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ABSTRACT OF DISSERTATION

Title	Fas/S1P$_1$ crosstalk via NF-κB activation in osteoclasts controls subchondral bone remodeling in murine TMJ arthritis 破骨細胞における NF-κB 活性化による Fas / S1P$_1$ クロストークがマウス TMJ 関節炎の骨リモデリングを制御する
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Rheumatoid arthritis (RA), a chronic inflammatory disease, mainly affects several joints of the body. Increased subchondral trabecular bone turnover due to imbalanced bone-resorbing and bone-forming activities is a hallmark of RA. It has been previously postulated that aberrant apoptosis or migration of cells from the subchondral bone may be responsible for the pathogenesis of RA. The temporomandibular joint (TMJ) is frequently affected in human RA. However, the involvement of TMJ in RA remains unclear. The aim of this study was to investigate the effects of the Fas and Sphingosine-1-phosphatase (S1P) signaling pathways on osteoclast precursors that participate in the pathogenesis of RA in the TMJ.

TMJs were resected from 8-22 weeks old female MRL/*lpr* (n=30) and MRL+/+ (n=25) mice. Micro-computed tomography (CT), histological, and immunohistochemical analyses were performed to assess subchondral bone resorption. Bone marrow macrophages (BMMs) from MRL/*lpr* and MRL+/+ femur were harvested for tartrate-resistant acid phosphatase (TRAP) stain and western blot analysis. RNA isolation from cartilage/subchondral bone interface of the TMJ was carried out to examine the expression of osteoclastogenic markers and S1P/S1P-receptor genes.

Abnormalities in the condylar subchondral bone, including dynamic changes in bone mineral density and microstructure, were observed in Fas-deficient MRL/*lpr* mice. Micro-CT and TRAP stain revealed more severe subchondral bone resorption in MRL/*lpr* than in MRL+/+ mice, with a greater number of osteoclasts in the subchondral bone. As MRL/*lpr* mice contain increased numbers of osteoclast *in vitro* compared with MRL+/+ mice, this increased number is due to the hypersensitivity of precursors receptor activator of nuclear factor-kappa B ligand (RANKL). The phosphorylation of NF-κB, as well as Akt and MAPK of RANKL-stimulated BMMs from MRL/*lpr* mice, were significantly upregulated. The western blot analysis

showed higher protein expression levels of osteoclastogenic markers (e.g NFATc1, c-Fos, and c-Src) in MRL/*lpr* than in MRL+/+. On the other hand, lower levels of collagen type II, aggrecan, and osteoprotegerin and higher levels of MMP9, MMP13, VEGF, and S1P-receptor-1 ($S1P_1$) were detected in the mandibular condyles of MRL/*lpr* mice.

Furthermore, expression of $S1P_1$, but not $S1P_2$ or $S1P_3$ was significantly upregulated in the condylar cartilage of MRL/*lpr* mice. $S1P_1$ was found to be required for S1P-induced migration of osteoclast precursors and downstream signaling via Rac1. When SN50, a synthetic NF-κB-inhibitory peptide, was applied to the MRL/*lpr* mice, subchondral trabecular bone loss was reduced and both productions of osteoclastogenesis markers and sphingosine kinase (Sphk) 1/ $S1P_1$ signaling were reduced. Taken together, these results indicate that Fas/$S1P_1$ signaling pathway via activation of NF-κB in osteoclast precursors plays an important role in the pathogenesis of TMJ-RA.