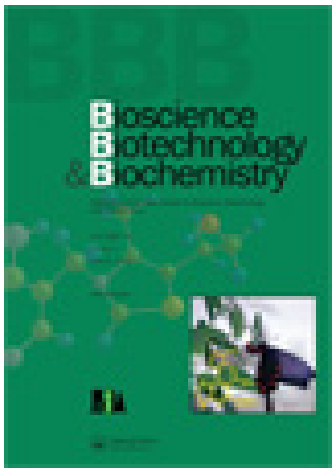

Bioscience, Biotechnology, and Biochemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb20

Curcumin enhances the production of major structural components of elastic fibers, elastin, and fibrillin-1, in normal human fibroblast cells

Shu-Mei Lee[a], Shu-Hua Chiang[b], Hsun-Yen Wang[c], Pey-Shiuan Wu[d] & Chih-Chien Lin[d]

[a] Department of Cosmetic Science and Management, Mackay Medicine, Nursing and Management College, Taipei, Taiwan, ROC

[b] Department of Food and Beverage Management, Taiwan Hospitality and Tourism College, Hualien, Taiwan, ROC

[c] Tri-Service General Hospital, Taipei, Taiwan, ROC

[d] Department of Cosmetic Science, Providence University, Taichung, Taiwan, ROC

Published online: 21 Oct 2014.

To cite this article: Shu-Mei Lee, Shu-Hua Chiang, Hsun-Yen Wang, Pey-Shiuan Wu & Chih-Chien Lin (2014): Curcumin enhances the production of major structural components of elastic fibers, elastin, and fibrillin-1, in normal human fibroblast cells, Bioscience, Biotechnology, and Biochemistry, DOI: 10.1080/09168451.2014.972324

To link to this article: http://dx.doi.org/10.1080/09168451.2014.972324

Bioscience, Biotechnology, and Biochemistry, 2014

Curcumin enhances the production of major structural components of elastic fibers, elastin, and fibrillin-1, in normal human fibroblast cells

Shu-Mei Lee[1], Shu-Hua Chiang[2], Hsun-Yen Wang[3], Pey-Shiuan Wu[4,*] and Chih-Chien Lin[4,*]

[1]*Department of Cosmetic Science and Management, Mackay Medicine, Nursing and Management College, Taipei, Taiwan, ROC;* [2]*Department of Food and Beverage Management, Taiwan Hospitality and Tourism College, Hualien, Taiwan, ROC;* [3]*Tri-Service General Hospital, Taipei, Taiwan, ROC;* [4]*Department of Cosmetic Science, Providence University, Taichung, Taiwan, ROC*

Received June 2, 2014; accepted September 8, 2014
http://dx.doi.org/10.1080/09168451.2014.972324

Curcumin is the major component of the yellow extract derived from the rhizome of the *Curcuma longa*, which is also a main bioactive polyphenol and has been generally used as a spice, food additive, and herbal medicine. In this presented study, we found that curcumin can enhance the production of major structural components of elastic fibers, elastin, and fibrillin-1, in normal human fibroblast cells via increasing *ELN* and *FBN1* promoters' activities. With 2 μM curcumin treatment, the enhanced tropoelastin and fibrillin-1 protein amounts in Detroit 551 cells were approximately 134 and 130% of control, respectively. Therefore, our results demonstrated that curcumin may be used as a functional compound and applied to drugs, foods, and cosmetics in the future.

Key words: curcumin; elastin; fibrillin-1; fibroblast cells; promoter activity

The constituents of skin, blood vessels, and lungs are required to withstand various mechanical environments. The mechanical properties of such elastic biological tissues are dominated by an extracellular matrix, in which elastic fibers offer both features of compliance and passive recoil.[1)] Although elastic fibers are complex assemblies with multiple parts, their structures comprise two main constituents: elastin and fibrillin microfibrils. Elastin is the most important structural element of the elastic fibers that is secreted from cells as a soluble protein (referred to as tropoelastin). The assembly of tropoelastin into a fibrillar matrix is a multi-step process.[2)] The super-family of fibrillin is a group of large glycoproteins. The most abundant constitution of fibrillin microfibrils backbone is fibrillin-1, which is also released from cells to form fibrillin microfibrils through a complex progression.[3)]

Denaturations of elastin structure, metabolism or assembly may cause both congenital and acquired diseases, such as the cases of cutis laxa, pseudoxanthoma elasticum, and elastosis perforans serpiginosa in the parts of connective tissues and skin.[4)] Similarly, fibrillin-1 gene mutations cause Marfan syndrome, which also leads to various connective tissue disorders in the skeletal, cardiovascular, and ocular systems.[5)] Therefore, the regulations of elastin and fibrillin-1 expression in cells are the key steps for the formation of elastic fibers. Moreover, discovery of functional compounds that regulate the expressions of elastin and fibrillin-1 in cells is a good method to treat related syndromes.

