Human dermal fibroblasts, Nuclear factor-κB, Cellular aging, Skin cell regeneration, Antioxidation

Background

More than 300 theories have been proposed to explain the basic mechanisms for the progression of aging (Medvedev 1990). Among the three major theories, the first theory of reactive oxygen is a key mechanism of innate immunity (Harman 2006). The theory of free radicals was first introduced by Dr. Gerschman in 1954, but was developed by Dr. Denham Harman. In the free radical theory, it is known that reactive oxygen species (ROS), which are generated abnormally, can induce DNA damage and oxidation of proteins, nucleic acids, and lipids (Nakayama et al. 2016). Secondly, in the programmed cellular senescence theory (Aubert and Lansdorp 2008), the major mechanisms involved in the senescence process, which include the insulin/IGF-1 signaling pathway and the mTOR pathway as well as the AMP kinase (AMPK), and Sirtuin protein were found to be aging factors. Thirdly, according to the inflammation theory, immune responses decrease with aging, contributing to the increased

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incidence of different chronic diseases with an inflammatory component (Coppe et al. 2010).

With increased levels of the pro-inflammatory cytokines, interleukin (IL)-1β, IL-6, and IL-8 are the senescence-associated secretory phenotype (SASP). While these theories aid the overall understanding of aging, the mechanism for skin disease and aging is difficult to define (Fagiolo et al. 1993).

Cells that are involved in skin wrinkles are fibroblasts, granulocytes, macrophages, and mast cells that are present in the dermis, which is a visible characteristic of skin aging that is of most importance to modern women. Decreased synthesis of collagen types I and III due to incomplete mechanical stimulation of the tissue forming the skin tissue and matrix metalloproteinase (MMP) activity forms skin sagging and deep wrinkles (Sardy 2009). Phenomenons caused by aging include changes in cell contact, insecure DNA replication at the end of chromosome, reduction of DNA repair ability, histone methylation modification, stem cell exhaustion, loss of quality of proteostasis ability of protein, mitochondrial dysfunction, nutritional control disorder, and increased senile senescence. As a typical phenomenon, cell senescence and telomere portions become shorter, and aging cells change the tissue microenvironment associated with matrix metalloproteinases, a kind of secretory phenotypes (Tchkonia et al. 2013), and induced matrix metalloproteinases, and in turn matrix metalloproteinases inhibit the synthesis of a generation of collagen in the body and thus represent an important feature of skin aging (Wondrak et al. 2003; Kimura et al. 2014).

Recent studies have shown that SIRT1 (silent mating type information regulation 2 homolog, Sirtuin 1), a SIR2 mutein that acts to enhance the viability of mammalian cells belonging to the longevity gene that regulates the activity of NF-κB, is a nicotinamide adenine dinucleotide (NAD +) which is a class III deacetylase that is involved in a number of biological processes including DNA repair, energy metabolism, tumor suppression, and mitochondrial stabilization (Michan and Sinclair 2007). SIRT1-associated SIR2 is a type of sirtuin, a deacetylase that is dependent on nicotinamide adenine dinucleotide (NAD +), and these genes have been shown to have an effect on life prolongation in yeast and *Drosophila* (Cohen et al. 2004). Recent studies have also shown that SIRT1 plays a positive role in stress, metabolism, apoptosis, and premature aging. Environmental stresses such as smoking and air pollution are reported to reduce the production of SIRT1 in lungs, causing lung diseases (Rajendrasozhan et al. 2008). In addition, activation of NF-κB and activation of JNK, ERK, p38, and MAP kinase through SIRT1 expression inhibition lead to increased production of ROS. SIRT1 is mainly found in the nucleus and is known to deacetylate various proteins including p53, FOXO, NK-κB, Ku70, and histone (Haigis and Guarente 2006; Kimura et al. 2014).

SIRT1 plays an important role in cell survival by acting on cell cycle molecules and deacetylation of apoptosis regulatory proteins (Noh et al. 2013). As a factor controlling the development and differentiation of neurons, it has been recently reported to effectively inhibit neuronal dysfunction and nerve cell death in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Finkel and Holbrook 2000).

SIRT1 inhibits the secretion of inflammatory cytokines TNF-α and IL-6 and increases the secretion of anti-inflammatory cytokine IL-10 (Lu et al. 2008) and has anti-inflammatory effects by downregulating the expression of various inflammatory cytokines in endothelial cells and macrophages (Michishita 2005; Stein and Matter 2011). As the expression level of SIRT1 decreases, the expression level of MMPs increases. Furthermore, by inhibiting the expression of MMP1, SIRT1 promotes the synthesis of extracellular matrix (Razaq et al. 2010).

