Mechanotransduction Pathways Regulating YAP Nuclear Translocation under Yoda1 and Vibration in Osteocytes

Chun-Yu Lin[1], Amel Sassi[1], Yuning Wu[2], Kimberly Seaman[1], Wentian Tang[1], Xin Song[1], Raphael Bienenstock[1], Yu Sun[1], Fei Geng[2], Liyun Wang[3], Lidan You[1]
[1]University of Toronto, Toronto, ON, [2]McMaster University, Hamilton, ON, [3]University of Delaware, Newark, DE
Email of Presenting Author: delphine.lin@mail.utoronto.ca

Disclosures: No disclosures

INTRODUCTION: Low-magnitude high-frequency (LMHF) vibration has shown anabolic benefits on bone cells, but inconsistent clinical outcomes in postmenopausal women indicate the need to enhance its efficacy in this population [1]. Our previous research demonstrated that Yoda1, an agonist of the Piezo1 calcium channel, enhances the mechanoresponse of MLO-Y4 osteocytes to LMHF vibration by promoting YAP (Yes-associated protein) nuclear translocation [2]. However, the underlying mechanotransduction pathways remain unclear. Studies suggest that F-actin disruption retains YAP in the cytoplasm [3], while mechanical compression on the nucleus increases the nuclear import of YAP [4]. We hypothesize that combined Yoda1 and LMHF vibration treatment regulates cytoskeletal and nuclear remodeling to activate YAP translocation in osteocytes. In this study, we first identified the mechanoregulation of F-actin and nuclear envelope (NE) under mono- or combined treatments, followed by investigating the associated transcriptional regulation.

METHODS: MLO-Y4 osteocytes-like cells were used in the study. We first validated the augmentation of YAP nuclear translocation in MLO-Y4, resulting from the synergistic application of combined treatment. Specifically, this involved subjecting the cells to a one-hour exposure of 1 μM Yoda1 along with LMHF vibration at 0.3g and 60 Hz, followed by a 1 h incubation in fresh media to facilitate translocation. We then elucidated the contributions of actin polymerization and SUN1/2 in the context of YAP nuclear localization regulated by combined treatment. Additionally, we analyzed F-actin intensity in MLO-Y4 right after subjecting them to treatments. Cells were stained with phalloidin, allowing measurements of F-actin mean fluorescent intensity and perinuclear actin intensity. We also investigated the influence of individual or combined treatment on NE morphology, specifically the induction of wrinkles, which was linked to YAP localization [5]. Previous study suggested that YAP shuttling occurs preferentially in conditions of low NE wrinkling and taut nuclei [5]. Finally, RNA sequencing (RNAseq) analysis was conducted on MLO-Y4 cells subjected to the treatments to identify the differential expressed genes (DEGs).

RESULTS: YAP nuclear translocation in MLO-Y4 subjected to combined treatment was significantly increased by 21.3% compared to DMSO control (Fig 1A). Furthermore, our observations demonstrated that both Yoda1 and combined treatment elicited an increase in the mean intensity of F-actin (Fig 1B), along with a shrinkage area of DAPI staining which represents DNA (Fig 1C). In NE morphology, Yoda1 decreased the percentage of cells with wrinkles (Fig 2A, B) and lowered the NE wrinkle by 6.7% in comparison to the DMSO control (Fig 2C). Finally, RNAseq revealed that LMHF vibration resulted in the upregulation of 460 DEGs compared to DMSO, while Yoda1 showed limited upregulation of DEGs. All treatments increased Csf1 and Hmga2 (Fig 3).

DISCUSSION: In the context of single treatments, Yoda1 effectively regulated F-actin intensity and NE wrinkling (Fig 1B, 2), whereas vibration had no impact. This is consistent with the macrophage observation where Yoda1 amplifies actin polymerization [6]. The heightened F-actin polymerization and reduced NE wrinkling imply that Yoda1-induced polymerization aids in transmitting contractile strain energy to the nucleus, leading to NE tension and reduced nuclear wrinkling. In combined treatment, Yoda1 dominants the effect in cytoskeleton regulation, thus contributing to the heightened YAP translocation.

