Mod Rheumatol (2008) 18:220–227
DOI 10.1007/s10165-008-0051-2

REVIEW ARTICLE

Osteoclast cell fusion: mechanisms and molecules

Masaru Ishii · Yukihiko Saeki

Received: 12 October 2007 / Accepted: 25 January 2008 / Published online: 19 April 2008
© Japan College of Rheumatology 2008

Abstract Osteoclasts are bone-resorbing multinuclear polykaryon that are essential for bone remodeling and are formed through cell fusion of mononuclear macrophage/monocyte-lineage hematopoietic precursors. In arthritic joints, a large number of activated osteoclasts can be detected, which are suggested to be causative of bone erosion in rheumatoid arthritis. It has been fully established that osteoclastogenesis is critically regulated by several key essential factors, such as M-CSF and RANKL. However, regarding their most characteristic property, i.e., cell fusion to form giant polykaryons, there are still miscellaneous questions to be clarified, although several molecules have been shown to be critically involved in this process. Here we review the latest knowledge about osteoclastogenic cell fusion and novel concepts underlying the characteristic phenomenon. Because cell fusion is a genuine property of mature osteoclasts, modulating this process will become a promising therapeutic tool for bone resorptive disorders in the future.

Keywords Rheumatoid arthritis · Osteoclast · Cell fusion · Tetraspanin

Electronic supplementary material The online version of this article (doi:10.1007/s10165-008-0051-2) contains supplementary material, which is available to authorized users.

M. Ishii (✉) · Y. Saeki
Department of Rheumatology and Clinical Research,
NHO National Osaka Minami Medical Center,
2-1 Kidohigashimachi, Kawachinagano,
Osaka 586-8521, Japan
e-mail: mishii@ommc-hp.jp

Introduction

Increasing evidence has suggested that osteoclast, a multinucleated giant polykaryon differentiated from monocyte-lineage precursors, plays a key role in bone erosion in rheumatoid arthritis (RA). In patients with RA, every part of systemic bones and joints is gradually destroyed over a long period of diseases, which finally leads to the significant loss of their activities for daily living [1, 2]. The current standard regimen of therapy against RA, e.g., administration with methotrexate and/or TNF blockers from the onset of the diseases, has been developed in order to enforce the prevention of this undesirable outcome. Based on the great advances in basic immunology in the last two decades, many promising molecule-targeting remedies have been emerging, although most of them target inflammatory cytokines and membrane molecules on active immune cells. However, for better prevention of arthritic bone destruction, it is indispensable to unmask the character of osteoclast, which is an only somatic cell type endowed with bone resorbing capacity, and to find out how to regulate its function efficiently.

Osteoclast is highly specialized for its function as a "bone destructor", which shows a marked contrast to the fact that many other hematopoietic cells have generally pleiotropic natures [3–5]. We can reasonably assume that resolving hard bone tissues is such a hard task that an expert fully skilled exclusively for the work must deal with it. For its specialized work, osteoclast is matured as a characteristic giant polykaryon through homotropic cell–cell fusion from their precursor monocytes [6, 7]. In this review article, we discuss the function and mechanism of osteoclastogenic cell fusion and discuss them as a possible future therapeutic target against RA.

Osteoclastogenic cell fusion: physiological and rational aspects

Cell fusion is one of the most characteristic properties of osteoclasts, however there are several other kinds of cell-fusion events in physiological conditions, including sperm–oocyte fusion and myoblast fusion to form skeletal muscle fiber. Although these phenomenon may share some common mechanisms (as shown later), they can be clearly discriminated from osteoclastogenic cell fusion. During fertilization, a sperm cell is fused into an oocyte whose volume is much larger than that of a sperm cell, and this (heterotropic) cell–cell fusion usually does not change the cell size [8, 9]. Moreover, sperm–oocyte fusion occurs only one time in principle. Myofibril of skeletal muscle is formed through longitudinal fusion of (homotropic) myoblasts (precursor), and this firm syncytium structure seems suitable for synchronized excitation–contraction coupling [10, 11]. The function of myofibril is restricted to the mechanical contraction triggered by neuromuscular junction, and therefore they are quiescent in light of the metabolic activity and thus they are longer-lived.

On the contrary, osteoclastogenic cell fusion occurs within homotropic monocyte-lineage precursors (discussion is necessary and described later) in 2D direction, and the activity of osteoclasts (as bone destroyers) is so high that they cannot survive very long (their half-life is about 3 days) [12]. Then what is the meaning of cell fusion and consequent multinucleation during osteoclastogenesis? The major effect of multinucleation is obviously to increase the cell size of osteoclasts, which enables them to resorb larger areas of bone tissues. While macrophages and neutrophils degrade internalized targets in lysosome, osteoclasts degrade their targets (bone tissues) extracellularly. They attach tightly to the bone surface with a sealing zone, to avoid leakage of low-pH compartment for dissolving bone minerals [4] (this is referred to as "extracellular lysosome"). In other words, they can only degrade and resorb their targets which they are covering firmly.