SIRT1 and NF-κB system regulation, one of the most important causes of various common skin problems in modern people, represents an ancient signaling pathway that regulates metabolism and metabolism. This reaction inhibits the inflammatory cells of mammals through opposite regulation mechanisms (Salminen 2008; Kauppinena et al. 2013). Prevention of skin aging, anti-pollution, and inflammation treatments are primarily aimed at the cosmetic and medical industries; however, they are still insufficient (Rajendrasozhan et al. 2008).

Recently, reported anti-aging molecules have been developed to minimize intrinsic or extrinsic aging factors and to produce intracellular signaling molecules involved in collagen synthesis and its degradation (Rajendrasozhan et al. 2008; Mukherjee et al. 2011; Hwang et al. 2014). These antioxidants include antioxidants of natural raw materials through in vitro experiments, such as *Ligularia fischeri*, *Rubus coreanus* Miquel, Korean pine nut shells, lotus leaf, and bolus terminated chitosan, which is a seawood.

Through in vivo or human application tests, citrus, *Oenanthe javanica*, dandelion, soybean, *Saururus chinensis*, and *Peucedanum japonicum* Thunberg were proven as natural raw material antioxidants (Na et al. 2016).

Naringenin (4′,5,7-trihydroxy flavanone), a yellow crystalline powder, is hydrolyzed from a form of rutinoside of flavanone to an aglycone form. When the convert is complete, absorption is promoted and present at a higher concentration in plasma, bile, urine, etc., thus exhibiting anti-inflammatory effects in RAW 264.7 cell lines and anti-cancer effects in cancer cells (Fuhr and Kummert 1995; Tripoli et al. 2007).

Naringenin has a polyphenol structure capable of eliminating active oxygen groups and active oxygen generated by hydrogen/electron transfer in the 4-hydroxyl group of

group B, and shows anti-inflammatory activity and anti-oxidant activity (Vafeiadou et al. 2009).

This study focused on the lack of research on naringenin's effects on skin, especially its potent as a cosmetic ingredient and its intracellular mechanism of action, and carried out experiments to investigate how naringenin regulates the activity of SIRT1, which in turn controls the activity of nuclear factor-κB, a major cause of modern skin aging, and has inhibitory effects on the reactive oxygen species production.

Methods

Cell culture

In this study, human dermal fibroblasts (HDFs) were purchased from Lonza Switzerland and cultured in Dulbecco's modified Eagle medium (DMEM; Hyclone, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone), 1% penicillin/streptomycin (100 IU/mL penicillin, 100 μg/mL streptomycin; Invitrogen, USA) and cultured at 37 °C and 5% CO_2.

Sample treatment

Naringenin (Sigma-Aldrich, USA) was used to dissolve the purified powder (> 99%) in a suitable concentration in dimethyl sulfoxide (DMSO; Sigma-Aldrich). Lipopolysaccharide (LPS), a cell-stimulating agent, was purchased from Sigma-Aldrich. For the experiment, the solution was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich) at a proper concentration. HDFs (1×10^6 cells/well) were incubated in a 60-mm cell culture dish for 24 h. An appropriate concentration of naringenin was added to the culture medium. Lipopolysaccharide was co-treated at a constant concentration for 24 h and analyzed 3 h later. The significance of PCR was validated using a melting curve. The gene expressions were compared for analysis by normalizing the β-actin expression.

Cell viability estimation

WST-1 Cell Proliferation Assay System used a chromogenic material, formazan, which is formed from tetrazolium salts (WST-1) by intracellular mitochondrial dehydrogenase, to quantify cell proliferation and cell viability. Human dermal fibroblasts were inoculated into metabolically active 96-well plates, at a concentration of 3×10^3 cells/well, and cultured for 24 h. HDFs were simultaneously treated with various samples such as naringenin and LPS and cultured for 24 h. Ten microliters of EZ-Cytox cell viability assay kit reagent (ItsBio, Korea) was added to the cultured cells and incubated for 1 h. The absorbance was measured at 490 nm using a microplate reader (Bio-Rad, USA). The mean and standard deviation of cell viability were calculated after repeating the procedure three times.

qRT-PCR analysis

Changes of gene expression caused by naringenin in the cells were quantitatively confirmed. qRT-PCR was performed by mixing 0.2 μM primers, 50 mM KCl, 20 mM Tris/HCl pH 8.4, 0.8 mM dNTP, 0.5 U Extaq DNA polymerase, 3 mM $MgCl_2$, and 1X SYBR green (Invitrogen). PCR was validated by melting curve. The melting curve confirmed the validity of PCR. Expression of each gene was standardized and compared. The primers used in this experiment are shown in Table 1.

