Curcumin upregulates Nrf2 nuclear translocation and protects rat hepatic stellate cells against oxidative stress

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Abstract. The present study aimed to investigate the protective role of curcumin against oxidative stress in rat hepatic stellate cells (HSCs)-T6, and to determine the possible underlying mechanisms. HSC-T6 cells were divided into three groups: Negative control group, oxidant-treated group and curcumin-treated group. Flow cytometry and spectrophotometry were used to measure the production of reactive oxygen species (ROS), and the levels of malondialdehyde (MDA) and glutathione (GSH). Immunocytochemistry and a radioimmunoassay were used to determine the expression of smooth muscle α-actin (α-SMA) and the secretion of extracellular matrix (ECM) molecules. In addition, western blotting and immunocytochemistry were used to determine the expression levels of nuclear factor-erythroid 2-related factor (Nrf2). Treatment with glucose oxidase (GO) significantly stimulated the formation of ROS and increased the production of MDA, as compared with the control cells; however, the production of GSH was only slightly increased. In addition, treatment with GO significantly promoted the expression of α-SMA and the secretion of ECM molecules. Conversely, treatment with curcumin significantly decreased the levels of ROS and MDA, and significantly increased the levels of GSH. Curcumin significantly inhibited the expression of α-SMA and decreased the secretion of ECM molecules. Furthermore, treatment with curcumin significantly increased the nuclear expression levels of Nrf2. These results indicated that curcumin may protect rat HSCs against oxidative stress and inhibit the GO-induced activation and secretion of ECM molecules *in vitro*. These effects were mediated by the upregulation of Nrf2 nuclear translocation.

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Abbreviations: Nrf2, nuclear factor-erythroid 2-related factor; HSC, hepatic stellate cell; GO, glucose oxidase; ROS, reactive oxygen species; MDA, malondialdehyde; GSH, glutathione; α-SMA, smooth muscle α-actin; ECM, extracellular matrix; PCIII, type III procollagen; C IV, type IV collagen; LN, laminin; HA, hyaluronic acid; DHE, dihydroethidium

Key words: curcumin, nuclear factor-erythroid 2-related factor, oxidative stress, hepatic stellate cell, fibrosis

Introduction

Previous studies have demonstrated that oxidative stress has a significant role in the occurrence and progression of hepatitis and hepatic fibrosis (1-4). Oxidative stress results from the excessive production of reactive oxygen species (ROS), and the inability of an organism to eliminate them. Excessive ROS lead to lipid peroxidation, protein and DNA damage, and injury to cellular structure and function (5). Numerous studies have reported the important role of ROS in various types of hepatic injury (6-10). In addition to ROS-induced inflammation, ROS may lead to loss of normal regulatory functions, resulting in tissue injury and excessive repair, and the development of hepatitis and hepatic fibrosis (11,12). It has previously been demonstrated that activation of hepatic stellate cells (HSCs) has a key role in the progression of hepatic fibrosis; therefore, HSCs are considered important target cells in hepatic fibrosis research (13). Furthermore, HSCs can be activated by oxidative stress and transformed into myofibroblasts. Myofibroblasts synthesize abundant extracellular matrix (ECM) molecules, which may lead to hepatic fibrosis (14); therefore, how to inhibit the activation of HSCs exposed to oxidative stress requires further investigation. Nuclear factor-erythroid 2-related factor (Nrf2) is a transcription factor that activates numerous antioxidant enzymes and phase II detoxifying enzymes (15). In addition, Nrf2 has an important role regulating oxidative stress (16,17); however, the majority of studies regarding Nrf2 have focused on the nervous and respiratory systems. The effects of Nrf2 on HSCs, and the underlying molecular mechanisms, have seldom been reported.

The present study hypothesized that upregulation of Nrf2 nuclear translocation would promote the expression of antioxidant enzymes and phase II detoxifying enzymes, thus protecting the liver against injury. The present study investigated the

alterations and regulatory mechanisms of the Nrf2 pathway, which is of great significance for understanding the pathogenesis of hepatic fibrosis and developing novel preventative strategies and curative therapies.

Curcumin, which is an ingredient of the spice turmeric, is present in the rhizomes of *Curcuma longa* Linn (Zingiberaceae). Curcumin has been reported to exert antioxidant, anti-inflammatory, anticancer and hepatoprotective effects (18). Furthermore, curcumin functions as an exogenous Nrf2 agonist, and can promote the nuclear translocation and biological effects of Nrf2 (19). Therefore, the present study used curcumin to upregulate Nrf2, and subsequently investigated the effects of Nrf2 on HSCs.

