HMGB1 Induces Sterile Inflammation in Rodent Tendons Subject to Excessive Mechanical Loading

Guangyi Zhao1, Yiqin Zhou1,2, James H-C. Wang1*

1MechanoBiology Laboratory, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA 2wanghc@pitt.edu

INTRODUCTION

The function of tendons is to transmit muscular forces to bones. However, excessive and/or repetitive mechanical stress induces tendinopathy, which is manifested by tendon inflammation and/or degeneration. Thus far, it is not known how mechanical stress translates into the development of tendinopathy.

High Mobility Group Box 1 (HMGB1) is a DNA-binding nuclear protein. After trauma or severe cellular stress, it is released into the extracellular matrix (ECM) and triggers sterile inflammation in injured tissues [1]. Whether HMGB1 is also present in injured tendons is not known. In this study, we performed in vitro and in vivo experiments to detect HMGB1 in tendon cells and matrix. HMGB1 was also implanted in vivo to determine its likely function.

METHODS

In vitro cell stretching experiment - Tendon cells were isolated from SD-rat patellar tendons and cultured in DMEM + 20% FBS. Cells at passage 3 were plated on custom designed silicone dishes with microgrooves to facilitate cell alignment along one direction. Cells were then cyclically stretched for 72 hrs at 4% (moderate) or 8% (excessive) stretching magnitudes [2]. Un-stretched cells in the dishes served as the controls. At the end of stretching, immunostaining was performed to detect HMGB1, and positively stained cells were quantified.

In vivo mouse treadmill running experiment - Mice were randomly divided into 3 groups: Moderate treadmill running (MTR) group: Mice ran for 50 min at the speed of 13m/min, 5 days/week, for 3 weeks; Intensive treadmill running (ITR) group: Mice ran for 3 hrs at the speed of 13m/min, 5 days/week, for 3 weeks. Control group: Mice were free in cages. After running, the Achilles and patellar tendons were harvested from 6 mice/group to measure HMGB1 levels in the tendon by ELISA, and stain for HMGB1 by immuno-histochemistry. The experiment was repeated twice with 36 mice.

RESULTS

In tendon cells under normal conditions, HMGB1 was observed in the nuclei (Fig. 1A-D). Most HMGB1 were still present in the nuclei after moderate stretching at 4% strain level (Fig. 1E). In contrast, HMGB1 was mostly absent in the nuclei of tendon cells stretched excessively to 8% (Fig. 1F). This suggests that excessive mechanical stretching triggers the release of HMGB1 from the nuclei. Consistently, HMGB1 was abundant in the tendon ECM of mice on the ITR regimen (Fig. 2A, B), unlike the control with minimum HMGB1 (Fig. 2D, E). In addition, staining for CD68 was higher in the ITR (Fig. 2C) than the control (Fig. 2F) group indicating more monocytes/macrophages in the ITR group. Besides, HMGB1 content was the maximum in the Achilles and patellar tendons of mice in the ITR group (Fig. 2G, H). Lastly, HMGB1 implantation resulted in hypercellularity (Fig. 3A), angiogenesis (Fig. 3C, D), and higher infiltration of inflammatory monocytes/macrophages (Fig. 3E) when compared to the controls (Fig. 3B, F).

DISCUSSION

Despite the prevalence of tendinopathy, the cellular and molecular mechanisms of this tendon disorder are poorly understood. In this study, we have shown for the first time that HMGB1 is present in the nuclei of tendon cells. Moreover, excessive mechanical loading induces the release of HMGB1 from tendon cells into the tendon ECM thereby triggering a sleuth of cellular changes including hypercellularity, hypervascularity and infiltration of inflammatory macrophages into the area. These findings indicate that sterile inflammation occurs in tendons subject to excessive mechanical loading, which may contribute to the development of tendinopathy and pain symptoms seen in tendinopathic patients.

SIGNIFICANCE

HMGB1 could be a potential therapeutic target for the management of tendinopathy.

REFERENCES


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Fig. 1 Excessive mechanical stretching induces the release of HMGB1 from the nuclei in tendon cells. HMGB1 is present in the nuclei of un-stretched tendon cells (A). Staining of nuclei with H33342 (B) and the overlay of both stainings confirm the presence of HMGB1 only in the tendon cell nuclei (C). HMGB1 is observed in > 95% of the un-stretched cells (D) and 4% or moderately stretched cells (E). In contrast, 8% stretching decreases the number of nuclei that stained positive for HMGB1 to ~30% (F). Bar - 50 µm.

Fig. 2 ITR increases HMGB1 levels in tendon ECM and the number of inflammatory cells. HMGB1 staining is robust in the Achilles tendon sections from mice on the ITR regimen (A, B). At 400x magnification, HMGB1 can be observed in the ECM (B, arrows). Similarly, robust staining of CD68 indicates the presence of infiltrated inflammatory monocytes/macrophages (C, arrows). In contrast, the control tendons are negative for HMGB1 (D, E) and are minimally stained for monocytes/macrophages (F), Bar - 50 µm. Quantification of HMGB1 in the Achilles and Patellar tendons of mice by ELISA (G, H). Clearly, HMGB1 in both tendons from the ITR group is significantly higher than the control and MTR groups. P < 0.05.

Fig. 3 HMGB1 bead implantation into rat patellar tendons induces hypercellularity, angiogenesis and infiltration of inflammatory cells after 2 weeks. In the HMGB1 gel implanted group, extensive cell proliferation (A, white square) is visible unlike the control group (B). Besides, the implanted group also has vessel like structures (C, arrow), which were confirmed as blood vessels by IHC staining for CD31 (D). Lastly, HMGB1 implantation increased CD68 staining (E) over the control (F). Bar - 100 µm.