Chondrocyte Migration is Modulated by Osmotic Environment

[1]Wen, S M; [2]Tang, A R; [2]Hung, C T; +[1]Chao, P G
[1]National Taiwan University, Taipei, Taiwan, [2]Columbia University, New York, NY
+pgchao@ntu.edu.tw

INTRODUCTION

In situ osmolarity of cartilage tissue is estimated to be 350-450 mOsm [1-2]. In joint injury and arthritis, breakdown of tissue macromolecules and increasing water content is estimated to result in a hypotonic environment (~300 mOsm), which can also be measured from the synovial fluid [3-4]. Many studies have investigated the influence of extracellular osmolarity on chondrocytes, which are usually cultured in medium of ~300 mOsm [5-7]. We previously reported the effects of medium osmolarity on chondrocyte cytoskeleton organization and hydraulic permeability [8-9]. In the current study, we hypothesize that extracellular osmotic conditions modulate chondrocyte migration.

SIGNIFICANCE

Motility is closely related to wound healing. Findings from this study can improve the current understanding of chondrocyte mechanobiology as well as migration behavior, providing possible new insights for development of novel strategies for cartilage repair.

METHODS AND MATERIALS

Cell Culture: Primary bovine chondrocytes were isolated via enzymatic digestion from calf wrist joints and cultured in DMEM with 10% FBS. Prior to experiments, cells were trypsinized from the culture plate and equilibrated in different osmotic solutions for 2 hours prior to experiments. Osmotic Solutions: Osmotic medium was made by adding sucrose to DMEM to attain an osmolarity of 410 mOsm, while the isotonic culture medium was 310 mOsm, as confirmed by an osmometer (Advanced Instruments). Image and Data Analysis: Cell size was analyzed with a custom segmentation program in Matlab [10]. Migration distance was quantified with MetaMorph and used to calculate the migration speed. Gene Expression Study: Cells were harvested and total RNA isolated with TRIzol reagent (Invitrogen) after equilibration in the osmotic medium. RNA was reverse-transcribed and the GAPDH, type II collagen (COLII), and aggrecan (AGC) expression levels were analyzed using the iCycler real-time PCR system and iQ SYBR Green supermix (Bio-Rad). Adhesion Strength Assay: The parallel-plate flow chamber as described for the migration study was also used to subject laminar flow to cultured cells. Fluid flow was controlled by a pulse-less pump (Fisher). Step shear flows of warm PBS from 0 to 800 dynes/cm^2 were applied with 40 dynes/cm^2 increments and cells were imaged at the end of each interval. In this manner, the percentage of cells detached as a function of shear stress was obtained. Cell adhesion strength was determined from the shear stress level where half of the cells were detached [11]. Cytoskeleton Organization: Chondrocytes were equilibrated in the respective osmotic medium for one hour and fixed and stained with Oregon Green-phalloidin (Invitrogen). Quantitative analysis of actin distribution was conducted by normalizing the intensity of the cortical and plasma regions to the nuclear region [8]. Statistical Analysis: All studies were repeated at least three times. Statistics were performed by Statistica (StatSoft) using ANOVA and LSD post hoc test with α=0.05.

RESULTS

Medium osmolarity changes chondrocyte volume and gene expression profile, which is indicative of the arthritic phenotype (Figure 1, [12]). Cell migration speed was also significantly altered with medium osmolarity, with higher migration speed in the more physiologic osmotic condition (410 mOsm, Figure 2). This increase in migration speed corresponds with decreased attachment strength (Figure 2, [13]). Examination of cytoskeleton organization revealed a more spread morphology for chondrocytes of the 310 mOsm group, which displayed more pronounced attachment plaques (Figure 3, arrows). In addition, chondrocytes from the 410 mOsm group exhibited a more peri-nuclear distribution of their actin cytoskeleton.

