Estrogen-Independent Sex Differences in Chondrocytes Response to Mechanical Stimulation

Zahra Barati[1], Ze Yu[1], Chao Xing[1], Paula A. Hernandez[1]
[1]University of Texas Southwestern Medical Center, Dallas, TX
Paula.Hernandez2@utsouthwestern.edu

Disclosures: P.A.H. serves on the ORS DEI Committee and the ORS Open Door 2024 Committee. Z.B. currently works for Reata Pharmaceuticals.

INTRODUCTION: The prevalence of knee osteoarthritis is higher in women. Although sex hormones might be key drivers for this disparity, they are not responsible for all sex-specific responses. We have previously shown sex differences in cartilage extracellular (ECM) and pericellular matrix (PCM) components [1] and in the gene expression of LOXL2, SOX9, and PIEZO1 after cyclic compression [2]. This was observed in regular culture media that has not been depleted from sex hormones. Here, we hypothesize that there are sex-specific biological responses of chondrocytes to mechanical stimulation, depending on their chromosomal constituency (i.e. XX vs XY) and independent of sex hormones, which can be further modulated by sex hormones.

METHODS: Cell culture: Articular chondrocytes were isolated from bovine knee articular cartilage (n = 5♂ + 5♀, 24-30 months old). Cells were expanded in monolayers for up to 14 days in complete media (DMEM + 10% FBS + 1x antibiotics-antimycotics + 50 µg/ml ascorbic acid), and transferred to alginate discs (1.2% alginate, 4 x 10^6 cells/mL alginate, 3mm thickness and 10 mm diameter). Alginate-embedded cells were grown for 5 days to allow for PCM synthesis, in absence of sex hormones (phenol red-free DMEM + 10% charcoal-stripped FBS + 1x GlutaMAX + 1 mM sodium pyruvate + 1x antibiotics-antimycotics + 50 µg/ml ascorbic acid). For some experiments, 3.7 x 10^{-10}M (100 pg/ml) 17-β Estradiol (E2) was added into the media 1 hour before the loading protocol. Loading: The Electroforce 5500 (TA Instruments) was used to apply cyclic compression. A sinusoidal 10% strain and 1 Hz frequency was applied for 6 cycles of 1 hour load and 3 hours rest. Control non-loaded were left untouched. RNA extraction and qPCR: 30 min after the final loading protocol, cells were extracted from the discs with 55mM sodium citrate. RNA was extracted using the RNeasy kit with DNAse I digestion. cDNA was converted using SuperScript IV VILO (Invitrogen) and qPCR was performed using Taqman (BioRad) with SDHA and YWHAZ as normalizers. Bulk RNAseq: RNAseq was performed by the Next Generation Sequencing Core Facility at UT Southwestern using the Illumina NextSeq 2000. Pathway analysis: Differentially Expressed Genes (DEGs) with p<0.05 between male and female chondrocytes under either control (non-loaded) or after mechanical loading were used to identify enriched molecular pathways using WebGestalt. Statistical analysis: The effect of sex in the response to mechanical stimulation on qPCR data was assessed with Repeated Measures two-way ANOVA using Prism GraphPad v10. Significance was set to 0.05.

RESULTS: Bulk-RNAseq was used to investigate sex differences before and after mechanical loading in absence of sex hormones. From the upregulated DEGs after loading, with fold change (FC) >2 and false discovery rate (FDR) <0.05, there were 104 genes shared between male and female chondrocytes, 88 genes upregulated only in females, and 32 genes upregulated only in males. From the downregulated DEGs with FC <0.5 and FDR <0.05, 76 DEGs were common between male and female, 60 were downregulated only in females, and 60 were downregulated only in males. For the pathway analysis, we first considered all DEGs with p<0.05 when comparing non-loaded control male vs female. The enriched pathways with FDR <0.05 were mostly related to ion channel activity and transmembrane transporter activity (Table 1). The gene ontology database showed that the most enriched biological processes were "biological regulation" and "metabolic process", while the most enriched molecular functions were "protein binding" and "ion binding". To evaluate the effect of mechanical loading, we subtracted those genes shared between males and females under control condition from the loaded condition. Pathway analysis of these subtracted DEGs with p<0.05 showed that the only enriched pathway with FDR <0.05 was calcium ion binding (Table 2). When E2 was added back into the media, qPCR analysis after mechanical loading showed that the expression of the calcium-permeant mechanosensitive channels TRPV4 and PIEZO1 was differential between male and female chondrocytes (Fig. 1A-B). Although the loading-dependent upregulation of LOXL-2 was sex- and E2-independent, the upregulation of SOX9 was observed only in females with or without E2 (Fig. 1C-D).