ROS assay

To investigate the effect of naringenin on the removal of reactive oxygen species, ROS scavenging assay was performed. Human dermal fibroblasts were seeded in a 60-mm culture dish at 2×10^5 cells/well and cultured for 24 h. Cells were treated appropriately, cultured for an additional 24 h, added 10 μM of dichlorofluorescein diacetate (DCF-DA), and then cultured for 30 min. After cells were harvested and analyzed for changes in ROS using a flow cytometer (BD Biosciences, USA). *N*-Acetyl-ʟ-cysteine (NAC; Calbiochem, USA) serving as a ROS scavenger was also measured in the same manner.

Nuclear factor-κB luciferase assay

To investigate the effect of naringenin on the nuclear factor-κB transcriptional activity, the nuclear factor-κB promoter luciferase assay was used. In this experiment, the nuclear factor-κB reporter NIH-3T3 stable cell line (Panomics, USA) containing the reporter gene with the nuclear factor-κB promoter consensus sequence was used in the promoter region. Nuclear factor-κB reporter NIH-3T3 stable cells were inoculated in a 60-mm culture dish at a concentration of 2×10^5 cells, cultured for 24 h, treated with appropriate conditions, and cultured for additional 24 h. The cultured cells were harvested; passive lysis buffer (Promega, USA) was added, put in ice for 10 min, dissolved, then centrifuged at 12,000 rpm at 4 °C for 30 min to recover the supernatant. A sample containing the same amount of protein was added to a black 96-well plate (80 μg each), followed by luciferin (Promega, USA). The luminescence of luciferin was measured using a luminometer (Veritas, USA).

Table 1 Lists of primers

Gene	Forward primer	Reverse primer
β-actin	GGATTCCTATGTGGGCGACGA	CGCTCGGTGAGGATCTTCATG
SIRT1	GCAGGTTGCGGGAATCCAA	GGCAAGATGCTGTTGCAAA
MMP1	TCTGACGTTGATCCCAGAGAGCAG	CAGGGTGACACCAGTGACTGCAC
MMP3	ATTCCATGGAGCCAGGCTTTC	CATTTGGGTCAAACTCCAACTGTG
HO-1	GCCTGCTAGCCTGGTTCAAG	AGCGGTGTCTGGGATGAACTA

Enzyme-linked immunosorbent assay (ELISA)

MMPs (Merck, USA; sandwich) and PGE2 (Cayman chemical, USA; competitive) were evaluated using an enzyme-linked immunosorbent assay (ELISA) kit according to the instruction manual for the HDF culture medium. Prostaglandin E2, MMPs, and monoclonal antibody were immobilized on plastic cell culture dish wells. One hundred microliters of each culture medium was dispensed (at room temperature for 2 h). After washing five times each with a single washing buffer, 100 μL horseradish peroxidase (HRP)-conjugated anti-MMP1 antibody was added for 1 h at room temperature, and 100 μL of tetramethylbenzidine (TMB) was added and incubated for 30 min in a dark room. The absorbance was measured at 405–420 nm (PGE2) and 450 nm (MMPs).

Nicotinamide adenine dinucleotide phosphate oxidase assay

Naringenin has an antioxidative effect on the oxidase activity of reduced NADP, NADPH, which directly influences superoxide anion (O2-) production, which in turn plays the most fundamental role in ROS production. To study the antioxidative effect of naringenin on NADPH oxidase activity, using HDFs, 100 μL of homogenates was added to each well and 900 μL of 50 mM phosphate buffer (1 mM EGTA, 150 mM sucrose, 5 mM lucigenin, 100 mM NADPH, pH 7.0) was added which was examined through lucigenin chemiluminescence assay.

Statistical process

All experiments were performed independently three times, and the results were expressed as mean ± standard deviation. Student's t test was used to analyze all findings, with a p value of 0.05, 0.01, or 0.001 below considered as statistically significant ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

