Macrophage fusion: the making of osteoclasts and giant cells

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The fusion of cells is a fundamental biological event that is essential for a variety of developmental and homeostatic processes. Fusion is required for the formation of multinucleated osteoclasts and giant cells, although the mechanisms that govern these processes are poorly understood. A new study now reveals an unexpected role for the receptor, dendritic cell–specific transmembrane protein (DC–STAMP), in this process. The potential mechanism by which DC–STAMP governs fusion and the implications of this finding will be discussed.

Introduction

Macrophages are present in all tissues and can fuse with other macrophages to differentiate into multinucleate osteoclasts (in bone) or giant cells (in multiple tissues), which play a central role in osteoporosis and chronic inflammatory diseases, respectively. Multinucleation is an essential step in the differentiation of osteoclasts, as mononucleated macrophages cannot resorb bone efficiently, and may also be essential in the differentiation of giant cells, which form in tissues in response to foreign particles. Macrophages might also fuse with somatic cells to promote tissue repair and with tumor cells to trigger metastasis.

On page 345 of this issue, Yagi et al. (1) show that DC-STAMP is required for the fusion of preosteoclasts and macrophages to yield osteoclasts and giant cells, respectively. DC-STAMP was first identified in dendritic cells, which can transdifferentiate and fuse to yield osteoclasts (2). Thus, clarification of the role of DC-STAMP in macrophage fusion will be a major step toward a better understanding of fusion and will bring together several areas of research.

The mechanics of fusion

The first clues about the nature of membrane fusion came from studies of

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viruses. The fusion of viruses with cells, in particular influenza virus and human immunodeficiency virus (HIV), provided strong evidence that fusion is mediated both by viral proteins and host cell surface molecules that function as viral receptors (3). Thus, a receptor–ligand interaction mediates the binding of viruses to the membrane of the host cell. For example, the HIV surface ligand gp120 binds to its receptor (CD4) on T lymphocytes and macrophages, and the hemagglutinin protein of influenza binds to its receptor (sialic acid) on epithelial cells. Most of the viral proteins that are responsible for the binding of the virus to the host cell also play a role in fusion. For example, the attachment of HIV to its target cell via gp120 leads to a conformational change that exposes the associated fusion protein gp41 (gp120 and gp41 are derived from a single protein, gp160, that is posttranslationally cleaved). These fusion proteins are integral membrane glycoproteins that exist as oligomers on the surface of each virion. Most viral fusion proteins contain a stretch of hydrophobic amino acids, known as a fusion peptide, which penetrates host cells like a sword, destabilizing the lipid bilayer of the host cell. The fusion protein then undergoes a conformational change, forming a hairpin-like α-helical bundle, which acts like a spring to propel the viral membrane close enough to the cell membrane to trigger fusion.

Another type of fusion occurs between distinct membranes within a cell, such as during intracellular trafficking between the endoplasmic reticulum and the Golgi apparatus. Fusion of an intracellular vesicle with its target membrane is mediated by a set of conserved proteins that are collectively referred to as SNAREs (soluble NSF-attachment protein [SNAP] receptors). Many vesicle (v)- and target (t)-SNAREs have been characterized in yeast, plants, and animals (4), and shown to form a bundle of α-helices (SNAREpins) that bring opposing membranes close enough to fuse. The fusion process between intracellular membranes occurs in a manner analogous to that between viruses and cells.

A number of putative fusion proteins have recently been identified in the plasma membrane of various types of fusing cells from different species, many of which belong to the immunoglobulin superfamily of proteins (see reference 5 for a review). As immunoglobulin proteins do not contain a fusion peptide or α-helical spring, these cannot be the universal tools that mediate membrane fusion. As thus far no two cell types share the same putative fusion protein, it seems plausible that alternative mechanisms for fusion might have evolved for each cell type, with fusion being cell type–specific and depending on different proteins in different species (5). Yet, these observations still support the idea that receptor–ligand interactions are universally required for fusion.

