Food Sci. Biotechnol. 25(S): 133-141 (2016)
DOI 10.1007/s10068-016-0110-y

Food Science
and
Biotechnology

Beneficial Role and Function of Fisetin in Skin Health via Regulation of the CCN2/TGF-β Signaling Pathway

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Received December 12, 2015
Revised January 23, 2016
Accepted January 24, 2016
Published online March 31, 2016

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pISSN 1226-7708
eISSN 2092-6456

Abstract Skin is composed of multiple layers, including the epidermis, dermis, and hypodermis. Although several biological activities of fisetin have been reported, beneficial effects and the functions of fisetin in skin remain unclear. B16F10 melanoma cells, human skin fibroblasts, and 3T3-L1 cells were used to examine the beneficial effects of fisetin in skin health. α-MSH- and IBMX-induced melanosis in B16F10 melanoma cells was inhibited by fisetin treatment, which also enhanced mRNA expression levels of skin fibril-related genes via the CCN2/TGF-β signaling pathway. Decreased intracellular lipid accumulation via down-regulation of transcriptional factors through activation of the CCN2/TGF-β signaling pathway was observed. A novel function of fisetin in skin health via down-regulation of melanosis and adipogenesis, and up-regulation of skin fibril-related genes was observed. Evidence for development of nutri-cosmetics for skin health is presented.

Keywords: CCN2, TGF-β, fisetin, skin, melanoma

Introduction

Skin is a largest integumentary body organ consisting of the epidermis, dermis, and hypodermis. The skin provides a vital barrier structure that protects vertebrates from external environmental antigens, solvents, ultraviolet light, microorganisms, toxins, and weather conditions. The epidermis, as the outermost layer, acts as a barrier against the external environment. The basal layer on the bottom protects the skin from UV light through melanin secreted by melanocytes (1). However, excessive melanin accumulation causes the skin pigmentation disorders hyperpigmentation, melasma, and freckles. Particularly excessive amounts of melanin produced from melanosomes in melanocytes cause a darker skin tone after translocation to keratinocytes in the epidermis (2). The dermis provides tensile strength and elasticity via activation of type I and III collagen fibrils, fibronectin, and elastin (3). Thus, enhancement of the structural matrix of the dermis is important in maintenance of healthy skin.

The hypodermis is located beneath the skin and plays important roles in storage of excessive fat, maintenance of body temperature, and providing protective padding (4). However, a diminished dermis component and excessive fat accumulation result in poor skin health (5). Inhibition of excessive accumulation of fat in the hypodermis is crucial for prevention of skin wrinkles and poor skin health. Therefore, controlling the epidermis, dermis, and hypodermis can be important targets for enhancement of skin health.

Connective tissue growth factor (CCN2) is a member of the CCN2 family of matricellular-secreted proteins. CCN2 plays biological roles in cell adhesion, migration, extracellular matrix (ECM) production, and tissue wound repair. Cell growth and proliferation are especially closely associated with production and formation of the ECM (6). Recent reports have suggested that CCN2 stimulates ECM synthesis via activation of the transforming growth factor-β (TGF-β) signaling pathway (7). Furthermore, TGF-β plays important role in synthesis and degradation of the ECM. Regulation of the CCN2/TGF-β signaling pathway may also reportedly exert beneficial effects on skin fibroblasts for production of ECM components and for wound healing.

Fisetin (3,7,3',4'-tetrahydroxyflavone) is a flavonol in many fruits and vegetables that belongs to the flavonoid group of polyphenols (8). Previous studies have suggested use of fisetin as a food functional material for anti-oxidant, anti-inflammatory, and anti-carcinogenesis activities (9). In addition, fisetin reportedly has anti-adipogenic activities based on inhibition of mRNA expression levels of PPARγ in 3T3-L1 cells, and *in vivo* in mice fed a high-fat diet (10). Inhibition of preadipocyte proliferation due to fisetin treatment in the early stage of 3T3-L1 differentiation was accompanied by alterations in cell cycle regulatory proteins due to effects on mitotic clonal expansion (11). Although several biological effects of fisetin have been reported, the

precise role and the molecular mechanism of fisetin in skin health both remain unknown.

Thus, this study focused on evaluation of fisetin effects on skin health via regulation of melanosis, skin-fibril related genes, and adipogenesis. Beneficial effects of fisetin for skin health were evaluated *in vitro* using B16F10 melanoma cells, human skin fibroblasts (HSFs), and 3T3-L1 cells. Fisetin was also shown to be a dietary regulator involved in activation of CCN2/TGF-β.

Materials and Methods

Materials and reagents Fisetin, Oil Red O (ORO), 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (Dex), insulin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), sodium deoxycholate, nonidet P-40 (NP-40), phosphatase inhibitor cocktail, and phenylmethane sulfonyl fluoride (PMSF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM), penicillin-streptomycin (PS), and bovine calf serum (BCS) were obtained from Welgene Inc. (Daegu, Korea). Fetal bovine serum (FBS) was purchased from GE Healthcare Bio-Sciences Co. (Piscataway, NJ, USA). TRIzol reagent was purchased from Life Technologies (Carlsbad, CA, USA) and PCR PreMix (i-Taq) was obtained from Intron (Seongnam, Korea). All anti-bodies were purchased from Santa Cruz Biotechnology (Dallas, TX, USA).