Results

Cytotoxicity of naringenin and lipopolysaccharide

Lipopolysaccharide acts as an endotoxin in cells, activating signals such as MAPK, NF-κB, and IRF-3 and promoting excessive secretion of inflammatory cytokines (Lu et al. 2008). Lipopolysaccharide induced nuclear factor-κB activity through IκB phosphorylation and its degradation. In this study, WST-1 assay was performed to determine the cytotoxicity of naringenin in human dermal fibroblasts. Human dermal fibroblasts were treated with untreated control group and naringenin-treated group at 1-, 2-, 5-, and 10-μM dose levels, each respectively, for 24 h.
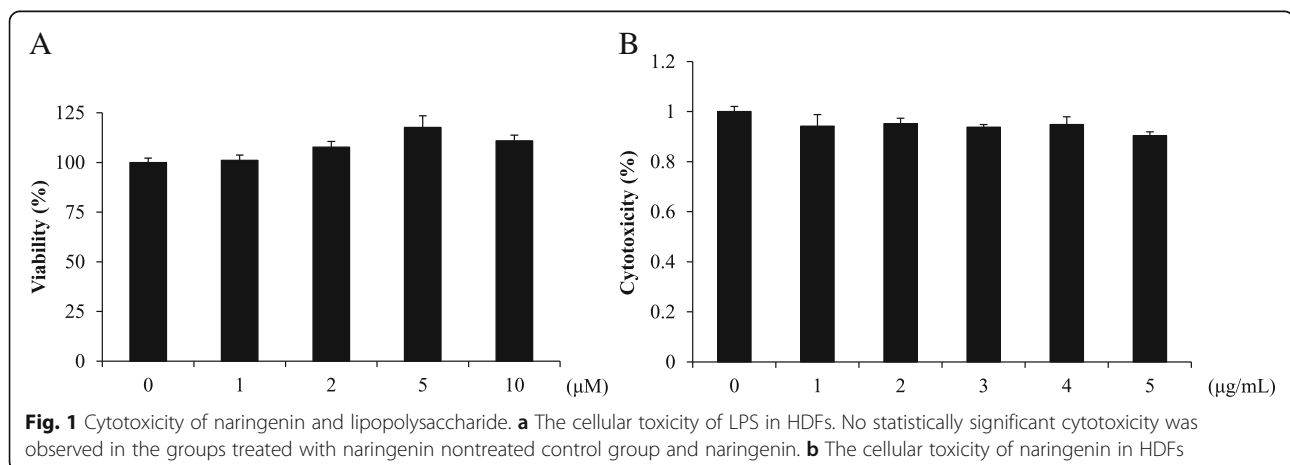
Cell viability of untreated control group was set as 100%, and up to 1-μM dose level, the survival rate was shown to be 100% or more. When naringenin was treated at 1-, 2-, 5-, and 10-μM dose levels, the cell viability levels of 101%, 107%, 117%, and 110% did not decrease, confirming almost no toxicity (Fig. 1a). To confirm the changes in the survival rate of cells, HDFs was treated with LPS at 1-, 2-, 3-, 4-, and 5-μg dose levels, respectively, and the survival rate was 94% at 1 μg/mL (Fig. 1b).
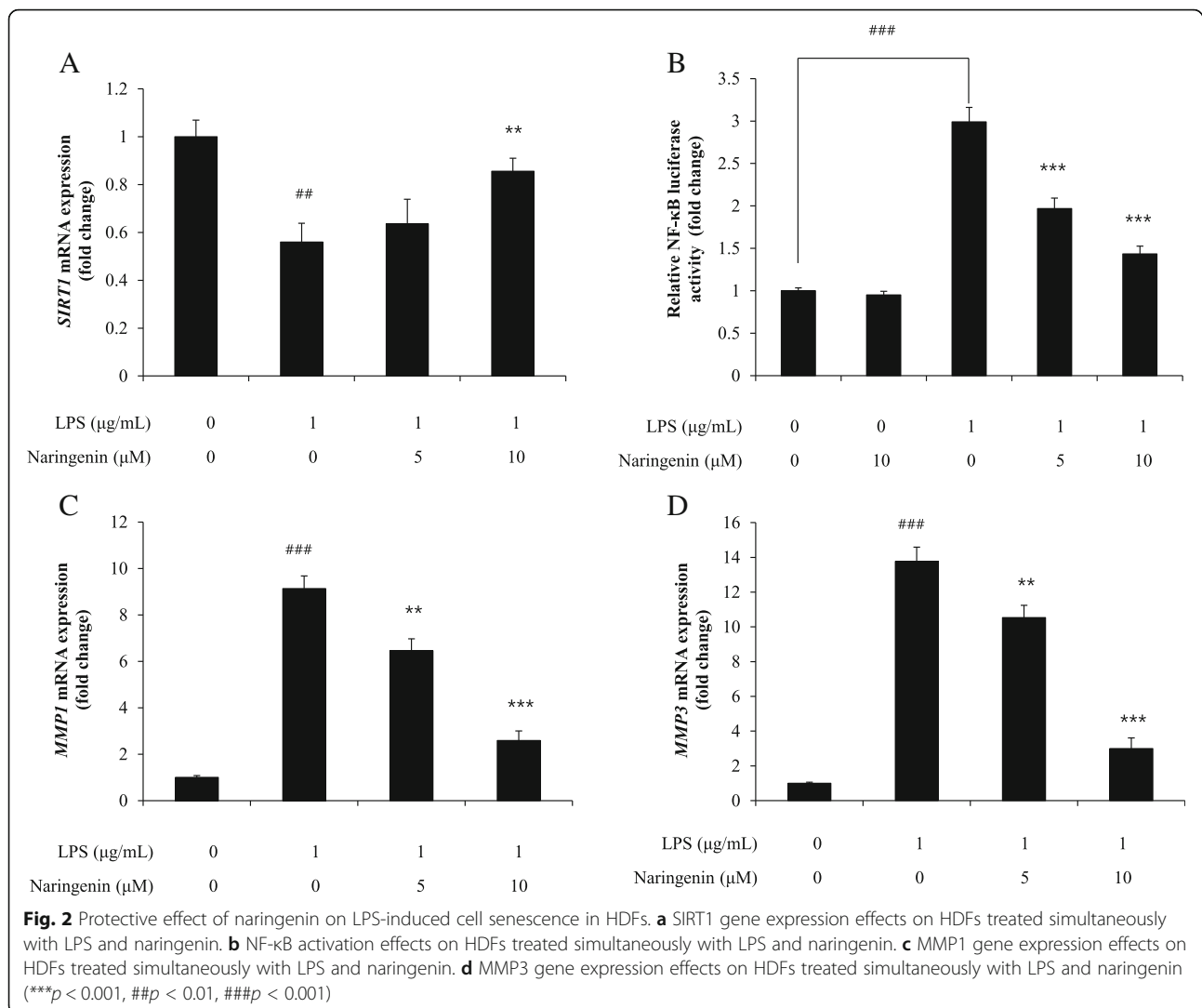
Protective effect of naringenin on LPS-induced cell senescence in HDFs