Curcumin (Fig. 1(A)) is the major component of the yellow extract derived from the rhizome of the *Curcuma longa* (turmeric, an Indian spice), which is also a main bioactive polyphenol and has been generally used as a spice, food additive, and herbal medicine in Asia.[6,7)] Many articles have discussed the molecular basis of curcumin for the antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal, and anticancer activities. In addition, many clinical trials have been conducted on this compound, which has effects on various chronic diseases including autoimmune, cardiovascular, neurological, psychological diseases, as well as diabetes and cancer.[8–10)] Previous studies have demonstrated that curcumin can ameliorate age-associated large elastic artery stiffening, and also protect the developing lung against long-term hyperoxic injury.[11,12)] However, to the best of our knowledge, no study has reported the effects of curcumin on the expressions of the major structural proteins of elastic fibers: elastin and fibrillin-1.

Therefore, in this study, we use a human cell-based antiaging compound assay system that analyses curcumin through the promoters of elastin (*ELN*) and fibrillin-1 (*FBN1*) genes combined with the reporters of secreted alkaline phosphatase (SEAP) in normal human fibroblast cells. Moreover, reverse transcription polymerase chain reaction (RT-PCR) and western blotting analyses were used to confirm the effects of curcumin on the regulations of elastin and fibrillin-1 in fibroblasts.

*Corresponding authors. Email: chchlin@pu.edu.tw (P. -S. Wu), jwu2@pu.edu.tw (C. -C. Lin)
Shu-Mei Lee and Shu-Hua Chiang contributed equally to this work.

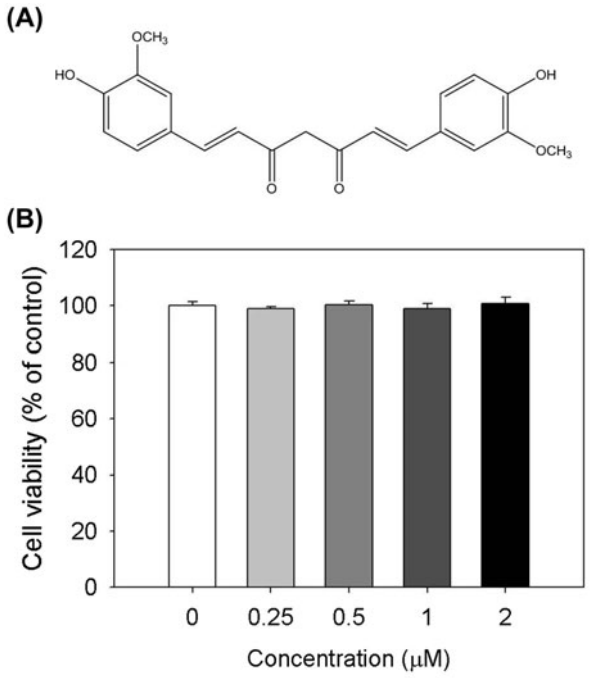

Fig. 1. Cytotoxic effect of curcumin on Detroit 551 cells.
Notes: (A) Chemical structure of curcumin. (B) Cell viability of curcumin treated Detroit 551 cells. Each value is expressed as the mean ± S.E. ($n = 3$).

Materials and methods

Materials. Curcumin, retinol, dimethyl sulfoxide (DMSO), diethanolamine, magnesium chloride ($MgCl_2$), L-homoarginine and para-nitrophenyl phosphate (pNPP), ethidium bromide, dithiothreitol (DTT), agarose, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from HyClone (Logan, UT, USA). Minimum essential medium alpha (α-MEM), L-glutamine, penicillin-streptomycin, deoxynucleotide triphosphate (dNTP), oligo(dT), *Pfu* and *Taq* DNA polymerase, and M-MLV reverse transcriptase were purchased from Gibco BRL/Invitrogen (Carlsbad, CA, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Affymetrix/USB (Cleveland, OH, USA). Xfect™ transfection reagent was purchased from Clontech Laboratories, Inc. (Mountain View, CA, USA). Restriction enzymes were purchased from New England BioLabs (Beverly, MA, USA). The antihuman tropoelastin, antihuman fibrillin-1, and antiβ-actin antibodies were purchased from GeneTex Inc. (Irvine, CA, USA). Deionized distilled water (ddH_2O) used to prepare solutions was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