Cell culture B16F10 melanoma and 3T3-L1 cells were obtained from the Korean Cell Line Bank (Seoul, Korea). HSFs were purchased from the American Type Culture Collection (Manassas, VA, USA). B16F10 melanoma cells and HSFs were maintained in DMEM supplemented with 10% FBS and PS at 100 unit/mL. 3T3-L1 cells were maintained in DMEM supplemented with 10% BCS and PS at 100 unit/mL. Cells were maintained at 37°C in a humidified 5% CO_2 atmosphere (TC115; Thermo Scientific, Pittsburgh, PA, USA) and the medium was replaced every 2 days.

Cell viability The effect of fisetin treatments on cell viability was evaluated using B16F10 melanoma cells and HSFs with previously reported MTT assay methods following slight modification (12). After reaching confluency, B16F10 melanoma cells and HSFs were treated with 10, 25, 50, 75, 100, 200, and 400 μM fisetin for 24 h and the DMEM medium was removed. Then, 1 mL of MTT-medium (DMEM including 0.2 mg/mL MTT) was added, for an additional 1 h. The MTT-medium was removed and insoluble formazan was dissolved in DMSO. 3T3-L1 cell viability was assessed using an EZ-Cytox assay kit. After reaching confluency, 3T3-L1 cells were treated with 10, 20, 40, 60, 80, 100, and 200 μM fisetin for 24 h. The DMEM was then removed and after 24 h of fisetin treatment, an EZ-Cytox solution was added. After 1 h of incubation, absorbance values were measured at a wavelength of 570 nm using a microplate reader (VersaMax; Molecular Devices, Sunnyvale, CA, USA). Cell viability was calculated as a percentage of viability vs. untreated cells.

Melanin content Melanin accumulation in B16F10 melanoma cells was induced using 1 μM α-MSH and 100 μM IBMX with treatments of 10, 50, 75, and 100 μM fisetin for 72 h for measurement of inhibitory activities of fisetin against melanosis. After washing with phosphate buffered saline (PBS), cells were detached from the bottom of cell culture plate using 200 μL of trypsin-EDTA. Collected cells were centrifuged at 18,472x*g* for 10 min for measurement of intracellular melanin contents. Pellets were dissolved in 200 μL of 1 N NaOH and 10% (v/v) dimethyl sulfoxide (DMSO) for 10 min at 100°C then the medium was collected for quantification of extracellular melanin amounts. Amounts of intracellular and extracellular melanin were quantified at 405 nm using a microplate reader and the melanin content was calculated as a percentage vs. fisetin untreated cells.

Evaluation of cell growth and proliferation Cells were scraped using a plastic needle upon reaching confluence for examination of the effect of fisetin treatment on cell growth and proliferation. Cells were then exposed to 0 and 50 μM fisetin. Cell growth and the proliferation activity were observed using an inverted microscope (KI2000; Korea Lab Tech, Seongnam, Korea) at magnifications of 200x for quantification of distances between cells.

3T3-L1 adipocyte differentiation Two-day post-confluent 3T3-L1 preadipocytes were cultured in DMEM supplemented with 10% BCS and 100 unit/mL PS at 37°C in a humidified 5% CO_2 atmosphere and the medium was replaced every 2 days. 3T3-L1 cells were treated with fisetin during Day 2 to Day 6. At 2 days after reaching confluence, designated as Day 0, 3T3-L1 preadipocytes were cultured in DMEM supplemented with 10% FBS and 100 unit/mL PS (FBS-medium) containing 500 μM IBMX, 5.2 μM dexamethasone, and 167 nM insulin. After Day −2, the medium was changed to FBS-medium with addition of only 167 nM insulin for another 2 days. Thereafter, 3T3-L1 adipocytes were cultured in FBS-medium with no additions.

Oil Red O (ORO) staining The DMEM medium was removed and cells were fixed with 3.7% (v/v) formaldehyde for 30 min at 25°C after washing with PBS for examination of anti-adipogenic effects of fisetin on 3T3-L1 adipogenesis. Intracellular lipid accumulation in differentiated adipocytes was assessed using ORO staining on Day 6. Formaldehyde fixed-adipocytes were then washed three times with water. Lipid droplets were stained with 3 mg/mL ORO in isopropanol for 15 min (CR300; FINEPCR, Gunpo, Korea). After stained cells were washed three times with distilled water, cells were photomicrographed. The ORO stained-lipid droplets were dissolved in DMSO and transferred at 100 μg/well to a 96 well plate. The absorbance value of each well was quantified using a microplate reader at 510 nm.

Isolation of total RNA and RT-PCR analysis mRNA expression levels of transcriptional factors were estimated using RT-PCR analysis.