Here we assume that the cell (both osteoclasts and their precursor mononuclear cells) is round and can degrade the hemisphere whose cross section is identical to the cell size (Fig. 1). The cell size (square) and its target volume are defined as πr^2, and $(2/3)\pi r^3$, respectively (where r is the radius of the cell). If the four cells work without cell fusion, the target volume increases to fourfold ($4 \times (2/3)\pi r^3$), whereas it increases to eightfold when the cell fuses to form multinucleated polykaryon ($((2/3)\pi(2r)^3 = 8 \times (2/3)\pi r^3$). In general, when n cells are fused, the volume which they can dig and absorb jumps to $\sqrt{n}$-fold. This means that osteoclasts can do their work more efficiently when they become multinucleated giant cells. A recent study has shown that cell fusion-incompetent osteoclasts isolated from DC-STAMP$^{-/-}$ mice (shown later) can still resorb the bone tissues although the activity is greatly impaired [13–15]. This study supports the notion that cell fusion is not essential for bone resorption but significantly affects the efficiency for the action.

Osteoclasts are formed through homotropic cell–cell fusion among monocyte-lineage precursors, although fusing two (or more) cells do not appear to be completely identical (Fig. 2, also see Supplemental movie S1). According to time-lapse analyses, large "founder" cells (which have already multinucleated) seem to catch mononuclear fusion-competent "follower" to fuse them (Iwai et al., unpubl. obs.). It is interesting that large "founder" cells are highly motile to seek their partners while mononuclear "follower" cells are rather stationary. This data clearly suggests that cell fusion during osteoclastogenesis has certain directionality and occurs among two distinct parts, i.e., "fuser (=founder)" and "to be fused (=follower)". This idea is also supported by a recent study showing that mixed culture of wild-type and DC-STAMP$^{-/-}$ (cell fusion-incompetent) precursors leads to formation of DC-STAMP$^{-/-}$ osteoclast polykaryons, suggesting that DC-STAMP is necessary only for one side of cell fusion ("fuser" or "to be fused") [14]. However, it is still unknown how these two kinds of cells are distinguishable, what is the character of "founder", and finally whether there is any difference between "fusion-competent cells" and other mononuclear cells that seem indifferent to cell fusion.

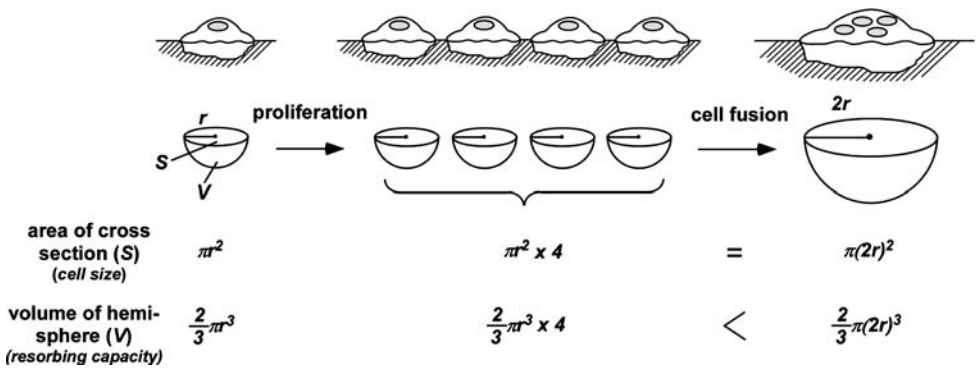

Fig. 1 Schematic representation of osteoclast cell fusion and their bone resorptive capacity. The capacity is represented as a volume of hemisphere whose cross section is identical to the cell size

