Local Administration of CD1530, Selective RARγ Agonist, Facilitates Tendon Healing by Modulating the Healing Environment Including Reduced Heterotopic Ossification in a Mouse Achilles Rupture Model

Dilimulati Yimiti[1], Kenta Uchibe[2], Minoru Toriyama[1], Yuta Hayashi[1,3], Yasunari Ikuta[1], Tomoyuki Nakasa[1,4], Haruhiko Akiyama[6], Chisa Shukunami[5], Nobuo Adachi[1], Shigeru Miyaki[1,7]

[1]Department of Orthopaedic Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan, [2]Department of Maxillofacial Anatomy and Neuroscience, Hiroshima University Graduate School of Biomedical and Health Sciences, Hiroshima, Japan, [3]Department of Musculoskeletal Traumatology and Reconstructive Surgery, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan, [4]Department of Artificial Joints and Biomaterials, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan, [5]Department of Molecular Biology and Biochemistry, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan, [6]Department of Orthopaedic Surgery, Graduate School of Medicine, Gifu University, Gifu, Japan, [7]Medical Center for Translational and Clinical Research, Hiroshima University Hospital, Hiroshima, Japan.

Email: dilmurat1205@gmail.com

Disclosures: Dilimulati Yimiti (N), Kenta Uchibe (N), Minoru Toriyama (N), Yuta Hayashi (N), Yasunari Ikuta (N), Tomoyuki Nakasa (N), Aki Takimoto (N), Haruhiko Akiyama (N), Chisa Shukunami (N), Nobuo Adachi (N), Shigeru Miyaki (N)

INTRODUCTION: Heterotopic ossification (HO) in Achilles tendon often arises due to endochondral ossification during the healing process following trauma. Understanding of HO pathological process in tendon healing could help in developing effective therapeutic strategies. Recently, in cell lineage studies using CreERT2/lox system, tendon stem/progenitor cells (TSPC) were demonstrated to be involved in tendon healing and HO[1,2,3]. Several reagents including retinoic acid receptor (RAR) agonists are attenuating HO in mouse models and are currently being investigated using various models[4,5]. Although clinical trials for FOP have been performed using several drugs, the underlying mechanism of pharmacological action for RAR agonists on Achilles tendon healing including HO has not been fully understood yet. The purpose of this study is to elucidate the therapeutic effects of CD1530, RARγ selective agonists, on Achilles tendon healing, and the involvement of cells using a cell lineage tracing system.

METHODS: Mouse Achilles tendon rupture model: To investigate the therapeutic effect of CD1530, the Achilles tendon rupture model was performed in 15 male C57BL6/J mice or 5 male *ScxCreERT2*-tdTomato mice at 10 weeks of age (Fig1A). Histological assessment: Four weeks after injury, the healing Achilles tendon tissues were collected and Hematoxylin and Eosin (H.E), Safranin-O and Fast Green (Saf-O), and Picro-Sirius Red (PSR) were performed. The histologic tendon healing was evaluated using a modified tendon healing scoring and chondrification scoring system[6]. Immunohistochemistry: The sections were immunostained with anti-COLLAGEN TYPE II antibody (Hybridoma, Ⅱ-Ⅱ6B3, 1:200) using Vectastain Elite ABC-HRP kit (Vector Laboratories, Inc, CA, USA) and DAB substrate kit (Vector Laboratories, Inc, CA, USA), and anti-SOX9 antibody (Millipore, AB5535, 1:500) with immunofluorescence staining. Chondrogenic differentiation of injured tendon-derived fibroblasts (iATF): iATFs were isolated from injured Achilles tendons of *ScxCreERT*-tdTomato mice one week after injury (Fig2A). After being characterized with tendon markers or tendon progenitor/stem cell markers by immunocytochemistry (Fig2B), the pellet culture of iATF was performed by treatment of 10 ng/mL TGF-β3 and 500 ng/mL BMP2 for 3 weeks. Statistical analysis: The results are expressed as means or means ± S.E. The data were analyzed with the paired t-test and Ordinary one-way ANOVA with the Tukey test. P values <0.05 were statically significant.