Table 1. Primer sequences for PCR analysis

Primer	Species	Primer sequence (5′→3′)
Pro-collagen 1α2	*Homo sapiens*	GCACCACTTGTGGCTTTTGA (sense) TACCACACCAAACTGCCCTT (antisense)
Collagen 1α2	*Homo sapiens*	ACTGGCGAAACCTGTATCCG (sense) ACCGATGTCCAAAGGTGCAA (antisense)
Fibronectin	*Homo sapiens*	GGCTTGAACCAACCTACGGA (sense) CATGAAGCACTCAATTGGGCA (antisense)
Smad2	*Homo sapiens*	CGCCGGGAGGTTCGATA (sense) ACTGTGAAGATCAGGCCAGC (antisense)
CCN2	*Homo sapiens*	TCCACCCGGGTTACCAATGA TCAAACCAGTGTCTGGGGAAGAT (antisense)
β-Actin	*Homo sapiens*	GCAGGAGTATGACGAGTCCG (sense) AGGGACTTCCTGTAACAATGC (antisense)
LPL	*Mus musculus*	AAACCCCAGCAAGGCATACA (sense) ATTTGTGGAAACCTCGGGCA (antisense)
PPARγ	*Mus musculus*	CCATTCTGGCCCACCAACTT (sense) CCTTCTCGGCCTGTCGATCC (antisense)
C/EBPα	*Mus musculus*	CCAGAGGATGGTTTCGGGTC (sense) TCCCCAACACCTAAGTCCCT (antisense)
FAS	*Mus musculus*	GTGCACACAGTGCTCAAAGG (sense) GGTATAGACGACGGGCACAG (antisense)
β-Actin	*Mus musculus*	AGGGAAATCGTGCGTGACAT (sense) AGCTCAGTAACAGTCCGCCT (antisense)

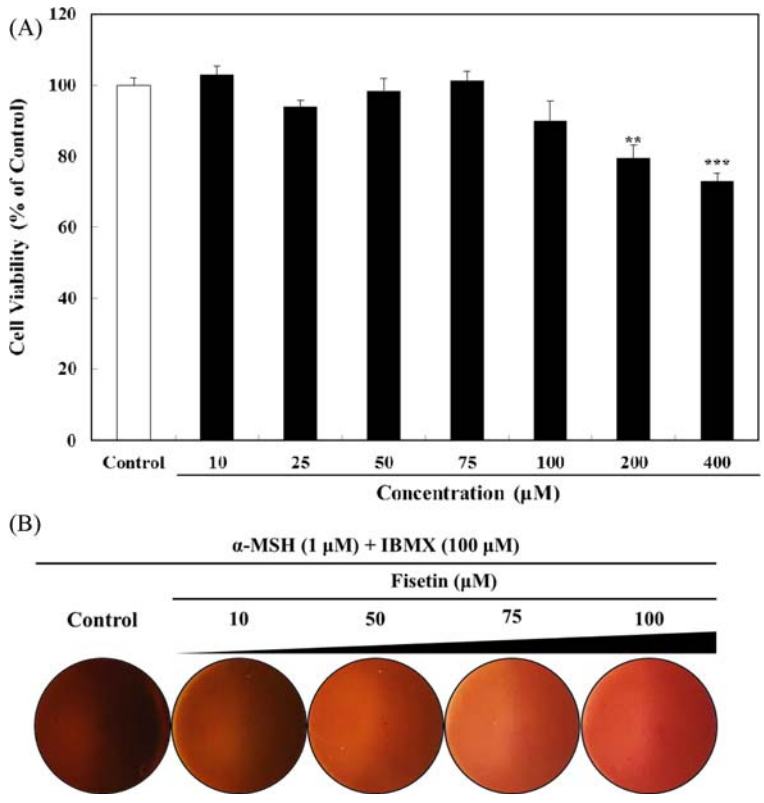

Fig. 1. Effects of fisetin treatment on melanin synthesis in B16F10 melanoma cells. Effects of fisetin treatment on cell viability of B16F10 melanoma cells. B16F10 melanoma cells were treated with indicated concentrations of fisetin for 24 h. Melanin accumulation in B16F10 cells was initiated using 1 μM α-MSH and 100 μM IBMX for 72 h (A). Observation of intra and extracellular melanin amounts secreted from B16F10 melanoma cells (B). Levels of intra (C) and extracellular melanin in B16F10 melanoma cells (D). Melanin contents were quantified using a microplate reader at 405 nm. Corresponding letters indicate significant differences between treated and control cells based on Student's *t*-test (**p<0.01 and ***p<0.001).

