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Biglycan mediates suture expansion osteogenesis via potentiation of Wnt/β-catenin signaling

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ABSTRACT

Mechanical force across sutures is known to modulate suture osteogenesis. However, the underlying mechanisms still remain poorly understood. Biglycan is a component of extracellular matrix (ECM) that is postulated to release from ECM and function as a signaling molecule. Biglycan stimulates the bone formation through Wnt/β-catenin signaling. To investigate the involvement of biglycan and Wnt/β-catenin signaling in suture expansion osteogenesis, we observed the expansion force-induced response in mouse midpalatal suture expansion model in vivo, and the mechanical strain-induced response of Wnt/β-catenin signaling in biglycan-deficient calvarial osteoblasts in vitro. Our data showed that expansion force significantly enhanced new bone formation at the edge of midpalatal sutures. Stronger biglycan positive staining was visible at the edge of expanding midpalatal sutures. The spatio-temporal expression of biglycan was highly consistent with ALP and COL-1, which also coincided with new bone formation throughout the midpalatal suture expansion process. Both protein and mRNA levels of biglycan, β-catenin, and osteogenic markers including Runx2, ALP and COL-1 were increased together. In addition, mechanical strain sufficiently induced upregulation of osteoblastic biglycan, which was paralleled with the strain-induced potentiation of Wnt/β-catenin signaling and Runx2 transcriptional activity. However, silencing osteoblastic biglycan resulted in an attenuated increase in the expression of nuclear active β-catenin and Runx2 in response to mechanical strain. Our data demonstrated that biglycan as a component of ECM mediates suture expansion osteogenesis through the activation of Wnt/β-catenin signaling.

1. Introduction

Mechanical force plays a crucial role in bone repair and regeneration (Huang and Ogawa, 2010). Orthodontists and dentofacial orthopedists always impose various forms of mechanical force to bony sutures to correct craniofacial deformities (Takahashi et al., 1996). Midpalatal suture expansion, or orthopedic maxillary expansion, is a preferred treatment to correct maxillary transverse skeletal deficiency in orthodontics (Gray and Brogan, 1972). However, even after a retention phase, the expanded maxillary arch still has a tendency back to its previous form (Vardimon et al., 1989; Zhong et al., 2011). The cellular response of midpalatal sutures to the expansion force still remains poorly understood (Hou et al., 2007). Therefore, understanding the underlying mechanisms of this remodeling process should contribute greatly to preventing this relapse.

Extracellular matrix (ECM) contains various important biochemical information (Frantz et al., 2010). ECM can sense extracellular mechanical stimuli and then convert them into biological signals thereby eliciting signal transduction and regulating cell behaviors (Khatiwala et al., 2006; Smith et al., 2011). Biglycan is an important structural component of ECM, which is able to release from ECM and function as a signaling molecule (Popovic et al., 2011). Biglycan can interact with various growth factors, including bone morphogenetic protein 4 (BMP4), transforming growth factor-beta (TGF-β), Wnt1-inducible-signaling pathway protein 1 (WISP-1) and tumor necrosis factor alpha (TNF-α) (Nastase et al., 2012; Nikitovic et al., 2012). Upon tissue stress or injury, biglycan is proteolytically released from the ECM and then becomes a signaling molecule to trigger an immune response in immune systems (Nastase et al., 2012; Schaefer et al., 2005). Biglycan is also enriched in the pericellular space of skeletal tissues. It is involved in the differentiation of osteoblast precursor cells and assembly of collagen fibrils (Bianco et al., 1990; Takagi et al., 1999). Recent researches show that biglycan activates the osteogenic program

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through the Wnt/β–catenin and BMP/TGF-β signaling pathway (Berendsen et al., 2011; Parisuthiman et al., 2005). Biglycan-deficient mice exhibit osteogenesis defect and collagen fibrils abnormalities (Corsi et al., 2002; Xu et al., 1998). However, scanty knowledge is available about the role of biglycan in bone remodeling process in response to mechanical stress.