DISCUSSION

The osmotic environment significantly impacts the phenotype and motility of chondrocytes. Analysis of the actin cytoskeleton reveals less prominent cytoskeletal organization in 410 mOsm medium, corroborated with decreased attachment strength (Figures 2 and 3). We previously demonstrated that the extracellular osmotic environment changes the membrane hydraulic permeability of chondrocytes [9], which is partly mediated by the water channel aquaporin (AQP, [14]). AQP expression is modulated in osmotic challenged chondrocytes as well as diseased tissues [15]. Moreover, AQP has been known to localize at the leading edge of lamellipodia [16] and co-localizes with Na^+/H^+ and Cl^-/HCO_3^- exchangers [17], which mediates regulatory volume increase/decreases in response to osmotic shock [18]. Taken together with these studies, our results suggest that the osmotic environment may modulate homeostasis of the plasma membrane, such as the quantity and activity of AQP and ion exchangers that lead to altered cytoskeleton organization, attachment and cell migration. Our finding may have implications in cartilage wound healing and degeneration progression.

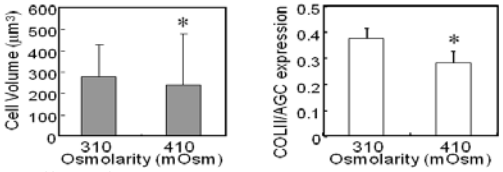

Figure 1. Effects of medium osmolarity on chondrocyte cell volume and phenotypic expression ratios (n=152-395 for cell volume, n=6 for gene expression, *p<0.05).

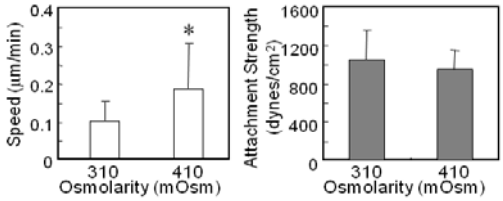

Figure 2. Effects of medium osmolarity on chondrocyte migration speed and attachment strength (n=102-138 cells for migration, n=201-230 cells for attachment, *p<0.05)

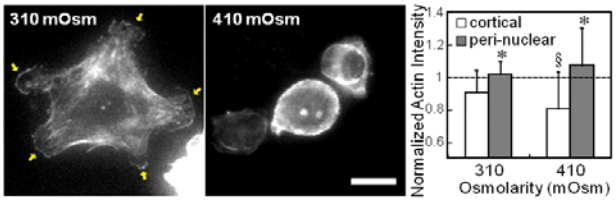

Figure 3. Chondrocyte actin cytoskeleton organization at different osmotic levels. Attachment plaques are indicated with yellow arrows. Scale bar = 10 μm. (n=36-53, *p<0.05 vs cortical region, §p<0.05 vs 310 mOsm group)

ACKNOWLEDGEMENT

This work was supported by NSC (PGC: 99-2221-E-002-044, 100-2221-E-002-142), the NHRI (PGC: EX100-10019EC), the NTU International Collaboration Grant, and the NIH (CTH: AR52871).

REFERENCES

[1] Maroudas A, Evans H (1972). Conn Tiss Res, (1): 69. [2] Urban JP, Hall AC (1992). Articular Cartilage and Osteoarthritis, 393. [3] Newman JP, Grana WA (1988). Arthroscopy, (4): 179. [4] Shanfield S+. (1988). Clin Orthop Relat Res, 289. [5] Urban JP+. (1993). J Cell Physio, (154): 262. [6] Erickson GR+. (2003). Osteoarthritis Cartilage, (11): 187. [7] Hall AC, Bush PG (2001). Pflugers Arch, (442): 771. [8] Chao P-HG+. (2006). Am J Physiol Cell Physiol, (291): C718. [9] Oswald E+. (2008). Cell Mol Bioeng, (1): 339. [10] Chao P-HG+. (2005). Journal of Biomechanics, (38): 1273. [11] Palecek S+. (1998). J Cell Sci, (111): 929. [12] Matyas JR+. (2002). Arthritis & Rheumatism, (46): 1536. [13] DiMilla PA+. (1993). J Cell Biol, (122): 729. [14] Elmoazzen HY+. (2002). Cryobiology, (45): 68. [15] Wang F, Zhu Y (2011). Journal of Orthopaedic Science, (16): 304. [16] Monzani E+. (2009). PLoS ONE, (4): e6167. [17] Schwab A (2001). Am J Physiol Renal Physiol, (280): F739. [18] Yamazaki N+. (2000). Acta Physiologica Scandinavica, (169): 221.