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NOVEL QUANTIFICATION OF MECHANICAL LOAD INDUCED LATENT TGF-BETA ACTIVATION IN ARTICULAR CARTILAGE

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INTRODUCTION

The long-term health of articular cartilage is dependent on the ability of its cells to effectively synthesize, repair, and replace extracellular matrix (ECM) constituents. It is well understood that cell matrix synthesis is mediated in large part by the powerful signaling action of growth factors. However, far less is understood about the mechanobiological mechanisms that regulate growth factor activity during periods of high mechanical demand (e.g., routine loading, overloading, or injury).

An intriguing characteristic of articular cartilage is its remarkably high content of the powerful signaling molecule transforming growth factor beta (TGF-β; up to 300ng/mL [1]). TGF-β is tethered to the cartilage ECM in a unique inactive latent form (LTGF-β), where it awaits a molecular activation trigger. Activation is the critical rate-determining step for bioavailability [2]. While it had traditionally been understood that physiologic activation of LTGF-β occurs exclusively via chemical pathways, a recent paradigm shift led by us [3] and others [4] has uncovered the critical role that physiologic mechanical joint loading plays in the activation of LTGF-β. Recent work demonstrated that loading can activate its ECM-bound LTGF-β stores in cartilage [5]. These discoveries suggest the existence of unique mechanobiological feedback mechanisms whereby mechanical load-induced activation of LTGF-β can promote the maintenance or repair of articular cartilage. This project aims to perform the first ever quantification of load-induced LTGF-β activation in articular cartilage.

Measuring LTGF-β activation in live tissues *in situ* is a major challenge due to the short half-life of activated TGF-β in cartilage (due to rapid receptor internalization/degradation [6]). As such, activation assessments typically require analysis of downstream events. However, assessments of intracellular TGF-β signaling molecules (Smad2/3 phosphorylation) yield mostly qualitative measures and reporter cell assays are not compatible with intact cartilage tissues. Alternatively, we propose the quantification of LTGF-β activation *in situ* through a novel assay that capitalizes on TGF-β's robust autoinduction behavior; active TGF-β activity induces a predictable increase in synthesis of soluble LTGF-β. The dominant fraction of newly synthesized LTGF-β is secreted from the tissue (not retained in ECM) and stable [7]. Accordingly, measurements of LTGF-β secretion into culture medium allows for quantifications of TGF-β activity in cartilage (Fig 1). Levels of activated endogenous TGF-β in medium are negligible due to ECM binding interactions [8] and as such do not confound measurements of newly synthesized LTGF-β. In order to confirm that LTGF-β secretion enhancements result from TGF-β activity (and not other load-initiated signaling cascades), a control group can readily be utilized, consisting of TGF-β activity inhibition from a TGF-β-receptor specific kinase inhibitor. In the current study, we explore the analytical capability of this assay system by examining: 1) the autoinduction relationship between active TGF-β activity and the LTGF-β secretion rate in cartilage explants, and 2) the specificity of TGF-β receptor kinase inhibitors in inhibiting LTGF-β secretion rate enhancements. Lastly, we use the assay to perform a novel quantification of the activity of TGF-β in cartilage explants from load-induced activation.

METHODS

Tissue source: Cylindrical immature bovine cartilage explants were procured within 24h of sacrifice and maintained in chondrogenic medium for a 1-week equilibration period before experiments.

Autoinduction Standards: Small cartilage explants (∅2×2mm) were used to generate auto-induction assay standards, relating activated TGF-β activity to the endogenous LTGF-β secretion rate. Explants were exposed to exogenous active TGF-β3 (aTGF-β3) at 0, 0.1, 0.3,1,3, or

10 ng/mL (n=4 explants per group) for 3 days. Conditioned media was collected daily and assayed for the endogenous LTGF-β1 secretion rate (normalized to explant volume) via an isoform-specific ELISA (Duoset, R&D) that avoids contamination from the exogenous aTGF-β3 (as described previously [1]).

Auto-Induction Assay Specificity: To assess the specificity of a TGF-β kinase inhibitor to inhibit the autoinduction effect, explants were exposed to aTGF-β3 (10ng/mL), FGF-2 (100ng/mL), or IGF-I (200ng/mL) while in the absence (-LY) or presence (+LY) of a TGF-β kinase inhibitor, LY364947 (3μg/mL). For all groups (n=3 explants per group), conditioned media was collected every 2 days and analyzed for endogenous LTGF-β1 secretion rate.

Load-Induced TGF-β Activity Measurements: Cartilage explants (∅3×2mm) were subjected to a daily dynamic loading (DL) regimen (10% compressive strain at 0.3Hz for 1-hour) via a custom bioreactor or maintained free swelling (FS) over 3 days. For both groups, explants were maintained in the absence (-LY) or continuous presence (+LY) of LY364947. Conditioned media of each explant was collected daily and analyzed for endogenous LTGF-β1 secretion rate. Resulting endogenous aTGF-β activity was computed using autoinduction standards of explants exposed to aTGF-β for 1-hour daily (to mimic exposure durations from DL).

RESULTS

Autoinduction Standards: In the absence of aTGF-β3 supplementation, explants exhibited a low, baseline LTGF-β secretion rate of 13.6 ± 2.0 ng/mL/day. This rate increased proportional to aTGF-β3 exposure, reaching 107.4 ± 25.0 ng/mL/day at 10ng/mL aTGF-β3 (Fig 2A).