Macrophage fusion: what we know

The molecular mechanisms that allow macrophages to fuse with each other and, possibly, with somatic and cancer cells, remain poorly understood. Several proteins have been identified that play a role in macrophage fusion. One of these proteins, macrophage fusion receptor (MFR), was identified by cloning the target of monoclonal antibodies that altered the fusion of rat al-

veolar macrophages in vitro (6, 7). MFR and its recently identified ligand CD47 both belong to the immunoglobulin superfamily (8). Expression of CD47 is ubiquitous, whereas that of MFR is restricted to myeloid cells and neurons. In addition, the expression of MFR is induced strongly but transiently in macrophages at the onset of fusion, whereas the expression of CD47 remains constant, supporting the hypothesis that fusion is a regulated event. CD44 is another cell surface receptor similar to MFR whose expression is induced strongly but transiently at the onset of fusion in macrophages. (9). No cell surface ligand for CD44 has yet been identified, perhaps because the extracellular domain of CD44 is cleaved by membrane type 1–matrix metalloproteinases (10), and may thus be shed from the plasma membrane of fusing macrophages. Such cleavage would allow plasma membranes from opposite cells to interact more closely and, hence, facilitate fusion. A long and a short form of MFR have been identified, leading to a model in which CD47 binds first to the long form of MFR to secure the attachment of macrophages, and then switches to the short form of MFR to bring the plasma membranes closer to one another. At the same time, the shedding of the extracellular domain of CD44 might allow plasma membranes to get closer (11).

It has been proposed that MFR–CD47 interactions also play a central role in cell–cell recognition, which might be essential for the survival of the fused macrophages (11). If this is the case, terminally differentiated macrophages—which are "professional" phagocytes—might recognize CD47 as a reciprocal signal of self. This would allow them to survive "cellocytosis" (cell–cell internalization), leading to multinucleation, rather than to the lysosomal degradation of the internalized cell. Hence, regulating the expression of MFR might be of central importance to fusion and the making of a new multinucleate cell.

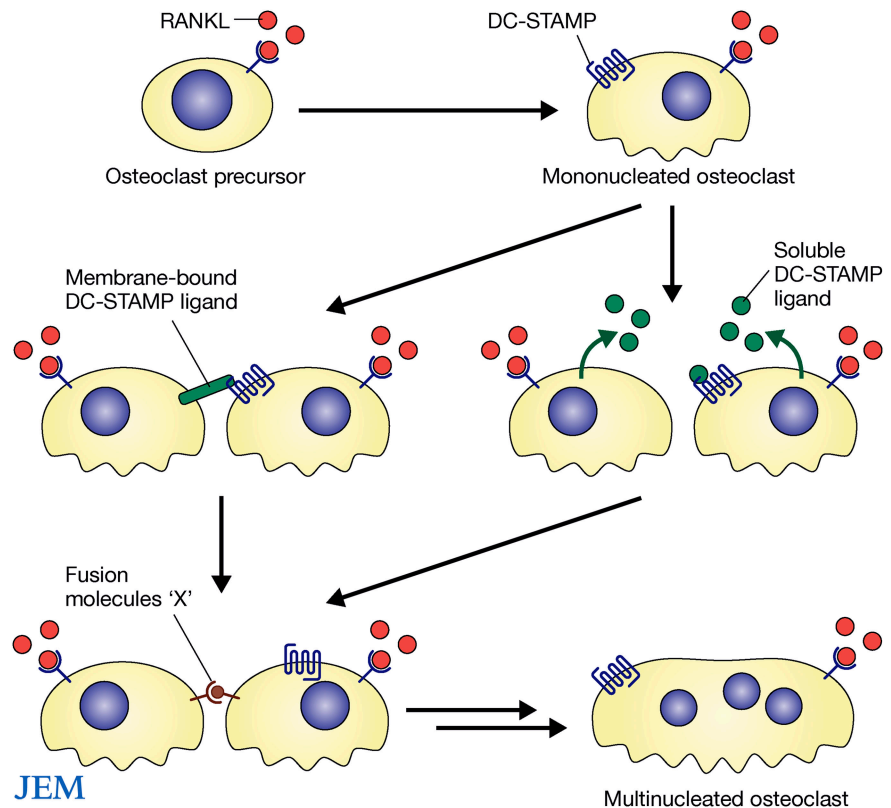MFR belongs to the family of signal regulatory proteins (SIRPs), which

