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Original Article

Curcumin promotes bone marrow stromal cell viability and attenuates TNF-α-induced prostaglandin E2 synthesis via cyclooxygenase-2 suppression

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Abstract

Background Prostaglandin E2 (PGE2) is the main mediator for receptor activator of nuclear factor-κB ligand (RANKL)-mediated osteoclastogenesis and bone resorption in periodontitis. Bone marrow stromal cells (BMSCs) secrete PGE2 in response to pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α). Curcumin, a polyphenolic compound from the turmeric plant, possesses an anti-inflammatory effect by downregulating key mediators of inflammation.

Objectives This study examined the effect of curcumin on the process of PGE2 biosynthesis in TNF-α-stimulated BMSCs.

Materials and methods To investigate curcumin cytotoxicity, mouse ST2 BMSCs were treated with 1-50 μM curcumin and cell viability was determined by the MTT assay. Next, BMSCs were treated with curcumin for 30 minutes followed by TNF-α stimulation. The level of PGE2 in the culture media and the expression of cyclooxygenase (COX)-2 and microsomal prostaglandin E synthase (mPGES)-1 was measured using enzyme-linked immunosorbent assay (ELISA) and quantitative polymerase chain reaction, respectively.

Results One to ten μM curcumin promoted cell viability, whereas 30-50 μM curcumin was cytotoxic. TNF-α dose-and time-dependently upregulated COX-2 expression, showing the highest increase by 20 ng/mL at 24 hours. Curcumin (10-20 μM) significantly reduced TNF-α-stimulated COX-2 expression. Curcumin (20 μM) almost completely inhibited the TNF-α-induced PGE2 synthesis. Although mPGES-1 expression was also upregulated by TNF-α, it was not affected by curcumin treatment.

Conclusion Curcumin enhanced cell viability and inhibited TNF-α-induced PGE2 synthesis in ST2 BMSCs via COX-2, but not mPGES-1, suppression. These findings suggest a therapeutic potential of curcumin for inflammation-induced bone diseases and tissue regeneration.

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Key words: *Bone marrow stromal cells; COX-2; Curcumin; mPGES-1; PGE2*

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Introduction

Excessive bone destruction is a consequence of chronic inflammatory diseases such as rheumatoid arthritis or periodontitis (Offenbacher, 1996; Shaw and Gravallese, 2016). Pro-inflammatory cytokines are crucial in the pathogenesis of bone loss in these conditions by uncoupling bone formation and bone resorption (Shaw and Gravallese, 2016). Osteoclasts are responsible for both physiologic and inflammation-induced bone resorption. Osteoclasts are multinucleated cells with a monocyte/macrophage lineage origin. Osteoclast precursors proliferate, fuse, and differentiate into mature osteoclasts when cultured with monocyte colony stimulating factor (MCF) and receptor activator of nuclear factor-κB ligand (RANKL) (Shaw and Gravallese, 2016).

Bone marrow stromal cells (BMSCs) and their derivative osteoblasts are important in stimulating osteoclast differentiation via RANKL/receptor activator of nuclear factor-κB (RANK) interaction (Boyce and Xing, 2008; Suda et al., 1999). RANKL, which is expressed on osteoblasts and stromal cells, binds to RANK on osteoclast precursors, inducing osteoclast differentiation (Suda et al., 1999). RANKL expression is upregulated by cytokines and inflammatory mediators, such as interleukin-1, tumor necrosis factor (TNF)-α, and prostaglandin E2 (PGE2) (Kim et al., 2010; Martinez-Calatrava et al., 2012; Singh et al., 2012).

TNF-α is a pro-inflammatory cytokine that plays a critical role in the destruction of alveolar bone in periodontitis (Hienz et al., 2015; Wajant et al., 2003). TNF-α is primarily secreted by macrophages following stimulation by lipopolysaccharide (LPS) and other bacterial products (Holden et al., 2014; Page, 1991). TNF-α indirectly stimulates osteoclast differentiation by promoting RANKL expression in BMSCs and osteoblasts (Quinn et al., 2000; Wei et al., 2005). TNF-α also increases the production of pro-inflammatory mediators, including prostaglandins, in various cell types (Lin et al., 2004; Nakao et al., 2002; Pettus et al., 2003).