To investigate the possible regulatory mechanisms that underlie the Nrf2 pathway, the present study examined the effects of curcumin on Nrf2. In addition, alterations in the levels of ROS, malondialdehyde (MDA) and glutathione (GSH) were detected. As an index of HSC activation, smooth muscle α-actin (α-SMA) and desmin levels were measured. Furthermore, ECM-secreted proteins, including type III procollagen (PCIII), type IV collagen (CIV), laminin (LN) and hyaluronic acid (HA), were measured following treatment of HSCs with curcumin. The results of the present study indicated that curcumin was able to protect HSCs against oxidative stress, and inhibit the activation of HSCs via induction of Nrf2 nuclear translocation.

Materials and methods

Materials. Curcumin and GO were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-α-SMA (1:200; cat. no. sc-53142) and anti-β-actin (1:500; cat. no. sc-47778) antibodies were obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Anti-Nrf2 (1:500; cat. no. BS6286) and anti-desmin (1:500; cat. no. BS1712) antibodies were purchased from Bioworld Technology, Inc. (St. Louis Park, MN, USA). Horseradish peroxidase-conjugated anti-rabbit (1:10,000; cat. no. bs-0295G-HRP) and anti-mouse (1:10,000; cat. no. bs-0296G-HRP) immunoglobulin (Ig)G, and fluorescein isothiocyanate (FITC)-conjugated anti-rabbit (1:1,000; cat. no. bs-0295G-FITC) and anti-mouse (1:1,000; cat. no. bs-0296G-FITC) IgG secondary antibodies were obtained from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Dihydroethidium (DHE) was purchased from Beyotime Institute of Biotechnology (Jiangsu, China). MDA and GSH kits were obtained from Nanjing Jiancheng Biotechnology, Inc. (Nanjing, China). PCIII, CIV, LN and HA kits were purchased from the Shanghai Naval Medical Institute (Shanghai, China).

Cell culture. The HSC-T6 immortalized rat HSC line exhibits a stable phenotype and biochemical characteristics (20). HSC-T6 cells were a generous gift from Dr Ding of the Medical College of Xi'an Jiaotong University (Xi'an, China). The cells were grown under standard conditions in a normoxic atmosphere in high-glucose Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Sijiqing, Hangzhou, China) in a humidified incubator containing 5% CO_2 at 37˚C. All subsequent experiments were conducted using cells at the exponential stage of growth. Cells were seeded into a 25 cm^2 plastic culture flask at a density of $1x10^6$ cells. The cells were separated into three groups: The negative control cells were incubated with 5 ml culture medium; the oxidant-treated cells were incubated with 5 ml culture medium supplemented with 100 mU/ml GO for 2 h before each experimental manipulation; and the curcumin-treated cells were pre-treated with 5 ml culture medium containing 0.15 μmol curcumin for 3 h, and then incubated in the same manner as the oxidant-treated cells.

Western blot analysis. Total, cytoplasmic and nuclear proteins were obtained from the cells using protein extraction kits (Beyotime Institute of Biotechnology, Shanghai, China), according to the manufacturer's protocol. The concentration of the protein samples was quantified using a Bradford Protein Assay kit (Beyotime Institute of Biotechnology), according to the manufacturer's protocol. Subsequently, the protein samples (15 μg) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and were transferred to nitrocellulose membranes (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membranes were blocked in buffer containing skim milk for 2 h, and were then incubated with anti-Nrf2 primary antibody at 4˚C overnight, and washed with phosphate-buffered saline (PBS). The membranes were then incubated with a horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h. Pierce Enhanced Chemiluminescence Western Blotting substrate (Thermo Fisher Scientific, Inc.) was used to develop the blots, and immunoreactivity was visualized following exposure to X-OMAT BT Film (Beyotime Institute of Biotechnology). β-actin was used as an internal control. The results were quantified and normalized to β-actin using Molecular Analyst software (version 1.4.1; Bio-Rad Laboratories, Inc.)

Immunocytochemistry. Following a 24 h cell culture, in which the cells became adherent, immunocytochemistry was conducted. Cell-coated dishes ($1x10^5$ cells/ml) were fixed with 4% paraformaldehyde for 30 min and washed with PBS. The cells were permeabilized using 0.3% Triton X-100 (Sigma-Aldrich) for 15 min. Endogenous peroxidases and biotins were quenched using 3% H_2O_2. The cells were then blocked with FBS and incubated with the indicated primary antibodies at 4˚C overnight. Sections stained for Nrf2 protein expression were washed and incubated with a horseradish peroxidase-conjugated secondary antibody at room temperature for 30 min. Bound secondary antibodies were detected using Histostain-SP kits (ZSbio, Beijing, China), according to the manufacturer's protocol. The reaction products were visualized using diaminobenzidine tetrahydro-chloride (Tiangen Biotech Co., Ltd., Beijing, China). Sections stained for α-SMA and desmin protein expression were incubated with FITC-conjugated secondary antibodies at room temperature for 30 min in the dark. The nuclei were stained using Evans Blue (Sigma-Aldrich) in sections stained for α-SMA. In the negative control group, primary antibodies were substituted with PBS. Stained sections were viewed under a Nikon Eclipse 800 fluorescent microscope (Nikon Corporation, Tokyo, Japan).