Figure 1. Potential mechanism of fusion of preosteoclasts and of macrophages. Osteoclast precursor cells respond to the osteoclast differentiation factor RANKL, which induces the expression of DC-STAMP. The DC-STAMP–expressing osteoclast becomes the master fusing cell, which can fuse with a DC-STAMP–negative follower cell. The ligand for DC-STAMP may be membrane bound or soluble; a soluble ligand might be released by either of the fusion partners. DC-STAMP ligation may trigger fusion of the two cells directly or may trigger the expression of as yet unknown membrane-bound molecules ('X') that mediate fusion. A similar scenario may occur during the formation of giant cells in which macrophages respond to interleukin-4 by inducing the expression of DC-STAMP, which then facilitates fusion.

have intrinsic signaling functions dictated by either a cytoplasmic domain that contains an immunoreceptor tyrosine-based inhibitory motif (ITIM), as is the case for MFR (also known as SIRPα), or a transmembrane region that associates with adaptor molecules that contain an immunoreceptor activating motif (ITAM), as is the case for SIRPβ (12). These ITAM-containing adaptor molecules (such as DAP12, CD3ζ, and the γ chain of the Fc receptor [FcRγ]), recruit tyrosine kinases (such as Syk or ZAP70) that trigger the activation of downstream signaling events. ITIMs recruit tyrosine phosphatases, such as Src homology 2 domain tyrosine phosphatase (SHP) 1 and SHP-2, that negatively regulate ITAM signaling.

Recently, it was reported that mice that lack DAP12 and/or FcRγ develop mild osteopetrosis, a disease associated with a defect in the differentiation or activation of osteoclasts, which leads to the thickening of bones (13, 14). It was concluded in that study that, upon activation of surface receptors, the ITAM domains of DAP12 and FcRγ become phosphorylated and initiate activating downstream signals. Conversely, mice with a mutation in SHP-1 develop severe osteoporosis (loss of bone), which is associated with an increase in the differentiation and activation of osteoclasts (15, 16). Given the link between fusion and osteoclast differentiation, it is possible that the intracellular signaling pathways downstream of MFR and SIRPβ, involving ITAM–ITIM cross

talk, might be functionally linked to modulate fusion.

DC–STAMP: a new kid on the block

DC-STAMP was identified from a screening of human monocyte cDNA libraries (17) as a putative seven-trans-membrane–spanning receptor with no homology to any other known protein or multimembrane-spanning receptor. DC-STAMP is expressed both in immature and mature dendritic cells (DCs), and its mRNA levels fall upon activation of DCs with CD40 ligand (CD40L). DC-STAMP is overexpressed in giant cell tumors together with receptor activator of NF-κB ligand (RANKL), a protein required for the development of osteoclasts (18). In a recent study, Kukita et al. showed, using small interfering RNAs and specific antibodies, that DC-STAMP is essential for osteoclastogenesis in mice (2). They reported that overexpression of DC-STAMP enhanced osteoclastogenesis and induced the expression of a marker of osteoclasts, tartrate-resistant acid phosphatase. In this issue, Yagi et al. (1) used gene targeting to demonstrate that DC-STAMP is also essential for the fusion of osteoclast precursor cells and macrophages. Mice that lack DC-STAMP had mononucleated osteoclasts and developed mild osteopetrosis. In contrast to the study by Kukita et al. (2), the mild osteopetrosis that developed in the DC-STAMP–deficient mice was attributed by Yagi et al. to the defect in the fusion of osteoclasts, rather than a defect in osteoclast differentiation, which occurred normally. Hence, osteoclasts from deficient mice were unable to resorb bone as efficiently as multinucleated osteoclasts. This phenomenon was observed despite the expression of *MFR*, *CD47,* and *CD44*—components of the putative fusion machinery—as well as *E-cadherin* and *meltrin-α*, which have been suggested to participate in multinucleation (19, 20). It is important to stress here that the level of *MFR* and *CD44* transcripts is not elevated in macrophages during fusion. Rather, expression of MFR and CD44 protein is induced transiently at the onset of fu-

sion, possibly by a mechanism that operates posttranscriptionally (7, 9). Thus, definition of the kinetics of expression of MFR, CD47, and CD44 protein in the mutant mice might provide clues to their various roles in fusion. It is possible that DC-STAMP promotes fusion via the transient induction of MFR and CD44 protein expression, or the induction of an as yet unknown protein.

