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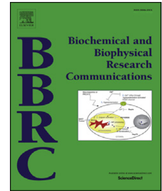

Effects of vimentin disruption on the mechanoresponses of articular chondrocyte

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ABSTRACT

Human articular cartilage is subjected to repetitive mechanical loading during life time. As the only cellular component of articular cartilage, chondrocytes play a key role in the mechanotransduction within this tissue. The mechanoresponses of chondrocytes are largely determined by the cytoskeleton. Vimentin intermediate filaments, one of the major cytoskeletal components, have been shown to regulate chondrocyte phenotype. However, the contribution of vimentin in chondrocyte mechanoresponses remains less studied. In this study, we seeded goat articular chondrocytes on a soft polyacrylamide gel, and disrupted the vimentin cytoskeleton using acrylamide. Then we applied a transient stretch or compression to the cells, and measured the changes of cellular stiffness and traction forces using Optical Magnetic Twisting Cytometry and Traction Force Microscopy, respectively. In addition, to study the effects of vimentin disruption on the intracellular force generation, we treated the cells with a variety of reagents that are known to increase or decrease cytoskeletal tension. We found that, after a compression, the contractile moment and cellular stiffness were not affected in untreated chondrocytes, but were decreased in vimentin-disrupted chondrocytes; after a stretch, vimentin-disrupted chondrocytes showed a lower level of fluidization-resolidification response compared to untreated cells. Moreover, vimentin-disrupted chondrocytes didn't show much difference to control cells in responding to reagents that target actin and ROCK pathway, but showed a weaker response to histamine and isoproterenol. These findings confirmed chondrocyte vimentin as a major contributor in withstanding compressive loading, and its minor role in regulating cytoskeletal tension.

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1. Introduction

The cytoskeleton of chondrocyte consists of actin microfilaments, tubulin microtubules, and vimentin intermediate filaments (VIFs), all of which play an important role in maintaining chondrocyte biological as well as mechanical function. Actin predominantly provides the cell with mechanical integrity (shape, stiffness, etc), and to some extent controls chondrocyte phenotype. It has been shown that the increase of actin stress fibers inhibits chondrogenic differentiation [1–3], and the disruption of stress fibers restores chondrocyte phenotype [4–6]. Tubulin is mainly responsible for intracytoplasmic transport [7]. Vimentin also contributes to chondrocyte phenotype, as disruption of vimentin in vitro results in reduction of type II collagen and aggrecan synthesis [8], and altered levels of vimentin are observed in situ in both human [9] and rat [10] osteoarthritic chondrocytes. Moreover, vimentin is thought to be involved in the mechanical function of chondrocyte, which is supported by the observation of increased vimentin expression in chondrocytes of weight-bearing cartilage [11].

However, the role of VIFs in the mechanical properties of chondrocytes is still unclear. Trickey et al. reported that disruption of VIFs doesn't affect the stiffness of chondrocytes in 2D culture [12]. In contrast, Haudenschild et al. found that disruption of vimentin substantially reduce the stiffness of chondrocytes in 3D culture [13]. These results suggest a different organization of vimentin cytoskeleton between 2D and 3D culture of chondrocytes. Moreover, the contribution of vimentin cytoskeleton to the mechanoresponses of chondrocytes is less studied. Ofek et al. found that the removal of VIFs causes chondrocytes to become incompressible to compressive strains [14]. Note that in their study chondrocytes were isolated from extracellular matrix (ECM), which

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might affect the force transmission from ECM to cytoskeleton. To systematically investigate the effects of vimentin disruption on chondrocyte mechanoresponses, in this study we seeded goat articular chondrocytes on polyacrylamide (PA) substrate, applied a transient stretch or compression, and compared the changes of cellular stiffness and traction force between acrylamide-treated cells and untreated control. In addition, effects of vimentin disruption on the intracellular force generation were examined by the real-time measurement of cellular stiffness upon administration of contractile or relaxation reagents. Our results would provide a comprehensive understanding in mechanoresponses of chondrocytes grown on a soft matrix to mechanical and chemical stimulation.

2. Materials and methods

2.1. Cell culture

Primary Spanish goat articular chondrocytes were kindly provided by Dr. Myron Spector (Harvard University). Cells were plated onto soft PA gel substrates at the density of 4000 per substrate in Advanced Dulbecco's Modified Eagle Medium (DMEM; Gibco 12491) supplemented with 10% (v/v) fetal bovine serum (FBS; Hyclone Technologies, Logan, UT), and 1% (v/v) penicillin/streptomycin (Life Technologies). Culture medium was changed every 2 days. Cells were serum starved overnight before measurement. To disrupt VIFs, chondrocytes were treated with 4 mM acrylamide for 24 h, as previous studies have shown that 4 mM acrylamide substantially disrupt vimentin network without interrupting other cytoskeletal components or inducing cytotoxic effect [12,13]. The following reagents were used to change cytoskeletal tension of chondrocytes: 20 μM histamine (Sigma−Aldrich), 10 ng/ml Interlukin-1β (IL-1β; R&D Systems), 10 ng/ml transforming growth factor-β1 (TGF-β1; R&D systems), 10 μM isoproterenol (Sigma−Aldrich), 10 μM Y-27632 (Sigma−Aldrich), and 0.1 μM latrunculin-A (Sigma−Aldrich).