RESULTS: To evaluate the therapeutic effects of CD1530, we performed macroscopic and histological analysis of Achilles tendons using a mouse Achilles tendon rupture model (Fig1A). At 4 weeks after transection, the Achilles tendon rupture model exhibited a high frequency of chondrification during tendon healing (Fig1B). Chondrification score and type 2 collagen-positive area were significantly lower in CD1530 treatment group than Control (Fig1C). Furthermore, CD1530 showed less fibrous tissue-like scar formation and better orientation of collagen fibers in the healing tendon site than Control (Fig1D). Indeed, tendon healing scores indicated a significant increase in the histological changes of Achilles tendon in CD1530 treatment group compared with Control (Fig1E). These results indicated that CD1530 accelerated the healing of Achilles tendon grossly and histologically through the inhibition of HO. In order to investigate the cells involved in HO during tendon healing, we further performed cell lineage analysis using *ScxCreERT*-tdTomato mouse. In the pre-injury labeled mouse model, a significant reduction in the number of Scx^+ cells was observed at 4 weeks after injury in the healing site. Remarkably, in the chondrification site, a major of Sox9-expressing cells were Scx^- cells. These results indicated that tendon resident Scx^+ cells in Achilles tendon are not actively contributing to HO and tendon healing following injury, but rather by TSPC-like cells. In pellet culture of injured tendon-derived fibroblasts (iATF), under induction for 3 weeks, the resulting iATF pellets were significantly smaller and exhibited no Saf-O staining in CD1530 treatment group (Fig2C). Furthermore, the expression of Col2a1 and Col10a1 was significantly lower in the presence of CD1530 compared to the Control (Fig2D). These results clearly indicated that CD1530 inhibited the chondrogenesis of TSPC-like iATF. This dual effect suggests the potential of CD1530 to effectively modulate the healing environment during tendon healing.

DISCUSSION: The present study demonstrates that the local administration of CD1530 accelerated tendon healing by modulating the healing environment, including reducing chondrification via targeting TSPC-like cells in a mouse Achilles tendon rupture model. The HO formation and tendon healing is not primarily driven by resident Scx^+ cells, but rather by TSPC-like cells. CD1530 may have the potential to be a novel tendon therapy that offers dual benefits via the inhibition of chondrogenesis and the induction of tenogenesis. For future clinical applications, developing an administration method involving a carrier, such as hydrogel, could be essential to enhance the therapeutic effects even further.

SIGNIFICANCE/CLINICAL RELEVANCE:

These results suggest that CD1530 may have the potential to be a novel tendon therapy that offers dual benefits via the inhibition of chondrogenesis and the induction of tenogenesis.

REFERENCES:

1: Huang et al. (2021) Front. Cell Dev. Biol.
2: Agarwal et al. (2017) Stem Cells.
3: Yea et al. (2023) Bone Res.
4: Shimono et al. (2011) Nat. Med.
5: Di Rocco et al. (2015) Am. J. Pathol.
6: Yuta et al. (2022) FEBS Lett.

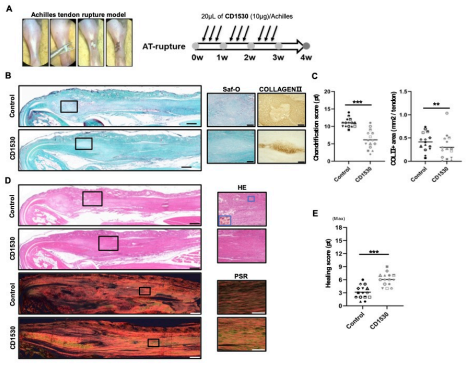

Fig 1. Histological analysis of Achilles tendon chondrification and healing

(A) A mouse Achilles Tendon rupture model and CD1530 administration. (B) Histological analysis of chondrification with Safranin O staining and IHC for Type 2 Collagen and evaluated with (C) Chondrification scoring system and Type 2 Collagen-positive area (n=15). The data are represented as mean. Comparison of mean values was performed using paired t-test. *P < 0.05, **P < 0.01, and ***P < 0.001 versus Control. Scale bars: 500 μm and 100 μm.

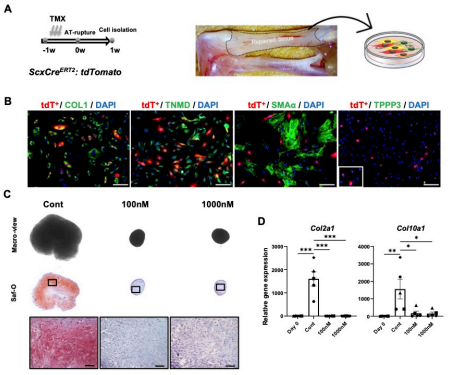

Fig 2. Effect of CD1530 on chondrogenic differentiation of iATF

(A) iATF were isolated from *ScxCreERT*-tdTomato mice at 1 week after injury. (B) Tendon markers, and tendon progenitor/stem cell markers in iATF. (C) Macro-view and Safranin O staining. (D) Cartilage-specific markers were significantly suppressed by CD1530 (n=5 each group). The data are represented as mean ± S.E. Comparison of mean values was performed using one-way ANOVA. *P < 0.05, **P < 0.01, and ***P < 0.001 versus Control. Scale bars: 500 μm and 100 μm.