Cell line and cell culture. The Detroit 551 cells (BCRC 60118, normal human fibroblast cells) were obtained from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan, ROC). Detroit 551 cells were cultured in α-MEM medium supplemented with 10% FBS and 1% penicillin–streptomycin (100 U/mL penicillin and 100 μg/mL streptomycin). The cells were maintained in a humidified incubator at 37 °C with 5% CO_2. The cells were subcultured every 3–4 days to maintain logarithmic growth and were allowed to grow for 24 h before transfection or treatment were applied.[13)]

MTT Assay for cell viability. The Detroit 551 cells were seeded in 96-well plates (8×10^3 cells/well) for 24 h incubation. The prepared cells were subsequently treated with different concentrations of curcumin in DMSO for 24 h. The used concentration of DMSO in medium is 0.1% (v/v). Subsequently, 100 μL (0.5 mg/mL) of MTT solution was added to cells, which were then incubated at 37 °C for 4 h and washed twice with phosphate buffered saline. Finally, the cleaned cells were lysed with 100 μL DMSO, and the absorbance was measured spectrophotometrically at 540 nm using an ELISA reader.[14)]

Plasmid construction and transfection. The genomic DNA from Detroit 551 human normal fibroblast cells were extracted by FavorPrep™ Blood/Cultured Cell Genomic DNA Extraction Mini Kit (Favorgen, PingTung, Taiwan, ROC). Human *ELN* and *FBN1* promoters DNA fragments were amplified through the extracted genomic DNA by *Pfu* DNA polymerase with designed primers (Table 1). The prepared promoter DNA fragments were then cloned into pSEAP2-control vectors (Clontech, Mountain View, CA, USA) by *Xho*I and *Eco*RI restriction sites to generate pSEAP2-pELN and pSEAP2-pFBN1 plasmids (Fig. 2(A)). The PCR reaction was performed using 1 μg plasmid DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 2.5 units of *Taq* DNA polymerase, and 0.1 μM each of the primers (Table 1). For transfection, Detroit 551 cells were cultured in 24-well plates with 8×10^4 cells/well and the prepared plasmids were transfected by Xfect™ transfection reagent into cells at concentration of 1 μg/well according to the protocol. After 4 h transfection at room temperature, the transfection reaction was terminated by replacing of fresh medium.[15)] The transfection efficiency of tested plasmid was measured by the co-transfected control plasmid (pEGFP-1, Clontech) at the ratio of 10:1.

SEAP activity assay. Detroit 551 cells were seeded onto the 24-well plates at a density of 8×10^4 cells/well. After incubating at 37 °C for 24 h in 5% CO_2 atmosphere incubator, cells were transfected with 1 μg/well plasmids using the Xfect™ transfection reagent. After transfection, the cells were then treated with or without (untreated group) curcumin for 24 h. Cell culture supernatants were collected and then analyzed for SEAP activity. For SEAP activity assay, the cell culture supernatants were placed at 65 °C for 10 min to eliminate endogenous alkaline phosphatase activities, which were then centrifuged at 14,000 *x g* for 1 min. Add 300 μL supernatants with an equal volume of 2X SEAP buffer (2 M diethanolamine, 1 mM $MgCl_2$, 20 mM L-homoarginine, pH 9.8) to each well with 20 mM pNPP substrate. The kinetic assay was performed by reading the absorbance at 405 nm at regular

Table 1. Information of the primers used in this study.

Target	Name	Sequence
ELN promoter	pELN-F	5′-GCTCGAGAAGAGAGGGCTCC-3′
	pELN-R	5′-GGAATTCCTCGGGGAGAAATGCCCCA-3′
FBN1 promoter	pFBN1-F	5′-GCTCGAGTTGGTGCAGGTGGAACAGC-3′
	pFBN1-R	5′-GGAATTCAGTCCCTGTCCCTGCCCT-3′
ELN mRNA	ELN-mRNA-F	5′-CTTTTGCTGGAATCCCAGG-3′
	ELN-mRNA-R	5′-CCAACACCAGGGAGGACT-3′
FBN1 mRNA	FBN1-mRNA-F	5′-CTGAAGACATGAAGACTTGTG-3′
	FBN1-mRNA-R	5′-AGTTCCAAAGACACAGATGTT-3′
GAPDH mRNA	GAPDH-mRNA-F	5′-CCCTTCATTGACCTCAACTA-3′
	GAPDH-mRNA-R	5′-AGATGATGACCCTTTTGGCT-3′