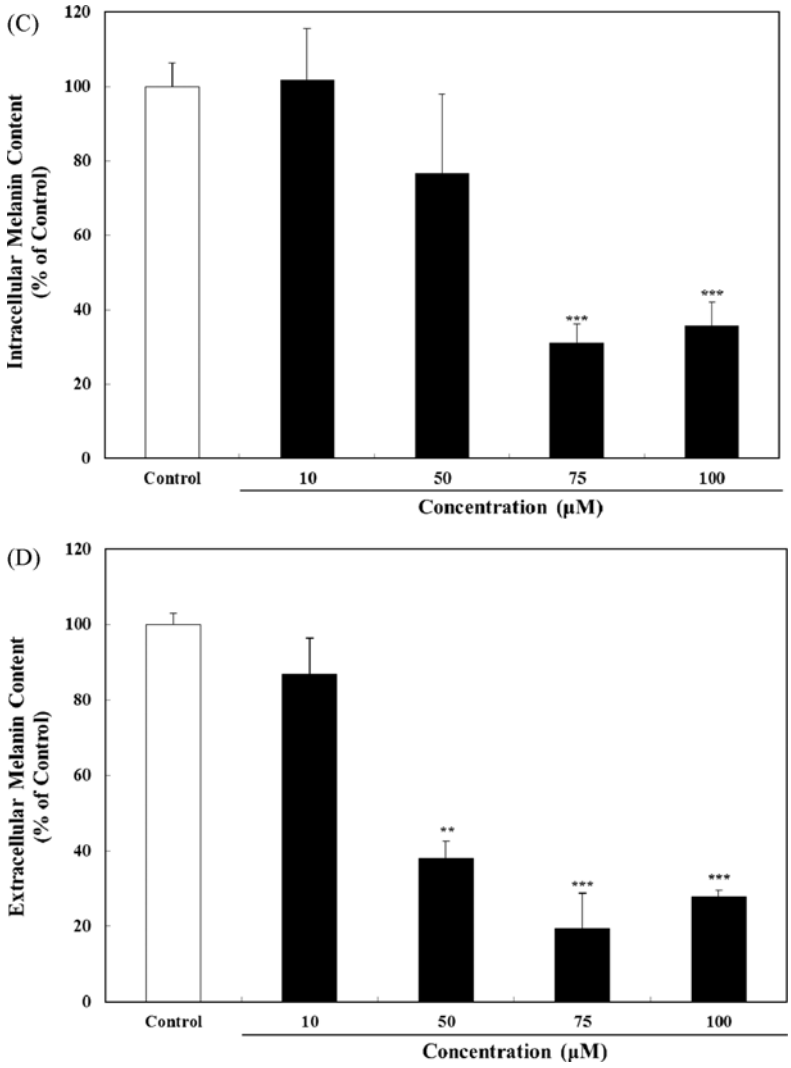

Fig. 1. Continued.

Total RNA was extracted from HSFs and 3T3-L1 cells treated with fisetin using TRIzol reagent. Total RNA was dissolved in 40 μL of RNase-free water. Two μg of cDNA was then subjected to reverse transcription using CycleScript RT PreMix for cDNA synthesis through 12 cycle reactions at 48°C. mRNA expression levels were analyzed using electrophoresis with agarose gel, and β-actin as a control. RT-PCR primers for C/EBPα, PPARγ, CCN2, FAS, LPL, procollagen 1α2, collagen 1α2, fibronectin, Smad2, and β-actin are shown in Table 1.

Western blotting HSFs and 3T3-L1 cells were lysed in RIPA lysis buffer containing 1 mM PMSF, 50 mM Tris-HCl at pH 8.0, 1% (w/v) NP-40, 0.5% sodium deoxycholate, 150 mM NaCl, and a phosphatase inhibitor cocktail. After centrifugation at 18,472*xg* for 20 min at 4°C (1730R; LaboGene, Seoul, Korea), total protein in supernatant was quantified using a Bradford assay. Quantified supernatants were then subjected to electrophoresis using SDS-PAGE and separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes, which were blocked with 5% (w/v) skim milk diluted in TBST, followed by reaction with primary antibodies for 12 h at 4°C. Membranes were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h. Target proteins were detected using a chemiluminescent detection kit and visualized using a ChemiDoc Molecular Imager (Bio-Rad, Hercules, CA, USA).

Statistical analysis All data are presented as mean value±standard deviation (SD). Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). Significance of differences between groups was assessed using a one-way analysis of variance (ANOVA) followed by either Duncan's or Student's *t*-test. A p value of <0.05 was considered statistically significant.

Results and Discussion

Fisetin treatment inhibits melanin synthesis in B16F10 melanoma cells Effects of fisetin on cell viability in B16F10 melanoma cells

