Mod Rheumatol (2006) 16:341–342
DOI 10.1007/s10165-006-0524-0

© Japan College of Rheumatology 2006

MINIREVIEW

Takeshi Miyamoto

The dendritic cell-specific transmembrane protein DC-STAMP is essential for osteoclast fusion and osteoclast bone-resorbing activity

Received: June 8, 2006 / Accepted: July 10, 2006

Abstract Osteoclasts are bone-resorbing cells that play a critical role for bone destruction in rheumatoid arthritis. It is well known that osteoclasts form multinuclear cells by cell–cell fusion of mononuclear osteoclasts; however, what molecules are required for osteoclast cell–cell fusion, and the role of multinucleation remain uncharacterized. We identified the dendritic cell-specific transmembrane protein DC-STAMP, a putative seven transmembrane protein, and generated DC-STAMP-deficient mice. The cell fusion of osteoclasts was completely abrogated in DC-STAMP-deficient mice, while the transcription factors required for osteoclast differentiation or osteoclast maturation markers were induced as wild type osteoclasts. Interestingly, bone-resorbing activity was reduced in DC-STAMP-deficient osteoclasts compared with wild-type osteoclasts, and DC-STAMP-deficient mice showed osteopetrosis. Thus, we identified DC-STAMP as an essential molecule for osteoclast cell–cell fusion, and found that multinuclear osteoclasts have a higher bone-resorbing activity than mononuclear osteoclastic cells seen in DC-STAMP-deficient mice.

Key words Cell fusion · DC-STAMP · Osteoclast · Rheumatoid arthritis

Identification of DC-STAMP

To date, various molecules, i.e., E-cadherin, macrophage fusion receptor (MFR), CD44, CD9, and CD81, have been implicated in the multinucleation of osteoclasts and macrophages; however, defects in multinucleation of osteoclasts and macrophages have not been shown by the analysis of gene targeting mice.[1–4] To isolate osteoclast cell–cell fusion molecules, we undertook DNA subtraction screens between multinuclear osteoclasts and mononuclear macrophages.

Previously, we had found that osteoclasts and macrophages were derived from common precursor cells.[5] Multinuclear osteoclasts were induced by macrophage colony-stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL), whereas mononuclear macrophages were induced by M-CSF alone.[6] Numbers of molecules, such as RANK, c-Fms, integrin$\alpha_v\beta_3$, and Mac-1, are expressed in both osteoclasts and macrophages equivalently, thus a DNA subtraction screen between osteoclasts and macrophages was considered useful in identifying osteoclast-specific molecules. Approximately 100 molecules were identified by the screen, and among them DC-STAMP was selected as an osteoclast-specific molecule by Northern blot and revere transcription–polymerase chain reaction analyses.

Characterization of DC-STAMP

DC-STAMP was originally identified in dendritic cells or interleukin (IL)-4 stimulated macrophages as IL-4 induced (FIND).[7,8] Recently, it has been identified in osteoclasts and reported to induce differentiation of osteoclasts.[9] However, the role of DC-STAMP in cell–cell fusion has not been described. To analyze the function of DC-STAMP in vivo, we generated DC-STAMP-deficient mice.[10] We found that osteoclast cell–cell fusion was completely abrogated in DC-STAMP-deficient mice in vivo and in vitro. Interestingly, the osteoclasts derived from DC-STAMP-deficient mice show higher tartrate-resistant acid phosphatase (TRAP) activity than wild-type osteoclasts. Osteoclasts derived from DC-STAMP-deficient mice expressed osteoclast differentiation markers and the transcription factors required for osteoclast differentiation at equivalent levels of wild-type osteoclasts. Furthermore, the formation of ruffled boarders was detected in *DC-STAMP*$^{-/-}$ osteoclasts by electron

T. Miyamoto (✉)
Departments of Cell Differentiation, Orthopedics, and Musculoskeletal Reconstruction and Regeneration Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
Tel. +81-3-5363-3475; Fax +81-3-5363-3475
e-mail: miyamoto@sc.itc.keio.ac.jp

Table 1. Impacts of the DC-STAMP deficiency

Cell–cell fusion	Completely blocked
Osteoclast differentiation	Not altered (except multinucleation)
Bone-resorbing activity	Reduced
Bone phenotype	Increased bone mass

microscopic analysis, suggesting that DC-STAMP is specifically required for osteoclast cell–cell fusion but not for mononuclear osteoclast differentiation. Indeed, the osteoclast cell–cell fusion was effectively rescued by retroviral transduction of DC-STAMP into DC-STAMP-deficient osteoclast progenitor cells.