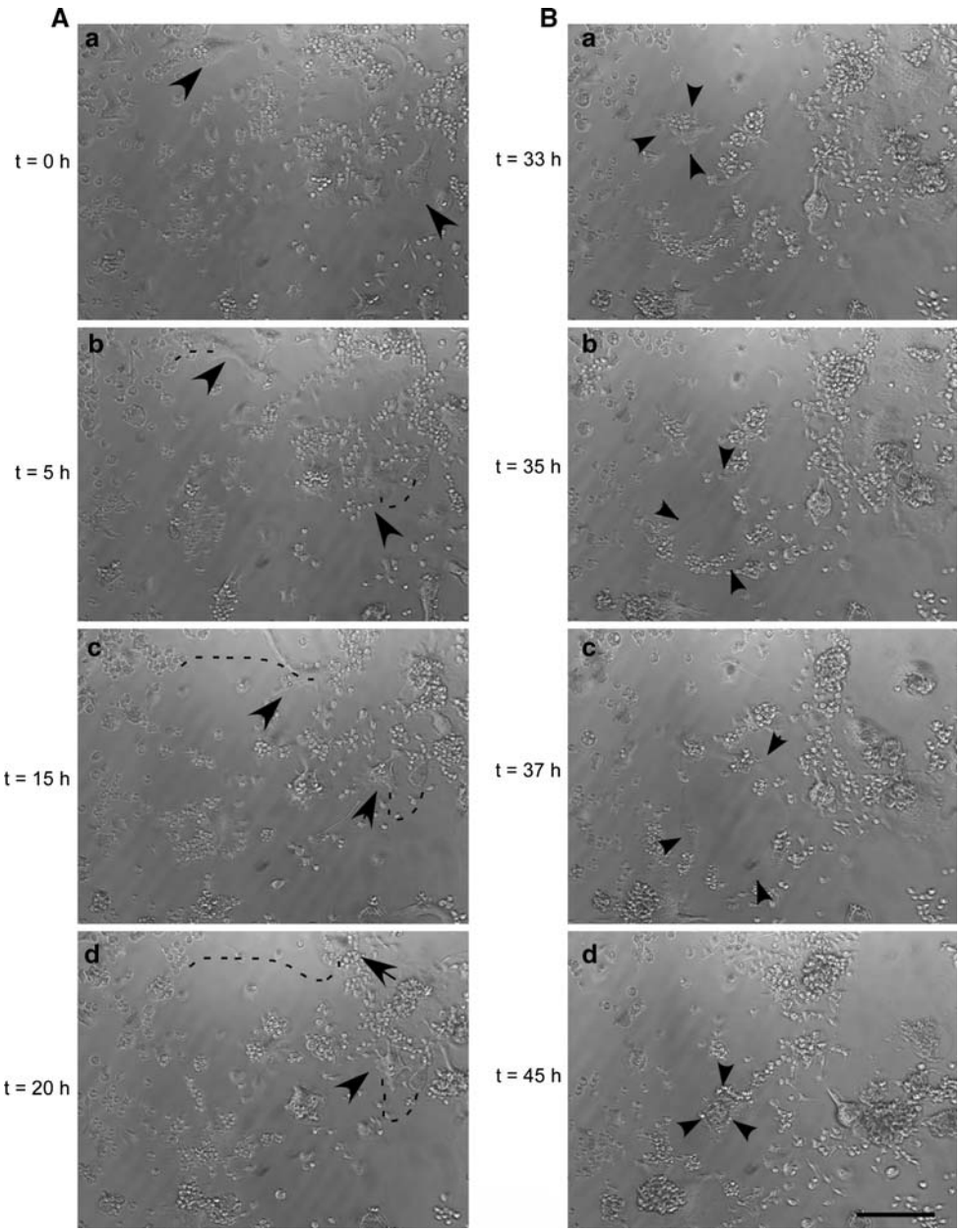

Fig. 2 Time-lapse analysis of osteoclast cell fusion. RAW264.7, a murine cell line having the character as osteoclast precursor monocytes, were cultured with 50 ng/ml of RANKL for 3 days. Nomarski images were acquired every 1 h by a timer-controlled CCD camera. "Founder" multinuclear cells moved rapidly to catch mononuclear "follower" cells (**A**, *arrows*) and they finally expanded aberrantly, leading to the death of fused polykaryon (**B**, *arrowheads*). *Dashed lines* (in **A**) represent trajectories of moving founder cells. *Scale bar* 100 μm

It has already been reported that two distinct role players, i.e., "founder cell" and "fusion competent cell" are present in the system of myoblast fusion [15, 16], although it is unknown whether there are some differences in the motility between these groups. In myoblast cell fusion, halting of cell movement for stable cell–cell contact was shown to be a prerequisite for subsequent cell fusion [17], which may contrast with the system in osteoclastogenic cell fusion.

Osteoclastogenic cell fusion: mechanistic aspects

To date, there have been many molecules reported to be involved in osteoclastogenic cell fusion. Because several excellent reviews have already been published for each molecule [6, 12, 18, 19], here we discuss some of the selected molecules instead of enumerating all the reported molecules.

MFR/SIRPα and CD47; a molecule for partner recognition

Macrophage fusion receptor (MFR), also called as SIRPα (signal regulatory protein α), is the first molecule identified to be crucial for osteoclastogenic cell fusion [20, 21]. MFR/SIRPα is a type I transmembrane protein that belongs to the immunoglobulin superfamily (IgSF) proteins, and monoclonal antibody against MFR/SIRPα interferes cell

fusion. MFR/SIRPα was subsequently shown to bind CD47, which also belongs to IgSF, and intercellular interaction via MFR/SIRPα–CD47 association is pivotal for osteoclastogenic cell fusion [22]. Interestingly, there are two isoforms (long and short) MFR/SIRPα depending on the length of extracellular domain, and it is hypothesized that CD47 first binding to long-form MFR/SIRPα might switch to the short form, which contributes to make plasma membranes of the two cells close enough to start fusion. Therefore, we may reasonably consider that MFR/SIRPα–CD47 interaction is involved in cell recognition before cell–cell fusion rather than in fusing process itself.

DC-STAMP: a master regulator of osteoclastic cell fusion

DC-STAMP (dendritic cell-specific transmembrane protein) has recently been emerging as a key molecule for cell fusion during osteoclastogenesis [13, 14, 18]. This seven-transmembrane protein is originally identified in dendritic cells or IL-4-activated macrophages, and subsequently it is shown to be expressed also in osteoclasts and to be up-regulated following stimulation by RANKL [13]. DC-STAMP-deficient osteoclasts cannot undergo cell fusion, although the expressions of osteoclast-specific molecules, such as V-type H^+ ATPase and cathepsin K, are intact [14]. The osteoclastic ability of DC-STAMP$^{-/-}$ mononuclear cells is largely impaired but not completely abrogated. These results clearly suggest that (1) DC-STAMP is important exclusively for cell fusion but not for osteoclastogenesis in general and that (2) multinucleation is not indispensable for bone resorbing activity but it does greatly enhance its activity (as discussed formerly). It was also shown that mutant monocytes expressing green fluorescent protein (GFP) in lieu of DC-STAMP can form multinucleated polykaryon with co-cultured wild-type monocytes, indicating the two distinct parts for cell–cell fusion, i.e., "fuser (=founder)" and "to be fused (=follower)", and the expression of DC-STAMP is necessary only for one side of fusion. In spite of these brilliant results, the question has not been answered how DC-STAMP is involved in the regulation of cell fusion. Because seven-membrane spanning DC-STAMP is structurally close to classical heterotrimeric G protein-coupled receptors, it is hypothesized that the role of DC-STAMP for osteoclastogenic cell fusion may be similar to that of the chemokine receptor CXCR4 and CCR5, co-receptors for HIV-T cell fusion [23].

