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Molecular Mechanisms of Osteoclast Differentiation: Relationships between Immune System and Bone Metabolism in the Cells

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Osteoclasts are multinucleated giant cells responsible for bone resorption. Monocytes/macrophages are capable of differentiating into osteoclasts. Therefore, characteristics of the precursor cells dramatically change during osteoclast differentiation (Fig. 1). Bone marrow-derived macrophages possess immune functions, such as inflammatory cytokine production, antigen presentation and phagocytosis. However, the cells lose those functions during osteoclast differentiation. All kinds of toll-like receptors (TLRs), sensors for the constituents of microorganisms, are expressed on macrophages, while osteoclasts express TLR2 and 4, suggesting that osteoclasts are less sensitive against microorganisms compared to macrophages. It is generally accepted that the differentiation of osteoclasts is induced by the receptor activator of NF- κ B ligand (RANKL) expressed on osteoblasts. To identify the central factors responsible for osteoclast differentiation, we analyzed mRNA expression levels in osteoclast precursors stimulated with RANKL using a DNA micro-array. We found that a transcription factor, DOF-1, was downregulated in the early phase of osteoclast differentiation (Fig. 1). Overexpression of the DOF-1 gene in the precursors using retrovirus vectors resulted in complete inhibition of osteoclast formation, suggesting that DOF-1 is an inhibitory factor of osteoclast differentiation. DOF-1-deficient mice exerted severe osteoporosis due to enhanced osteoclast formation. The differentiation of osteoclasts from bone marrow cells derived from DOF-1-deficient mice was significantly enhanced compared to that derived from wild-type mice. Subsequently, we molecularly analyzed the relationships between DOF-1 and NFATc1, a central transcription factor of osteoclast differentiation. It was revealed that DOF-1 interacts with NFATc1 and inhibits NFATc1 activity and its auto-amplification. Furthermore, when lipopolysaccharide (LPS)

Differentiation stage	Precursors (Macrophages)	Osteoclasts
Function	Immune response • Cytokine production • Phagocytosis • Antigen presentation	Bone resorption
Toll-like receptors expressed on the cells	TLR1-9	TLR2, 4
Expression level of DOF-1	High	Low

Fig. 1 Macrophages are capable of differentiating into osteoclasts in response to RANKL and macrophage colony-stimulating factor (M-CSF). Macrophages, osteoclast precursors, possess immune functions such as cytokine production, antigen presentation and phagocytosis, whereas osteoclasts possess a bone-resorbing function. All kinds of toll-like receptors (TLRs), a sensor of microorganisms, are expressed on macrophages, while TLR2 and 4 are expressed on osteoclasts. Although DOF-1 mRNAs are highly expressed in osteoclasts, the expression levels of DOF-1 mRNA are downregulated after differentiating into osteoclasts.

was administered to the calvaria of mice, more severe bone destruction was induced in the DOF-1-deficient mice as compared to the wild-type mice. These results suggest that DOF-1 plays an inhibitory role in osteoclast formation, which in turn protects the bone from osteoporosis and inflammatory bone destruction *in vivo*.

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