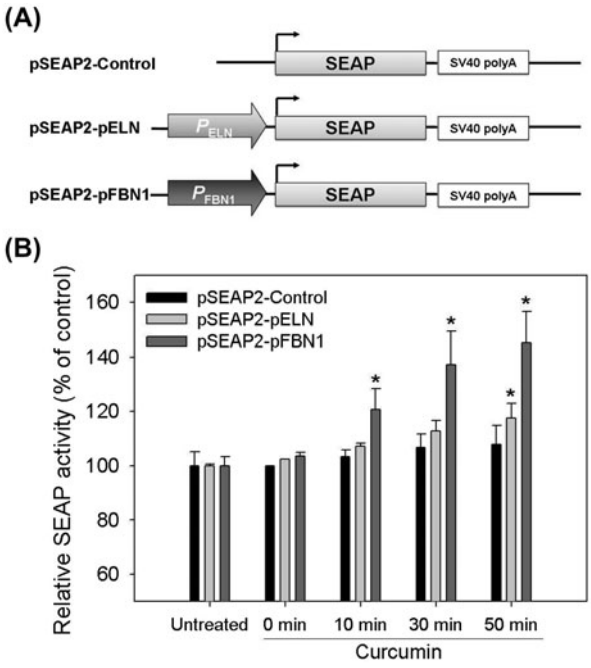

Fig. 2. Effects of curcumin on the activities of human *ELN* and *FBN1* promoters in the transfected Detroit 551 cells.

Notes: (A) Illustrations of pSEAP2-Control, pSEAP2-pELN, and pSEAP2-pFBN1 plasmids and (B) Relative SEAP activities of curcumin treated Detroit 551 cells. 0–50 min indicates the reaction time of SEAP activity analysis. Each value is expressed as the mean ± S.E. ($n = 3$). Tukey's *post hoc* tests, *Significant difference ($p < 0.05$) compared with the control group.

intervals over a 10 min period using an ELISA microplate reader (BioTek, Seattle, WA, USA).[16)]

RT-PCR analysis. Detroit 551 cells were seeded onto the 6-cm dish at a density of 5×10^5 cells. After 24 h incubation, cells were with or without (untreated group) curcumin. After 24 h treatment, total RNAs were extracted using illustra RNAspin mini RNA isolation kit (GE Healthcare, Piscataway, NJ, USA) and cDNA synthesis was performed using the RevertAid^{TM} first strand cDNA synthesis kit (Fermentas, Amherst, NY, USA). Primers used for RT-PCR analyses are listed in Table 1. The reverse transcription reaction was performed using 5 μg of total RNA, 1 μL of oligo(dT), 1 μL of dNTP mix (10 mM), and up to 12 μL of ddH_2O. The mixture was heated for 5 min at 65 °C and quickly chilled on ice. Subsequently, 4 μL of first strand buffer, 2 μL of 0.1 M DTT, and 1 μL of ribonuclease inhibitor (40 U/μL) were added to the mixture. The mixture was incubated at 37 °C for 2 min, and 1 μL of reverse transcriptase was added. The reaction was stopped by heating the solution at 70 °C for 15 min. A 1 μL aliquot of cDNA mixture was used in the subsequent enzymatic amplification. PCR was performed using 1.5 mM $MgCl_2$, 0.2 mM dNTP, 2.5 units of *Taq* DNA polymerase, and 0.1 μM each of the primers (Table 1). The amplified products were separated in 2% agarose gel in Tris–borate-EDTA (TBE) buffer and stained with ethidium bromide. The GAPDH (glyceraldehyde 3-phosphate dehydrogenase) gene was used as an internal control for normalization.[17)]

