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Full paper

Curcumin protects human adipose-derived mesenchymal stem cells against oxidative stress-induced inhibition of osteogenesis

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ABSTRACT

The detrimental effects of oxidative stress on the skeletal system have been documented, and understanding the mechanisms is important to design a therapeutic strategy. As an antioxidant and anti-inflammatory agent, the active ingredient of turmeric curcumin has been used as medication for numerous complications including bone loss. However, it is unclear if curcumin could influence the osteogenic potential of mesenchymal stem cells (MSCs), particularly in oxidative injuries. Here we demonstrate that curcumin treatment protects cell death caused by hydrogen peroxide (H_2O_2) exposure in human adipose-derived MSCs in vitro. Importantly, curcumin is able to enhance the osteoblast differentiation of human adipose-derived MSCs that is inhibited by H_2O_2. Notably, both oxidative stress and the inhibition of Wnt/β-catenin signaling are attenuated by curcumin treatment. These results suggest that curcumin can promote osteoblast differentiation of MSCs and protect the inhibitory effect elicited by oxidative injury. The findings support potential use of curcumin or related antioxidants in MSC-based bone regeneration for disease related with oxidative stress-induced bone loss.

1. Introduction

Mesenchymal stem cell (MSC) is one of the major candidates to treat human degenerative diseases (1–3). With potential to differentiate into all bone cell types, MSCs may improve healing of bone defects and even reverse clinically significant abnormalities in the skeleton system (4–6). In order to regenerate the complex collagen structure with appropriate mechanical properties, MSC-based approaches need to be capable of facilitating beneficial bone remodeling and mitigating bone injury. It has been reported that MSC can induce the repair process in fracture by replenishing bone cells including chondrocytes and osteoblasts. The increased numbers or activity of bone marrow MSCs have also been proposed to act effectively with parathyroid hormone (PTH) for treating osteoporosis (7). Furthermore, anti-inflammatory paracrine functions of MSCs have been suggested in several animal models of inflammation-associated diseases (8), which might play critical roles in modulating the inflammatory environment for optimal bone repair. The recent development of technique has greatly improved the in vitro isolation and expansion of adult mesenchymal stem cells (9–11). However, challenges remain for effective treatment. How to maintain the survival, growth, and osteogenic potential of transplanted MSCs are particularly limiting factors. As critical mediators of pathophysiological responses, reactive oxygen species (ROS) and free radicals have been implicated as negative regulators for bone homeostasis. For instance, the activation of bone resorption by oxidative stress has been extensively studied (12–14). In patients with inflammatory bone diseases, oxidative stress often downregulates bone formation by decreasing osteoblast number and differentiation (13). These detrimental effects can also be extended to MSCs, including the induction of apoptosis and delay of osteogenesis (15,16). Based on these considerations, an

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antioxidant agent may augment MSC treatment efficiency, by preventing ROS damage on osteogenic progenitors.

Curcumin (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3,5-dione), a phenolic antioxidant derived from Indian herbal medicine turmeric, is a potent free radical scavenger. The cytoprotection roles of curcumin have been demonstrated in alleviating tissue damages elicited from lipid peroxidation, nitric oxide (NO)-based free radicals, and hypoxia. In general, the pleiotropic activities of curcumin include the antitumor property and the mainly protective functions to oxidative injuries during inflammation or aging related scenarios (17). A low dose of dietary curcumin significantly decreased inflammation and β-amyloid plaque in the brains of a mouse model of Alzheimer's disease (18). Curcumin was shown to protect the hepatic injury and liver fibrosis (19). It was also reported that curcumin administration prevented diabetes-induced retina inflammation (20). In addition, curcumin treatment can prevent hippocampal neurons from traumatic injury such as cerebral ischemia (21). Recent findings suggest the possibility that curcumin may influence progenitor/stem cell. Besides extensively reported inhibitory effects on cancer stem cell populations, curcumin can induce embryonic stem cell differentiation through NO-cyclic guanosine monophosphate (GMP) pathway (22,23). A stimulating effect on neurogenesis by curcumin has also been found (24). The effects of curcumin in bone metabolism have been investigated as well. Some studies have suggested that curcumin can reduce osteoclast activation (25). On the other hand, the actions of curcumin on osteoblast cells are mixed (26,27), and it has been suggested curcumin can promote osteoblast differentiation of MSCs in vitro (26). Nevertheless, it is still unclear whether the suppression of bone formation during oxidative cell damages could be reversed by curcumin and which signaling pathway may be related to the function of curcumin in MSCs.

We hypothesized that curcumin can protect MSCs from oxidative stress-induced injury. In human adipose-derived MSCs (aMSCs), we found curcumin was able to prevent the cell death, ameliorate oxidative stress, and reverse the inhibition on osteogenic differentiation induced by H_2O_2 treatment in vitro. Interestingly, a critical signaling in bone formation pathway, Wnt/β-catenin-regulated cyclin D1 is reduced in H_2O_2-challenged aMSCs, which can be prevented by curcumin. An inhibitor of Wnt pathway blocked such protection by curcumin. These data suggested a novel mechanism involved in the protective roles of curcumin against oxidative injuries in mesenchymal stem cells under osteoblast differentiation.