Flow cytometry. HSC-T6 cells were maintained under standard conditions, and were then transferred to culture dishes. Following a 24 h culture, $1x10^6$ cells from each group were

placed in tubes. The cells were washed with PBS and separated by centrifugation at 432 x g for 10 min at 4˚C. Serum-free media supplemented with 2-μM DHE were added, and the tubes were incubated at 4˚C for 30 min in the dark. The remaining cells were washed with PBS and subjected to further centrifugation at 432 x g for 10 min at 4˚C. Paraformaldehyde (2 ml; 4%) was added to each tube and the tubes were incubated at 4˚C for 30 min in the dark, followed by centrifugation at 432 x g for 10 min at 4˚C. Subsequently, 300 μl PBS was added to the preparations and mixed gently. DHE fluorescence was measured by flow cytometry (FACSCanto II; BD Biosciences, Franklin Lakes, NJ, USA).

MDA and GSH assays. HSC-T6s were incubated in culture dishes and the supernatants were collected for the detection of MDA and GSH by spectrophotometry. Measurement of the product (MDA-TBA adduct) of a reaction between MDA and 2-thiobarbituric acid, and of the product (2-nitro-5-sulphur benzoic acid) of a reaction between GSH and dithio-dinitrobenzoic acid allow the levels of MDA and GSH to be analyzed using colorimetric assays. MDA and GSH levels were determined using kits, according to the manufacturers' protocols. Absorbance was measured at 532 and 412 nm using a spectrophotometer (UV-2450; Shimadzu Corporation, Kyoto, Japan) for MDA and GSH, respectively. The concentrations of MDA and GSH were calculated according to the equation provided in the kits.

Analysis of ECM secretion. Cells were initally separated into three groups and cultured in serum-free medium overnight. Subsequently, the cells were treated as mentioned previously (negative control, oxidant-treated and curcumin-treated cells). Following centrifugation at 1,000 x g for 20 min at 4˚C, the supernatant was collected and maintained at -80˚C until further analysis. The levels of PCIII, CIV, LN and HA secreted into the supernatant were analyzed using commercially available radioimmunoassay kits, according to the manufacturers' protocols.

Statistical analysis. The data are presented as the mean ± standard deviation and significance was assessed using SPSS 12.0 software (SPSS, Inc., Chicago, IL, USA). Statistical comparisons were performed using one-way analysis of variance. Paired comparisons were conducted using Student Newman Keuls-q test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Curcumin protects HSCs against GO-induced oxidative stress injury. As a marker of oxidative stress, GO may react with glucose in the culture media and subsequently promote the generation of glucuronic acid and hydrogen peroxide (H_2O_2). It is well known that DHE, which is a superoxide radical-specific fluorescent probe, is able to enter viable cells freely where it is oxidized by superoxide to form ethidium, which binds to DNA and exhibits red fluorescence. Therefore, the present study detected fluorescence intensity in order to estimate the relative levels of ROS production. As shown in Fig. 1A, compared with the negative control cells, treatment of the cells with GO significantly increased fluorescence intensity, whereas pretreatment with curcumin significantly decreased fluorescence intensity. However, the fluorescence intensity in the curcumin-treated cells was stronger, as compared with in the negative control cells.

Furthermore, ROS may react with lipids in cell and mitochondrial membranes, resulting in the production of MDA, which can be used to measure lipid peroxidation. Due to its strong cytotoxicity, MDA is able to alter membrane permeability or disrupt membrane integrity, leading to oxidative injury. However, even in the case of increased lipid peroxidation, oxidative stress only occurs in cells that are unable to defend and protect against free radical injury or chemically induced damage. GSH is an important endogenous antioxidant, which reduces levels of ROS. GSH acts as a free radical scavenger, a coenzyme for various antioxidant enzymes, a regulator of thiol-disulfide status, and is involved in the detoxification of electrophilic xenobiotics via conjugation. Therefore, the present study aimed to detect the levels of MDA and GSH in the supernatant. Significantly enhanced levels of MDA were detected in the oxidant-treated cells, as compared with the negative control cells, which were correlated with the levels of ROS. The MDA levels were markedly attenuated in response to curcumin pretreatment, however they remained higher than in the control cells. Furthermore, compared with the negative control cells, the levels of GSH were slightly increased in the oxidant-treated cells, whereas they were significantly elevated in the curcumin-treated cells (Fig. 1B).