Prostaglandins are arachidonic acid-derived lipid compounds pivotal in the acute inflammatory response (Smith et al., 2000; Williams and Peck, 1977). PGE2 biosynthesis requires the function of members of the phospholipase A2, cyclooxygenase (COX), and prostaglandin E synthase (PGES) enzyme groups (Murakami and Kudo, 2004). Two isoforms of the COX family, COX-1 and COX-2, transform arachidonic acid to prostaglandin H2, which is a substrate for prostaglandin synthase and thromboxane synthase to produce prostaglandins and thromboxanes. COX-1 is constitutively expressed in most cells and serves a tissue protective role. In contrast, COX-2 is induced by cytokines and bacterial LPS, leading to increased PGE2 levels in inflamed tissues (Ricciotti and FitzGerald, 2011; Smith et al., 2000).

PGE2 is a key player in bone metabolism with both anabolic and catabolic effects on bone. Intermittent exposure to exogenous PGE2 resulted in increased osteoblastogenesis and bone formation. However, continuous PGE2 administration stimulated bone resorption in rats (Tian et al., 2008). PGE2 is the main mediator for RANKL-dependent osteoclastogenesis induced by periodontogenic bacteria (Choi et al., 2005). LPS and macrophage-derived pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6, induce PGE2 production in BMSCs and their derivative osteoblasts (Agarwal et al., 1995; Chen et al., 1997; Hegyi et al., 2012; Inada et al., 2006; Tai et al., 1997). Elevated PGE2 in gingival crevicular fluid may reflect the involvement of PGE2 in the pathogenesis of alveolar bone destruction in periodontitis (Blackwell et al., 2010; Kats et al., 2013; Noguchi and Ishikawa, 2007; Preshaw and Heasman, 2002). Inhibition of PGE2 synthesis by nonsteroidal anti-inflammatory drugs (NSAIDs) results in decreased osteoclastogenesis *in*

vitro and a reduced rate of bone loss in periodontitis patients (Choi et al., 2005; Noguchi and Ishikawa, 2007; Williams et al., 1989). However, the side effects of NSAIDs, such as gastrointestinal irritation, have limited their long-term use for treating chronic periodontitis (Williams et al., 1989).

Curcumin, a polyphenol compound found in *Curcuma Longa,* is known for its antioxidant, anti-carcinogenic, and anti-inflammatory properties (Duvoix et al., 2005; Punithavathi et al., 2000). Curcumin downregulated several inflammatory mediators, including IL-1β, IL-6, TNF-α, and PGE2, in several cell types (Cho et al., 2007; Lee et al., 2012; Lev-Ari et al., 2006; Singh and Aggarwal, 1995). A clinical study reported the promising use of curcumin as an adjunct to conventional periodontal therapy (Nagasri et al., 2015). In addition, the systemic administration of curcumin reduced bone loss in experimental periodontitis (Correa et al., 2016). However, little is known about the effects of curcumin on BMSCs, a key player in osteoclast differentiation and bone resorption. Therefore, we investigated whether curcumin treatment affected TNF-α-stimulated COX-2 expression and PGE2 synthesis in ST2 BMSCs.

Materials and Methods

Dulbecco's modified Eagle's medium (DMEM) and curcumin were purchased from Sigma-Aldrich, St. Louis, USA. Fetal bovine serum (FBS), Antibiotic-Antimycotic (penicillin, streptomycin and amphotericin), Trypsin-EDTA were obtained from Gibco™, Thermo Fisher Scientific, Waltham, USA. MTT and TNF-α were from Invitrogen, Thermo Fisher Scientific, Waltham, USA

Cell culture Mouse ST2 BMSCs were maintained in DMEM containing 10% FBS, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (10% FBS DMEM) at 37°C in a humidified 5% CO_2 atmosphere. When confluent, the cells were passaged using 0.125% Trypsin-EDTA The culture medium was changed every other day.

Cell viability assay

ST2 BMSCs were seeded in 96-well plates at 5×10^3 cells/well. The next day, the cells were treated with 0-50 μM curcumin in 1% FBS DMEM. After 24 hours, cell viability was determined using the MTT assay (Attari et al., 2015). The absorbance was measured using a microplate reader (EZ Read 400; Biochrom, Cambridge, UK) at 570 nm. Cell viability in each group was calculated as a percentage of the untreated control.