2. Materials & methods

2.1. Human adipose-derived mesenchymal stem cell culture

Human adipose tissues that were collected by needle biopsy or liposuction aspiration in the First Affiliated Hospital of Zhengzhou University. Tissue samples were first extensively washed with phosphate-buffered saline (PBS) containing penicillin/streptomycin followed by digestion using 0.075% collagenase Type I for 30 min at 37 °C. The stromal vascular fractions (SVF), containing the MSCs (28), were obtained by centrifugation at 2000 rpm for 5 min. After the separation of the stromal cells from the primary adipocytes, the pellets were resuspended in 1 ml lysis buffer and incubated for 10 min on ice. The cell pellet was resuspended in stromal medium (alpha-MEM, supplemented with 20% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin/streptomycin) and filtered through 70 μm cell strainer. Cells were plated and washed three days later with fresh stromal medium. The cells were maintained in a humidified tissue culture incubator at 37 °C with 5% CO_2, and media was changed every second day until the cells reach 80% confluence. This study was approved by The Local Ethical Review Board of the First Affiliated Hospital of Zhengzhou University and written informed consent was obtained from all patients.

2.2. Induction of osteoblast differentiation of human aMSCs

Human aMSCs were induced with osteogenic differentiation medium: Ham's F12 Coon's modification medium supplemented with 10% FBS (Gibco, Grand Island, NY), 100 IU/ml penicillin, 100 μg/ml streptomycin, 10 nM dexamethasone, 0.2 mM sodium L-ascorbyl-2-phosphate, and 10 mM β-glycerol phosphate. Differentiation medium was changed every third day. Vehicle controls and H_2O_2 (0.1, 0.2, 0.5, 1 or 2 mM) were added in aMSCs for the first 24 h of culturing, and curcumin (1, 5, 10, 20, 50 or 100 μM) was added in the differentiation media throughout the differentiation processes until harvest for assays. The alkaline phosphatase (ALP) activity was determined at day 8, and Alizarin red-sulfate (ARS) staining was performed at day 16 of the differentiation.

2.3. Flow cytometry

The surface markers for human aMSCs were confirmed by flow cytometry. Undifferentiated MSCs were trypsinized and counted. The same number of cells was resuspended in FACS buffer (PBS containing 2% FBS), and aliquots were stained by FITC or PE-conjugated antibodies for human CD34, CD45, CD29, CD44, or CD105, respectively (eBioscience, San Diego, CA). Negative-stained cells were used to optimize the settings of analyzing in BD-FACScan (Franklin Lakes, NJ), and data were analyzed with FlowJo.

2.4. RT-PCR

We used RNeasy kit (Qiagen, Valencia, CA) or Trizol (Invitrogen, Pleasanton, CA) to isolate total RNA from cells at the indicated time points. The RNAs were reverse transcribed to complementary cDNAs using Superscript III (Invitrogen) according to manufacturer's instructions. A SYBR Green dye-based detection method was used by master mixes for quantitative real-time PCR (Applied Biosystems, Waltham, MA). The PCR reactions were performed in triplicated samples, and serial dilutions of control cDNA were used to make a standard curve for each gene expression. The specific primers were used including: *OPN*, forward: 5′-TTGCAGCCTTCTCAGCCAA-3′, reverse: 5′-GGAGGCAAAAGCAAATCACTG-3′; *Collagen I*, forward: 5′-GAGGGCCAAGACGAAGACATC-3′, reverse: 5′-CAGATCACGTCATCGCACAAC-3′; *OPG*, forward: 5′-GTGTGCGAATGCAAGGAAGG-3′, reverse: 5′-CCACTCCAAATCCAGGAGGG-3′; *RANKL*, forward: 5′-CAACATATCGTTGGATCACAGCA-3′, reverse: 5′-GACAGACTCACTTTATGGGAACC-3′; *β-catenin*, forward: 5′-AGCTTCCAGACACGCTATCAT-3′, reverse: 5′- CGGTACAACGAGCTGTTTCTAC-3′; *Cyclin D1*, forward: 5′-GCTGCGAAGTGGAAACCATC-3′, reverse: 5′-CCTCCTTCTGCACACATTTGAA-3′ and *GAPDH*, forward: 5′-CTGGGCTACACTGAGCACC-3′, reverse: 5′-AAGTGGTCGTTGAGGGCAATG-3′. The results were normalized by the expression of *GAPDH*.

2.5. Western blotting

Cells were lysed (10 mM HEPES, pH 7.4, 1.5 mM $MgCl_2$, 10 mM KCl, 1% NP40, with protease inhibitors) followed by centrifugation at 12,000 rpm for 10 min at 4 °C. The supernatants were collected and protein concentrations were determined by BCA protein assays. The same amounts of proteins were subjected to SDS-PAGE, followed by electric transfer into PVDF membrane. The

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