These results suggest that an oxidative stress model of HSC-T6 was established with GO treatment, as demonstrated in the increased levels of ROS and MDA. Furthermore, pretreatment with curcumin was able to significantly suppress the degree of oxidative stress, at least partially due to the induced expression of endogenous GSH.

Curcumin promotes the nuclear translocation of Nrf2. GSH synthesis is governed by Nrf2 via the regulation of the rate-limiting enzymes glutamate cysteine ligase (GCL) catalytic subunit (GCLC) and GCL modifier subunit (GCLM). To elucidate the molecular mechanism by which curcumin protects HSCs against oxidative stress injury, the present study detected the expression of Nrf2 in HSC-T6 cells by immunocytochemistry (Fig. 2A) and western blot analysis (Fig. 2B and C). As expected, the expression of total Nrf2 did not differ between the groups; however, the expression levels of cytoplasmic Nrf2 were slightly decreased in the oxidant-treated cells, and significantly decreased in the curcumin-treated cells, as compared with the negative control cells. Conversely, the expression of nuclear Nrf2 was absent in the negative control cells and was only slightly expressed in the oxidant-treated cells; however, the expression levels of nuclear Nrf2 were markedly increased in the curcumin-treated cells, as compared with in the oxidant-treated cells. Immunocytochemistry detected similar results to the western blot analysis; only minimal positive brown staining was detected in the nuclei of the oxidant-treated cells, as compared with the control cells, in which no positive brown nuclear staining was detected. However, in the curcumin-treated cells, the amount of positive brown staining was abundant in the nucleus, as compared with in the oxidant-treated cells.

These results indicate that oxidative stress may activate the Nrf2 regulatory pathway, and pretreatment with curcumin

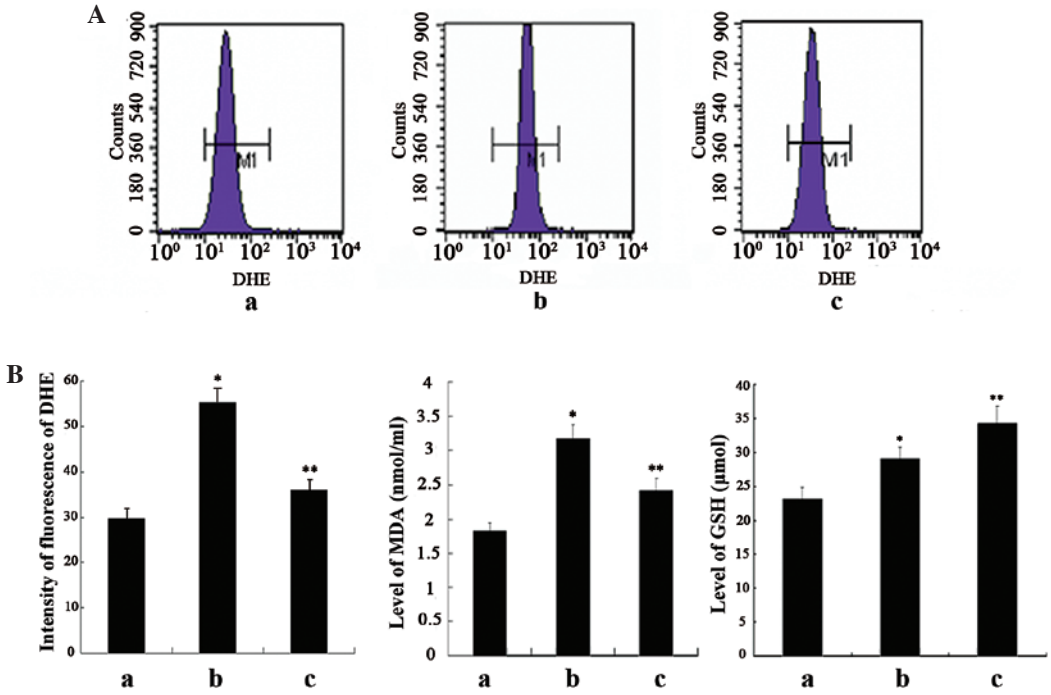

Figure 1. Levels of reactive oxygen species (ROS), as determined using dihydroethidium (DHE); malondialdehyde (MDA); and glutathione (GSH) in various hepatic stellate cell-T6 groups. (A) Analysis of DHE by flow cytometry. (B) Comparison of the levels of ROS, as determined using DHE; MDA; and GSH between the various groups. a, negative control cells; b, oxidant-treated cells; c, curcumin-treated cells. Data are presented as the mean ± standard deviation. *P<0.01 vs. the negative control cells; **P<0.01 vs. the oxidant-treated cells.

could protect HSCs against oxidative stress via promoting the translocation of Nrf2 from the cytoplasm to the nucleus.