Cell treatment

To determine the time course and dose response of TNF-α on COX-2 gene expression, ST2 cells were plated in 60-mm dishes at 6×10^5 cells/dish. The next day, the cells were washed with PBS and the medium was changed to serum-free DMEM. After being serum starved for 24 h, the cells were treated with 20 ng/mL TNF-α in serum-free media for 3, 6, 9, and 24 h to determine the induction time course. For the dose response assay, the cells were cultured in serum-free media containing 1, 10, or 20 ng/mL TNF-α for 24 hours. Total RNA was isolated and reverse transcribed, and real-time PCR analysis was performed.

To determine whether curcumin affected COX-2 and mPGES-1 expression in TNF-α-stimulated ST2 BMSCs, the cells were seeded in 60-mm plates at 6×10^5 cells/plate in DMEM containing 10% FBS overnight. The next day, the cells were washed with PBS and the culture media was changed to serum-free DMEM for 24 h. The cells were then treated with 1, 10, or 20 μM curcumin in serum-free DMEM for 30 min. After curcumin pretreatment, TNF-α (20 ng/mL) was added to the culture media and the cells were incubated for 24 h. Total RNA was isolated and reverse transcribed, and real-time PCR analysis was performed.

RNA isolation and reverse transcription

RNA was isolated using the Total RNA Mini kit (Geneaid, New Taipei City, Taiwan). Total RNA (2 μg) was reverse transcribed using the SuperScript III Reverse Transcriptase kit (Invitrogen, Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instructions.

Real-time polymerase chain reaction

Real-time PCR was performed on a LightCycler® 480 system (Roche Life Sciences, Indianapolis, USA) using the LightCycler® 480 SYBR Green I Master kit (Roche Life Sciences, Indianapolis, USA) per the manufacturer's directions. The primer sequences used are shown in Table 1 (Kong et al., 2014; Li et al., 2009; Sanuki et al., 2010). The PCR reaction was 95°C for 5 min, followed by 45 cycles of 30 sec at 95°C, 30 sec at 52°C (COX-2), 50°C (mPGES-1), or 56°C (GAPDH), and 30 sec at 72°C.

Enzyme-Linked Immunosorbent Assay (ELISA)

ST2 BMSCs were seeded in 24-well plates at 6×10^5 cells/well. The next day, the cells were washed with PBS and the culture media was changed to serum-free DMEM. After 24 h, the cells were pretreated with 1, 10, 20 μM curcumin, or vehicle (dimethylsulfoxide; DMSO) for 30 min. Subsequently, 20 ng/mL TNF-α was added to each experimental group. Twenty-four hours after TNF-α treatment, the supernatant was collected for PGE2 concentration analysis using an ELISA kit (R & D Systems,

Table 1. Primer sequences for real-time PCR

Gene	Primer sequences	References
COX-2	Forward 5'-TTCGGGAGCACAACAGAGTG-3' Reverse 5'-TAACCGCTCAGGTGTTGCAC-3'	(Sanuki et al., 2010)
mPGES-1	Forward 5'- CTTTCTGCTCTGCAGCACACT-3' Reverse 5'-AGATTGTCTCCATGTCGTTGC-3'	(Li et al., 2009)
GAPDH	Forward 5'-TGAACGGGAAGCTCACTGG-3' Reverse 5'-TCCACCACCCTGTTGCTGTA-3'	(Kong et al., 2014)

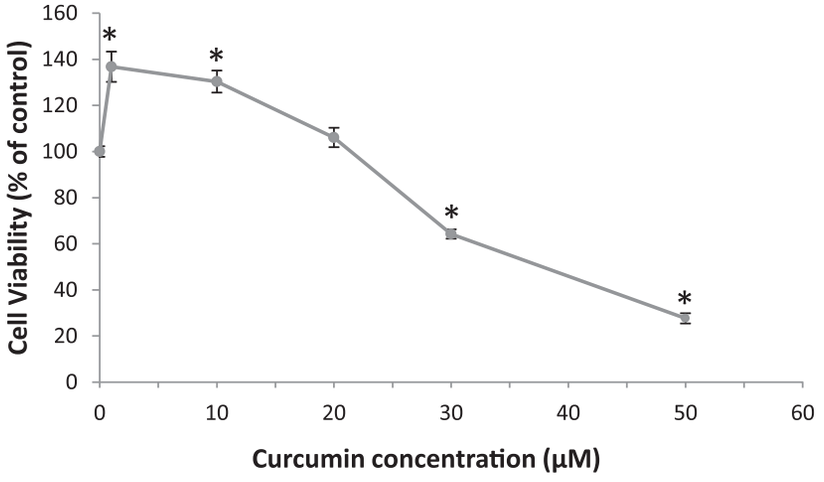

Fig. 1 MTT assay showing the effect of curcumin on ST2 cell viability (mean ± SE, $^*p < 0.05$ vs. control)

