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In vitro anti-osteoclastogenic activity of p38 inhibitor doramapimod via inhibiting migration of pre-osteoclasts and NFATc1 activity

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ABSTRACT

The mitogen activated protein kinase p38 plays a role in the receptor activator of NF-κB ligand (RANKL)-induced osteoclast differentiation. In this study, we investigated the effect of p38 inhibitor doramapimod on the osteoclast differentiation. Doramapimod significantly inhibited the osteoclastogenesis of bone marrow macrophages (BMMs) via attenuating the activation of p38 induced by M-CSF and RANKL. Importantly, doramapimod blocked the migration and fusion in pre-osteoclasts via the down-regulating NFATc1. The inhibitory effect of doramapimod on the migration/fusion of pre-osteoclasts via inhibiting NFATc1 activity were confirmed by measuring NFATc1 luciferase activity and evaluating the mRNA expression of NFATc1-responsive genes related to the osteoclastic migration/fusion. These results suggested anti-osteoclastogenic activity of doramapimod via inhibiting migration/fusion of pre-osteoclasts and NFATc1 activity.

1. Introduction

The p38 mitogen-activated protein (MAP) kinase is one of the best characterized kinases in the inflammatory process and many pharmaceutical companies have tried to develop p38 inhibitors as potential therapeutics for inflammatory diseases and cancers since a decade ago (1, 2). As results of their effort, there are several p38 inhibitors under the clinical trials (Supplementary Table 1), but relatively many p38 inhibitors have experienced to be failed in the stage of development due to a lack of efficacy in the clinical trials or even withdrawn in the market. Lack of efficacy has been also suggested that they did not effectively target the disease or indication for which they were intended (3). Therefore, 'indication switching' of failed drugs has been proposed in order to reduce the cost and time required for new drug development. This approach is also called to 'drug repositioning', 'drug repurposing', or 'drug reprofiling', and through this strategy, existing drugs and potential drug candidates have been repositioned and evaluated for new indications (4–6). The p38 MAPK pathway plays a crucial role in the receptor activator of nuclear factor-κB ligand (RANKL)-mediated osteoclast differentiation and its druggability as a potential therapeutic target for inflammatory osteolysis have been suggested (7, 8). Apparently, the pharmacological inhibition of p38 has been shown to strongly block the RANKL-induced differentiation of bone marrow macrophages (BMMs) into osteoclasts (7).

Bone is dynamic tissue with continuous remodeling process by osteoclastic bone resorption and osteoblastic bone formation. Therefore, the tight regulation of bone remodeling is important to maintain bone homeostasis. However, an imbalance by over-activated osteoclasts or increased number of osteoclasts causes bone resorption-related skeletal diseases including osteoporosis. Bone resorption-related diseases are characterized by low bone mass and microarchitectural deterioration of bone tissue, with a high risk of bone fracture.

Strong p38 inhibitor doramapimod (Fig. 1A) has been originally developed by Boehringer Ingelheim Pharmaceuticals Inc. for mitigating rheumatoid arthritis and Crohn's disease, but there was no evidence for its clinical efficacy in Crohn's disease (9). Since the effect of doramapimod on the osteoclast differentiation has not been studied yet, therefore, whether doramapimod could be

