Iguratimod inhibits the production of sclerostin and RANKL through the ERK/EGR1/TNFα pathway in osteocytes and improves disuse osteoporosis in mice.

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DISCLOSURES: Taihei Miura (Toyama Chemical Co., Ltd), Yuki Etani (Taisho Pharmaceutical Co., Ltd), Makoto Hirao (N), Kenji Takami (N), Atsushi Goshima (N), Takuya Kurihara (N), Yuji Fukuda (N), Takashi Kanamoto (N), Ken Nakata (Taisho Pharmaceutical Co., Ltd), Seiji Okada (N), Kosuke Ebina (Eisai Co., Ltd, Taisho Pharmaceutical Co., Ltd)

INTRODUCTION: Disuse osteoporosis is a prevalent complication among patients with rheumatoid arthritis (RA). A meta-analysis has reported that the antirheumatic drug iguratimod (IGU) improves osteoporosis in patients with RA [1]. However, its detailed effects on osteocytes remain unclear. Moreover, to the best of our knowledge, there are currently no osteoporosis medications that specifically target osteocytes. Therefore, in this study, we focused on osteocytes and aimed to investigate the effects of IGU on disuse osteoporosis in mice.

METHODS: Eight-week-old C57BL/6J male mice were randomly divided into three groups: normal saline (NS), hindlimb unloading + NS (HLU + NS), and HLU + IGU (HLU + IGU). HLU mice underwent 21 days of tail suspension, while 200 µl of NS and IGU (30 mg/kg) was intraperitoneally injected 5 times per week over the same period of 21 days. Micro-computed tomography (µCT) was utilized to analyze the trabecular and cortical bone of the distal femurs. Bone histomorphological features were assessed by tartrate-resistant acid phosphatase (TRAP) staining, osteocalcin, sclerostin, EGR1, and TNFα immunostaining. Additionally, the effect of IGU on osteocytes and its effect on bone metabolism were investigated in vitro. The relationship between disuse osteoporosis and the effects of IGU on osteocytes was analyzed by RNA sequencing. pCMV6-EGR1 and EGR1 small interfering RNA (siRNA) were used to assess the role of EGR1 in osteocytes.

RESULTS: Bone mass was significantly decreased in HLU + NS compared to NS, while IGU treatment in HLU mice attenuated the bone mass reduction. Additionally, osteoclast numbers and sclerostin-positive osteocyte rates were significantly increased in HLU + NS while suppressed by IGU treatment. Osteocalcin-positive cells were significantly decreased in HLU + NS while attenuated the reduction by IGU treatment. In vitro, IGU suppressed the gene expression of receptor activator of NF-κB ligand (RANKL) and sclerostin in MLO-Y4 and Saos-2 cells, leading to the inhibition of osteoclast differentiation in co-cultures with MLO-Y4 cells. We found that IGU down-regulated the expression of mechanical stress-related factor, early growth response protein 1 (EGR1) by RNA sequencing of Saos-2 cells. EGR1- and TNFα-positive osteocyte rates were significantly increased in HLU + NS while suppressed by IGU treatment. Furthermore, IGU suppressed ERK expression by western blotting. Moreover, gene expression of RANKL and sclerostin in osteocytes was increased by overexpression of EGR1 and suppressed by siRNA-mediated suppression of EGR1.

DISCUSSION: Our research revealed that IGU regulates the ERK/EGR1/TNFα pathway in osteocytes, resulting in an improvement of bone metabolism. It is known that mechanical unloading activates EGR1 through the ERK pathway [2]. Furthermore, it is recognized that EGR1 activates TNFα expression, and TNFα induces the expression of RANKL and sclerostin [3]. Moreover, in disuse osteoporosis, the expression of TNFα, sclerostin, and RANKL/OPG has been reported to be high in cortical bone osteocytes, suggesting a close relationship between unloading and EGR1 in osteocytes [4]. Our study indicates that IGU may exert suppression on the EGR1/TNFα pathway by downregulating ERK expression. Furthermore, within the scope of this study, IGU effectively attenuated the upregulated osteoclastogenesis due to increased RANKL expression in disuse osteoporosis, while concurrently improving the suppressed osteoblastogenesis associated with heightened sclerostin expression.

SIGNIFICANCE: IGU inhibits the production of sclerostin and RANKL from osteocyte, and has the potential to be an unique and effective treatment option for disuse osteoporosis targeting osteocytes.

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Fig. 1 Representative µCT images of the distal femur of three groups.
Scale bars: 500 µm

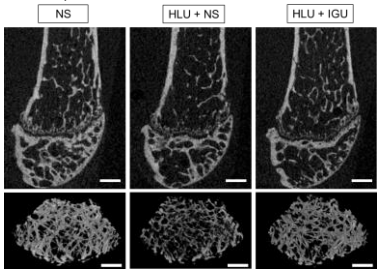

Fig. 3 RNA-seq analysis between the BMP2 and BMP2+IGU-treated groups.
The volcano plot displays the differentially expressed transcripts.

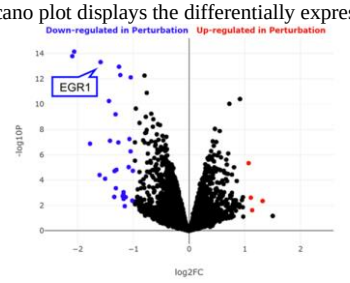

Fig. 2 Bone histomorphological features.
(TRAP staining, osteocalcin, sclerostin, EGR1, and TNFα immunostaining. Red arrows indicate positive cells)
Scale bars: 50 µm.