Curcumin blocks GO-induced α-SMA expression and HSC activation. Desmin, which is a type of cytoskeletal intermediate filament, has been widely used as a marker for distinguishing HSCs. Desmin was expressed in all cell groups, as detected using immunofluorescence (Fig. 3A), and there were no differences in desmin expression between the groups, as determined by western blot analysis (Fig. 3B). α-SMA is produced by activated HSCs, and is a characteristic signal of HSC activation. As shown in Fig. 3A, no fluorescence expression of α-SMA was detected in the negative control cells, whereas following treatment with GO for 2 h abundant fluorescence expression of α-SMA was observed. Furthermore, the fluorescence expression of α-SMA in the cells pretreated with curcumin was markedly decreased, as compared with the oxidant-treated cells. Consistent with the alterations in immunofluorescence, the protein expression levels of α-SMA were significantly increased in the oxidant-treated cells, as compared with the negative control cells, whereas the expression levels of α-SMA were significantly decreased in the curcumin-treated cells, as compared with the oxidant-treated cells. However, the expression levels remained higher in the curcumin-treated cells, as compared with in the negative control cells (Fig. 3B and C).

These results suggest that GO-induced oxidative stress may enhance α-SMA expression in HSCs, and the transformation of HSCs to myofibroblast-like cells. Furthermore, treatment with curcumin may activate the Nrf2 regulatory pathway and subsequently suppress α-SMA expression and HSC activation, which may be associated with its antifibrotic effects.

Curcumin inhibits the expression of ECM molecules in GO-treated HSCs. Serum levels of PCIII, CIV, LN and HA are often considered indices of liver fibrosis; therefore, the present study aimed to detect the levels of PCIII, CIV, LN and HA in the supernatant (Fig. 4A-D). Compared with the negative control cells, HSCs treated with GO for 2 h exhibited significantly increased levels of ECM molecules. Conversely, the levels of ECM molecules were markedly reduced following pretreatment with curcumin; however, the levels remained higher, as compared with in the negative control cells.

These results indicate that treatment with GO may markedly increase the expression of ECM molecules in HSCs. Furthermore, the activation of Nrf2 by curcumin may exert inhibitory effects on the expression of ECM molecules in HSC, which may be associated with its antifibrogenic effects.

Discussion

The Nrf2 pathway is regarded as the most important pathway with regards to cellular protection against oxidative stress (21). As a pivotal modulator of the response to oxidative stress, the activation of Nrf2 induces the expression of various protective antioxidant genes (22,23). Under normal conditions, Nrf2 is sequestered in the cytoplasm where it is bound to the cytoskeleton-associated protein Keap1 (24). Oxidative stress promotes the dissociation of the Nrf2-Keap1 complex, thus resulting in Nrf2 stabilization and translocation to the nucleus (25). In the nucleus, Nrf2 associates with dimerization partners and binds antioxidant-response element sequences, in order to induce the expression of various detoxification and antioxidant enzyme genes that contribute to the protective response (26).