Western blot analysis. Detroit 551 cells were seeded onto the 10-cm dish at a density of 8×10^5 cells. After 24 h incubation, the cells were with or without (untreated group) curcumin. After 24 h treatment, the lysates were collected and then quantified. For western blotting, each well was loaded with 20 ng of protein and resolved by 7% or 10% SDS PAGE (7% for fibrillin-1, 10% for tropoelastin), and then electrotransferred on to a polyvinylidene difluoride membrane using the Bio-Rad MiniProtean II apparatus (Bio-Rad Laboratories, Carlsbad, CA, USA). The blots were subsequently incubated with an antihuman fibrillin-1, antihuman tropoelastin or antiβ-actin antibody as the primary antibody, and the immune complexes were visualized using an ECL reagent. The β-actin was used as an internal control for data normalization. The bands were scanned and then quantified by measuring the optical densities using the VIpro Platinum 1.1 software package (Version 12.9; UVItec, UK).[18)]

Statistical analysis. The quantitative data for the present study were analyzed using Tukey's *post hoc* tests or Dunnett's multiple comparison test. The quantitative data are presented as the means ± S.E. for three independent experiments.

Results and discussion

Cytotoxic effect of curcumin on Detroit 551 cells

First of all, the cytotoxic effect of curcumin on normal human fibroblast cells (Detroit 551 cells) was examined by a standard MTT assay. The result is shown in Fig. 1(B). All tested curcumin concentrations

(0.25–2 μM) had no cytotoxic effects on Detroit 551 cells. The presented cell viabilities are all around 100% of control. Thus, we used the highest concentration of curcumin (2 μM) for the following experiments.

Many researchers have confirmed that curcumin possesses antioxidant, anti-inflammatory, antibacterial, anti-amyloid properties, and suppresses proliferation of a wide variety of tumor cells.[19–21] In our presented study, the concentrations of curcumin lower than 2 μM might be no cytotoxic effect on Detroit 551 cells (Fig. 1(B)). Therefore, this result also suggested that proper concentrations of curcumin have potential to be used as an active ingredient in the applications of drugs, foods, and cosmetics.

Effects of curcumin on the activities of human ELN and FBN1 promoters in Detroit 551 cells

To analyze the effects of curcumin on the expression of elastin and fibrillin-1 in fibroblasts, human *ELN* and *FBN1* promoters combining with the SEAP reporter were constructed for the assay and shown in Fig. 2(A). Previous studies have verified that the functional human *ELN* promoter's region is at the positions between −1 and −2260 bp.[22] Besides, for the promoter of human *FBN1*, an efficient promoter's region has been identified from −690 to −1351 bp.[23] Therefore, according to the results from previous researches, in this study, we constructed a human cell-based assay method to analyze the effects of curcumin on the activity of *ELN* and *FBN1* promoters.

The results are shown in Fig. 2(B). Our results indicated that curcumin can both increase the promoter activities of *ELN* and *FBN1*. From 10 to 50 min analytic conditions, all of the improved SEAP activities for *FBN1* in curcumin treated cells are higher than 122% of control. Under the 50 min analytic condition, the enhanced SEAP activities in curcumin treated cells can increase to approximately 120 and 145% of control for *ELN* and *FBN1* activity, respectively (Fig. 2(B)). Although the enhanced activity of *ELN* promoter is not clearer than that of *FBN1* promoter in other analytic conditions, the improvement effects of curcumin on both target genes are still recognized in this experiment. In addition, such enhancement results are not significantly revealed in the group of pSEAP2-control transfected Detroit 551 cells (Fig. 2(B)). To our knowledge, the effects of curcumin on the promoter activities of human elastin and fibrillin-1 are never discussed in any earlier study. Therefore, the improvement effects of curcumin should be further confirmed.

Effects of curcumin on mRNA levels of tropoelastin and fibrillin-1 in Detroit 551 cells

To confirm the effects of curcumin on both mRNA and protein levels of tropoelastin and fibrillin-1 in Detroit 551 cells, RT-PCR, and western blotting analyses were used in the experiments. The well-known enhancer for elastic fibers, retinol, is used as a positive control. Besides, retinol has no cytotoxicity for Detroit 551 cells if the concentrations are lower than 10 μM (data not shown), hence, 10 μM retinol is used for the following experiment. For RT-PCR assay, the image of gel electrophoresis is shown in Fig. 3(A) and the quantified result is presented in Fig. 3(B). The GAPDH mRNA was also analyzed and used as an internal control. For tropoelastin, the mRNA level was evidently increased by the treatment of curcumin in fibroblast cells to about 138% of control. However, the quantity of fibrillin-1 mRNA was slightly increased to approximately 110% of control by the treatment of curcumin, but which is not a significant improvement (Fig. 3(B)). In addition, retinol can evidently increase both the levels of tropoelastin and fibrillin-1 mRNA in Detroit 551 cells. Comparing the results of Figs. 3(B)–2(B), it showed that curcumin strongly activate FBN1 promoter compared to ELN, however the expression of mRNA level of tropoelastin were higher than that of fibrillin-1. This situation might be due to the activities of used ELN and FBN1 promoters are different. Therefore, although the enhanced effect of curcumin on FBN1 promoter is obvious, but the improved mRNA level of fibrillin-1 is still slight.