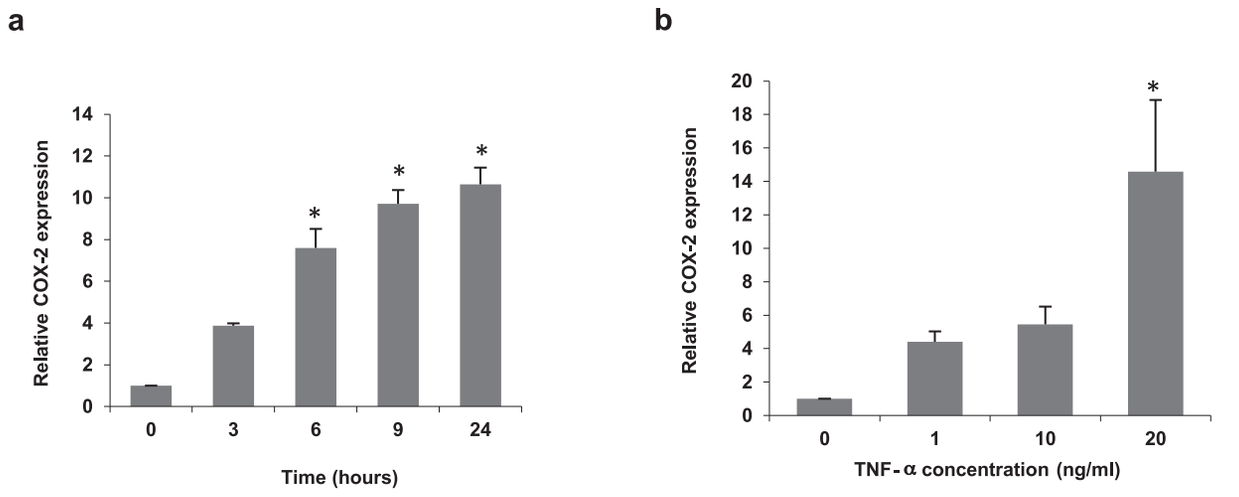

Fig. 2 TNF-α induced COX-2 expresssion. **a** qRT-PCR showing the time course of COX-2 induction by TNF-α; **b** qRT-PCR showing the dose response of COX-2 induction by TNF-α (mean ± SE, $^{*}p < 0.05$ vs. control)

Minneapolis, USA) per the manufacturer's instructions. The optical density was read using a microplate reader (EZ Read 400; Biochrom, Cambridge, UK) at 450 nm with a wavelength correction set to 570 nm. The PGE2 concentration of each sample was calculated from a standard curve.

Statistical analysis

Each assay was performed in triplicate and repeated at least 3 times. All values are shown as mean ± SE. Statistical analysis was performed using ANOVA followed by the Tukey test. Differences were considered statistically significant at $p < 0.05$.

Results

Curcumin affected ST2 cell viability

The effect of curcumin (1-50 μM) on ST2 BMSC viability was determined using the MTT assay (Fig. 1). The results showed that 1 and 10 μM curcumin significantly increased ST2 viability compared with the control ($p < 0.05$), whereas 20 μM curcumin did not significantly affect the number of viable cells. In contrast, 30 and 50 μM curcumin dose-dependently decreased ST2 cell viability ($p < 0.05$).

TNF-α induced COX-2 expression

Stimulating ST2 BMSCs with TNF-α resulted in increased COX-2 expression. We found that 20 ng/mL TNF-α time-dependently upregulated COX-2 expression with the highest induction seen at 24 h (Fig. 2a). Furthermore, 1-20 ng/mL TNF-α dose-dependently increased COX-2 expression in ST2 BMSCs ($p < 0.05$) with the maximal upregulation observed using 20 ng/mL (Fig. 2b).

Curcumin attenuated TNF-α-induced COX-2 expression and PGE2 synthesis

Pretreatment with 10 or 20 μM curcumin significantly reduced TNF-α-stimulated COX-2 expression compared with TNF-α treatment alone ($p < 0.05$) (Fig. 3a). Cell morphology was not affected by curcumin or TNF-α treatment (Fig. 3b).

