Biochimica et Biophysica Acta 1863 (2016) 1633–1642

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Compression regulates gene expression of chondrocytes through HDAC4 nuclear relocation via PP2A-dependent HDAC4 dephosphorylation

Chongwei Chen [a], Xiaochun Wei [a], Shaowei Wang [a,b], Qiang Jiao [a], Yang Zhang [a,b], Guoqing Du [b], Xiaohu Wang [a], Fangyuan Wei [c], Jianzhong Zhang [c], Lei Wei [a,b,*]

[a] Department of Orthopaedics, The Second Hospital of Shanxi Medical University, Shanxi Key Lab of Bone and Soft Tissue Injury Repair, Taiyuan 030001, China
[b] Department of Orthopaedics, Warren Alpert Medical School of Brown University/Rhode Island Hospital, Providence, RI 02903, USA
[c] Foot and Ankle Orthopaedic Surgery Center, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China

ARTICLE INFO

Article history:
Received 11 September 2015
Received in revised form 13 April 2016
Accepted 17 April 2016
Available online 19 April 2016

Keywords:
Mechanical loading
Compression
Chondrocytes
HDAC4
Gene expression

ABSTRACT

Biomechanics plays a critical role in the modulation of chondrocyte function. The mechanisms by which mechanical loading is transduced into intracellular signals that regulate chondrocyte gene expression remain largely unknown. Histone deacetylase 4 (HDAC4) is specifically expressed in chondrocytes. Mice lacking HDAC4 display chondrocyte hypertrophy, ectopic and premature ossification, and die early during the perinatal period. HDAC4 has a remarkable ability to translocate between the cell's cytoplasm and nucleus. It has been established that subcellular relocation of HDAC4 plays a critical role in chondrocyte differentiation and proliferation. However, it remains unclear whether subcellular relocation of HDAC4 in chondrocytes can be induced by mechanical loading. In this study, we first report that compressive loading induces HDAC4 relocation from the cytoplasm to the nucleus of chondrocytes via stimulation of Ser/Thr-phosphoprotein phosphatases 2A (PP2A) activity, which results in dephosphorylation of HDAC4. Dephosphorylated HDAC4 relocates to the nucleus to achieve transcriptional repression of Runx2 and regulates chondrocyte gene expression in response to compression. Our results elucidate the mechanism by which mechanical compression regulates chondrocyte gene expression through HDAC4 relocation from the cell's cytoplasm to the nucleus via PP2A-dependent HDAC4 dephosphorylation.

Published by Elsevier B.V.

1. Introduction

Cartilage covers the surfaces of articulating joints, and is composed of chondrocytes and extracellular matrix, the latter of which includes mainly collagen and proteoglycans [1]. Load-bearing is the fundamental function of cartilage and results in direct compression of articular cartilage. The effects of mechanical loading on chondrocytes are complex. Under physiological mechanical loading, the chondrocytes residing in articular cartilage are subjected to an intricate combination of strains: hydrostatic pressure, compression, tension, and shear stress [2]. Among these strains, compression plays a particularly important role in the regulation of articular chondrocyte functions [1,2]. Mechanical stimuli contribute to chondrogenesis and limb formation during embryogenesis and cartilage maturation, and maintain chondrocytic phenotype in adult cartilage [2–5]. Normal biomechanical loading increases cartilaginous gene expression and matrix protein production [6–10]. Alternatively, abnormal mechanical loading (excessive or insufficient loading) can promote the onset of cartilage degeneration, and lead to osteoarthritis [11]. In spite of our understanding of cause-and-effect relationships between mechanical loading and cartilage metabolic responses, the mechanisms underlying chondrocyte mechanotransduction, i.e., how chondrocytes sense and respond to mechanical stimuli, remain largely unknown [12,13].