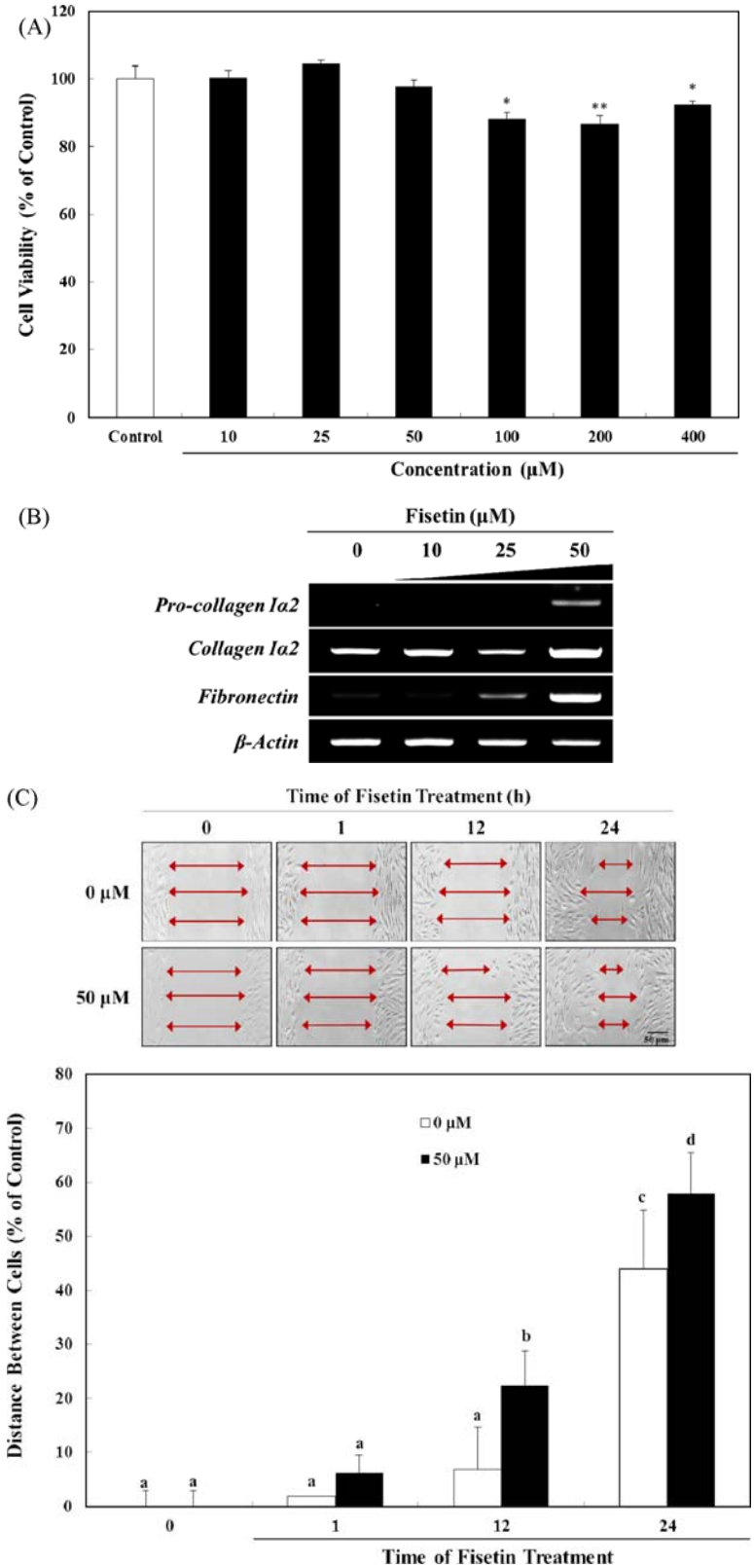

Fig. 2. Effects of fisetin treatments on expression levels of fibril-related genes and cell growth in HSFs. Cells were treated with indicated concentrations of fisetin for 24 h (A). HSFs were treated with 10, 25, and 50 μM fisetin for 2 h. mRNA expression levels of skin fibril-related genes were determined using RT-PCR (B). Effects of fisetin treatment on growth and proliferation of HSFs. Cells were treated with 50 μM fisetin for 1, 6, 12, and 24 h. (C). mRNA expression levels of Smad2 and CCN2 and protein expression levels of CCN2, TGF-β1, TGF-β2, and TGF-β3 in HSFs were determined using RT-PCR and Western blotting, respectively (D). Corresponding letters indicate significant differences based on either Duncan's multiple range test (p <0.05) or Student's t-test (*p<0.05 and **p<0.01)

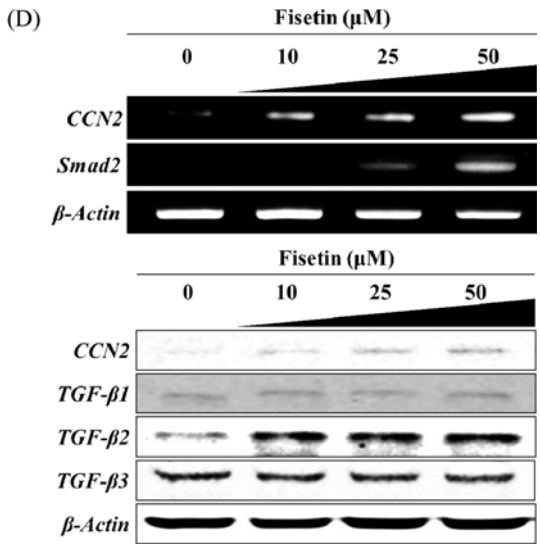

Fig. 2. Continued.

were studied using MTT assays. B16F10 melanoma cells were treated with 10, 25, 50, 100, 200, and 400 μM fisetin for 24 h. Exposure to fisetin had no toxic effect on the viability of B16F10 melanoma cells up to a concentration of 100 μM for 24 h (Fig. 1A). However, viability of B16F10 melanoma cells significantly (p<0.05) decreased upon exposure to 100, 200, and 400 μM fisetin treatments, compared with controls. Thus, fisetin concentrations of 10 to 50 μM were used for subsequent experiments.

Melanin accumulation was initiated via treatment with 1 μM α-MSH and 100 μM IBMX in the presence or absence of 10, 50, 75, and 100 μM fisetin for 72 h for examination of the effects of fisetin on B16F10 melanosis. Melanin in dermal tissues originates from mature melanosomes, which are specialized cellular vesicles of the epidermis. Thus, evaluation of total melanin contents, including both intra and extracellular melanin concentrations, is important.

