Establishing Ovine MSCs as a Translational Model: Continuous Low-Intensity Ultrasound Induces Comparable Chondroprotective Responses in Human and Ovine MSCs

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INTRODUCTION: Continuous low-intensity ultrasound (cLIUS) has emerged as a biophysical stimulus with chondro-inductive and anti-inflammatory potential. To translate cLIUS into clinically relevant models of post-traumatic osteoarthritis, it is necessary to evaluate its MSC chondrogenesis potential effects in an inflammation-rich environment. While inflammatory conditions can be induced *in-vitro* using cytokines, *in-vivo* studies require large-animal models. Sheep are commonly employed for cartilage repair research, but it remains unknown whether ovine MSCs (oMSCs) exhibit responses to cLIUS comparable to human MSCs (hMSCs) at molecular and transcriptomic levels. We hypothesize that cLIUS would induce conserved chondrogenic and anti-inflammatory responses in oMSCs and hMSCs. To test this, we designed a three-stage study: Stage 1 establishes MSC responses to cLIUS in 2D culture, Stage 2 extends analysis to alginate hydrogel culture with transcriptomic profiling under homeostatic and inflammatory conditions, and Stage 3 will be *in-vivo* ovine joint models.

METHODS: Bone marrow–derived oMSCs and hMSCs were cultured in 2D and exposed to IL-1β (10 ng/mL) with or without cLIUS (5 MHz, 2.5 Vpp, 14 kPa, 10 min). qRT-PCR quantified anabolic (SOX9, COL2A1, ACAN) and catabolic (NF-κB, MMP13) markers. Immunofluorescence (IF) measured SOX9 and phosphorylated NF-κB (pNF-κB) localization using corrected nuclear cell fluorescence (CNCF). The hydrogel experiments were performed by encapsulating oMSCs and hMSCs in 1.2% alginate hydrogels and culturing for 28 days with cLIUS ± IL-1β. Samples were collected at days 7 and 14 for qRT-PCR and IF analysis, and on day 28 for qRT-PCR, RNA-seq, and IF (analysis ongoing).

RESULTS: IF staining and quantification showed that in presence of cytokine cLIUS increased SOX9 expression from 0.5-fold to 2-12 fold and reduced pNFκb expression from 8-150-fold to 5-fold in both hMSCs and oMSCs. qRT-PCR analyses (data not shown) supported the IF results, revealing that oMSCs and hMSCs followed similar expression trends under cLIUS stimulation. This data was validated by multiple oMSCs and hMSCs cell lines.

DISCUSSION: These findings demonstrate that both ovine and human MSCs preserve chondrogenic potential under cLIUS stimulation despite inflammatory environment, supporting the hypothesis that cLIUS activates shared anabolic and anti-inflammatory pathways. Ongoing Stage 2 hydrogel culture studies (28 days, RNA-seq pending) will determine transcriptomic concordance between species, and Stage 3 *in-vivo* ovine studies will validate translational relevance for cartilage repair.

SIGNIFICANCE: This study provides proof-of-concept that ovine MSCs respond to cLIUS in a manner comparable to human MSCs, supporting their use as a translational preclinical model. Establishing this cross-species similarity justifies ongoing hydrogel transcriptomic studies and sets the stage for subsequent *in-vivo* ovine experiments, advancing the path toward clinical translation of cLIUS as a non-pharmacologic therapeutic for chondrogenesis.

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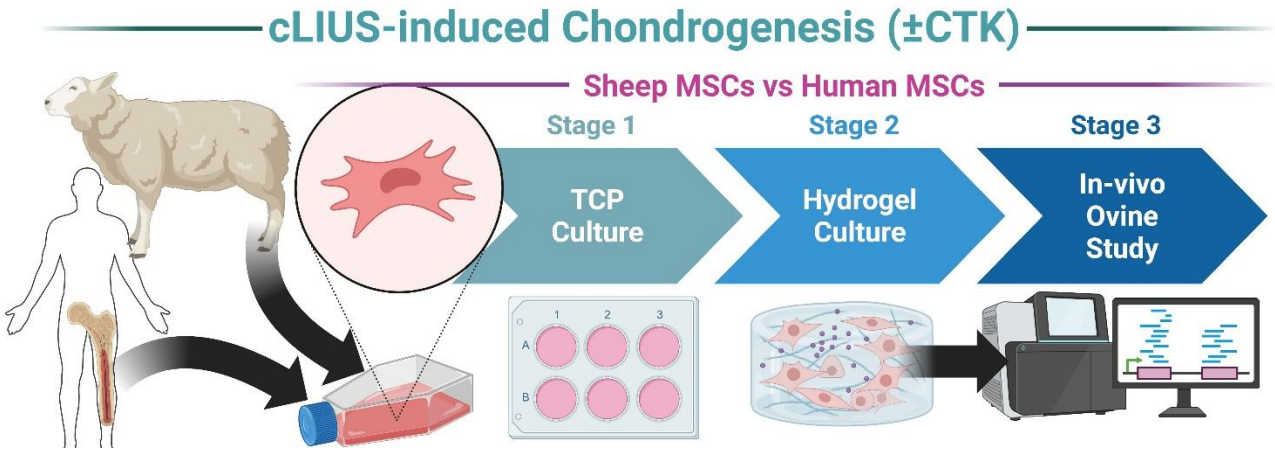

Figure 1: Schematics of Experimental Design for comparing cLIUS-induced chondrogenesis in oMSCs and hMSCs.

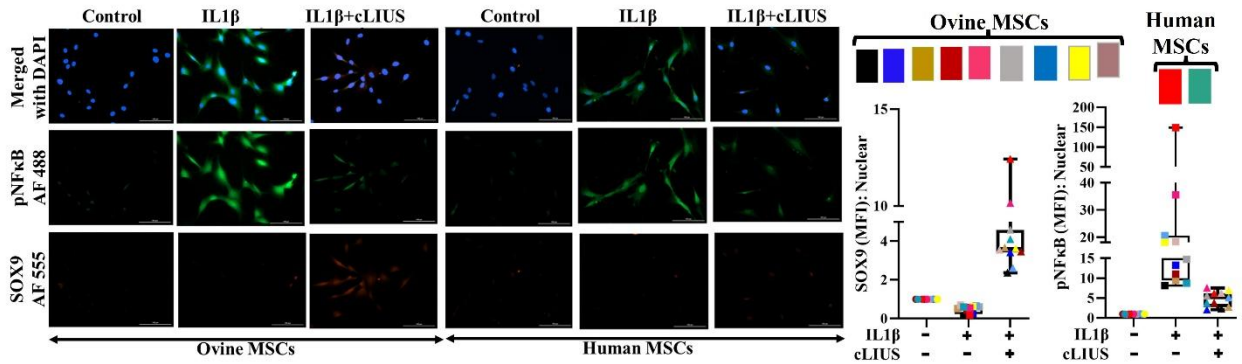

Figure 2: Immunofluorescence staining and quantification of oMSCs and hMSCs showing SOX9 and pNFκb protein expression.