Histone acetylation mediates decondensation of the nucleosome structure, alters histone and DNA interactions, and facilitates access and binding of transcription factors. Epigenetic evidence thus indicates that gene expression can be regulated by dynamic control of histone acetylation [14,15]. Histone acetylation by histone acetylase (HATs) promotes chromatin relaxation, whereas histone deacetylation by histone deacetylase (HDACs) condenses the structure of nucleosomes, thus altering histone and DNA interactions that control access and binding of transcription factors, and leads to transcriptional repression or activation [14,16,17]. In mammalian cells, three major classes of HDACs, comprising at least 18 HDACs have been described so far [18]. Class III HDACs (consisting of a large family of sirtuins) and class I HDACs (HDAC1, 2, 3 and 8) are ubiquitously expressed. Conversely, class II HDACs exhibit a tissue-specific pattern of expression and are further divided into two subgroups: class IIa (HDAC4, 5, 7 and 9) and class IIb

Abbreviations: HDAC4, histone deacetylase 4; GFP, green fluorescent protein; PI, propidium iodide; PP2A, Ser/Thr-phosphoprotein phosphatases 2A; OA, okadaic acid; Ps-HDAC4, phosphorylated-HDAC4; LK1, polo-like kinase 1; CDKN1A, cyclin-dependent kinase inhibitor 1A.

* Corresponding author at: Department of Orthopaedics, The Warren Alpert Medical School of Brown University/Rhode Island Hospital, Coro West, Suite 402H, 1 Hoppin Street, Providence, RI 02903, USA.
E-mail address: lei_wei@brown.edu (L. Wei).

http://dx.doi.org/10.1016/j.bbamcr.2016.04.018
0167-4889/Published by Elsevier B.V.

(HDAC6 and 10). Genetic studies demonstrate that class IIa HDACs act as crucial regulators in tissue-specific developmental and differentiation processes [18–20]. HDAC4, a key member of the class IIa HDACs, is highly expressed in the heart, brain, skeletal muscle and cartilage [18,21]. Mice lacking HDAC4 display ectopic and premature ossification of endochondrial bones due to abnormal onset chondrocyte hypertrophy and die early during the perinatal period [22]. A surprising feature of HDAC4 is its ability to translocate between the nucleus and cytoplasm of the cell. This feature could be unique to the class II HDACs since class I and III enzymes are not capable of subcellular shuttling [18,21]. Studies have shown that HDAC4 subcellular relocation plays a prominent role in muscle cell differentiation [23], neuronal cell death [24], and regulation of growth plate chondrocyte differentiation [21]. Since mechanical loading is critical for chondrogenesis, limb formation and gene expression, HDAC4 subcellular translocation might couple extracellular biomechanics signals to chromatin. On these grounds, we put forth the hypothesis that biomechanics regulates gene expression via promoting HDAC4 relocation from the cytoplasm to the nucleus of chondrocytes.

In this study we demonstrate that compressive stimulus promotes HDAC4 relocation from the cytoplasm to the nucleus in chondrocytes by dephosphorylation of HDAC4 in a PP2A-dependent manner, and that this in turn regulates the expression of proliferation and differentiation genes. Thus, HDAC4 plays an essential role in the mechanical regulation of gene expression of chondrocytes.

2. Materials and methods

2.1. DNA constructs and antibodies

Green fluorescent protein (GFP)-HDAC4 and Flag-HDAC4 plasmids were provided by T.A. Bolger [23] and G. Paroni [25], respectively. Flag-HDAC4 S246/467/632A triple mutant (serine/alanine mutations) expression vector was a generous gift from Dr. X.J. Yang [26]. The GFP-HDAC4 plasmids were cloned to adenovirus vectors, and the viral null vectors were propagated in human embryonic kidney 293 (HEK 293) cells. Viral titer was determined with standard plaque assays on HEK293 cells. The resulting titers for ad-(GFP)-HDAC4 were 1×10^{11} pfu/mL. The PP2A immunoprecipitation phosphatase assay kit was purchased from Upstate (Lake Placid, NY). Phosphoserine antibody was purchased from Zymed (90-0200, Carlsbad, CA). All other antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The experimental protocol was approved by the Institutional Animal Care and Use Committee of Second Hospital of Shanxi Medical University.

2.2. Primary cell culture, transfection and encapsulation in alginate disks

Murine chondrocytes were isolated from the ventral parts of the rib cages of 6-day-old mice (C57Bl/6) and cultured in F-12 media with 10% FBS (Gibco BRL) as previously described [13,27,28]. Briefly, the pieces of murine rib cartilage were subjected to enzymatic treatment with 3% collagenase D (Roche, cat. no. 11088882001), the chondrocytes were seeded on polystyrene tissue culture dishes (Becton Dickinson Labware, Franklin Lakes, NJ) at a density of 1×10^5 cells/cm^2 in Ham's F-12 medium with 10% FBS, and cultured at 37 °C in a thermal incubator under 5% CO_2. Through the experimental culture, the medium was refreshed every other day.