Inhibitory effects of fisetin treatments on melanin accumulation in B16F10 cells were evaluated via measurement of intra and extracellular melanin levels in B16F10 cells. Fisetin treatment for 72 h resulted in inhibition of the B16F10 melanosis induced by α-MSH and IBMX in a dose-dependent manner (Fig. 1B). Treatment with 10, 50, 75, and 100 μM fisetin suppressed both intra and extracellular melanin accumulation by 0, 22.27, 68.92, and 64.33% (Fig. 1C), and by 13.05, 61.97, 80.53, and 72.17%, respectively (Fig. 1D).

Melanins are natural pigments synthesized in the epidermis. However, hyperpigmentation in melasma, acanthosis nigricans, and freckles is caused by excessive production of melanin in melanocytes (13). Extracts from *Reynoutria elliptica* reportedly showed inhibition through α-MSH-mediated tyrosinase activity and melanogenesis in SK-MEL-2 cells (14). Furthermore, ethyl acetate fractions of *Taraxacum platycarpum* inhibited tyrosinase activity and melanin synthesis in B16F10 melanoma cells (15). Therefore, exploration of skin whitening materials is important for prevention of skin impairment and aging caused by excessive pigment accumulation from melanocytes. Thus, fisetin apparently plays an important role via inhibition of melanin synthesis in B16F10 melanoma cells.

Fisetin treatment enhances expression levels of skin fibril-related genes and cell growth through activation of the CCN2/TGF-β signaling pathway Effects of fisetin treatments on viability of HSFs were evaluated using MTT assays. HSFs were treated with 10, 25, 50, 100, 200, and 400 μM fisetin for 24 h. However, fisetin treatments at concentrations from 10 to 50 μM for 24 h had no effect on the viability of HSFs (Fig. 2A). HSFs were exposed to 10, 25, and 50 μM fisetin for examination of fisetin treatment contribution to development of skin-fibril-related genes. Fisetin treatment increased mRNA expression levels of pro-collagen 1α2, collagen 1α2, and fibronectin in a dose-dependent manner (Fig. 2B). The ECM, the interstitial matrix, and the basement membrane are all composed fibrillar collagen and fibronectin extracellular proteins (16). Sustainment and development of the ECM is a crucial factor for maintenance of healthy skin. Collagen is abundant in the ECM of human skin. Procollagen is a precursor of collagen that is secreted by exocytosis from skin fibroblasts and is converted to collagen via cleavage of end-terminal propeptides by procollagen proteases. Fibronectin is a high Mw glycoprotein of the ECM that binds the ECM components of collagen and fibrin. Cellular fibronectin is assembled into the ECM and fibronectin plays an important role in wound healing. Thus, fisetin has potential to serve as a positive regulator of skin health via activation of the ECM proteins procollagen, collagen, and fibronectin.

Cell growth and proliferation were observed after 0, 1, 12, and 24 h of fisetin treatment of HSFs for determination of fisetin treatment contribution to recovery of skin fibril damage and collapse. The distance between HSFs treated with fisetin after artificial scratching with a plastic needle was closer than for cells experiencing no treatment (Fig. 2C). Furthermore, cell growth and proliferation were significantly (p<0.05) stimulated by fisetin treatments in a time-