To analyze the role of cell–cell fusion in osteoclast function, bone-resorption assay was performed. The bone-resorbing area by DC-STAMP-deficient osteoclasts was significantly small compared with that of wild-type osteoclasts, even though the total number of nuclei was equal, suggesting that multinuclear osteoclasts have a higher bone-resorbing efficiency compared with mononuclear osteoclastic cells derived from DC-STAMP-deficient mice. In fact, an elevated radio-opacity was seen in *DC-STAMP*$^{-/-}$ mice by soft X-ray analysis. Furthermore, the bone mineral density (BMD) and bone volume per tissue volume (BV/TV) was increased in *DC-STAMP*$^{-/-}$ mice compared with wild-type mice. Thus, loss of osteoclast cell fusion increases bone volume in vivo. The impacts of the loss of DC-STAMP in osteoclasts are listed in Table 1.

It is well known that bone resorption by osteoclasts stimulates bone formation by osteoblasts. Thus complete block of osteoclast differentiation or function inhibits bone remodeling. Multinuclear osteoclasts are formed in rheumatoid arthritis, and multinucleation of osteoclast upregulates the efficiency of bone-resorbing activity, indicating that regulation of osteoclast cell–cell fusion might be a novel therapeutic target of joint destruction in rheumatoid arthritis, and that inhibition of cell–cell fusion alone may not inhibit bone remodeling.

References

1. Mbalaviele G, Chen H, Boyce BF, Mundy GR, Yoneda T. The role of cadherin in the generation of multinucleated osteoclasts from mononuclear precursors in murine marrow. J Clin Invest 1995;95:2757–65.
2. Saginario C, Sterling H, Beckers C, Kobayashi R, Solimena M, Ullu E, et al. MFR, a putative receptor mediating the fusion of macrophages. Mol Cell Biol 1998;18:6213–23.
3. de Vries TJ, Schoenmaker T, Beertsen W, van der Neut R, Everts V. Effect of CD44 deficiency on in vitro and in vivo osteoclast formation. J Cell Biochem 2005;94:954–66.
4. Takeda Y, Tachibana I, Miyado K, Kobayashi M, Miyazaki T, Funakoshi T, et al. Tetraspanins CD9 and CD81 function to prevent the fusion of mononuclear phagocytes. J Cell Biol 2003;161:945–56.
5. Arai F, Miyamoto T, Ohneda O, Inada T, Sudo T, Brasel K, et al. Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor kappaB (RANK) receptors. J Exp Med 1999;190:1741–54.
6. Miyamoto T, Ohneda O, Arai F, Iwamoto K, Okada S, Takagi K, et al. Bifurcation of osteoclasts and dendritic cells from common progenitors. Blood 2001;98:2544–54.
7. Hartgers FC, Vissers JL, Looman MW, van Zoelen C, Huffine C, Figdor CG, et al. DC-STAMP, a novel multimembrane-spanning molecule preferentially expressed by dendritic cells. Eur J Immunol 2000;30:3585–90.
8. Staege H, Brauchlin A, Schoedon G, Schaffner A. Two novel genes FIND and LIND differentially expressed in deactivated and Listeria-infected human macrophages. Immunogenetics 2001;53:105–13.
9. Kukita T, Wada N, Kukita A, Kakimoto T, Sandra F, Toh K, et al. RANKL-induced DC-STAMP is essential for osteoclastogenesis. J Exp Med 2004;200:941–6.
10. Yagi M, Miyamoto T, Sawatani Y, Iwamoto K, Hosogane N, Fujita N. et al. DC-STAMP is essential for cell–cell fusion in osteoclasts and foreign body giant cells. J Exp Med 2005;202:345–51.