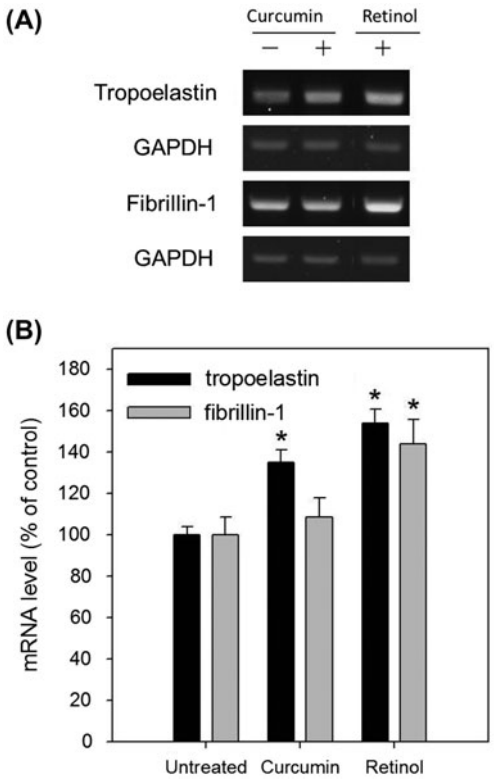

Fig. 3. Effects of curcumin on the mRNA levels of elastin and fibrillin-1 in Detroit 551 cells.

Notes: (A) Image of gel electrophoresis and (B) The quantified results. Each value is expressed as the mean ± S.E. ($n = 3$). Dunnett's multiple comparison test, *Significant difference ($p < 0.05$) compared with the control group.

Effects of curcumin on protein levels of tropoelastin and fibrillin-1 in Detroit 551 cells

For western blotting analysis, the results, as shown in Fig. 4(A) and (B) (quantified results), demonstrated that curcumin is similar to retinol, which can clearly increase both the protein levels of tropoelastin and fibrillin-1 in Detroit 551 cells. The β-actin protein was also analyzed and used as an internal control. For tropoelastin, the enhanced protein amount was

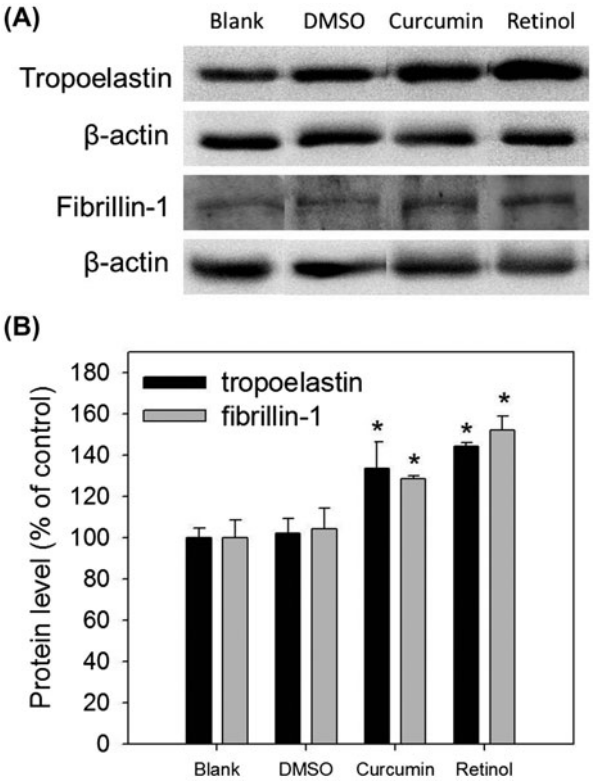

Fig. 4. Effects of curcumin on the protein levels of elastin and fibrillin-1 in Detroit 551 cells.