Other miscellaneous molecules

In addition to MFR/SIRFα and DC-STAMP, various kinds of membrane proteins have been so far shown to be responsible for osteoclastogenic cell fusion, including CD44 (hyaluronan receptor) [24], CD98 [25], CD200 [26], cadherin [27], a purinergic receptor P2X7 [28], V-type ATPase Vo subunit d2 [29]. However, the mechanisms by which they practically regulate cell fusion are less understood.

Tetraspanin superfamily proteins (TM4SF): association with membrane microdomains

Crucial roles and controversies

Transmembrane-4 superfamily (TM4SF) proteins, also called "tetraspanins", have long been known to be involved in cell–cell fusion. The tetraspanins are characterized by their four transmembrane domains and comprise at least 32 distinct members in mammals, including CD9, CD37, CD53, CD63, CD81, and CD151 [30–34]. Among them, one of the tetraspanins, CD9, a membrane glycoprotein expressed in a variety of cell types, has been best understood and implicated in the regulation of cell motility and cell–cell fusion, including gamete membrane fusion [35–37], fusion of muscle cells [38], and formation of myelinated axons [39]. Recently several reports have shown the crucial role of tetraspanin CD9 in osteoclastogenic cell fusion, although there are still controversies about its detailed functions.

By using neutralizing antibody and RNA interference, CD9 has been shown to be a positive regulator of cell fusion during osteoclastogenesis [40, 41]. The expression of CD9 on the cell surface, but not at the transcriptional level, is enhanced by osteoclastogenic RANKL stimulation, and functional blockage of CD9 by neutralizing monoclonal antibody or RNA interference inhibits cell fusion. On the contrary, forced expression of CD9 in a monocyte lineage cell line induces spontaneous cell fusion without stimulating expressions of osteoclast specific molecules, such as TRAP and cathepsin K. These results have suggested that tetraspanins CD9 is a downstream molecule of osteoclastogenic RANKL signaling pathway specialized for cell–cell fusion. It was also shown that the expression of CD9 is augmented at the site of bone erosion in arthritic joints or cancellous bone tissues in osteoporosis, which may suggest the pathogenic role of tetraspanins CD9 in bone resorptive disorders [42], although further studies are definitely needed, such as examining the effect of neutralizing antibody for collagen-induced arthritis or ovariectomy-induced osteoporosis models.

On the other hand, another report [43] showed a contradictory result: Genetic deletion of tetraspanin CD9 enhanced osteoclastogenic cell fusion, and it was claimed that tetraspanins CD9 rather inhibits cell fusion during osteoclastogenesis. It is difficult to explain here the reason of this discrepancy, although we should consider the following points;

1. Redundant expression of different tetraspanins: expression of tetraspanins proteins is highly redundant, and it has already been shown that 12 different kinds of mammalian tetraspanins, such as CD9, CD37, CD53, CD63, CD81, CD82, CD151, NAG-2, SAS, Tspan-3, Tspan-5, and NET-6/Tspan-12, are expressed in osteoclasts and its precursors at least mRNA levels [44]. Among them, Tspan-5 is up-regulated and NET-6/Tspan-12 is down-regulated during osteoclastogenesis. Targeted inhibition of Tspan-5 and suppressed RANKL-induced cell fusion, whereas inhibition of NET-6/Tspan-12 augmented the event. These results suggest that Tspan-5 and NET-6 have reciprocal role for osteoclastogenic cell fusion [44]. Taken together, redundancy and diversity of tetraspanins contribute to fine control of cell-fusion processes during osteoclastogenesis. Therefore, gene ablation of a single type of tetraspanin may be compensated (sometimes "over-compensated") by some alternative tetraspanins and may not necessarily bring straightforward results. To further elucidate the aspects, it may be helpful to examine the expression levels of different tetraspanins in CD9-global knockout mice [43].
2. Expression of CD9 in other cell types (bone marrow stromal cell or osteoblasts): tetraspanins are known to be expressed in various kinds of cell types in bone marrows as well as in hematopoietic cells. As for CD9, it has been already reported that CD9 is expressed in bone marrow stromal cells and controls osteoclastogenesis via conversation with osteoclast precursors [45, 46]. This means that CD9-global knockout mice may exhibit a compound phenotype, i.e., derived from genetic deletion of CD9 both in osteoclasts and in stromal cells, and this may not be appropriate for solely studying the function of CD9 expressed on osteoclasts. In order to clarify the functional impact of CD9 on osteoclasts in vivo, it is desirable to generate osteoclast-lineage specific conditional CD9 knockout mice or to reconstitute CD9-ablated osteoclasts in osteoclast-deficient mice strains by generating bone marrow chimeras.

Interestingly, because granuloma-forming macrophage fusion, which has long been considered to be a close phenomenon to osteoclastogenic cell fusion, was not enhanced in the same CD9-global knockout mice [47], there must be a matter specific for osteoclastogenesis. Although most of the reports published so far supported the positive (stimulatory) functions of tetraspanins for cell–cell fusion in various biological systems, one more report has recently emerged showing their inhibitory role in cell fusion (HIV-induced cell fusion) [48], and now the actual functions of tetraspanin CD9 on osteoclastogenic cell fusion still remain elusive.