The PGE2 ELISA results demonstrated that TNF-α significantly stimulated PGE2 production compared with the control. Curcumin (1 or 10 μM) did

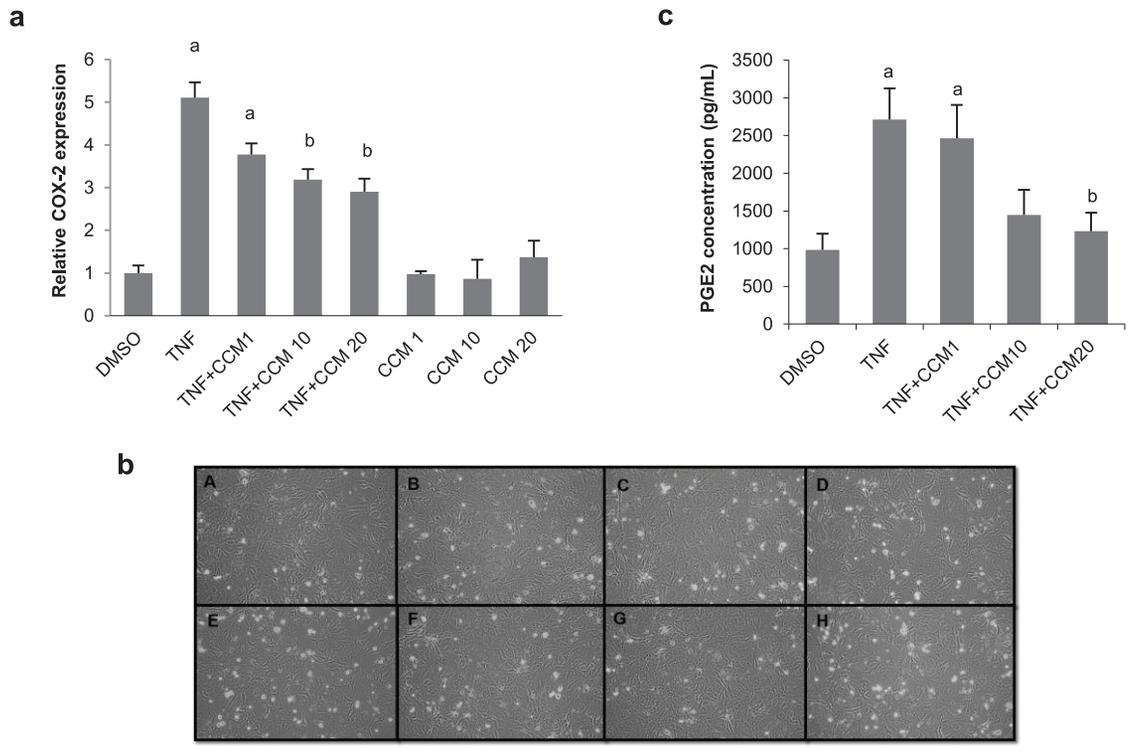

Fig. 3 Curcumin inhibited TNF-α-induced COX-2 expression and PGE2 synthesis. **a** qRT-PCR showing the effect of 1-20 μM curcumin (CCM) on TNF-α-induced COX-2 expression (mean ± SE, $^{a}p < 0.05$ vs. dimethylsulfoxide (DMSO), $^{b}p < 0.05$ vs. TNF-α); **b** ST2 morphology following curcumin pretreatment and TNF-α (20 ng/mL) stimulation. (A) DMSO (B) DMSO+TNF-α, (C) 1 μM curcumin + TNF-α, (D) 10 μM curcumin + TNF-α, (E) 20 μM curcumin + TNF-α, (F) 1 μM curcumin, (G) 10 μM curcumin, (H) 20 μM curcumin; **c** ELISA showing the inhibition of TNF-α-induced PGE2 synthesis by 1-20 μM curcumin (CCM). (mean ± SE, $^{a}p < 0.05$ vs. control, $^{b}p < 0.05$ vs. TNF-α)

not significantly alter TNF-α-stimulated PGE2 production. However, 20 μM curcumin inhibited TNF-α-induced PGE2 synthesis to approximately baseline level ($p < 0.05$) (Fig. 3c).

mPGES-1 expresssion was not affected by curcumin treatment

Two inducible enzymes, COX-2 and mPGES-1, are responsible for PGE2 synthesis during inflammation (Park et al., 2006; Samuelsson et al., 2007). We investigated the effect of TNF-α and curcumin on mPGES-1 expression. We determined that TNF-α time-dependently upregulated mPGES-1 expression with maximum induction seen at 24 h (Fig. 4a). However, curcumin pretreatment did not significantly affect TNF-α-stimulated mPGES-1 expression ($p > 0.05$) (Fig. 4b).