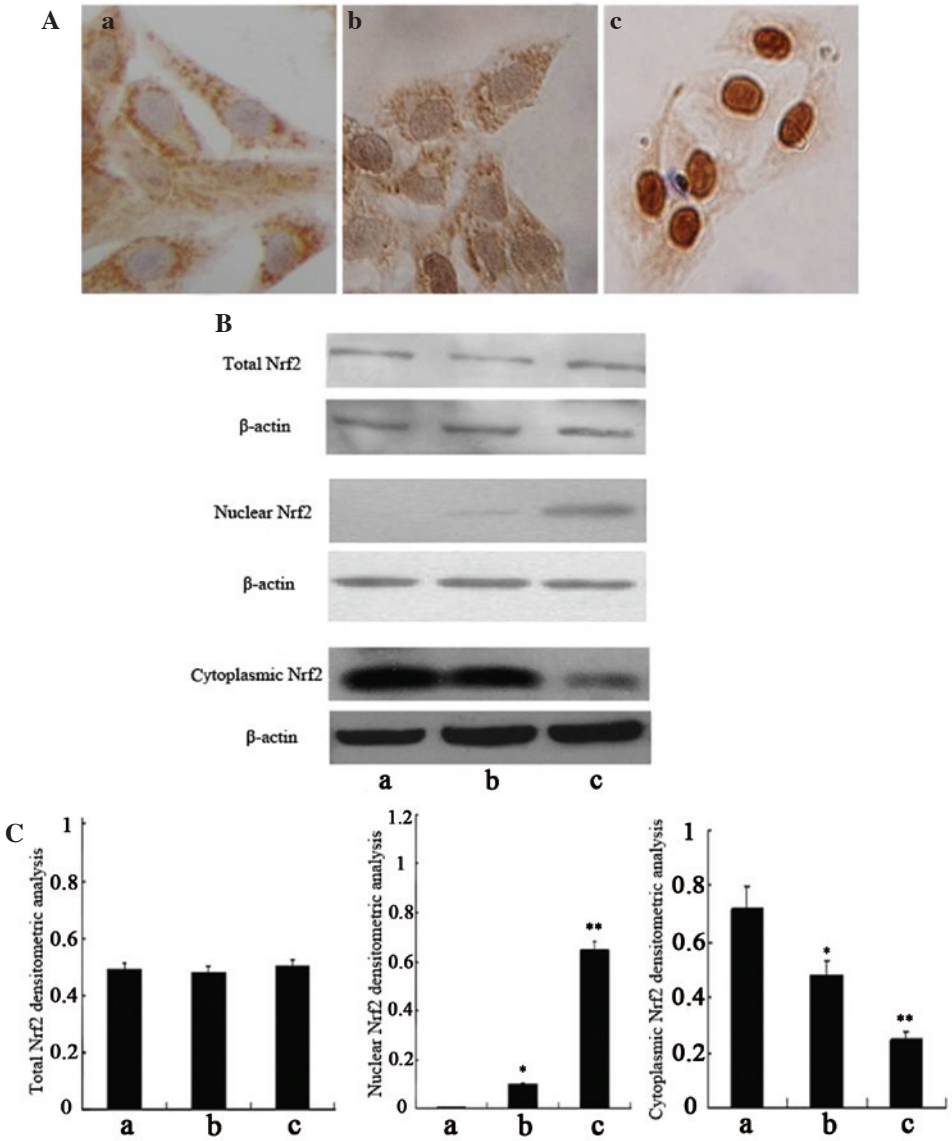

Figure 2. Expression of nuclear factor-erythroid 2-related factor (Nrf2) in various hepatic stellate cell-T6 groups. (A) Expression of Nrf2 in the various groups, as detected by immunocytochemical analysis (original magnification, x400). (B) Expression of Nrf2 in the various groups, as detected by western blot analysis. β-actin was used as a loading control. (C) Densitometric analysis of western blot results. a, negative control cells; b, oxidant-treated cells; c, curcumin-treated cells. Data are presented as the mean ± standard deviation. *P<0.01 vs. the negative control cells; **P<0.01 vs. the oxidant-treated cells.

Curcumin is a natural polyphenol product that is derived from the rhizome of *Curcuma longa*. Numerous studies have detected the various bioactivities of curcumin, including anti-oxidant, anti-inflammatory, cell apoptosis-inducing and cell proliferation-inhibiting activities (27-30). In addition, it has been suggested that curcumin may be used clinically against numerous types of cancer, inflammatory bowel disease, irritable bowel syndrome (IBS), rheumatoid arthritis and atherosclerosis (31). Cheng *et al* (32) detected the effects of curcumin on 25 patients with various types of high-risk or premalignant lesions; following 3 months of treatment with curcumin, some patients exhibited histological improvement in premalignant lesions. Dhillon *et al* (33) studied the efficacy of curcumin on patients with advanced pancreatic cancer, and demonstrated that oral curcumin administration exerted biological activity in some patients. Hanai *et al* (34) conducted a double-blind, placebo-controlled trial in 89 patients with ulcerative colitis (UC); curcumin was shown to reduce the incidence of UC and may be considered a promising treatment for UC. In addition, a blind pilot study demonstrated that abdominal pain and the discomfort score of patients with IBS were significantly reduced following treatment with curcumin (35).

Previous studies have detected various functions of curcumin. Notably, curcumin has been shown to function as an exogenous agonist of Nrf2 (19). A previous study suggested that curcumin may alter the conformation of Keap1, and promote the dissociation of Nrf2 from Keap1 and its subsequent nuclear translocation (36). The present study evaluated the effects of curcumin on Nrf2 regulation in HSCs; Nrf2 was localized to the cytoplasm under normal conditions and little Nrf2 was localized to the nucleus following the induction of oxidative stress. However, pretreatment with curcumin induced a substantial localization of Nrf2 to the nucleus in HSCs.