DISCUSSION: Our results show that there are significant differences between male and female chondrocytes at basal levels, this is, in the absence of mechanical stimulation and in the absence of sex hormones. Those differences appear to be mostly related to the regulation of ion channels activity. Interestingly, calcium ion binding is the only significantly enriched pathway comparing male and female cells after mechanical loading. Supporting this finding, we found that the expression of the calcium-permeant mechanosensitive channels TRPV4 and PIEZO1 was sex-specific. To our knowledge, this is the first indication of basal and intrinsic differences between male and female chondrocytes, depending only on their chromosomal constituency (XX vs XY). Future experiments will identify sex-specific downstream factors linked to calcium ion binding pathways that can be further modulated by sex hormones.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding cellular sex-specific responses to mechanical stimulation in absence of sex hormones can help determine a baseline to study specific hormonal modulation of mechanotransduction. This information can be used to identify key drivers for the sex differences in the prevalence of knee osteoarthritis.

GeneSet	Description	Enrichment Ratio	pValue	FDR
GO:0046873	metal ion transmembrane transporter activity	3.99	2.76E-11	3.13E-08
GO:0015267	channel activity	3.85	6.96E-11	3.13E-08
GO:0022803	passive transmembrane transporter activity	3.84	7.36E-11	3.13E-08
GO:0005244	voltage-gated ion channel activity	5.85	8.33E-11	3.13E-08
GO:0022832	voltage-gated channel activity	5.85	8.33E-11	3.13E-08
GO:0022839	ion gated channel activity	4.42	1.08E-10	3.38E-08
GO:0005216	ion channel activity	3.96	1.39E-10	3.71E-08
GO:0005261	cation channel activity	4.48	1.78E-10	4.09E-08
GO:0022836	gated channel activity	4.30	1.96E-10	4.09E-08
GO:0022857	transmembrane transporter activity	2.63	2.94E-10	5.52E-08

Table 1: Enriched pathways of DEGs between males and females. Control, non-loaded cells

GeneSet	Description	Enrichment Ratio	pValue	FDR
GO:0005509	calcium ion binding	2.68	1.66E-05	3.12E-02
GO:0022857	transmembrane transporter activity	1.92	1.32E-03	9.88E-01
GO:0015085	calcium ion transmembrane transporter activity	4.10	1.67E-03	9.88E-01
GO:1990939	ATP-dependent microtubule motor activity	7.29	2.16E-03	9.88E-01
GO:0005215	transporter activity	1.75	3.10E-03	9.88E-01
GO:0005160	transforming growth factor beta receptor binding	6.29	3.72E-03	9.88E-01
GO:0005262	calcium channel activity	3.97	4.11E-03	9.88E-01
GO:0008083	growth factor activity	3.44	4.40E-03	9.88E-01
GO:0031748	D1 dopamine receptor binding	17.81	5.26E-03	9.88E-01
GO:0070700	BMP receptor binding	17.81	5.26E-03	9.88E-01

Table 2: Enriched pathways of DEGs between males and females, unique from mechanical stimulation.

REFERENCES: [1] Hernandez et al., Cartilage 2022; [2] Moreno et al, ORS 2023, Paper 304

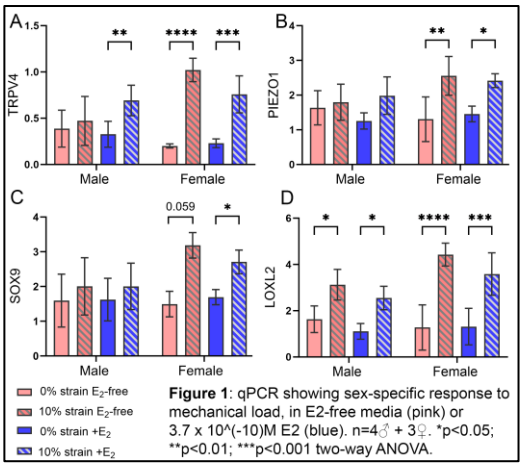

Figure 1: qPCR showing sex-specific response to mechanical load, in E2-free media (pink) or 3.7 x 10^(-10)M E2 (blue). n=4♂ + 3♀. *p<0.05; **p<0.01; ***p<0.001 two-way ANOVA.