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Brief report

Nanomechanical phenotype of chondroadherin-null murine articular cartilage

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ABSTRACT

Chondroadherin (CHAD), a class IV small leucine rich proteoglycan/protein (SLRP), was hypothesized to play important roles in regulating chondrocyte signaling and cartilage homeostasis. However, its roles in cartilage development and function are not well understood, and no major osteoarthritis-like phenotype was found in the murine model with CHAD genetically deleted ($CHAD^{-/-}$). In this study, we used atomic force microscopy (AFM)-based nanoindentation to quantify the effects of CHAD deletion on changes in the biomechanical function of murine cartilage. In comparison to wild-type (*WT*) mice, CHAD-deletion resulted in a significant ≈70–80% reduction in the indentation modulus, E_{ind}, of the superficial zone knee cartilage of 11 weeks, 4 months and 1 year old animals. This mechanical phenotype correlates well with observed increases in the heterogeneity collagen fibril diameters in the surface zone. The results suggest that CHAD mainly plays a major role in regulating the formation of the collagen fibrillar network during the early skeletal development. In contrast, CHAD-deletion had no appreciable effects on the indentation mechanics of middle/deep zone cartilage, likely due to the dominating role of aggrecan in the middle/deep zone. The presence of significant rate dependence of the indentation stiffness in both *WT* and $CHAD^{-/-}$ knee cartilage suggested the importance of both fluid flow induced poroelasticity and intrinsic viscoelasticity in murine cartilage biomechanical properties. Furthermore, the marked differences in the nanomechanical behavior of *WT* versus $CHAD^{-/-}$ cartilage contrasted sharply with the relative absence of overt differences in histological appearance. These observations highlight the sensitivity of nanomechanical tools in evaluating structural and mechanical phenotypes in transgenic mice.

1. Introduction

The mechanical function of articular cartilage is determined by its extracellular matrix (ECM). Cartilage ECM is mainly composed of highly negatively charged aggrecan proteoglycans enmeshed within the type II/IX/XI heteropolymeric collagen (Eyre et al., 2006) fibrillar network (Maroudas, 1979; Han et al., 2011b). Proper assembly and organization of this ECM *in vivo* are regulated by a variety of secondary matrix proteins and proteoglycans, including collagen VI, thrombospondins, small leucine rich proteoglycans/proteins (SLRPs) and matrilins (Heinegård, 2009). Despite their low concentrations in native cartilage, several of these molecules directly bind to the chondrocyte cell surface receptors to govern cell signaling, and others form important networks within the pericellular matrix. They can also bind pro-collagen molecules and, in many cases, remain bound to the newly formed fibers to provide additional stability and connectivity to other structural networks (Heinegård, 2009; Kalamajski and Oldberg, 2010; Iozzo et al., 2011).

Our study focuses on the roles of one particular regulatory molecule, chondroadherin (CHAD) (Larsson et al., 1991), a non-canonical class IV SLRP (Schaefer and Iozzo, 2008). CHAD is a 38 kD protein with 11 leucine-rich repeats (LRR) (Neame et al., 1994). It is localized within the epiphyseal growth plate during skeletal development and in the pericellular and territorial matrices in mature cartilage (Shen et al., 1998). In cartilage, CHAD mediates signaling between chondrocytes

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and the ECM through binding to the α2β1 integrin (Camper et al., 1997; Haglund et al., 2011) and to cell surface proteoglycans such as syndecans (Haglund et al., 2013) (Fig. 1). CHAD also binds type II collagen and interacts with both the N- and C-terminal globular domains of type VI collagen (Camper et al., 1997; Månsson et al., 2001). It has thus been hypothesized that CHAD plays a critical role in regulating linkages between collagens and other ECM molecules *in vivo*, as well as the communication between chondrocytes and their surrounding matrices. Recently, gross histological and protein compositional analyses of CHAD-null murine joints have provided evidences that genetic deletion of CHAD can result in a distinct skeletal phenotype characterized by alterations in trabecular and cortical bone, widening of the epiphyseal growth plate, and variation in the molecular composition of cartilage, though no major osteoarthritis-like phenotype was found (Hessle et al., 2013). However, there is still a lack of understanding of whether deletion of the CHAD gene affects the biomechanical properties of articular cartilage, which are essential to the proper joint tissue function. Such changes in biomechanical properties may in the long term be linked to the initiation and progression of osteoarthritis.

In this study, we applied atomic force microscopy (AFM)-based nanoindentation to directly quantify the nanomechanical phenotype of CHAD-null murine articular cartilage. We assessed and compared the local biomechanical and collagen nanostructural properties of CHAD-null ($CHAD^{-/-}$) and wild-type (*WT*) murine knee cartilage in different depth-dependent zones (superficial and middle/deep zones) and at different ages. We found that the absence of CHAD significantly changed the collagen network assembly and mechanical properties of the superficial zone cartilage. These findings support the hypothesis that CHAD plays a critical role in the proper organization and function of cartilage ECM. In contrast, no differences in middle/deep zone cartilage were found. This study provides an important mechanics-based insight into how quantitatively minor ECM molecules, such as CHAD, may affect the biophysical functioning of cartilage otherwise thought to be dominated by collagens and aggrecan.