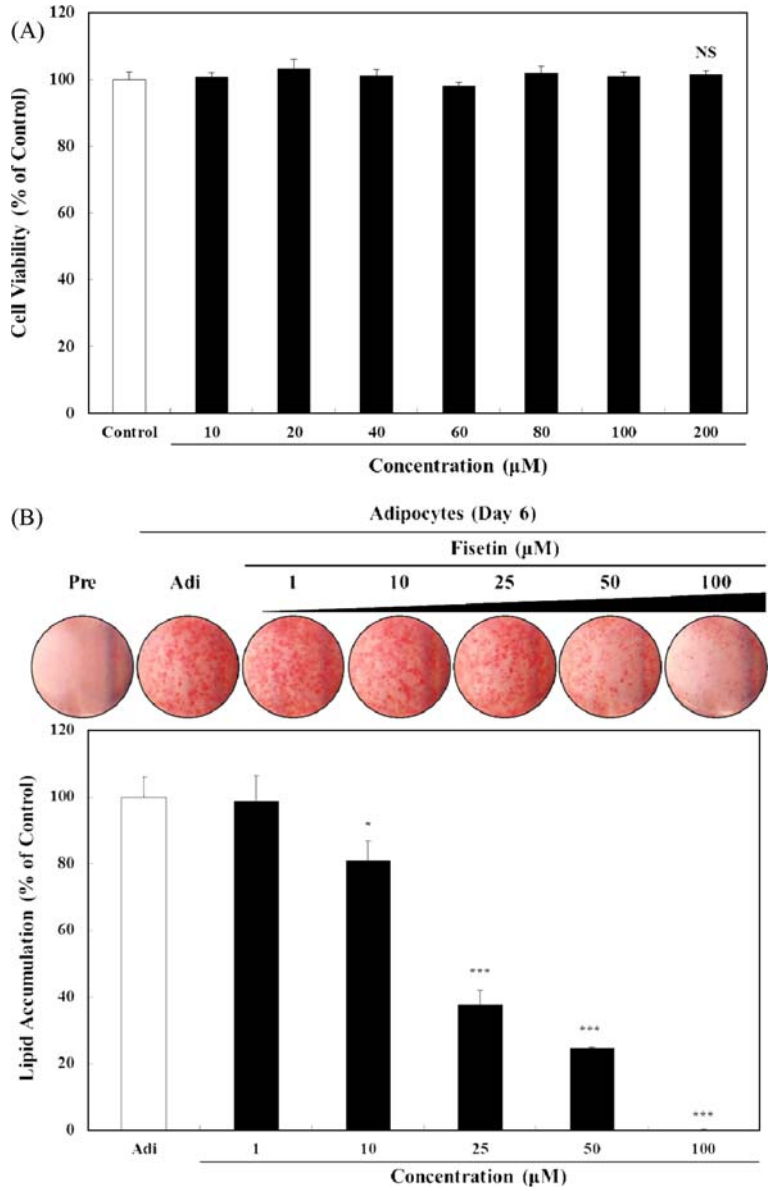

Fig. 3. Effects of fisetin treatment on 3T3-L1 adipogenesis. Cell viability of 3T3-L1 preadipocytes after treatment with indicated concentrations of fisetin for 24 h (A). 3T3-L1 preadipocytes were subjected to adipogenesis in the presence or absence of 1, 10, 25, 50, and 100 μM of fisetin for 8 days. Levels of lipid accumulation in mature 3T3-L1 adipocytes were quantified using ORO staining on Day 6 (B). Microscopic image analysis (C). Effects of fisetin treatment on mRNA expression levels of adipogenic transciptional factors and adipocyte-specific genes in 3T3-L1 cells (D). Corresponding letters indicate significant differences based on Student's *t*-test (**p*<0.05 and ****p*<0.001). NS: not significant

dependent manner, compared with controls.

Wound healing in skin is a complex process that includes the inflammatory response, formation of renewal tissue, and remodeling of the ECM (17). Skin fibroblasts that migrate into wounds proliferate for synthesize of collagen types I and III, and proteoglycans for facilitation of ECM recovery around the wound. Some fibroblasts differentiate into myofibroblasts and synthesize α-smooth muscle actin, commonly used as a marker of myofibroblast formation for stimulation of wound repair via contraction of the would edge (18). Incision wounds in ICR mice recovered after treatment with total ginseng saponin, and collagen deposition in mice treated with total ginseng saponin was stimulated by ginseng during wound healing (19). Thus, fisetin treatment contributed to cell growth and proliferation via up-regulation of skin fibril-related genes in response to physical damage.

Effects of fisetin on mRNA expression levels of CCN2 and Smad2 were examined using RT-PCR for determination of effect of fisetin on CCN2/TGF-β signaling. Fisetin treatment increased mRNA expression levels of CCN2 and Smad2, a CCN2 downstream mediator, in a dose-dependent manner (Fig. 2D). Protein expression levels of CCN2, TGF-β1, TGF-β2, and TGF-β3 were assessed using Western blotting for determination of effects of fisetin on the CCN2/TGF-β signaling

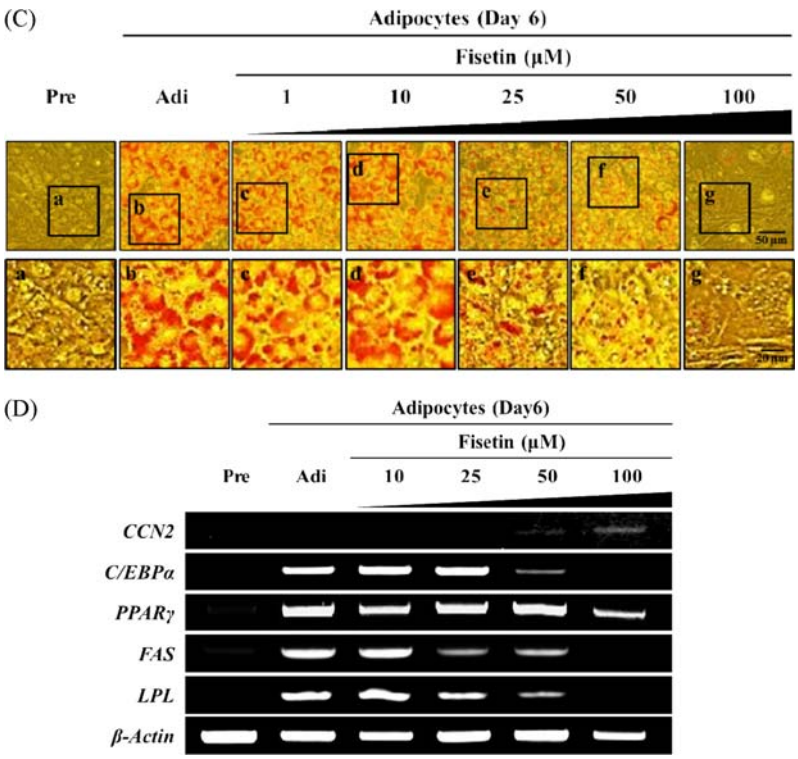

Fig. 3. Continued.

pathway. Protein expression levels of CCN2 and TGF-β2 were increased by fisetin treatment for 6 h. In contrast, fisetin treatment had no significant (p>0.05) effect on protein expression levels of TGF-β1 and TGF-β3, compared with controls (Fig. 2D).