Changes in Sirt1 expression

SIRT1 inhibits NF-κB, a protein complex that promotes inflammatory responses. Furthermore, by inhibiting the MMP1 production, SIRT1 promotes the synthesis of extracellular matrix (Wang et al. 2012).

qRT-PCR was performed to study the SIRT1 expression. Compared to the control group, SIRT1 expression was decreased to 0.56 level at 1-μg dose of LPS. However, when naringenin treatment was applied, SIRT1 gene expression increased back in a dose-dependent manner, resulting in 0.64 level at 5 μM and 0.86 level at 10 μM (Fig. 2a).

Fig. 1 Cytotoxicity of naringenin and lipopolysaccharide. **a** The cellular toxicity of LPS in HDFs. No statistically significant cytotoxicity was observed in the groups treated with naringenin nontreated control group and naringenin. **b** The cellular toxicity of naringenin in HDFs

Fig. 2 Protective effect of naringenin on LPS-induced cell senescence in HDFs. **a** SIRT1 gene expression effects on HDFs treated simultaneously with LPS and naringenin. **b** NF-κB activation effects on HDFs treated simultaneously with LPS and naringenin. **c** MMP1 gene expression effects on HDFs treated simultaneously with LPS and naringenin. **d** MMP3 gene expression effects on HDFs treated simultaneously with LPS and naringenin (***$p < 0.001$, ##$p < 0.01$, ###$p < 0.001$)

Changes in nuclear factor-κB expression

In NF-κB signaling pathway, NF-κB is activated by LPS through phosphorylation and is then translocated to the nucleus, promoting the transcription of inflammatory cytokines and mediators, initiating various diseases (Lvashkiv 2011; Schett et al. 2013).

When LPS was treated with NF-κB luciferase assay, cell expression level increased to 3.0. When naringenin was treated with 5- and 10-μM dose levels, cell expression level each decreased to 2 and 1.4. Therefore, cell interval level was dose-dependently reduced by naringenin (Fig. 2b).

Changes in MMP1 and MMP3 expression

ELISA was performed to determine the effect of naringenin on the dermis by assaying the antigen or antibody using antigen-antibody reaction after binding the enzyme to the antibody, in order to investigate the effect of naringenin on the dermal structure of the skin through the changes of MMPs, an anti-aging index, and to examine the changes in gene expression of MMP1 and MMP3, which break down type I collagen of dermis related to skin wrinkles.

When HDFs were not treated with naringenin and LPS, the level of MMP1 gene expression was 1.0, and when treated by LPS, the level increased to 9.1. However, the level of MMP1 gene expression was decreased by naringenin in a dose-dependent manner to 6.4 at 5 μM treatment and 2.5 at 10 μM treatment (Fig. 2c). In addition, when HDFs were not treated with naringenin and LPS, MMP3 gene expression level was 1.0 and when treated with LPS, MMP3 gene expression level increased to 14. However, MMP3 expression decreased in a dose-dependent manner with naringenin, with MMP3 gene expression level decreasing from 11 to 3 when pretreatment of 5 μM and 10 μM were each applied (Fig. 2d). Therefore, naringenin was shown to inhibit MMP1 and MMP3 gene expression in the dermis.