Although vibration had no impact on regulating F-actin intensity and NE wrinkle, the increased YAP translocation could potentially result from alternative signaling pathways. In addition to mechanical cues, studies have indicated that YAP translocation is influenced by biochemical cues. The YAP/TAZ complex serves not only as a downstream component of Hippo signaling pathways but also engages with other pathways like Notch, Wnt/β-catenin, and TGFβ [7]. Vibration had potent transcriptional effects, upregulating 460 DEGs compared to DMSO (Fig 3). Additionally, within the 417 upregulated DEGs in the combined treatment, 350 overlapped with vibration, highlighting vibration's major role in transcriptional regulation in combined treatment. Analyzing these shared DEGs could reveal the signaling mechanisms contributing to YAP translocation. Indeed, it is surprising that Yoda1 had limited effects on transcriptional regulation, possibly due to various factors. First, we revealed that Yoda1 led to a decrease in DAPI-stained area (Fig 1C), suggesting chromatin condensation. The result aligns with findings in epithelial cells, where Yoda1 triggered DAPI shrinkage via calcium signaling, independently of cytoskeletal reorganization [8]. This could restrict the accessibility of DNA for transcriptional activity. Secondly, unlike vibration where mechanical stimuli were directly transmitted to the nucleus, the effects of Piezo1 activation on transcriptional regulation involve downstream signaling cascades triggered by calcium influx and require longer duration. Hence, we expect Yoda1 to exhibit stronger gene regulation at extended post-treatment time points. Finally, all treatments increased Csf1 (M-CSF), a regulator of osteoclast precursor. Csf1 was recognized as a YAP downstream gene in response to compression, where YAP/TAZ knockdown blunted the compression-induced Csf1 increase [9].

In summary, the enhanced YAP nuclear translocation through combined treatment results from the distinct regulatory roles of Yoda1 and vibration, where Yoda1 influences actin and nuclear dynamics, while vibration exerts its effects through unknown mechanisms, as indicated by potent transcriptional regulation.

SIGNIFICANCE/CLINICAL RELEVANCE: With osteocytes being the predominant and highly mechanosensitive cells in bone tissue, uncovering their mechanotransduction pathways offer insights into focused strategies for bone disease management.

REFERENCES: [1] Marin-Cascales et al., *Medicine* (2018); [2] Lin et al., *Cancers* (2022); [3] Zhao et al., *Gene & Dev* (2011); [4] Elosegui-Artola et al., *Cell* (2017); [5] Cosgrove et al., *Biomaterials* (2021); [6] Atcha et al., *Nat Comm* (2021); [7] Zarka et al., *Front. Cell Dev. Biol*. (2022); [8] Jetta et al., *J. Cell Sci.* (2019); [9] Zarka et al., *Lab Invest* (2021)

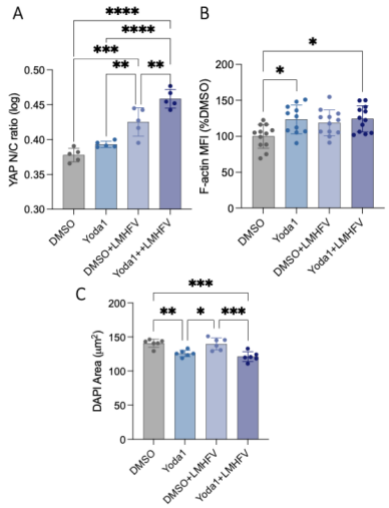

Figure 1. (A) YAP nuclear-cytoplasmic ratio, (B) F-actin mean fluorescent intensity, and (C) DNA area of MLO-Y4 subjected to mono- or combined treatments

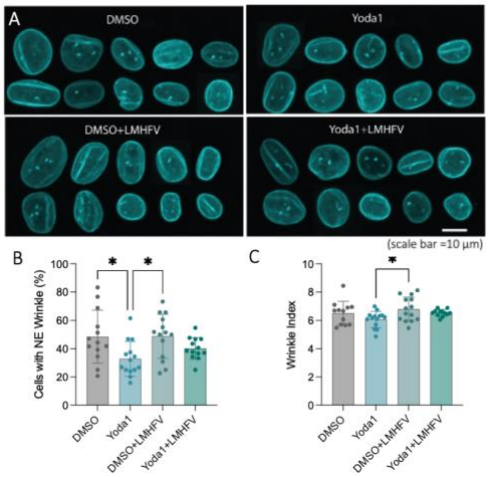

Figure 2. (A) Representative Lamin-A/C max projection images of MLO-Y4 under treatments. Quantifications of (B) percentage of cells with NE wrinkle and (C) wrinkle index of MLO-Y4 under treatments. Wrinkle index was calculated using algorithm developed by [5], which relies on the mean bright pixel intensity

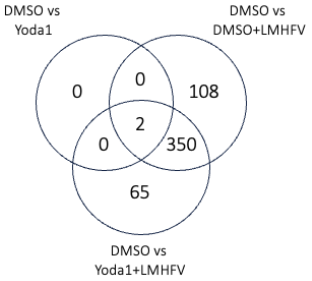

Figure 3. Venn Diagram of the RNAseq results. Top left circle represents the upregulated genes of Yoda1 compared to DMSO. Top right circle represents the upregulated genes of vibration compared to DMSO. The bottom circle represents the upregulated genes of combined treatment compared to DMSO