Extracts of cuttlebone reportedly enhanced wound healing in burn injuries via down-regulation of pro-inflammatory cytokines levels of IL-6 and TNF-α (20). β-Glucans derived from cereals and microorganisms reportedly have beneficial effects of anti-wrinkle, anti-oxidant, and wound healing activities, and moisturizing effects via regulation of vascular endothelial growth factor, platelet-derived growth factors, and neurotrophin 3. However, the underlying molecular mechanisms by which regulation of the CCN2/TGF-β signaling pathway treated with fisetin occurs have not been investigated. This study suggests that up-regulation of skin fibril-related genes is mediated via activation of CCN2 and TGF-β2.

Fisetin treatment suppresses 3T3-L1 adipogenesis via down-regulation of adipogenic transcriptional factors Adipose tissue is mainly located immediately beneath the skin, around internal organs, and in breast tissue, and has beneficial effects of insulation against heat and cold, maintenance of the energy balance, and homeostasis (21). However, excessive accumulation of fat can be a cause of subcutaneous cellulite that causes wrinkles in skin (22). Natural materials are known to inhibit adipogenesis; however, the anti-adipogenic activity of fisetin for skin health and the underlying molecular mechanism both remain unclear. 3T3-L1 preadipocytes were treated with 10, 20, 40, 60, 80, 100, and 200 μM fisetin for 24 h. Fisetin treatment had no toxic effect on the viability of 3T3-L1 preadipocytes at concentrations of 1 to 200 μM for 24 h (Fig. 3A). Therefore, concentrations of 1, 10, 25, 50, and 100 μM fisetin were used for subsequent experimentation. Cells were exposed to an adipogenic differentiation medium to initiate adipogenesis in the presence and absence of 1, 10, 25, 50, and 100 μM fisetin for examination of fisetin inhibition of 3T3-L1 adipogenesis. The inhibitory effect of fisetin on 3T3-L1 adipogenesis was evaluated based on ORO staining at Day 6. Fisetin treatment significantly (p<0.05) inhibited 3T3-L1 adipogenesis in a dose-dependent manner, compared with controls. Fisetin treatments at concentrations of 1, 10, 25, 50, and 100 μM inhibited 3T3-L1 adipogenesis by 1.31, 19.04, 62.31, 75.44, and 100.00%, respectively (Fig. 3B). In addition, numbers and sizes of intracellular lipid droplets dramatically decreased upon fisetin treatment (Fig. 3C). Thus, fisetin treatments inhibited intracellular lipid accumulation via down-regulation of 3T3-L1 adipogenesis.

Adipogenesis is defined as differentiation of preadipocytes into adipocytes (23) and is dependent on coordinated regulation of hormones, nutrients, adipokines, and adipogenic transcription factors, such as the CCAAT/enhancer-binding protein (C/EBP) family and peroxisome proliferator-activated receptors (PPARs), which are known to play crucial roles in adipogenesis (24). C/EBPβ and C/EBPδ are immediately induced after differentiation, whereas C/EBPα and PPARγ are expressed much later (25,26). C/EBPα and PPARγ are necessary for expression of the adipocyte-specific genes adipogenectin, fatty acid synthase (FAS), and lipoprotein lipase (LPL), which results in

morphological changes and lipid accumulation within cells.

Expression levels of C/EBPα and PPARγ were analyzed using RT-PCR for assessment of fisetin-inhibited adipogenesis involvement in mRNA expression of transcription factors and adipocytes-specific genes. 3T3-L1 adipocytes were exposed to 10, 25, 50, and 100 μM fisetin for −2 to 6 days. mRNA expression levels of the adipogenic transcription factors C/EBPα, PPARγ, and CCN2 were suppressed by fisetin treatment in a dose-dependent manner (Fig. 3D). Furthermore, fisetin treatments led to inhibition of adipocytes-specific gene expression, including FAS and LPL. Anti-adipogenic activities of extracts from the natural materials *Rumex crispus* L. (a perennial flowering plant in the family Polygonaceae) and *Aster scaber* Thunb (a perennial herb of the Compositae family) were reportedly associated with inhibited mRNA expression levels of the transcription factors C/EBPs and PPARγ (27,28). Regulation of adipogenic transcriptional factors and adipocyte-specific genes reportedly plays an important role for inhibition of lipid accumulation (29,30). Furthermore, activation of the CCN2/TGF-β signaling pathway plays an important role via inhibition of expression levels of C/EBP-β and C/EBP-α during adipocyte differentiation (31,32). Thus, mRNA expression levels of transcription factors are probably associated with activation of the CCN2/TGF signaling pathway that is induced by fisetin treatment.

Fisetin treatment inhibited melanin synthesis in B16F10 melanoma cells. In addition, the CCN2/TGF-β signaling pathway is important for development and formation of skin fibril-related genes in HSFs. Inhibition of lipid accumulation during 3T3-L1 adipogenesis via down-regulation of the transcription factors C/EBPα and PPARγ is also regulated by this signaling pathway.

In conclusion, a novel role of fisetin in skin health via down-regulation of melanosis and adipogenesis, and upregulation of skin fibril-related genes was shown herein. Useful information is provided for development of nutri-cosmetics and functional foods using fisetin for skin health.

Acknowledgments The Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2014R1A1A2058956) provided support.

Disclosure The authors declare no conflict of interest.

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