Protective effect of naringenin on oxidative stress by LPS in HDFs

Expression changes in ROS production

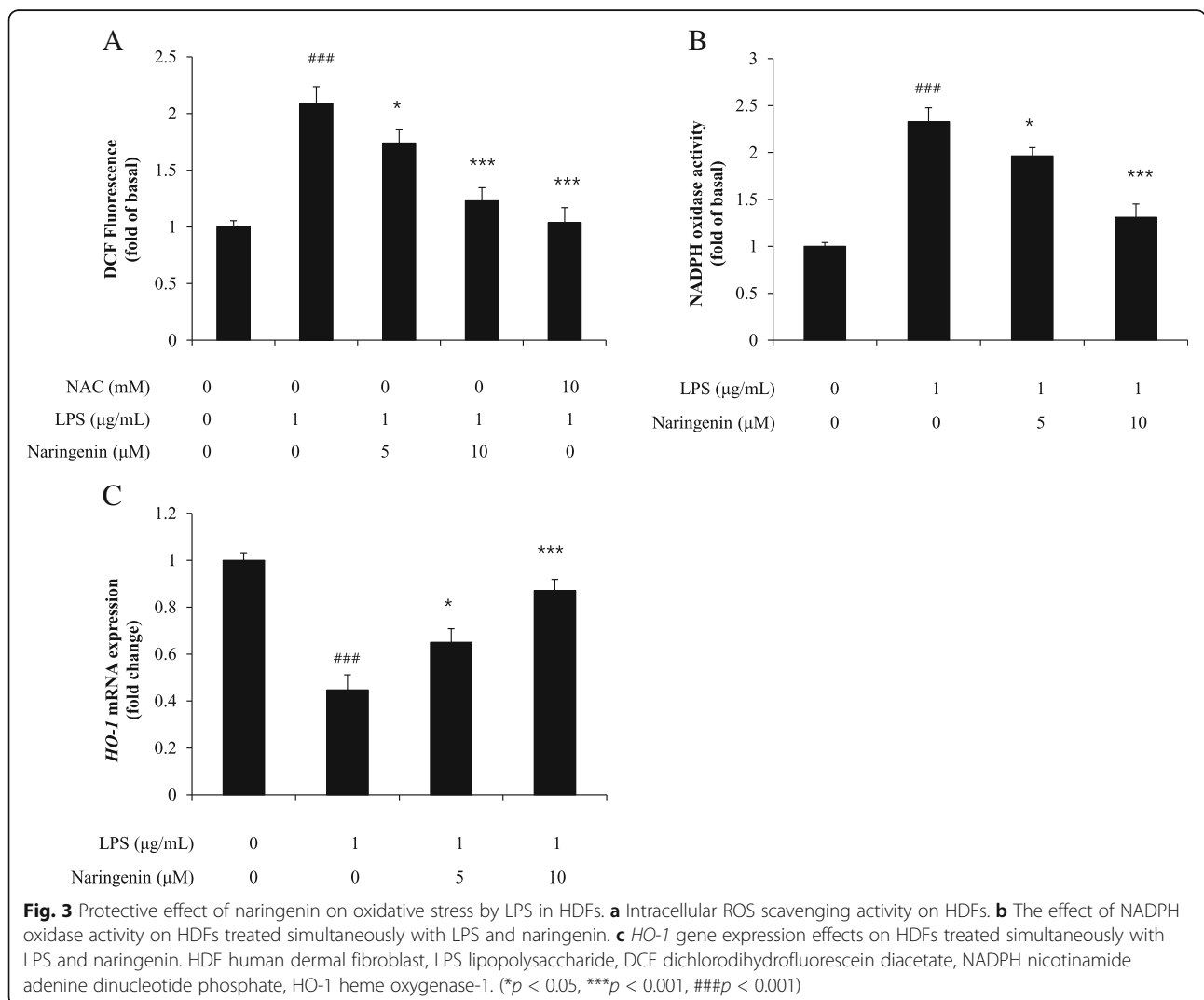ROS activates NF-κB, a transcriptional factor of inflammatory response through LPS stimulation, and in turn activates pro-inflammatory factors such as cytokines, NO, PGE2, iNOS, COX2, IL-1β, MCP-1, MIP-1, IL-16, IL-18, and M-CSF, which contributes to the development of chronic diseases (Levi et al. 1998; Knott et al. 2000; Rivest 2003).