Discussion

Curcumin has been studied for its potential use as an anti-inflammatory, antioxidant, and anti-tumor agent. This natural compound regulates multiple cellular functions, including cell proliferation, apoptosis, gene expression, and cytokine production in a variety

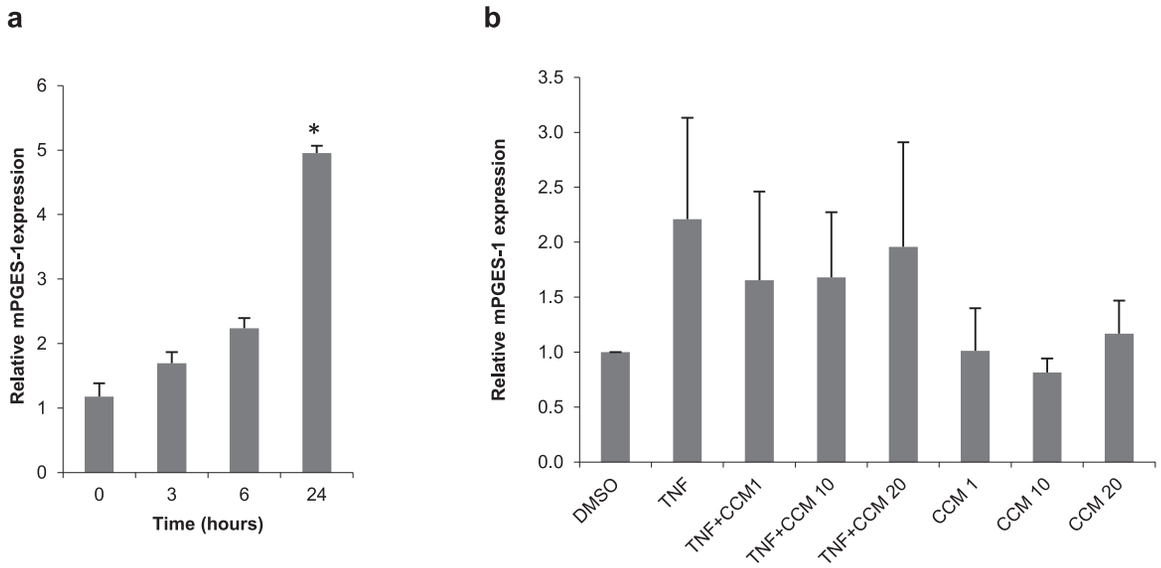

Fig. 4 Curcumin did not affect mPGES-1 expression. **a** qRT-PCR showing time course of mPGES-1 induction by TNF-α (mean ± SE. $^{*}p < 0.05$ vs. control); **b** qRT-PCR showing the effect of 1-20 μM curcumin (CCM) or dimethylsulfoxide (DMSO) on TNF-α-induced mPGES-1 expression (mean ± SE).

of cell types (Cho et al., 2007; Duvoix et al., 2005; Lee et al., 2012; Lev-Ari et al., 2006; Tan et al., 2011). PGE2 is a key player in inflammatory bone resorption (Choi et al., 2005). BMSCs and osteoblasts are the main source of PGE2 in response to pro-inflammatory cytokines, including TNF-α, leading to alveolar bone destruction in periodontitis (Chen et al., 1997; Inada et al., 2006; Tai et al., 1997). Curcumin inhibited PGE2 production and COX-2 expression in both normal and malignant cells (Guimaraes et al., 2013; Lev-Ari et al., 2006; Miyahara et al., 2004). However, there have been no reports regarding how curcumin regulates PGE2 biosynthesis in TNF-α stimulated BMSCs.