Another important finding reported by Yagi et al. (1) is that fusion is not required for bone resorption, as DC-STAMP–deficient macrophages differentiated into osteoclasts but remained mononucleated. Although it has been shown previously that mononucleated osteoclasts resorb bone poorly, mice deficient in DC-STAMP provide a clear confirmation of this phenomenon. Their findings demonstrate that fusion is not required for the differentiation of osteoclasts and giant cells and that both cell types require the expression of DC-STAMP to become multinucleated.

The most elegant experiment performed by Yagi et al. involved a cofusion assay, in which bone marrow macrophages that originated from mutant mice that expressed green fluorescent protein (GFP) in lieu of DC-STAMP were mixed with those from wild-type mice. In this assay, multinucleate bone marrow cells expressed GFP, suggesting that fusion occurred between a "founder" fusing cell that expressed DC-STAMP and a "follower" cell that did not. This observation confirms the hypothesis that one macrophage must take the lead in "cellocytosing" another one (11). Once fusion of two cells has been initiated and completed, the two cells form a binucleate cell which then becomes the "leader" or "master" fuser and can fuse with other mono- or multinucleated cells. The results reported by Yagi et al. suggest that expression of DC-STAMP is not required in every fusing cell, but rather that DC-STAMP must be expressed by the master fusing cell (Fig. 1). It is now important to identify the ligand for DC-STAMP and to determine whether its expression is required by both members of a pair of fusing cells. Also, although Yagi et al. conclude that

the ligand for DC-STAMP is a surface protein expressed by macrophages, one cannot exclude the possibility that it is a soluble protein that is released by macrophages in a constitutive or regulated manner. Such a ligand might be released by the master cell, the follower cell, or both. An intriguing possible ligand is the chemokine CCL2 (monocyte chemoattractant protein 1), which has recently been shown to participate in the formation of foreign body giant cells and in osteoclast fusion (21, 22). This possibility is supported by the structural similarity between DC-STAMP and chemokine receptors, which are also seven-transmembrane–spanning proteins. This also suggests the possibility that DC-STAMP may function as a fusion co-receptor on macrophages. Chemokine receptors on macrophages and T cells are known to play critical roles as coreceptors for viral entry during infection (23). For HIV, the chemokine receptors CXCR4 and CCR5 are the principal coreceptors for T cell–tropic and macrophage–tropic 1 isolates of HIV, respectively. Yagi et al. argue that DC-STAMP is not a receptor for a soluble chemokine, as DC-STAMP–deficient macrophages plated at high density still failed to fuse. Nonetheless, it cannot be excluded that a soluble factor, released by macrophages, might activate DC-STAMP to promote fusion.

It will also be important to determine whether expression of DC-STAMP is required for fusion of macrophages with somatic and cancer cells (11). If tissue macrophages express DC-STAMP, this might explain why these cells can fuse and express osteoclast markers independently of the essential osteoclast growth factor RANKL (24).

The finding that DC-STAMP is required for the fusion of both osteoclasts and giant cells leads us to question the long-standing dogma that osteoclasts and giant cells are different entities and are unlikely to share the same fusion machinery, even though both cell types originate from the fusion of mononucleate precursor cells that belong to the monocyte–macrophage lineage, both resorb the substrate onto which they

adhere (bone for osteoclasts; foreign bodies for giant cells), and no molecule has been identified that is expressed in one cell type and not in the other. Hence, the primary differences between these two types of cell are likely to be found in the nature of their targets, and the nature of their microenvironments. Establishment of a role for DC-STAMP in macrophage fusion is extremely important and suggests that osteoclasts and giant cells use similar machinery for fusion (24, 11).

Concluding thoughts

The elegant and conclusive work of Yagi et al. both breaks down barriers and raises new questions. It remains to be determined where DC-STAMP is localized during macrophage fusion—possibly clustering in specific domains within the plasma membrane or in the endoplasmic reticulum (25). It will also be critical to define the signals that both regulate the expression of DC-STAMP and act downstream of DC-STAMP. We know that its expression is induced by both RANKL and interleukin-4, which paradoxically stimulate and inhibit fusion, respectively. We also know that the gene for DC-STAMP contains several putative binding sites for NF-κB and octamer factors (26), but there are undoubtedly many regulatory mechanisms that remain undefined. It is also unclear whether DC-STAMP associates with MFR, CD47, and/or CD44, as proposed for HIV interaction with chemokine receptors during fusion (23). Hence, a careful dissection of the signaling events initiated by DC-STAMP ligation will lead to a clearer understanding of how fusion is initiated.

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