Such ambivalent evidence lets us know the nature of tetraspanins as the modulators of signaling (for cell fusion). It may be plausible that tetraspanins are not essential factors for cell fusion (e.g., DC-STAMP) but modulating the other essential machinery. Nevertheless, this fact does not necessarily mean that tetraspanins are unimportant ones, but on the contrary, they are suitable as therapeutic targets. It is generally risky to alter pharmacologically the action of some "essential" molecules because the outcome easily exceeds the therapeutic range. Anyway, further studies are necessary to clarify the detailed function of tetraspanins in cell fusion during osteoclast formation and to test the therapeutic potentials for anti-bone resorptive treatments.

Association with membrane domain and cis-interaction with other molecules

Although there are one or two examples showing the interaction of tetraspanins with counter-receptors on other cells or soluble ligands (Fig. 3Aa), it is well accepted that tetraspanins exert their functions by interacting in *cis* (laterally) with various other membrane proteins, such as integrins [38, 49, 50] or B cell receptor complexes [51, 52], and by modulating their activities (Fig. 3Ab). Lateral interaction of tetraspanins with various membrane proteins and lipids contributes to organization of multimolecular complexes ("tetraspanins web") on a certain membrane microdomain, named "tetraspanins-enriched microdomain (TEM)" [52–55]. Biochemically, TEMs are mostly soluble in stringent non-ionic detergents, such as 1% Triton X-100, although they are resistant to solubilization by milder detergents such as 1% CHAPS or Brij-99 [53]. Therefore, TEM can be clearly discriminated from the conventional "lipid raft" microdomains that are characterized by their insolubility in the stringent detergents such as 1% Triton X-100. It is suggested that lipid raft microdomain and TEM occupy different areas on eukaryotic cell membrane, however, the respective functions and their relationship between these two microdomains have remained to be elusive. It has been demonstrated that RANKL induces the translocation of tetraspanin CD9 to conventional "lipid raft" microdomains from non-raft microdomains [40]. This suggests that CD9 may interact with some membrane molecules responsible for cell fusion and carry them to lipid raft microdomain [56], which is the platform for cell signal transduction (Fig. 3B). It would be interesting in further studies to examine whether tetraspanins and DC-STAMP can communicate "softly" with each other (not by "hard" direct protein-protein interaction), utilizing a certain membrane microdomain as a signaling platform.

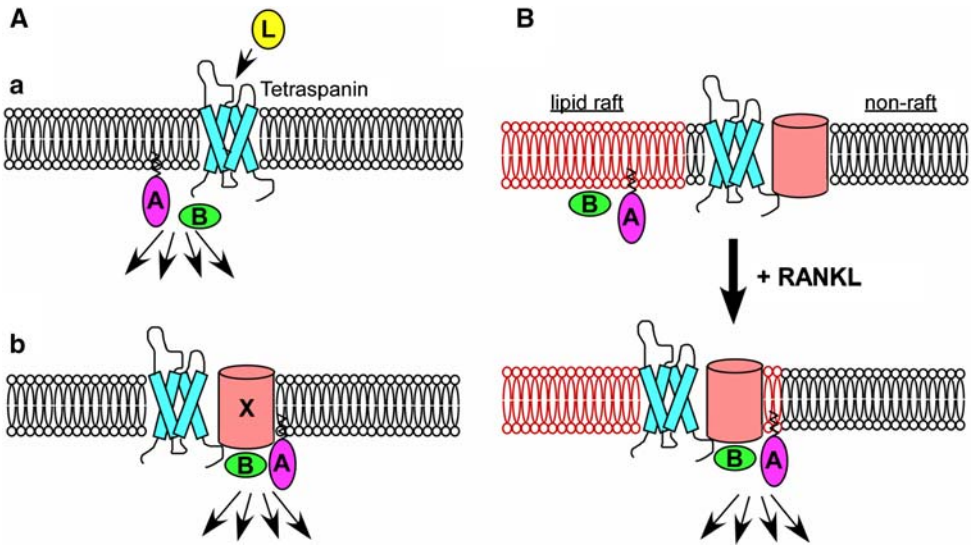

Fig. 3 Possible mode of action of tetraspanin proteins. **A** Tetraspanin mediates signal transduction either by directly activating intracellular signal modules (**A**, **B**) by itself (**A***a*), or by interacting in *cis* (laterally) with other partner proteins (X) and by modulating its activity (**A***b*). **B** RANKL-induced lipid raft localization of tetraspanin CD9 from non-raft fraction. This may be coupled with partners (X), facilitating their signal transduction

The relationship between tetraspanins and membrane microdomain is intriguing because it has been shown that particular lipid composition of plasma membrane, such as in lipid raft, is important for membrane fusion [57–59]. The concept has been best understood in intracellular vesicle membrane fusion during exocytosis. Raft membrane highly contains phosphorylated phosphoinositides, such as phosphatidylinositol-4,5-bisphosphate (PIP_2) [60], which is known to have an "inverted cone-shaped" structure (Fig. 4). The interaction of membrane PIP_2 with Ca^{2+}, elevated by the signal stimulating exocytosis, convert the structure into "cone-shaped" structure, whose biophysical property favors the membrane curvature to form stalks within two membranes [61, 62]. Membrane fusion for vesicular trafficking is initiated from inner leaflet, whereas the process starts from the outer leaflet in the case of cell–cell fusion. Nevertheless, we can speculate that there may be some shared mechanisms involving lipid raft membrane domains for membrane fusion. Tetraspanin-dependent localization of signaling molecules in lipid raft fraction may contribute to the initiation of membrane fusion by changing some biophysical properties of membranes, although further studies regarding this aspect must be performed to get the whole picture of this theme.