After 5–6 days, when reaching 100% confluency, chondrocytes were subcultured for 12 h at 45% confluency. Then, the culture medium was replaced with fresh medium, and chondrocytes were incubated with adenoviral vectors containing GFP-HDAC4 for 20 h at a multiplicity of infection (MOI) of 30. The transfected cells were resuspended in 2% w/v alginate gels (Sigma, St. Louis, MO) solution at 1×10^7 cells/mL. Using a cylindrical mold (4.5 mm inter-diameter and 3 mm height), chondrocyte–alginate solution was cross-linked in 102 mM $CaCl_2$ solution for 10 min to form identical cylindrical 3D cell/alginate constructs (Φ 4.5 mm × 3 mm) [10]. The cell/alginate constructs were cultured for 7 days in F-12 media plus 10% FBS at 37 °C and 5% CO_2 atmosphere to allow pericellular matrix deposition to occur before introducing mechanical compression [13,28,29]. The culture medium was changed every other day. Transfection efficiency was confirmed with observation of the expression of GFP in infected chondrocytes using Olympus FV1000 confocal laser scanning microscope (Olympus, Japan). Cell nuclei were counterstained with Hoechst 33342 (Pierce, Rockford, IL, USA).

In addition, the chondrocytes were also transfected with Flag-HDAC4 or Flag-HDAC4 S246/467/632A triple mutant expression vector to further confirm the nuclear location of HDAC4 regulates the gene expression by using Lipofectamine™ 2000 (Invitrogen) as described in the manufacturer's protocol. Transfection efficiency was confirmed by western blot.

2.3. Mechanical stimulation

Before loading, the cell/alginate constructs were placed within the 5 mm diameter foam ring of Biopress™ compression plate wells (Flexcell International Corporation), and 4 mL F-12 media with 10% FBS was added to each well. Dynamic unconfined compression was applied by a computer-controlled Flexcell® FX-5000™ Compression System (Flexcell International Corporation) as described in the manufacturer's manual (www.flexcellint.com). The compression testing regimen consisted of a sinusoidal strain from 0 kPa to 20 kPa amplitude at 0.5 Hz as indicated (Fig. 1A). Control cell/alginate constructs were maintained under uncompressed conditions. After compressive stimulation, 3D cell culture constructs were washed with phosphate buffered saline (PBS; Sigma), and a 1-mm thickness sample was vertically cut from each construct to observe HDAC4 location by confocal laser scanning microscope. The remaining cell/alginate constructs were collected to evaluate the HDAC4 protein, metabolic and biosynthetic activities of chondrocytes.

2.4. Fluorescent microscopy

To detect HDAC4 subcellular localization, 1-mm thickness cell/alginate constructs were incubated immediately after compression at room temperature for 15 min with 10 μg/mL of Hoechst 33342 (Pierce, Rockford, IL, USA) while avoiding exposure to light. Stained cells were examined with a Olympus FV1000 confocal laser scanning microscope (Olympus, Japan).

2.5. Evaluation of cell viability following compression

The viability of the chondrocytes in the alginate hydrogels after different compressive stimulation regimes was evaluated using Hoechst 33342/Propidium Iodide (PI) Double Stain Apoptosis Detection Kit (Cat. L00309, GenScript, Piscataway, NJ, USA). Fourty-eight hours after compression, the samples were vertically sectioned, and incubated with Hoechst 33342 for 10 min at room temperature and protected from light, then washed with PBS, and then the dye reagent (containing 1000 μL of 1× buffer A and 5 μL of PI prepared according to the manufacturer's instruction) was loaded into each sample. After 10 min incubation, images of live and dead (red) cells were captured using a confocal microscope (Olympus, Japan). Cell viability was then quantified by counting the dead (red) cells in proportion the live ones. Samples that had been frozen at −20 °C were thawed and served as positive controls.

2.6. Immunoprecipitation and western blot analysis

Immunoprecipitation was performed as previously described [21]. Briefly, after being washed in ice-cold PBS, chondrocytes were lysed in