In the present study, we determined the toxicity of curcumin on ST2 BMSCs, with 1-10 μM curcumin increasing ST2 cell survival and 30-50 μM curcumin demonstrating a dose-dependent cytotoxicity. However, 20 μM curcumin did not significantly affect the number of viable cells. Thus, we used the non-cytotoxic concentrations (1-20 μM) in our subsequent experiments. Our results are similar to those of a study by Attari et al., where 0.1-10 μM curcumin increased rat BMSC viability at 48 hours (Attari et al., 2015). However, these investigators found that longer exposure to 5-10 μM curcumin decreased the survival of these cells. Studies in other cell types have also shown a differential effect of curcumin concentration on cell survival (Abuelba et al., 2015; Attari et al., 2015; Huang et al., 2015; Qin et al., 2012; Yamauchi et al., 2014). Curcumin decreased the viability of a variety of cancer cells (Abuelba et al., 2015; Shanmugam et al., 2015; Yamauchi et al., 2014). In contrast, curcumin showed a cytoprotective effect on primary cell cultures of several cell types (Huang et al., 2015; Qin et al., 2012; Reyes-Fermin et al., 2012). Therefore, the effect of curcumin on cell survival may be cell-type specific and dependent on its concentration and treatment duration. Our ongoing studies involve elucidating the molecular basis for the differential effect of curcumin concentration on cell viability.

BMSCs can differentiate into several cell types and have therapeutic potential in treating degenerative

diseases, including cell-based periodontal regeneration (Yang et al., 2010). However, a major obstacle in cell therapy is poor cell viability at the administration site (Lee et al., 2015). Various strategies have been used to manipulate BMSCs *ex vivo* to overcome the low cell survival rates, including pretreatment with growth factors or cytokines (Lee et al., 2015). Our results suggest that pretreating BMSCs with 1-10 μM curcumin for 24 h may be a promising strategy to improve BMSC survival in clinical applications to increase the success rate of cell-based tissue regeneration.

TNF-α regulates key biological phenomena such as inflammation, gene expression, cell differentiation and cell death (Brenner et al., 2015). TNF-α can bind to tumor necrosis factor receptor type I (TNFR1) and tumor necrosis factor receptor type II (TNFR2). The intracellular domains of TNFR1 contains the "death domain" which can recruit the adaptor molecule TNFR1-associated death domain protein (TRADD) (Lavrik et al., 2005). TNFR1-TRADD signaling can induce either cell survival or cell death. TNFR2, on the other hand, lacks the cytoplasmic death domain, and recruits TNFR-associated factor 1 (TRAF1) and TRAF2. TNFR2-TRAF signaling leads to activation of nuclear factor (NF)-κB pathway which promotes cell survival. Almost every mammalian cells express TNFR1 while only endothelial cells and immune cells express both types of TNFRs (Faustman and Davis, 2010). Therefore, the relative expression of TNFR1 and TNFR2 on the cell surface partially determines the cell fate. TNF-α cytotoxicity varies significantly depending upon cell types The level of TNF-α in the serum of endotoxin-injected mice is not toxic to normal mouse embryo fibroblasts but induce cell death in tumor cells in vitro (Carswell et al., 1975). It has been shown that 100 ng/mL TNF-α is cytotoxic to the TNF-α-sensitive L929 mouse fibrosarcoma cell line (Humphreys and Wilson, 1999). Physiologic level of TNF-α in the serum of healthy adults differs from one study to another ranging from 2.13-50 pg/mL (Kleiner et al., 2013; Singhal et al., 2016). Furthermore, TNF-α level can be induced by various stimuli including infection. Patients with chronic periodontal diseases have the average level of TNF-α at 5.32 pg/mL in gingival crevicular fluid, compared to 1.57 pg/mL in healthy individuals (Singhal et al., 2016). *In vitro* stimulation of murine macrophages by DNA from periodontopathic bacteria dose-dependently induced TNF-α production from 2-8 ng/mL (Nonnenmacher et al., 2003).

In this study, we identified that TNF-α (1-20 ng/mL) dose-dependently upregulated COX-2 expression and curcumin significantly inhibited both TNF-α-stimulated COX-2 induction and PGE_2 production. However, curcumin had a more profound effect on inhibiting TNF-α-induced PGE_2 production compared with its effect on COX-2 expression. Therefore, we investigated the effect of curcumin on the expression of prostaglandin E synthase (PGES), the terminal enzyme that converts PGH_2 to PGE_2 (Kudo and Murakami, 2005).