2.2. Fabrication of PA substrates

PA gel substrates were prepared according to our previous studies [15,16]. The glass bottom of 35 mm dishes (P25-G-020-C, MatTek Corporation, MA) was treated with bind silane for 30 min, washed and air dried. Then, 300 uL gel solution consisting of 5% (v/v) acrylamide (Bio-Rad, Hercules, CA), 0.1% (v/v) bis-acrylamide (Bio-Rad), 0.6% (v/v) fluorescent bead suspension (0.5 μm, pink, Invitrogen, Eugene, OR) and ultrapure water was mixed with 5% (v/v) ammonia persulphate (Bio-Rad) and 0.05% (v/v) TMMED (Bio-Rad) successively. The mixture was then added to the center of glass bottom of each dish and covered by a 25 mm circular coverslip (VWR) to yield gel with a final thickness of ~700 μm. After gel polymerization, the coverslips were gently removed with a forceps, and gels were surface-activated using 200 μL of 1 mM sulpho-succinimidyl-6-[4-azido-2-nitrophenyl-amino]hexanoate (Sulpho-SANPAH; Pierce, Rockford, IL) under UV light for 10 min. These gel substrates were then coated with type I collagen (0.1 mg/mL in PBS; Advanced BioMatrix, San Diego, CA) and stored overnight at 4 °C. On the next day, the collagen solution was removed, and the gels were kept in PBS at 4 °C till use. We set the ratio of acrylamide to bis-acrylamide at 5%: 0.1% to generate gel substrates with Young's modulus of 4 kPa, as 4 kPa PA substrates showed good results in maintaining chondrocyte phenotype in vitro [17].

2.3. Optical Magnetic Twisting Cytometry

The stiffness of chondrocytes was probed using Optical Magnetic Twisting Cytometry (OMTC). Detailed descriptions of this method have been given elsewhere [18,19]. RGD-coated ferrimagnetic beads (4.5 μm in diameter) were incubated with cells for 20 min to allow the beads bind to cell surface receptors that link to the underlying cytoskeleton. Then, a gel substrate was mounted to microscope stage equipped with bead twisting setup. The beads were magnetized horizontally and then twisted in an oscillatory magnetic field with a frequency of 0.75 Hz. This exerted a sinusoidal torque that caused the beads to twist, with resulting back-and-forth horizontal translation. The motions of beads were recorded with Leica DMIRB CCD camera. The specific torque (T) applied to a bead was computed as $T = mB/V$, where V is the bead volume, m is the bead magnetic moment, and B is the applied magnetic field. The complex elastic modulus (G^*) of the cell was computed from the Fourier transforms of the applied torque T^* and of the resulting bead displacement (d^*), as given by $G^* = T^*/d^* = G' + jG''$, where G' is the storage modulus, which we referred to as cellular stiffness (in Pa/nm), G'' is the loss modulus, and $j^2 = -1$. Baseline cellular stiffness was denoted as G'_0. Due to variability in G'_0 from different experimental batches and groups, G' was normalized to G'_0 in each experiment so that the stiffness response to interventions could be compared.

2.4. Traction force microscopy

As described previously in Butler et al. [20], the traction field was calculated from a displacement vector map of changes in bead positions using Fourier transform traction cytometry (FTTC). This field was used to calculate the root-mean-square traction (RMST), which is a scalar measure of the cell's net contractile strength. Compression/stretch experiments were conducted as follows: baseline images of the cell and beads were recorded and then homogeneous isotropic compression/stretch of 10% strain was applied. Bead images were collected immediately after and 10 min after compression, followed by a final reference image of the gel after the cell's removal by trypsin. Compression and stretch were applied with annular indenters as described in Krishnan et al. [21].

2.5. Fluorescent staining

Cells were fixed in 4% paraformaldehyde in PBS for 10 min, and permeabilized in PBS containing 0.25% Triton X-100 (Sigma−Aldrich) for 10 min. Non-specific bindings were blocked with 1% bovine serum albumin (BSA; Sigma−Aldrich) in PBS for 30 min. For actin, fixed cells were stained with Alexa 594 Phalloidin (Molecular Probes) for 30 min at room temperature; for vimentin, fixed cells were incubated with mouse anti-vimentin primary antibody (1:100, Sigma−Aldrich) for 1 haat room temperature, and Alexa 488 donkey anti-mouse secondary antibody (1:400 dilution, Abcam) for another 1 h at room temperature in the dark. Nuclei were stained with 0.1 μg/mL 4′6-diamidino-2-phenylindole (DAPI; Sigma−Aldrich) in PBS for 1 min. Immunostained preparations were observed by Leica DM 6000 B microscope, and images were acquired using Leica Application Suite Advanced Fluorescence (LAS AF). To quantify the intensity of stained actin and vimentin, at least three staining images for each experimental group were analyzed using ImageJ.

2.6. Statistical analysis

Statistical analysis was performed with SigmaStat 3.5 (Systat Software, Chicago, USA). Data of cell stiffness measurement were presented as mean ± SEM, and other data were presented as mean ± SD. A two-tailed Student t test was performed to determine the statistical significance between two groups. A value of $p < 0.05$

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