

# Cyclin-Dependent Kinase Inhibitor-1-Deficient Mice Are Susceptible To Osteoporosis In Mice Arthritis Model.

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**INTRODUCTION:** Rheumatoid arthritis (RA) is a chronic inflammatory disease that causes irreversible joint damage and significant disability. We recently reported that cyclin-dependent kinase inhibitor 1 (p21) deficiency induces joint cartilage destruction and severe synovitis in mice arthritis model (1). In RA joint, the osteoclast formation and bone resorption activity were increased (2). However, it remains unsolved whether p21 is associated with osteoclast formation and activity. We hypothesized that p21 deficiency would lead to osteoporosis in inflammatory disease such as RA.

**METHODS:** This study was approved by the Animal Studies Committee of Kobe University, Japan (permit number P230407). Male p21- knockout (p21<sup>-/-</sup>) mice and wild-type (p21<sup>+/+</sup>) mice backcrossed against C57BL/6 more than 10 generations were used in this study. We studied 10-week-old male mice. p21<sup>+/+</sup> littermates were used as wild type (WT) controls. The tibiae of p21<sup>-/-</sup> mice and WT mice served as in vivo models of collagen antibody-induced arthritis (CAIA). A cocktail of five monoclonal antibodies recognizing conserved epitopes on various species of type II collagen was prepared as previously described (3). On days 7, 14, and 28, four mice each from the p21<sup>-/-</sup> and WT groups were euthanized using CO<sub>2</sub>. We defined the mice without injection of monoclonal antibodies as the control mice. Hematoxylin and eosin (H&E) staining and tartrate-resistant acid phosphatase (TRAP) staining were performed, and radiological assessment was performed using a microfocus X-ray CT system.

**RESULTS:** Histological assessments with H&E staining was shown in Figure 1. In p21<sup>-/-</sup> mice, quantification of H&E staining demonstrated that the bone volume/tissue volume (BV/TV) was decreased compared with WT mice on days 7, 14, and 28. Bone morphometric study was shown in Figure 2. In p21<sup>-/-</sup> mice, bone volume was decreased significantly compared to WT mice. Quantitative analysis of bone parameters also confirmed decreased bone BV/TV, trabecular thickness (Tb.Th), trabecular number (Tb.N), and increased trabecular separation (Tb.Sp) on days 28.

TRAP staining was shown in Figure 3. The number of TRAP positive osteoclasts was significantly increased on the surface of bone in p21<sup>-/-</sup> mice compared with WT mice on days 7, 14, 28.

**DISCUSSION:** We demonstrated that p21-deficient CAIA model mice exhibited progressed osteoporosis associated with enhanced osteoclastogenesis. Our results suggest that p21 may have a regulatory role in osteoclast formation and osteoporosis in arthritis joint such as RA.

**SIGNIFICANCE:** The regulation of p21 expression may be the possible therapeutic target for osteoporosis in RA.

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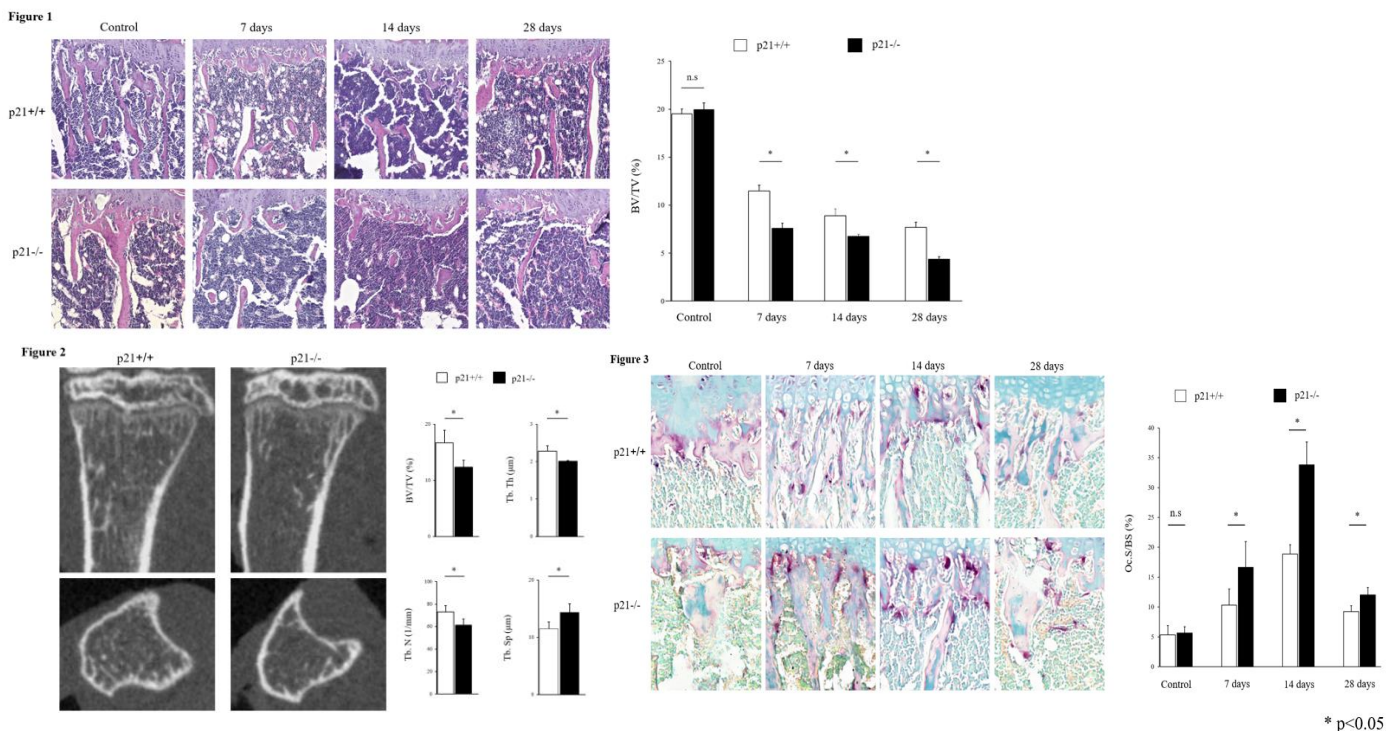
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**IMAGES:**

**Figure 1:** H&E staining of p21<sup>+/+</sup> and p21<sup>-/-</sup> tibia of control and on days 7, days 14, and days 28.

**Figure 2:** Radiological morphological evaluation of p21<sup>+/+</sup> and p21<sup>-/-</sup> tibia on days 28.

**Figure 3:** TRAP staining of p21<sup>+/+</sup> and p21<sup>-/-</sup> tibia of control and on days 7, days 14, and days 28.



\* p<0.05