To investigate the effect of naringenin on the total intracellular amount of ROS in LPS-treated HDFs, fluorescent probe DCF-DA was performed. When naringenin and NAC treatment were not present, LPS treatment increased the total amount of ROS level to 2.1. At 5 μM dose, naringenin decreased the level to 1.7 and further decreased the level to 1.2 at 10 μM. When the control group NAC was treated with 10 mM, the total amount of ROS level decreased to 1.0.

The results showed that at a low concentration of 10 μM, naringenin reduced ROS in a dose-dependent manner compared to the 10 mM of NAC applied on the positive control group, showing inhibiting effects on normal skin cell function impairment which resulted in excellent antioxidant activity (Fig. 3a).

Changes in NADPH oxidase expression

NADPH oxidase exists in vascular smooth muscle cells and endothelial cells and is also a major source of peroxidation that causes vascular disease and atherosclerosis. NADPH oxidase is a multiple enzyme complex consisting of large gp91phox known as Nox2, a small protein p22phox, and three cytosolic components p40phox, p47phox, p67phox, and Rac (Zalba et al. 2005). Cellular stimulation directly produces superoxide (O2-) and is produced by the activity of NADPH oxidase.

Fig. 3 Protective effect of naringenin on oxidative stress by LPS in HDFs. **a** Intracellular ROS scavenging activity on HDFs. **b** The effect of NADPH oxidase activity on HDFs treated simultaneously with LPS and naringenin. **c** *HO-1* gene expression effects on HDFs treated simultaneously with LPS and naringenin. HDF human dermal fibroblast, LPS lipopolysaccharide, DCF dichlorodihydrofluorescein diacetate, NADPH nicotinamide adenine dinucleotide phosphate, HO-1 heme oxygenase-1. (*$p < 0.05$, ***$p < 0.001$, ###$p < 0.001$)

When LPS was treated with naringenin, cell interval level increased to 2.3. When naringenin was treated with 5 and 10 μM concentrations, the cell interval levels decreased to 2.0 and 1.3. Therefore, naringenin was shown to have dose-dependent reducing effect (Fig. 3b).

Changes in HO-1 expression

Heme oxygenase-1 (HO-1), one of the representative protective enzymes in the human body, shows protective effects through strong anti-apoptosis and antioxidant action during hepatic ischemia and reperfusion (40, 41).

To study the effect naringenin has on the dermal structure, the changes in HO-1 expression was analyzed through qRT-PCR assay. When LPS was treated with naringenin, the cell interval level increased to 0.4. When naringenin was treated at 5 and 10 μM concentrations, the level increased to 0.7 and 0.9. Therefore, it was confirmed that naringenin increased HO-1 expression again in a dose-dependent manner (Fig. 3c).

Discussion

Inhibitory effect of naringenin on aging HDFs in SIRT1

Aging cells release several factors called SASPs, which have deleterious effects on surrounding cells as well as tissues. Molecular activation of the inflammation-inducing gene by the redox signal pathway ultimately leads to inflamed tissues and organs, or aging (Cheng et al. 2011; Hall et al. 2003). We found that an increase in NF-κB activation and expression of inflammatory molecules, in addition to an increase in oxidative stress, causes exogenous aging and photoaging. We also found that endogenous aging occurs due to a combination of various internal factors over time and is mainly represented by cellular senescence. The main cause of cellular senescence is oxidative stress, which stops cell division and growth by blocking the cell cycle. MMP1, which is widely distributed in human tissues, is secreted by fibroblasts and mainly degrades type II collagen, a major component of cartilage matrix, as well as types VII and VI collagen (Roberts et al. 2006; Donmez 2012). In addition, when MMP1 cleaves the middle portion of collagen fibers, MMP3 and MMP9 subdivide and cleave the collagen further. HDFs regulate collagen metabolism through interaction of MMP1, tissue inhibitor of metalloproteinase 1 (TIMP1), type I collagen, transforming growth factor beta 1 (TGF-β1), and various cytokines. SIRT1, a major anti-senescence agent that inhibits the activity of these MMPs, is activated through nuclear histones, transcription factors, and protein deacetylation of cell-signaling mediators, which causes cell signaling, anti-aging, gene expression of cell protection, and neuronal differentiation (Wang et al. 2012; Correia et al. 2017). Recently, SIRT1 activation has been reported to suppress endoplasmic reticulum stress in renal tubular cells. In addition, resveratrol, polyphenol, and

SIRT1 activators mimic the anti-aging effects of calorie restriction in lower organisms, mitigate insulin resistance, and increase mitochondrial content in rats with a high-fat diet, resulting in their prolonged survival. SIRT1 also inhibits NF-κB, a protein complex that promotes inflammatory responses. Furthermore, by inhibiting MMP1 expression, SIRT1 promotes ECM synthesis (Wang et al. 2012). The degree of skin wrinkles and thickened skin formed by ROS controls MMP activity, which is highly correlated with aging features such as skin sagging and may lead to tumors due to their imbalance. It has been reported that ROS inhibits the transcriptional activity of Smad by inhibiting TGF-β, a growth-promoting factor, and so inhibits the expression of downstream collagen genes (Quan et al. 2010; Otterbein et al. 2003).