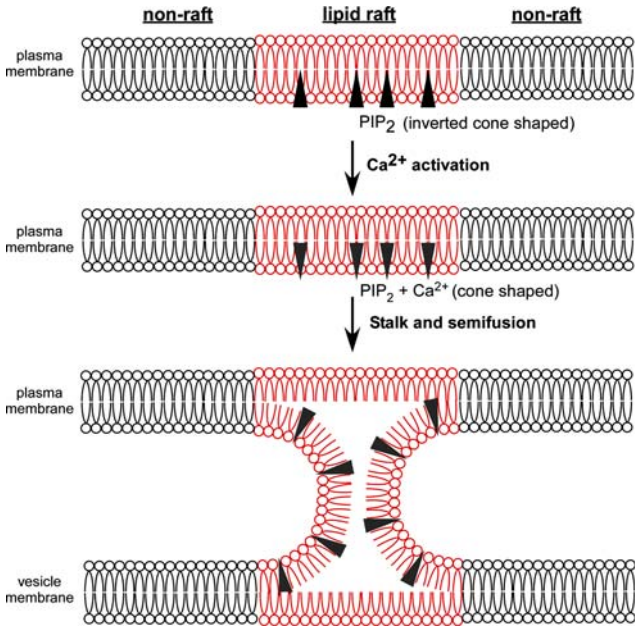

Fig. 4 "Stalk and pore model" and lipid raft membrane domain. Lipid raft contains plenty of "inverted cone-shaped" lipids, such as PIP_2 (*upper panel*). Membrane fusion-stimulating signals, such as Ca^{2+} elevation, convert the lipid structure into "cone shaped" (*middle panel*), which favors the bend of membrane to form stalk and pore initiating cell fusion (*lower panel*)

Closing remarks

Recent progress has gradually unraveled the enigmatic mechanisms underlying osteoclastogenic cell fusion. Because osteoclasts play central roles in arthritic bone destruction, it is sure to become a promising therapeutic tool in the future to control the osteoclast function by modulating their cell–cell fusion, the most characteristic property of this cell type.

Acknowledgments We thank Ms. Kaori Iwai (Department of Clinical Research, Osaka Minami Medical Center) for providing imaging data and helpful discussions. This work was supported by a Grant-in-Aid for the Encouragement of Young Scientists (15790133 and 17790170) from the Ministry of Education, Science, Sports and Culture of Japan, and by a Grant-in-Aid from Takeda Science

Foundation, and by a Grant-in-Aid from Kanae Foundation for Socio-Medical Sciences.

