Integrin α1β1 Is Necessary For Primary Cilia Shortening In Response To Hypo-osmotic Stress

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Disclosures:

Introduction: Osteoarthritis (OA) is a degenerative joint disease characterized by the degradation of articular cartilage and inflammation of the synovium. During the progression of OA, negatively charged proteoglycans are lost from the cartilage decreasing the charge to water ratio and leaving the extracellular matrix chronically hypo-osmotic [1]. During cartilage loading, chondrocytes experience fluctuations in their osmotic environment due to water exudation and reabsorption by the extracellular matrix [2].

Integrins are heterodimeric transmembrane glycoproteins that bind collagens in the extracellular matrix and cytoskeletal elements in the cytoplasm. Chondrocytes express eight different integrins including integrin α1β1 that binds collagen II and VI [3]. Importantly, expression of integrin α1β1 is upregulated during the early stages of OA and integrin α1-null mice develop spontaneous OA 2-3 months earlier than wild type controls [4].

Primary cilia are microscopic organelles that are also involved in signal transduction events. The cation channel primarily responsible for chondrocyte response to osmotic stress, transient receptor potential vanilloid 4 (TRPV4), is known to be present on the cilia [5,6]. Wild type chondrocytes respond to hypo-osmotic challenge with intracellular calcium transients [7] and by shortening their primary cilia [8]. Integrin α1-null chondrocytes however, are insensitive to hypo-osmotic stress as measured by intracellular calcium transients [7]. It is currently unknown if integrin α1β1 is necessary for primary cilia shortening after osmotic challenge.

Therefore, the purpose of this study was to measure cilia length changes in response to osmotic stress in ex vivo wild type and integrin α1-null chondrocytes. We hypothesized that integrin α1β1 is necessary for osmotically induced cilia length change to occur.

Methods: All animal procedures were approved by the University of Calgary Animal Care Committee. Female (age=4±0.5 months(mean±sd)) wild type (mass=25±4 g) and integrin α1-null mice (mass=30±3 g) were sacrificed and femora were isolated. The femora were incubated with 125 nM Tubulin Tracker and 4 μM Ethidium homodimer-1 to stain chondrocytes for polymerized tubulin and the nuclei of dead cells respectively. Stained femora were washed, glued to a glass coverslip, placed in a heated (37°C) perfusion chamber and submerged in iso-osmotic (300mOsM) media. Iso-osmotic media was withdrawn and replaced with new iso-, hypo- (200mOsM) or hyper-osmotic (400mOsM) media. After one minute, chondrocytes from the centre of the medial condyle were imaged using a 60x/0.9 N.A. dipping objective lens (Olympus). Confocal microscopy (Olympus Fluoview FV1000, excitation 488 nm, emission 500-600 nm and 630-730 nm) was used to take Z-stack images (step size 200nm, sampling frequency > 3x Nyquist frequency in X,Y and Z) of cilia (Figure 1). 3D images of the cells were created from the Z stacks using FV10-ASW 4.0 Viewer software (Olympus) and cilia length was measured. Statistics were conducted using either multivariate ANOVA with Fisher LSD post hoc or Chi-squared tests.

Results: Independent of genotype, the majority of chondrocytes (>70%) had two cilia and cilia found in pairs were shorter (1.53μm) than single cilia (1.80μm) (p<0.001) (Figure 1). Cilia were predominately located close to the nucleus on the basal side of the chondrocyte, oriented in the axial direction (Figure 1). Cilia on wild type chondrocytes were 1.68μm long under iso- or hyper-osmotic conditions, and shortened 0.2μm after hypo-osmotic stress (p=0.05) (Figure 2).

Discussion: Here we show that integrin α1β1 plays a critical role in controlling cilia shortening in response hypo-osmotic stress. Previous studies have shown that integrin α1-null and TRPV4-null chondrocytes fail to respond to hypo-osmotic stress with intracellular calcium transients [6,9]. Altogether these data suggest an interplay between the collagen receptor integrin α1β1 and TRPV4 in chondrocyte transduction of osmotic stress.

In this study we have measured the cilia length of live chondrocytes in 3D for the first time. The range of primary cilia lengths reported here are consistent with previous measurements of adult bovine (1.1-1.5μm) [10] and infant canine articular cartilage explants (1.76μm) [11]. The cilia of wild type chondrocytes shortened 0.2μm under hypo-osmotic challenge, which is 0.7μm less than previous studies measuring compression induced cilia shortening of chondrocytes in agarose constructs [12]. It is important to note however, that chondrocytes in agarose have longer cilia (2.2μm) under control conditions compared to those measured ex vivo (1.68μm) [12].

In addition to the aforementioned changes in cilia length, we also report that more than 70% of chondrocytes had two cilia, independent of genotype. This contrasts previous findings of the majority of chondrocytes having only one cilia [10,13]. By reconstructing the 200 nm confocal Z stacks in 3D, rather than working with 2D histological slices we have been able to
distinguish the abundance of pairs of cilia in an axial orientation for the first time.

**Significance:** Current treatments for OA enable patients to manage the symptoms, but do not modify the damage to joint tissues and structures. By improving our understanding of the molecular events underlying this debilitating disease, we hope to identify novel and effective targets for pharmaceutical intervention that may slow or ideally stop the progression of OA.

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