Increased levels of GSH are an index of Nrf2 activation, and increased GSH may be considered a major factor underlying the protection associated with Nrf2 activation (37,38). GSH not

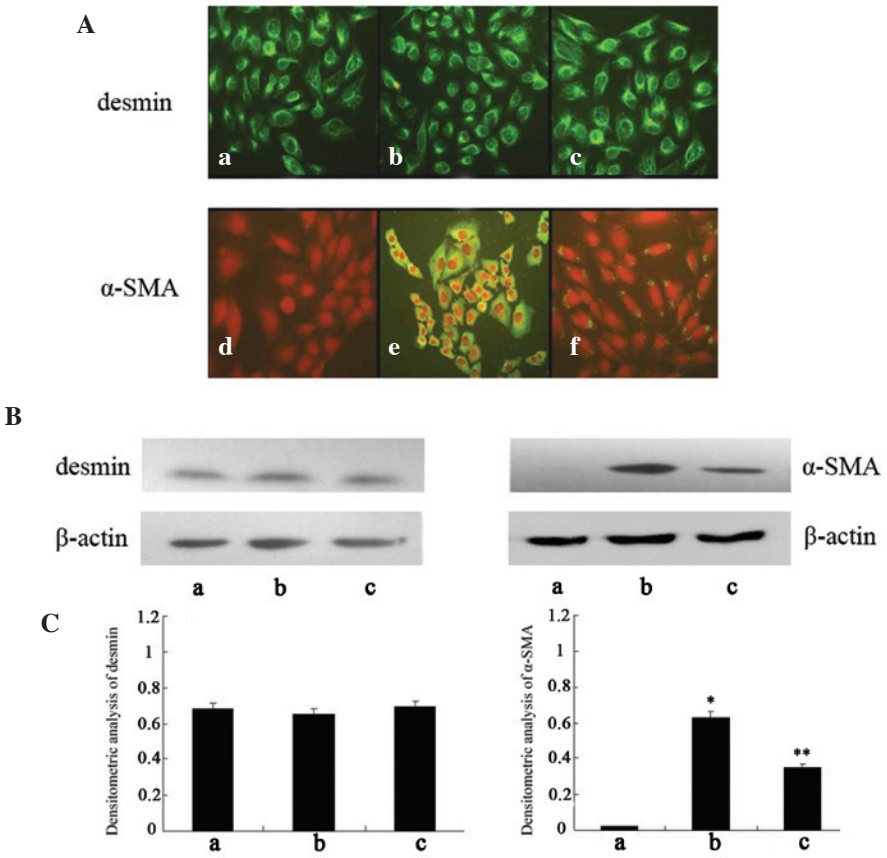

Figure 3. Expression of smooth muscle α-actin (α-SMA) and desmin in various hepatic stellate cell (HSC)-T6 groups. (A) Activation of HSC-T6 in the various groups, as determined by immunofluorescence (original magnification, x400). (a-c) Staining assay of desmin in negative control cells, oxidant-treated cells and curcumin-treated cells, respectively; (d-f) staining assay of α-SMA in negative control cells, oxidant-treated cells and curcumin-treated cells, respectively. Red staining indicates nuclei, green staining indicates α-SMA expression. (B) Expression of desmin and α-SMA, as determined by western blot analysis. β-actin was used as a loading control. (C) Densitometric analysis of western blotting. a, negative control cells; b, oxidant-treated cells; c, curcumin-treated cells. Data are presented as the mean ± standard deviation. *P<0.01 vs. the negative control cells; **P<0.01 vs. the oxidant-treated cells.