References

1. Bromley M, Bertfield H, Evanson JM, Woolley DE. Bidirectional erosion of cartilage in rheumatoid knee joints. Ann Rheum Dis. 1985;44:676–81.
2. Kuratani T, Nagata K, Kukita T, Hotokebuchi T, Nakashima A, Iijima T. Induction of abundant osteoclast-like multinucleated giant cells in adjuvant arthritic rats with accompanying disordered high bone turnover. Histol Histopathol. 1988;13:751–9.
3. Teitelbaum SL. Bone resorption by osteoclasts. Science. 2000;289:1504–8.
4. Teitelbaum SL, Ross FP. Genetic regulation of osteoclast development and function. Nat Rev Genet. 2003;4:638–49.
5. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature. 2003;423:337–42.
6. Vignery A. Macrophage fusion: are somatic and cancer cells possible partners? Trends Cell Biol. 2005;15:188–93.
7. Chen EH, Olson EN. Unveiling the mechanisms of cell–cell fusion. Science. 2005;308:369–73.
8. Nixon B, Aitken RJ, McLaughlin EA. New insights into the molecular mechanisms of sperm-egg interaction. Cell Mol Life Sci. 2007;64:1805–23.
9. Kaji K, Kudo A. The mechanism of sperm–oocyte fusion in mammals. Reproduction. 2004;127:423–9.
10. Taylor MV. Muscle differentiation: signaling cell fusion. Curr Biol. 2003;13:R964–6.
11. Prisk V, Huard J. Muscle injuries and repair: the role of prostaglandins and inflammation. Histol Histopathol. 2003;18:1243–56.
12. Chen EH, Grote E, Mohler W, Vignery A. Cell–cell fusion. FEBS Lett. 2007;581:2181–93.
13. Kukita T, Wada N, Kukita A, Kakimoto T, Sandra F, Toh K, Nagata K, Iijima T, Horiuchi M, Matsusaki H, Hieshima K, Yoshie O, Nomiyama H. RANKL-induced DC-STAMP is essential for osteoclastogenesis. J Exp Med. 2004;200:941–6.
14. Yagi M, Miyamoto T, Sawatani Y, Iwamoto K, Hosogane N, Fujita N, Morita K, Ninomiya K, Suzuki T, Miyamoto K, Oike Y, Takeya M, Toyama Y, Suda T. DC-STAMP is essential for cell–cell fusion in osteoclasts and foreign body giant cells. J Exp Med. 2005;202:345–51.
15. Baylies MK, Bate M, Ruiz-Gomez M. Myogenesis: a view from Drosophila. Cell. 1998;93:921–7.
16. Beckett K, Baylies MK. 3D analysis of founder cell and fusion competent myoblast arrangements outlines a new model of myoblast fusion. Dev Biol. 2007;309:113–25.
17. Bondesen BA, Jones KA, Glasgow WC, Pavlath GK. Inhibition of myoblast migration by prostacyclin is associated with enhanced cell fusion. FASEB J. 2007;21:3338–45.
18. Vignery A. Macrophage fusion: the making of osteoclasts and giant cells. J Exp Med. 2005;202:337–40.
19. Vignery A. Osteoclasts and giant cells: macrophage-macrophage fusion mechanism. Int J Exp Pathol. 2000;81:291–304.
20. Saginario C, Qian HY, Vignery A. Identification of an inducible surface molecule specific to fusing macrophages. Proc Natl Acad Sci USA. 1995;92:12210–4.
21. Saginario C, Sterling H, Beckers C, Kobayashi R, Solimena M, Ullu E, Vignery A. MFR, a putative receptor mediating the fusion of macrophages. Mol Cell Biol. 1998;18:6213–23.
22. Han X, Sterling H, Chen Y, Saginario C, Brown EJ, Frazier WA, Lindberg FP, Vignery A. CD47, a ligand for the macrophage fusion receptor, participates in macrohage multinucleation. J Biol Chem. 2000;275:37984–92.
23. Berger EA, Murphy PM, Farber JM. Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism and disease. Annu Rev Immunol. 1999;17:657–700.
24. Sterling H, Saginario C, Vignery A. CD44 occupancy prevents macrophage multinucleation. J Cell Biol. 1998;143:837–47.
25. Ohgimoto S, Tabata N, Suga S, Nishio M, Ohta H, Tsurudome M, Komada H, Kawano M, Watanabe N, Ito Y. Molecular characterization of fusion regulatory protein-1 (FRP-1) that induces multinucleated giant cell formation of monocytes and HIV gp160-mediated cell fusion. FRP-1 and 4F2/CD98 are identical molecules. J Immunol. 1995;155:3585–92.
26. Cui W, Cuartas E, Ke J, Zhang Q, Einarsson HB, Segdwick JD, Li J, Vignery A. CD200 and its receptor, CD200R, modulate bone mass via the differentiation of osteoclasts. Proc Natl Acad Sci USA. 2007;104:14436–41.
27. 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.
28. Lemaire I, Falzoni S, Leduc N, Zhang B, Pellegatti P, Adinolfi E, Chiozzi P, Di Virgilio F. Involvement of the purinergic P2X7 receptor in the formation of multinucleated giant cells. J Immunol. 2006;177:7257–65.
29. Lee SH, Rho J, Jeong D, Sul JY, Kim T, Kim N, Kang JS, Miyamoto T, Suda T, Lee SK, Pignolo RJ, Koczon-Jaremko B, Lorenzo J, Choi Y. v-ATPase V0 subunit d2-deficient mice exhibit impaired osteoclast fusion and increased bone formation. Nat Med. 2006;12:1403–9.
30. Boucheix C, Rubinstein E. Tetraspanins. Cell Mol Life Sci. 2001;58:1189–205.
31. Hemler ME. Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. Annu Rev Cell Dev Biol. 2003;19:397–422.
32. Hemler ME. Tetraspanin functions and associated microdomains. Nat Rev Mol Cell Biol. 2005;6:801–11.
33. Tarrant JM, Robb L, van Spriel AB, Wright MD. Tetraspanins: molecular organizers of the leukocyte surface. Trends Immunol. 2003;24:610–7.
34. Wright MD, Moseley GW, van Spriel AB. Tetraspanin microdomains in immune cell signaling and malignant disease. Tissue Antigens. 2004;64:533–42.
35. Kaji K, Oda S, Shikano T, Ohnuki T, Uematsu Y, Sakagami J, Tada N, Miyazaki S, Kudo A. The gamete fusion process is defective in eggs of Cd9-deficient mice. Nat Genet. 2000;24:279–82.
36. Le Naour F, Rubinstein E, Jasmin C, Prenant M, Boucheix C. Severely reduced female fertility in CD9-deficient mice. Science. 2000;287:319–21.
37. Miyado K, Yamada G, Yamada S, Hasuwa H, Nakamura Y, Ryu F, Suzuki K, Kosai K, Inoue K, Ogura A, Okabe M, Mekada E. Requirement of CD9 on the egg plasma membrane for fertilization. Science. 2000;287:321–4.
38. Tachibana I, Hemler ME. Role of transmembrane 4 superfamily (TM4SF) proteins CD9 and CD81 in muscle cell fusion and myotube maintenance. J Cell Biol. 1999;146:893–904.
39. Ishibashi T, Ding L, Ikenaka K, Inoue Y, Miyado K, Mekada E, Baba H. Tetraspanin protein CD9 is a novel paranodal component regulating paranodal junctional formation. J Neurosci. 2004;24:96–102.
40. Ishii M, Iwai K, Koike M, Ohshima S, Kudo-Tanaka E, Ishii T, Mima T, Katada Y, Miyatake K, Uchiyama Y, Saeki Y. RANKL-induced expression of tetraspanin CD9 in lipid raft membrane microdomain is essential for cell fusion during osteoclastogenesis. J Bone Miner Res. 2006;21:965–76.
41. Yi T, Kim HJ, Cho JY, Woo KM, Ryoo HM, Kim GS, Baek JH. Tetraspanin CD9 regulates osteoclastogenesis via regulation of