Numerous studies indicate that Wnt/β-catenin signaling plays an important role in the response of bone to mechanical stimuli (Bonewald and Johnson, 2008; Papachristou et al., 2009). Appropriate mechanical stimuli improve the stability of β-catenin in the cytoplasm and then promote its translocation into the nucleus, which subsequently interacts with T cell factor (TCF) and lymphoid enhancer factor (LEF) to activate downstream responsive target genes (Case and Rubin, 2010). Furthermore, activation of Wnt/β-catenin signaling enhances mechanical stimulus-induced osteoblastic osteogenesis (Robinson et al., 2006). Wnt/β-catenin signaling regulates osteoblastic osteogenesis by directly stimulating Runx2 gene expression (Gaur et al., 2005). Given that biglycan stimulates bone formation through Wnt/β-catenin signaling, we propose a mechanism that biglycan may mediate suture expansion osteogenesis through Wnt/β-catenin signaling.

In this study, we assessed the expansion force-induced response of biglycan and Wnt/β-catenin signaling in a mouse midpalatal suture expansion model in vivo. Meanwhile, we continued to examine the mechanical strain-induced response of Wnt/β-catenin signaling in biglycan-deficient calvarial osteoblasts in vitro. Collectively, we demonstrated that biglycan as a component of ECM mediates suture expansion osteogenesis via potentiation of Wnt/β-catenin signaling.

2. Materials and methods

2.1. Animal models

The mouse midpalatal suture expansion model was established as described previously (Zhang and Wang, 2009). Briefly, sixty male C57BL/6 mice (6-week-old) were used. Under chlora hydrate anesthesia, expansive wires with opening loops were bonded to the maxillary molars for various time points: 1, 3, 7 or 14 day (Fig. S1). All procedures were approved by the Ethics Committee at Nanjing Medical University, China (Approval ID 2008-00318).

2.2. Cell isolation and culture

Osteoblasts were derived from fetal C57BL/6 mouse calvarias as described before (Ichikawa et al., 1995). The cells were cultured in DMEM (Gibco) containing 10% FBS (Gibco), 1% glutamine (Gibco), and 1% penicillin/streptomycin (Gibco). All cells used were after the third passage. Pharmacological agent Wnt3a (100 ng/ml, R&D Systems.) was preincubated for 1 h.

2.3. Histology

Specimens were dissected and then fixed in PLP (Periodate-Lysine-Paraformaldehyde) fixative. Then specimens were decalcified, dehydrated and paraffin-embedded in turn. 5 μm paraffin sections were stained with hematoxylin and eosin (HE), or histochemically for total collagen, alkaline phosphatase (ALP) activity and tartrate-resistant acid phosphatase (TRAP), or immunohistochemically for biglycan and collagen type I (COL-1).

2.4. Histochemical staining for total collagen, ALP and TRAP

Histochemical staining was performed as described previously (Sun et al., 2010). For total collagen, de-waxed sections were subjected to 1% Sirius red in saturated picric acid for 1 h, followed by counterstained with hematoxylin and mounted with biomount medium. For ALP activity, de-waxed sections were preincubated overnight in 100 mM $MgCl_2$ in 100 mM tris-maleate buffer (pH 9.2), and then incubated in 100 mM tris-maleate buffer containing naphthol AS-MX phosphate (0.2 mg/ml, Sigma) and Fast Red TR (0.4 mg/ml, Sigma) for 2 h. For TRAP, de-waxed sections were preincubated for 20 min in buffer containing 50 mM sodium acetate and 40 mM sodium tartrate (pH 5.0), and then incubated for 15 min at room temperature in the same buffer containing 2.5 mg/ml naphthol AS-MX phosphate in dimethylformamide and 0.5 mg/ml Fast Garnet GBC (Sigma). After washing with distilled water, the sections were counterstained with Methyl Green and mounted in Kaiser's glycerol jelly.