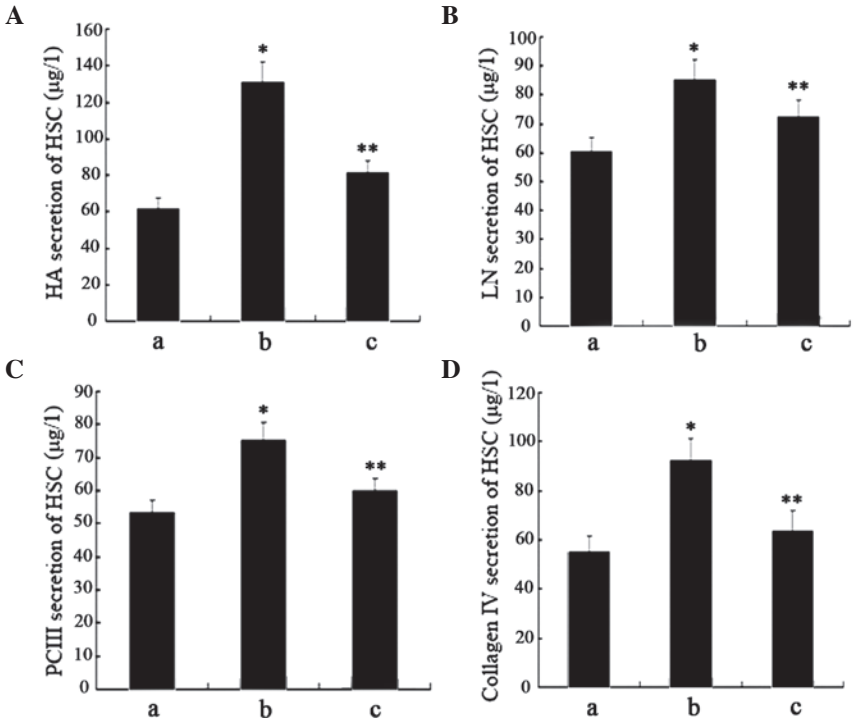

Figure 4. Secretion of components of the extracellular matrix in various hepatic stellate cell-T6 groups. Secretion of (A) hyaluronic acid (HA), (B) laminin (LN), (C) procollagen III (PCIII) and (D) collagen IV from the various cell groups, as determined by radioimmunoassay. a, negative control cells; b, oxidant-treated cells; c, curcumin-treated cells. Data are presented as the mean ± standard deviation. *P<0.01 vs. the negative control cells; **P<0.01 vs. the oxidant-treated cells.

only produces reducing equivalents, which are necessary for the conversion of H_2O_2 and lipid peroxides to water and lipid alcohols (39), but also has an important role in the protection of protein sulfhydration against oxidation (40). The rate-limiting reaction in GSH biosynthesis is catalyzed by GCL, which comprises two subunits: GCLC and GCLM. Nrf2 is able to increase the expression of GCLC and GCLM (41); therefore, the preferential activation of Nrf2 leads to more efficient GSH biosynthesis and improved antioxidant status (42). In the present study, the levels of GSH were increased alongside increasing Nrf2 nuclear translocation following curcumin treatment. GSH elevates the antioxidant ability of cells against oxidative stress.

The pathogenesis of hepatic fibrosis has yet to be completely clarified; however, it is generally accepted that the activation of HSCs is central to the process (43). In the prophase of liver fibrosis, quiescent HSCs transform into myofibroblasts, which are characterized by the assembly of α-SMA stress fibers, loss of cytosolic retinol and increased proliferation (44). This activation subsequently results in the synthesis of cytokines and the accumulation of ECM molecules (45). Considerable attention has been focused on elucidating the mechanistic triggers of HSC myofibroblast transformation. In addition, it is hypothesized that oxidative stress may contribute to HSC activation (46).

In the present study, GO reacted with glucose in the culture media resulting in the generation of glucuronic acid and H_2O_2. These increased levels of ROS may stimulate HSCs to undergo oxidative stress (47). The results of the present study demonstrated that following treatment with GO, the levels of ROS in the oxidant-treated cells were significantly increased, as compared with in the negative control cells. The negative control cells were quiescent, whereas the oxidant-treated cells were activated by oxidative stress. In addition to the upregulation of Nrf2 nuclear translocation in the curcumin-treated cells, the levels of ROS and HSC activation were decreased, as compared with in the oxidant-treated cells.

In-depth research regarding the mechanisms underlying liver fibrosis has revealed the role of free radicals and membrane lipid peroxidation in the process of liver injury (48). Oxygen radicals attack unsaturated fatty acids in cellular membranes and initiate lipid peroxidation. Lipid peroxidation leads to alterations in the permeability of cellular membranes, subsequently aggravating cell dysfunction and promoting the secretion of ECM molecules (49,50). Not only does oxidative stress stimulate HSC proliferation and collagen synthesis, it also further damages cells by promoting lipid peroxidation. MDA and other peroxidation products increase collagen synthesis via HSC activation, and stimulate Kupffer cells to release cytokines that promote fibrosis (51). Lipid peroxidation has an important role in regulating collagen gene expression, and is associated with cell injury and fibrosis. ROS and lipid peroxidation have been implicated as profibrogenic mediators (52), whereas Nrf2 is effective at suppressing cell damage resulting from lipid peroxidation (53). In the present study, the levels of MDA were increased alongside ROS levels in the oxidant-treated cells; however, following pretreatment with curcumin, the levels of MDA were reduced, as compared with in the oxidant-treated cells.

Liver fibrosis is the excessive deposition of ECM molecules following liver injury. ECM accumulation is associated with increased collagen synthesis and decreased matrix degradation, contributing to liver fibrosis and remodeling (54). Active HSCs are the primary source of the excessive production of ECM components (55), and the ECM enhanced density leads to increased matrix stiffness, which is a significant stimulus for the activation of HSCs (56). In the present study, oxidative stress induced HSC activation, which was followed by the enhanced synthesis of ECM components. Following curcumin pretreatment, the activation of HSCs was inhibited, and the secretion of ECM components was suppressed.

In conclusion, the present study demonstrated that curcumin-induced Nrf2 activation may protect HSCs against oxidative stress-induced injury, and this effect was characterized by enhanced Nrf2 nuclear translocation and antioxidant capacity. The underlying mechanism remains to be elucidated; however, the present study proposes a broader application for Nrf2 in the prevention and treatment of hepatic damage.

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