p44/42 MAPK activity. Biochem Biophys Res Commun. 2006;347:178–84.
42. Iwai K, Ishii M, Ohshima S, Miyatake K, Saeki Y. Abundant expression of tetraspanin CD9 in activated osteoclasts in ovariectomy-induced osteoporosis and in bone erosions of collagen-induced arthritis. Rheumatol Int. 2008;28:225–31.
43. Takeda Y, Tachibana I, Miyado K, Kobayashi M, Miyazaki T, Funakoshi T, Kimura H, Yamane H, Saito Y, Goto H, Yoneda T, Yoshida M, Kumagai T, Osaki T, Hayashi S, Kawase I, Mekada E. Tetraspanins CD9 and CD81 function to prevent the fusion of mononuclear phagocytes. J Cell Biol. 2003;161:945–56.
44. Iwai K, Ishii M, Ohshima S, Miyatake K, Saeki Y. Expression and function of transmembrane-4 superfamily (tetraspanin) proteins in osteoclasts: reciprocal roles of Tspan-5 and NET-6 during osteoclastogenesis. Allergol Int. 2007;56:457–63.
45. Tanio Y, Yamazaki H, Kunisada T, Miyake K, Hayashi SI. CD9 molecule expressed on stromal cells is involved in osteoclastogenesis. Exp Hematol. 1999;27:853–9.
46. Hayashi S, Miyake K, Kinkade PW. The CD9 molecule on stromal cells. Leuk Lymphoma. 2000;38:265–70.
47. Yamane H, Tachibana I, Takeda Y, Saito Y, Tamura Y, He P, Suzuki M, Shima Y, Yoneda T, Hoshino S, Inoue K, Kijima T, Yoshida M, Kumagai T, Osaki T, Eishi Y, Kawase I. Propionibacterium acnes-induced hepatic granuloma formation is impaired in mice lacking tetraspanin CD9. J Pathol. 2005;206:486–92.
48. Gordón-Alonso M, Yañez-Mó M, Barreiro O, Alvarez S, Muñoz-Fernández MA, Valenzuela-Fernández A, Sánchez-Madrid F. Tetraspanins CD9 and CD81 modulate HIV-1-induced membrane fusion. J Immunol. 2006;177:5129–37.
49. Yáñez-Mó M, Alfranca A, Cabañas C, Marazuela M, Tejedor R, Ursa MA, Ashman LK, de Landázuri MO, Sánchez-Madrid F. Regulation of endothelial cell motility by complexes of tetraspan molecules CD81/TAPA-1 and CD151/PETA-3 with $\alpha_3\beta_1$ integrin localized at endothelial lateral junctions. J Cell Biol. 1998;141:791–804.
50. Sterk LM, Geuijen CA, van den Berg JG, Claessen N, Weening JJ, Sonnenberg A. Association of the tetraspanin CD151 with the laminin-binding integrins $\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$ and $\alpha_7\beta_1$ in cells in culture and in vivo. J Cell Sci. 2002;115:1161–73.
51. Cherukuri A, Shoham T, Sohn HW, Levy S, Brooks S, Carter R, Pierce SK. The tetraspanin CD81 is necessary for partitioning of coligated CD19/CD21-B cell antigen receptor complexes into signaling-active lipid rafts. J Immunol. 2004;172:370–80.
52. Cherukuri A, Carter RH, Brooks S, Bornmann W, Finn R, Dowd CS, Pierce SK. B cell signaling is regulated by induced palmitoylation of CD81. J Biol Chem. 2004;279:31973–82.
53. Claas C, Stipp CS, Hemler ME. Evaluation of TM4SF protein complexes and their relation to lipid rafts. J Biol Chem. 2001;276:7974–84.
54. Brown DA, London E. Structure and function of sphingolipid- and cholesterol-rich membrane rafts. J Biol Chem. 2000;275:17221–4.
55. Kropshofer H, Spindeldreher S, Röhn TA, Platania N, Grygar C, Daniel N, Wölpl A, Langen H, Horejsi V, Vogt AB. Tetraspan microdomains distinct from lipid rafts enrich select peptide-MHC class II complexes. Nat Immunol. 2002;3:61–8.
56. Simons K, Ikonen E. Functional rafts in cell membranes. Nature. 1997;387:569–72.
57. Jahn R, Sudhof TC. Membrane fusion and exocytosis. Annu Rev Biochem. 1999;68:863–911.
58. Chernomordik LV Kozlov MM. Protein-lipid interplay in fusion and fission of biological membranes. Annu Rev Biochem. 2003;72:175–207.
59. Pike LJ, Han X, Chung K-N, Gross RW. Lipid rafts are enriched in arachidonic acid and plasmenylethanolamine and their composition is independent of caveolin-1 expression: a quantitative electrospray ionization/mass spectrometric analysis. Biochemistry. 2002;41:2075–88.
60. Hope HR, Pike LJ. Phosphoinositides and phosphoinositide-utilizing enzymes in detergent-insoluble lipid domains. Mol Biol Cell. 1996;7:843–5.
61. Muller M, Zschornig O, Ohki S, Arnold K. Fusion, leakage and surface hydrophobicity of vesicles containing phosphoinositides: influence of steric and electrostatic effects. J Membr Biol. 2003;192:33–43.
62. Salaun C, James DJ, Chamberlain LH. Lipid rafts and the regulation of exocytosis. Traffic. 2004;5:255–64.