2.5. Immunohistochemical staining

Immunohistochemical staining for biglycan and COL-1 was carried out as described previously (Sun et al., 2010). Briefly, de-waxed and rehydrated paraffin-embedded sections were incubated with methanol: hydrogen peroxide (1:10) and then washed in tris-buffered saline (pH 7.6). The sections were then incubated with primary antibodies (biglycan (ab49701) and COL-1 (ab6308) from Abcam) overnight at room temperature. After rinsing with tris-buffered saline, sections were incubated with appropriate secondary antibody. Then sections were washed and incubated with the Vectastain Elite ABC reagent (Vector Laboratories) for 45 min. Staining was developed using 3,3-diaminobenzidine (2.5 mg/ml) followed by counterstaining with Mayer's hematoxylin.

2.6. Mechanical strain and preparation of cell extracts

Cells were plated on 6-well Bioflex plates (Flexcell International). Cyclic sinusoidal continuous tensile strain (2% magnitude, 0.5 Hz) was applied using Flexcell® FX-5000™ Tension System (Flexcell International). Static control cells were cultured under identical conditions except the tensile strain. Whole cell lysates were prepared according to the manufacturer's protocol (Keygen China). Nuclear or cytoplasmic lysates were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce, Rockford, IL, USA). Protein concentrations were measured using a Bio-Rad protein assay kit (Pierce, Rockford, IL, USA).

2.7. RNA interference studies

Small interfering RNAs (siRNA) (sc-45735) against mouse biglycan and control siRNA (sc-44230) were purchased from Santa Cruz. After reaching approximately 70% confluence, cells were transfected with siRNA according to the manufacturer's protocol. The cells were then cultured for another 24 h before mechanical strain experiments.

2.8. Western blot

Proteins were extracted from midpalatal sutures or osteoblasts. The immunoblotting was carried out as described before (Wang et al., 2014). Briefly, primary antibody (the antibody against active β-catenin (05–665) or β-actin (04–1116) from Merck Millipore; the antibody against Runx2 (ab76956), biglycan (ab49701), Lamin B1 (ab16048) or osteopontin (OPN) (ab8488) from Abcam; the antibody against β-catenin (610154) from BD Biosciences) was incubated overnight at 4 °C. The blots were incubated with appropriate secondary antibody (anti-mouse (04-18-06) or anti–rabbit (04-15-06) from Kirkegaard & Perry Laboratories) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescence system (Supersignal West Pico Trial Kit, Pierce). Digital imaging of Western blots was collected using ImageQuant™ LAS 4000 biomolecular imager (GE Healthcare), and the density of each band was quantified with a Fluor-S MultiImager (Bio-Rad).

2.9. RNA isolation and quantitative real-time PCR

Total RNA was extracted using Trizol reagent based on manufacturer's protocols (Invitrogen). After detecting the concentration and purity of the RNA samples, reverse transcription reaction was carried out using the First Strand Synthesis Kit (Takara). Relative transcript levels were measured by quantitative PCR using ABI PRISM 7300 (Applied Biosystems), according to the manufacturer's protocol for SYBR-Green (Roche), and normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels. Sense and anti-sense primers (Invitrogen) were listed in Supplemental Table. The results were obtained using the comparative CT method and the arithmetic formula $2^{-\Delta\Delta CT}$.

2.10. Immunofluorescence

After washing with phosphate-buffered saline (PBS), cells were fixed in 4% paraformaldehyde and permeabilized in 0.25% Triton X-100/PBS at room temperature for 30 min. Cells were blocked in blocking solution containing 2% normal goat serum for 1 h. Primary antibody was incubated overnight at 4 °C with diluted 1:50 in blocking solution. Secondary antibody Alexa Fluor® 594 Goat Anti-Mouse IgG (H+L) (A11005) (Invitrogen) conjugating with appropriate dilution in PBS were performed in darkness for 1 h. Finally, nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI, Invitrogen). Fluorescence microscopy was viewed using a Zeiss LSM 510 confocal microscope.