Notes: (A) Images of western blotting and (B) The quantified results. Each value is expressed as the mean ± S.E. ($n = 3$). Dunnett's multiple comparison test, *Significant difference ($p < 0.05$) compared with the control group.

approximately 134% of control. In contrast, the improved fibrillin-1 protein level was also up to about 130% of control (Fig. 4(B)). This result is not similar to the result in Fig. 3(B) for mRNA level. This circumstance might be caused by the analytic time point of fibrillin-1 mRNA, which was 24 h. The enhancing effect of curcumin on fibrillin-1 mRNA level may be earlier than that of the protein amount in cells. Therefore, even the improvement effects of curcumin on mRNA and protein have a little difference and we still believe that curcumin can evidently up-regulate the productions of elastin and fibrillin-1 in fibroblasts.

It is well known that cAMP response element binding protein (CREB) is widely expressed in many cells and can be activated by phosphorylation. When CREB is activated, it will bind to the CREB binding protein and thus activate the target genes. Curcumin has a modulatory effect on the transcription factor CREB expression.[24] Curcumin increases the phosphorylated CREB and which has been mediated by mitogen-activated protein kinase (MAPK) and phosphoinositide-3 kinase (PI-3 K) pathways in cells.[25] Moreover, a cAMP response element was found between −1811 and −1818 bp within human *ELN* promoter region.[22] In addition, curcumin also has effect on gene expression through the regulation of CCAAT/enhancer-binding protein (C/EBP) factor function.[26] Similarly, a binding site for C/EBP has been identified in *FBN1* promoter region between −1107 and −1114 bp.[23] Thus, these relationships may be a good reason that explains why curcumin can regulate the production of elastin and fibrillin-1 in human fibroblasts. Curcumin is a broad functional compound, which may execute many biological effects. However, this study is the first report about the effects of curcumin on the production of elastin and fibrillin-1 in fibroblast cells.

In summary, our results supposed that curcumin can evidently up-regulate the productions of elastin and fibrillin-1 in fibroblasts. Thus, curcumin may be used as an active compound and may be applied to drugs, foods, and cosmetics to solve problems relating to elastic fibers in the future.

Conflict of interest

The authors declare no conflict of interest.

Funding

The authors are grateful for the financial support from the Ministry of Science and Technology of the Republic of China (Taiwan) awarded to Chih-Chien Lin [grant number NSC102-2313-B-126-001-MY3].

References

[1] Sherratt MJ. Age-related tissue stiffening: cause and effect. Adv. Wound Care (New Rochelle). 2014;2:11–17.
[2] Nonaka R, Sato F, Wachi H. Domain 36 of tropoelastin in elastic fiber formation. Biol. Pharm. Bull. 2014;37:698–702.
[3] Tiedemann K, Boraschi-Diaz I, Rajakumar I, Kaur J, Roughley DP, Reinhardt J, Komarova SV. Fibrillin-1 directly regulates osteoclast formation and function by a dual mechanism. J. Cell Sci. 2013;126:4187–4194.
[4] Baldwin AK, Simpson A, Steer R, Cain SA, Kielty CM. Elastic fibres in health and disease. Expert Rev. Mol. Med. 2013;15: e8:1–30.
[5] Robinson PN, Booms P, Katzke S, Ladewig M, Neumann L, Palz M, Pregla R, Tiecke F, Rosenberg T. Mutations of FBN1 and genotype-phenotype correlations in Marfan syndrome and related fibrillinopathies. Hum. Mutat. 2002;20:153–161.
[6] Sadowska-Bartosz I, Bartosz G. Effect of antioxidants supplementation on aging and longevity. Biomed. Res. Int. 2014;2014:404680.
[7] Brondino N, Re S, Boldrini A, Cuccomarino A, Lanati N, Barale F, Politi P. Curcumin as a therapeutic agent in dementia: a mini systematic review of human studies. Scientific World J. 2014;2014:174282:1–6.
[8] Zhang DW, Fu M, Gao SH, Liu JL. Curcumin and diabetes: a systematic review. Evid. Based Complement. Alternat. Med. 2013;2013:636053:1–16.
[9] Son Y, Lee JH, Cheong YK, Chung HT, Pae HO. Antidiabetic potential of the heme oxygenase-1 inducer curcumin analogues. Biomed. Res. Int. 2013;2013:918039:1–7.
[10] Norris L, Karmokar A, Howells L, Steward WP, Gescher A, Brown K. The role of cancer stem cells in the anti-carcinogenicity of curcumin. Mol. Nutr. Food Res. 2013;57:1630–1637.
[11] Sakurai R, Villarreal P, Husain S, Liu J, Sakurai T, Tou E, Torday JS, Rehan VK. Curcumin protects the developing lung against long-term hyperoxic injury. Am. J. Physiol. Lung Cell Mol. Physiol. 2013;305:L301–L311.
[12] Huang CS, Ho CT, Tu SH, Pan MH, Chuang CH, Chang HW, Chang CH, Wu CH, Ho YS. Long-term ethanol exposure-induced hepatocellular carcinoma cell migration and invasion through lysyl oxidase activation are attenuated by combined treatment with pterostilbene and curcumin analogues. J. Agric. Food Chem. 2013;61:4326–4335.