Using quantitative real-time polymerase chain reaction (qRT-PCR) assay and enzyme-linked immunosorbent assay (ELISA), we examined how naringenin affects the expression of the NF-κB upstream gene, *SIRT1*, and the downstream genes, *MMP1* and *MMP3*. The results showed that naringenin increases the expression of *SIRT1* and decreases the expression of *MMP1* and *MMP3* in a dose-dependent manner.

In conclusion, naringenin regulates the expression of *NF-κB*; regulates signal transduction in the ECM, which has important functions in the dermis; and alters *SIRT1*, *MMP1*, and *MMP3* expression. Therefore, naringenin has a suppressing effect on skin cell senescence, DNA protection, and cell protection, indicating that it can be an important cosmetic ingredient.

Inhibition of oxidation by naringenin through inhibition of ROS activity

NADPH oxidase directly influences the production of the superoxide anion (O^{2-}), which plays the most fundamental role in ROS production, and the inducible form of nitric oxide synthase (iNOS), which is related to the immune response in NOS and produces NO, which is important for immune cell signaling. $ONOO^-$, a potent oxidant that forms naturally in cells by the reaction of NO and O^{2-}, induces apoptosis and inflammation.

O^{2-} is an oxygen molecule produced in cells. It is then converted to H_2O_2 through a spontaneous reaction or enzymatic catalysis. In addition, when a transition metal (Fe, Cu) is present, Fenton's reaction reduces O^{2-} to H_2O_2 which are eliminated by the antioxidative enzymes superoxide dismutase (SOD) and catalase. However, the body lacks controlling enzymes for OH^-, which is harmful and is known to the human body (Chen and Ames 1994). Heme oxygenase-1 (HO-1) removes cause stains, wrinkles, and freckles through oxidation of inflammatory, toxic-free heme (Fe^{2+}), treats damaged cells, and protects the body from oxidative stress by increasing bilirubin, a bile pigment that removes ROS. HO-1 is also a cell-protecting

enzyme that produces biliverdin and iron and is a potent antioxidant that protects the body against various oxidative stress-related diseases. HO-1 can also inhibit endoplasmic reticulum stress (Hu et al. 2015).

In conclusion, naringenin inhibits NADPH oxidase activity, which produces O^{2-}, removes ROS, and increases *HO-1* gene expression. The results of this study suggested that naringenin inhibits the damaging and aging effects of toxic substances that cause aging and skin inflammation.

Conclusions

Naringenin is a flavanone known to have physiological antioxidant and active oxygen-scavenging properties. It has a polyphenol structure capable of eliminating active oxygen groups and active oxygen generated by hydrogen/electron transfer in the 4-hydroxyl group of group B and has anti-inflammatory effects and antioxidant properties (Lee et al. 2016; Hee et al. 2018). SIRT1 inhibits NF-κB, a protein complex that promotes inflammatory responses and is correlated with common skin diseases caused by harmful particulate matter (PM) and environmental pollutants. Furthermore, by inhibiting the expression of MMP1, SIRT1 promotes ECM synthesis.

This study analyzed the anti-aging and antioxidative effects of naringenin by observing changes in the expression of the longevity gene *SIRT1* and the downstream genes *MMP1* and *MMP3* in the cell senescence model of LPS-induced HDFs. The results showed that naringenin increases *SIRT1* expression and decreases *NF-κB*, *MMP1*, and *MMP3* expression in a dose-dependent manner. Additional experiments confirmed the ability of NADPH oxidase to inhibit O^{2-} activity, remove ROS, and increase *HO-1* gene expression.

In conclusion, this in vitro research confirmed that naringenin not only increases SIRT1 activity but, by blocking the NF-κB pathway, also inhibits the gene expression of inflammatory factors (which are major components of SASPs) and substances that produce ROS. Naringenin can be used as an effective cosmetic ingredient to prevent age-induced skin elasticity reduction and wrinkled skin, troublesome skin diseases, and premature skin aging caused by harmful environmental pollutants, PM, etc.

Authors' contributions
KHL and GRK did all of the research background such as experiments, data collecting, and statistical analysis as well as drafting the manuscript. Both authors read and approved the final manuscript.

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