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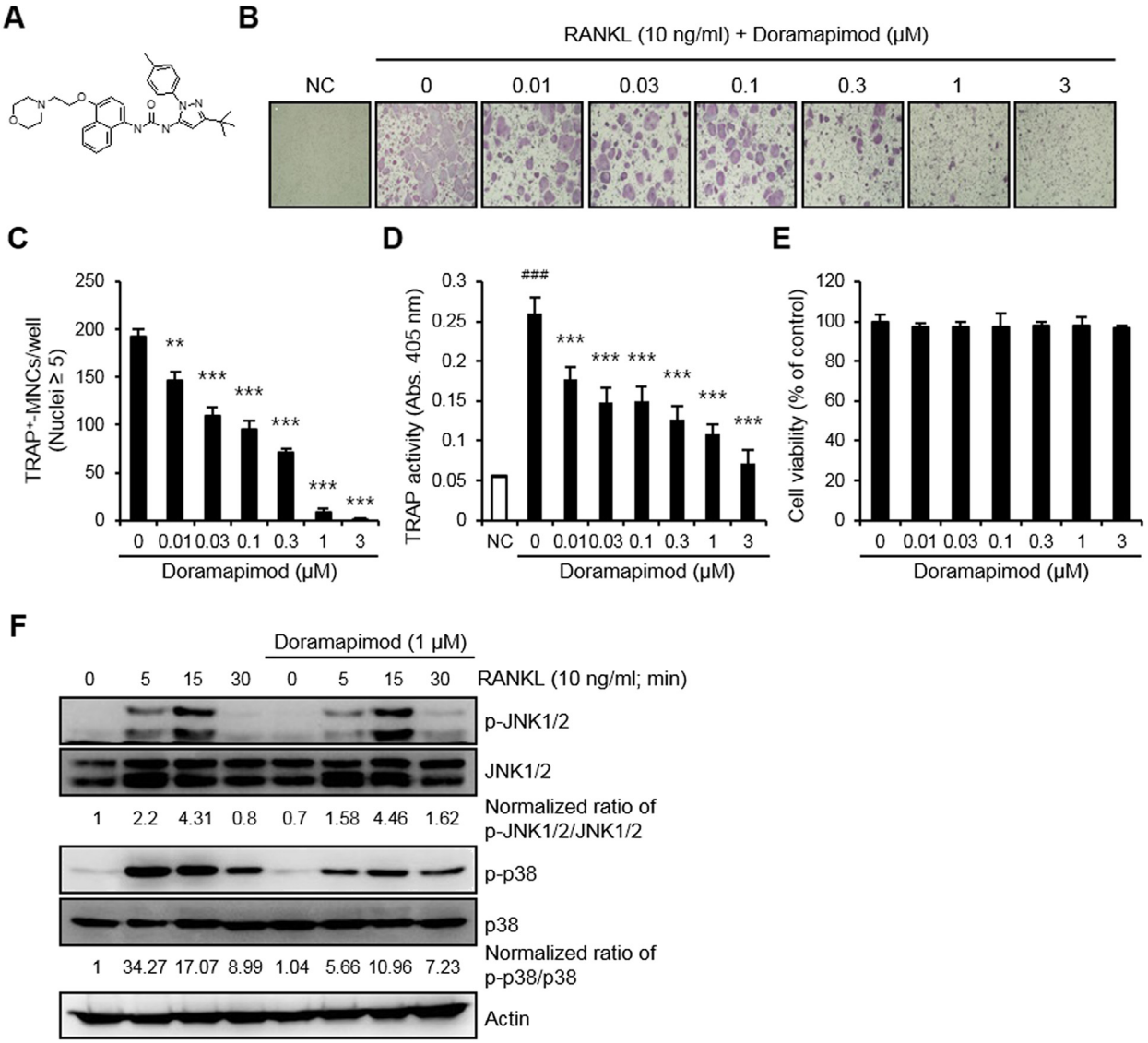

Fig. 1. Doramapimod inhibits RANKL-induced osteoclast differentiation by attenuating p38 activation. (A) The chemical structure of doramapimod. (B) BMMs were cultured with the vehicle (DMSO) or indicated dose of doramapimod in the presence of RANKL (10 ng/ml) and M-CSF (30 ng/ml) for 4 days. Multinucleated osteoclasts were visualized to purple-colored giant cells by TRAP staining. Stained cells were photographed under a light microscope (Magnification × 100). (C) The number of TRAP-positive multinucleated osteoclasts (TRAP-positive MNCs) with more than 5 nuclei ($N \geq 5$) were counted. (D) TRAP activity was measured. (E) Viability of BMMs was evaluated by CCK-8 assay. (F) Effects of doramapimod on RANKL-induced phosphorylation of JNK1/2 and p38 were evaluated by Western blot analysis. BMMs were cultured for 1 days in the presence of M-CSF (30 ng/ml) and starved for 2 h. After pretreated with DMSO or doramapimod (1 μM) for 2 h, cells were stimulated with RANKL (10 ng/ml) for indicated time. Total protein lysates (20 μg) were separated in 10% SDS-PAGE gel and immunoblotted with indicated primary antibodies respectively. All data are representative of at least three independent experiments. Densitometric analysis was performed using NIH ImageJ 1.43u software and the relative, normalized ratios of p-JNK1/2/JNK1/2, p-p38/p38 were presented. ###, $p < 0.001$ (versus 'the group treated with the vehicle only'), **, $p < 0.01$; ***, $p < 0.001$ (versus 'the group treated with RANKL only').

applied to inhibit osteoclast differentiation was investigated in this study.

2. Materials and methods

2.1. Materials

Doramapimod (BIRB796) was purchased from LC labs. (Woburn, MA, USA) and was diluted with DMSO to make 10 mM DMSO stock solution that was diluted with PBS before cell treatment. Therefore, 0.03% of DMSO was used as a vehicle control in all experiments. Penicillin, streptomycin, cell culture medium, and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Mouse soluble macrophage colony-stimulating factor (M-CSF) and recombinant mouse or human RANKL were purchased from R&D Systems (Minneapolis, MN, USA) and diluted with sterile phosphate buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) according to the manufacturer's protocol. Stock dilution was carried out by PBS. Cell viability assay kit, CCK-8 was purchased from Dojindo Molecular Technologies (Rockville, MD, USA). Primary antibodies against nuclear factor of activated T cells c1 (NFATc1) and actin, and the secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primary antibodies against phosphorylated (p)-JNK1/2, JNK1/2, p-p38, p38 were purchased from Cell Signaling Technology (Beverly, MA, USA). NFATc1 luciferase reporter vector (NFAT-luc) and the control pRL-Renilla control vector were purchased from Clontech (Mountain View, CA, USA) and Promega (Fitchburg, WI, USA), respectively.