[13] Kato T, Tanaka M, Oba M. Protein transfection study using multicellular tumor spheroids of human hepatoma Huh-7 cells. PLoS One. 2013;8:e82876:1–8.

[14] Chou ST, Peng HY, Hsu JC, Lin CC, Shih Y. *Achillea millefolium* L. essential oil inhibits LPS-induced oxidative stress and nitric oxide production in RAW 264.7 macrophages. Int. J. Mol. Sci. 2013;14:12978–12993.

[15] Yang CH, Kuo WT, Chuang YT, Chen CY, Lin CC. Cyclin B1 destruction box-mediated protein instability: the enhanced sensitivity of fluorescent-protein-based reporter gene system. Biomed. Res. Int. 2013;2013:732307:1–8.

[16] Lai YK, Hsu JT, Chu CC, Chang TY, Pan KL, Lin CC. Enhanced recombinant protein production and differential expression of molecular chaperones in *sf*-caspase-1-repressed stable cells after baculovirus infection. BMC Biotechnol. 2012;12:83:1–10.

[17] Chen YS, Lee SM, Lin CC, Liu CY. Hispolon decreases melanin production and induces apoptosis in melanoma cells through the down regulation of tyrosinase and microphthalmia-associated transcription factor (MITF) expressions and the activation of caspase-3, -8 and -9. Int. J. Mol. Sci. 2014;15:1201–1215.

[18] Peng HY, Lai CC, Lin CC, Chou ST. Effect of *Vetiveria zizanioides* essential oil on melanogenesis in melanoma cells: downregulation of tyrosinase expression and suppression of oxidative stress. Scientific World J. 2014;2014:213013:1–9.

[19] Lo CY, Liu PL, Lin LC, Chen YT, Hseu YC, Wen ZH, Wang HM. Antimelanoma and antityrosinase from *Alpinia galangal* constituents. Scientific World J. 2013;2013:186505:1–5.

[20] Dairaku I, Han Y, Yanaka N, Kato N. Inhibitory effect of curcumin on IMP dehydrogenase, the target for anticancer and antiviral chemotherapy agents. Biosci. Biotechnol. Biochem. 2010;74:185–187.

[21] Gururaj AE, Belakavadi M, Venkatesh DA, Marme D, Salimath BP. Molecular mechanisms of anti-angiogenic effect of curcumin. Biochem. Biophys. Res. Commun. 2002;297:934–942.

[22] Kähäri VM, Fazio MJ, Chen YQ, Bashir MM, Rosenbloom J, Uitto J. Deletion analyses of 5′-flanking region of the human elastin gene. Delineation of functional promoter and regulatory cis-elements. J. Biol. Chem. 1990;265:9485–9490.

[23] Summers KM, Bokil NJ, Baisden JM, West MJ, Sweet MJ, Raggatt LJ, Hume DA. Experimental and bioinformatic characterisation of the promoter region of the Marfan syndrome gene, FBN1. Genomics. 2009;94:233–240.

[24] Kumar TP, Antony S, Gireesh G, George N, Paulose CS. Curcumin modulates dopaminergic receptor, CREB and phospholipase c gene expression in the cerebral cortex and cerebellum of streptozotocin induced diabetic rats. J. Biomed. Sci. 2010;17:43:1–11.

[25] Wang R, Li YH, Xu Y, Li YB, Wu HL, Guo H, Zhang JZ, Zhang JJ, Pan XY, Li XJ. Curcumin produces neuroprotective effects via activating brain-derived neurotrophic factor/TrkB-dependent MAPK and PI-3 K cascades in rodent cortical neurons. Prog. Neuropsychopharmacol. Biol. Psychiatry. 2010;34:147–153.

[26] Balasubramanian S, Eckert RL. Green tea polyphenol and curcumin inversely regulate human involucrin promoter activity via opposing effects on CCAAT/enhancer-binding protein function. J. Biol. Chem. 2004;279:24007–24014.