2. Results

AFM-based nanoindentation measures the indentation force, *F*, as a function of depth into the tissue, *D*, as the microspherical tip indents into the tested sample (i.e., cartilage) at a given rate (μm/s) (Fig. 2a). The *F–D* indentation curves were fit to the linear elastic Hertz model to account for the spherical indentation geometry and calculate the effective indentation modulus, E_{ind}. The values of E_{ind} thus depict the effective resistance of cartilage to indentation at the measured rate. For both $CHAD^{-/-}$ and *WT* cartilage, the *F–D* data were fit well by the predictions of the Hertz model (e.g., Fig. 2a, with least squares linear regression giving $R^2 > 0.96$). For each mouse type and age group, significant variation in E_{ind} was found between different mice within the same cohort (Kruskal–Wallis test, $p < 0.05$, Fig. 2b). In addition, heterogeneity in E_{ind} was also found between different locations on the same joint. All these variations are likely associated with differences in proteoglycan content and local collagen cross-link density within and between different joints given the known heterogeneous nature of cartilage (Hunziker et al., 2007; Han et al., 2011b). From each mouse, there was no significant difference between the E_{ind} measured on cartilage from left versus right knee (Fig. S1). Therefore, the average value of E_{ind} for the indents on cartilage of both knees from the same mouse was used to compare the effects of age and CHAD deletion (Fig. 3).

For all tested age groups, CHAD deletion resulted in a significant ≈70–80% reduction in E_{ind} of the superficial layer cartilage at all tested rates ($p < 0.0001$, two-way ANOVA on the global rank transforms) (Conover and Iman, 1981) (Fig. 3). This effect was even more prominent at the faster rate and younger ages (Fig. 3). This biomechanical difference correlates well with greater heterogeneity in the type II collagen-dominated fibril diameter distribution (*F*-test, $p < 0.0001$) observed on $CHAD^{-/-}$ mice at both 11 week and 4 month age groups measured via SEM (Fig. 4). In comparison to the distinctive nanomechanical phenotype in the superficial layer, no significant effect of CHAD deletion was found on the E_{ind} of the middle/deep zone cartilage cross-section at all tested rates ($p > 0.05$, two-way ANOVA on the global rank transforms) (Conover and Iman, 1981) (Fig. S2). In addition, significant indentation rate dependence was observed for both *WT* and $CHAD^{-/-}$ mice (Friedman's test, $p < 0.05$, Figs. 3, S3). Interestingly, in contrast to the distinct biomechanical phenotype of $CHAD^{-/-}$ mice measured by AFM nanoindentation, histological analysis did not yield any appreciable differences between *WT* and $CHAD^{-/-}$ mice with respect to the gross-level morphology and toluidine blue proteoglycan staining (Fig. 5).

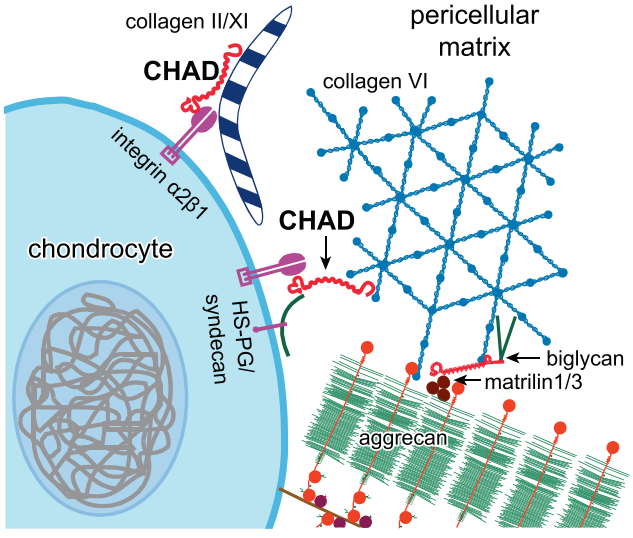

Fig. 1. Schematic of the roles of chondroadherin in mediating chondrocyte signaling through bindings to the α2β1 integrin (Camper et al., 1997; Haglund et al., 2011) and surface proteoglycan syndecans (Haglund et al., 2013).

3. Discussion

3.1. Roles of CHAD in murine cartilage mechanical properties

The weakening of cartilage upon CHAD deletion (Fig. 3) appears to be consistent with our hypothesis that CHAD has a biomechanically important function in the formation of an appropriately assembled fibrillar collagen network (Månsson et al., 2001) despite its low abundance (compared to collagen and aggrecan) and spatial localization within the territorial matrix (Shen et al., 1998). Lack of CHAD appears to slow down the development of the load bearing ECM, possibly due to alterations in both the chondrocyte cell signaling, as well as the assembly and linkages of the fibrillar collagen network. These effects could alter the cross-linking of the collagen network and, in turn, the local osmotic swelling and hydraulic permeability of the resident aggrecan. The fact that we observed increased heterogeneity in cartilage surface collagen fibrils (Fig. 4) further supports this statement. This effect is most salient in the cartilage superficial layer where the concentration of aggrecan is relatively low. Furthermore, the linkages between the collagen networks (types II and VI) and chondrocytes provided by CHAD (Camper et al., 1997; Haglund et al., 2011; Haglund et al., 2013) could have an effect of on the pericellular matrix. Indeed, in the superficial zone, a region with rather dense cell concentration (Stockwell, 1971), we detected significant differences in biomechanical properties (Fig. 3).

In comparison, we found CHAD deletion had no effects on the E_{ind} of the middle/deep zone cartilage (Fig. S2), where aggrecan concentration is substantially higher (Maroudas, 1979). Given the dominating role of aggrecan in cartilage middle/deep zone nanomechanics (Han et al., 2011a), the effects of changes in collagen fibril assembly on tissue