At least three PGES isoforms are involved in PGE_2 synthesis: cytosolic PGES (cPGES), microsomal PGES-1 (mPGES-1), and mPGES-2 (Kudo and Murakami, 2005). cPGES is ubiquitously expressed and transforms COX-1-derived PGH_2 to PGE_2 for immediate release (Murakami et al., 2002; Tanioka et al., 2000). However, mPGES-1 levels are markedly increased by pro-inflammatory stimuli and converts COX-2-derived PGH_2 to PGE_2 for delayed release (Murakami et al., 2002). mPGES-2, which is also constitutively and ubiquitously expressed, catalyzes COX-1-and COX-2-derived PGH_2 during PGE_2 biosynthesis. Unlike mPGES-1, mPGES-2 expression is not increased markedly during inflammation (Mattila

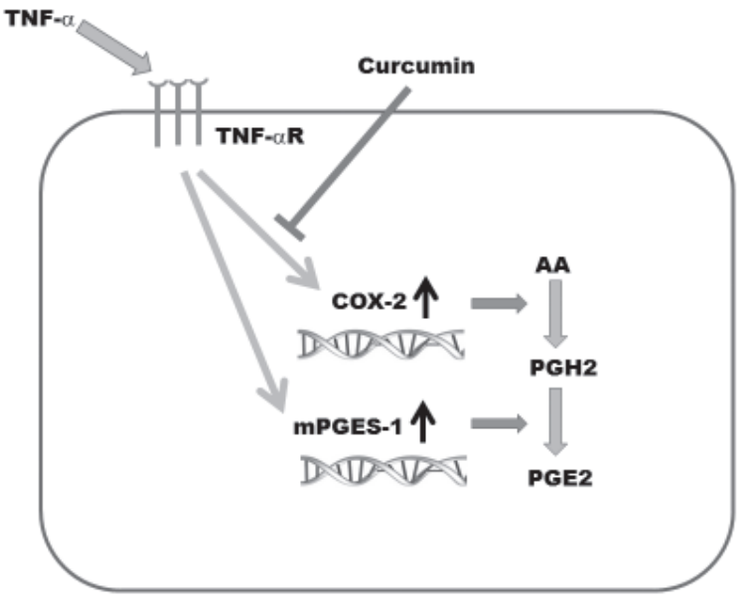

Fig. 5 Schematic model of the mechanism by which curcumin inhibits TNF-α-induced PGE2 synthesis in ST2 BMSCs. TNF-α induced PGE2 synthesis by upregulating COX-2 and mPGES-1 expression. Curcumin blocks TNF-α induced PGE2 synthesis by suppressing COX-2, but not mPGES-1, expression. AA Arachidonic acid; PGH2 Prostaglandin H2; TNF-αR Tumor necrosis factor-α receptor

et al., 2009). Previous studies reported that in addition to inhibiting COX-2 expression, curcumin also blocked PGE2 synthesis by inhibiting mPGES-1 expression in IL-1β-stimulated A549 lung carcinoma cells (Koeberle et al., 2009; Moon et al., 2005). In contrast, curcumin induced COX-2 and mPGES-1 mRNA expression without increasing PGE2 synthesis in human coronary artery endothelial cells (Tan et al., 2011). In our study, 20 μM curcumin completely blocked TNF-α-induced PGE2 synthesis and significantly inhibited COX-2 mRNA expression. However, curcumin did not significantly alter the TNF-α-induced mPGES-1 mRNA expression. Therefore, we hypothesize that the main mechanism of curcumin on inhibiting TNF-α-induced PGE2 production in ST2 BMSCs occurs via inhibiting COX-2 expression as shown in our model in Figure 5. Although curcumin did not significantly decrease TNF-α-induced mPGES-1 mRNA expression, the effect of curcumin on mPGES-1 protein level and enzyme activity is currently unknown. A previous study reported that curcumin strongly inhibited human recombinant mPGES-1 activity (Ahmad et al., 2014). Therefore, in addition to COX-2 suppression, curcumin may block PGE2 synthesis in ST2 BMSCs by inhibiting mPGES-1 activity without affecting its mRNA expression level. Further studies are needed to determine if curcumin regulate mPGES-1 activity in ST2 BMSCs.

In conclusion, we found that 1-10 μM curcumin promoted ST2 cell viability, 10-20 μM curcumin attenuated TNF-α-induced COX-2 expression, and 20 μM curcumin inhibited TNF-α-induced PGE2 production in ST2 BMSCs. These results support the use of curcumin for promoting cell viability and inhibiting PGE2 synthesis in tissue regeneration and the treatment of inflammation-induced bone diseases. However, the mechanisms whereby curcumin regulates cell viability, TNF-α-induced COX-2 expression, and PGE2 synthesis in ST2 BMSCs require further investigation.

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