Auto-Induction Assay Specificity: LTGF-β secretion rate increased with TGF-β3 and FGF-2 exposure. LY inhibited LTGF-β1 secretion enhancements from aTGF-β3 exposure, but did not inhibit LTGF-β1 secretion enhancements from FGF-2, thus confirming the specificity of LY exposure as a negative control of the autoinduction effect (Fig 2B).

Load-Induced TGF-β Activity Measurements: The LTGF-β1 secretion rate increased with DL at days 2 and 3 (Fig 3A). Conversion of LTGF-β1 secretion rates via our autoinduction standard curve, yielded a measure of the load-induced endogenous TGF-β activity at 0.15 ±0.12 ng/mL on average over 3 days (Fig 3B).

DISCUSSION

TGF-β Autoinduction Assay: Results demonstrate that cartilage explants exhibit a robust TGF-β autoinduction response whereby their LTGF-β secretion rate increases proportionally to aTGF-β exposure (Fig 2A). LTGF-β secretion rates can increase ~10-fold from basal levels, supporting a high sensitivity of the assay. Accordingly, LTGF-β secretion measurements can readily be used to quantify *in situ* activity levels of TGF-β in articular cartilage in response to external stimuli.

Load-Induced TGF-β Activation Measurements: Results demonstrate that LTGF-β secretion rates do indeed increase with cartilage mechanical loading (Fig 3A). Upon LY exposure, LTGF-β secretion rates return to basal control levels, thus confirming that LTGF-β secretion enhancements can be predominantly attributed to TGF-β activity in the tissue. Upon standard curve conversion, autoinduction assay results demonstrate that mechanical load-induced activation of ECM-bound LTGF-β gives rise to ~0.15ng/mL of TGF-β activity in cartilage (Fig 3B). Importantly, this measure represents the first quantitative assessment of TGF-β activity in articular cartilage. While these levels represent the activation of only a small fraction of the total LTGF-β stores in the cartilage ECM (~300ng/mL), they are indeed capable of giving rise to considerable chondrocyte biosynthesis enhancements in the tissue [1]. As such, these measurements support the mechanobiological role of load-induced LTGF-β activation in maintaining articular cartilage integrity. The assay platform advanced in this study sets the foundation for considerable advances in our understanding of the mechanistic details and physiologic importance of load-induced LTGF-β activation in cartilage. In the future, we plan to use this quantitative platform to assess: 1) the influence of varying loading regimens on LTGF-β activation rates (e.g. physiologic exercise, elevated stresses, high-impact trauma), and 2) changes to load-induced LTGF-β activation with aging or joint degeneration.

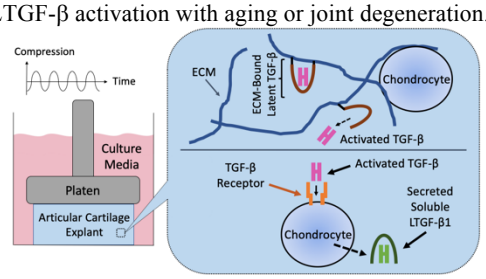

Fig 1: TGF-β autoinduction assay. Endogenous TGF-β activity in cartilage increases the synthesis and secretion of soluble LTGF-β from the tissue. As such, TGF-β activity can be accessed via measurements of LTGF-β secretion rates in culture medium.

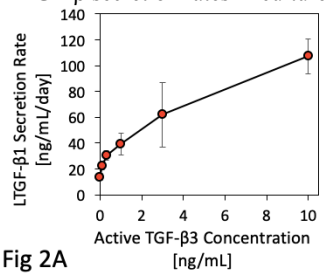

Fig 2: (A) Autoinduction assay standard curve. Explant LTGF-β1 secretion rate increases proportionally with supplemented dose of active TGF-β3. (B) TGF-β-induced enhancements of LTGF-β1 secretion are inhibited by TGF-β kinase inhibitor (LY). Enhancements from other signaling cascades (e.g. FGF-2) are not inhibited by LY. *p<0.05: significance above corresponding ctrl level. *p<0.05: significance from corresponding '-LY INH' value.

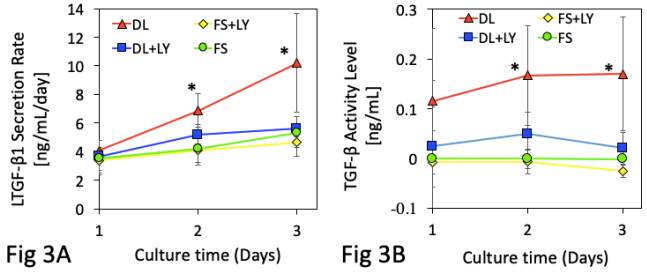

Fig 3: (A) Explant LTGF-β1 secretion rate increases with DL at days 2 and 3 but is suppressed with LY. (B) Explant TGF-β activity levels from standard curve conversion of LTGF-β1 secretion rates. *p<0.05: significant increase above DL+LY levels.

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REFERENCES: [1] Albro+ 2013 Biophys J 104(8):1794-804; [2] Xu+ 2018 Bone Res. 6:2; [3] Albro+ 2012 Osteoarthr. Cartil. 20(11): 1374-82; [4] Ahamed+ 2008 Blood 112(9): 3650-60; [5] Madej+ 2014 Osteoarthr. Cartil. 22(7):1018-1025 [6] Albro+ 2016 Biomaterials 77: 173-185; [7] Wang+ 2019 ORS Abstract No.0453