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The elementary fusion modalities of osteoclasts

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ABSTRACT

The last step of the osteoclast differentiation process is cell fusion. Most efforts to understand the fusion mechanism have focused on the identification of molecules involved in the fusion process. Surprisingly, the basic fusion modalities, which are well known for fusion of other cell types, are not known for the osteoclast. Here we show that osteoclast fusion partners are characterized by differences in mobility, nuclearity, and differentiation level. Our demonstration was based on time-laps videos of human osteoclast preparations from three donors where 656 fusion events were analyzed. Fusions between a mobile and an immobile partner were most frequent (62%), while fusion between two mobile (26%) or two immobile partners (12%) was less frequent ($p < 0.001$). In general, the immobile fusion partner contained more nuclei than the mobile one ($p < 0.01$). Furthermore, enrichment in nuclei of an osteoclast with three or more nuclei resulted from fusion with a mono-nucleated cell in 67% of the cases ($p < 0.001$), while mono-nucleated cells fused with a multinucleated cell in 61% of the cases ($p < 0.05$). This observation suggested that a more mature osteoclast prefers to fuse with a less mature pre-osteoclast. This hypothesis was supported by a nucleus-tracing approach in a co-culture of more and less differentiated pre-osteoclasts/osteoclasts. Furthermore, we found that osteoclast fusion proceeds through primarily two different types of cell contacts: phagocytic-cup and broad-contact-surfaces (>80% of all fusions). We conclude that osteoclasts most often gain nuclei by addition of one nucleus at a time, and that this nucleus is most often delivered by a moving cell to an immobile cell. These characteristics fit the in vivo observations where mono-nucleated precursors migrating from the bone marrow fuse with more mature osteoclasts sitting on the bone surface. They also fit the fusion modalities of other cell types.

Introduction

Cell fusion is an extremely rare event in the body, and it occurs only under very specific conditions. Well known examples are the fusion of egg and sperm during fertilization [1], fusion of trophoblasts in the placenta [2,3], fusion of myoblasts into myotubes and further into myofibers [4,5] and fusion of pre-osteoclasts (preOCs) into multinucleated osteoclasts (OCs) [6]. OCs are the only cells in the body that are capable of resorbing bone, and a direct relation between their resorptive activity and the number of nuclei per OC has been reported [7]. OCs with an unusual higher number of nuclei are formed in pathologic conditions such as Paget's disease [8] or in response to bisphosphonates [9]. There is no explanation for this increased number of nuclei.

Nevertheless, great progress has been made in understanding the molecular mechanism of OC fusion. Various knock-out mouse models and in vitro approaches with primary cell cultures or cell lines allowed to identify factors important for preparing fusion, such as DC-STAMP [10–12], CD47 [13–15], OSCAR [16], dynamin [17], OC-STAMP [18] and ATP6v0d2 [19]. Furthermore, syncytin-1 was so far the only factor found involved in the actual fusion of the lipid bilayers of the plasma membranes [20]. More and more attention is also paid to the respective partners involved in a single fusion event. This has led to the concept of donor and acceptor cells, that also rules the fusion of other cell types according to complementary properties of the partners [12,14,20–23]. An important aspect of this concept concerns the basic fusion modalities. These are well established for the fusion events generating muscle and placenta. In both these tissues, fusion occurs between a mobile and an immobile cell and multi-nucleation is primarily obtained by repeated fusions with mono-nucleated precursors, thereby adding one nucleus at a time [24,25]. However, in the case of the OCs, these basic fusion modalities have not received attention, probably because they are not put in focus by the current experimental approaches used to investigate OC fusion. We address this issue in the present study, and generated video recordings of fusing OCs in order to determine the characteristics of the fusion partners with respect to their mobility, number of nuclei, differentiation level, and type of cell surface contact prior to fusion.

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Materials and methods

Time-laps recordings of osteoclast fusion

$CD14^+$ monocytes were purified from the blood of human donors (approved by the local ethical committee, 2007-0019, and informed written consent was obtained from each donor) as previously described [14,20].

For time-laps recordings of fusing OCs $CD14^+$ cells were seeded in culture flasks in αMEM (Invitrogen, Taastrup, Denmark) containing 10% FCS (Biological Industries, Kibbutz Beit Haemek, Israel) and 25 ng/ml rhM-CSF (R&D Systems, Abingdon, UK) for 2 days at 37 °C in a humidified incubator at 5% CO_2. Subsequently the cells were detached and reseeded into 4 wells of a Nunc Lab-Tek II chambered coverglass (Nunc — Thermo Fisher Scientific, Roskilde, Denmark) at a density of 100,000 cells/well in αMEM, 10% FCS, 25 ng/ml M-CSF and 25 ng/ml rhRANKL (R&D Systems). The chambered coverglass was placed in the incubation chamber of a confocal Olympus Fluoview FV10i microscope (Olympus Corporation, Shinjuku, Tokyo, Japan) in a humidified atmosphere with 5% CO_2 and 37 °C for three days. The media were renewed and using the software of the microscope four random sites for each of the four wells were marked and stored (total of 16 sites), and time-laps images were made every 15 to 17 min for 20 to 24 h using phase contrast. This was repeated for four consecutive days. For each new recording session four new sites were chosen for each well and medium plus supplements were refreshed after 2 days. The size of each area recorded varied between 0.07 and 1.6 mm^2.

After the time-laps recording had been generated they were all analyzed by two observers and the recordings were carefully checked for fusion events. When a fusion event was identified the following characteristics were recorded: the number of nuclei for each fusion partner, the motility of each fusion partner in the hours prior to fusion (immobile, mobile) and the type of fusion (phagocytic cup, broad contact surface, filopodia/tube or from the top) using the FV10-ASW 4.1 Viewer software (Olympus). The first observer went through the entire video material and recorded the aforementioned details of the fusion and marked the individual fusion events on the video. The second observer verified the marked fusion events and the data recorded, and inspected the videos for further events that may have been missed by the first observer. A mobile cell was defined as a cell that in the hours preceding fusion moved the entire cell body from one location to another where after fusion occurred. An immobile cell was defined as a cell that in the hours preceding fusion did not move the cell body but may have sent out cell extensions/protrusions towards another cell in order to facilitate fusion. In order to determine whether cells are permanently or transiently mobile/immobile, we observed 100 randomly chosen mono-nucleated cells from the time-laps videos of days 3–4 with RANKL, over a period of 20 h. Data was collected from three separate experiments performed with cells isolated from 3 different female donors. A total of 174 videos reflecting a total of 3468 h were analyzed and a total of 656 fusion events were observed.

Fusion partner selectivity assay

$CD14^+$ monocytes from a female blood donor were purified and cultured for 2 days with M-CSF. Two different types of co-cultures were

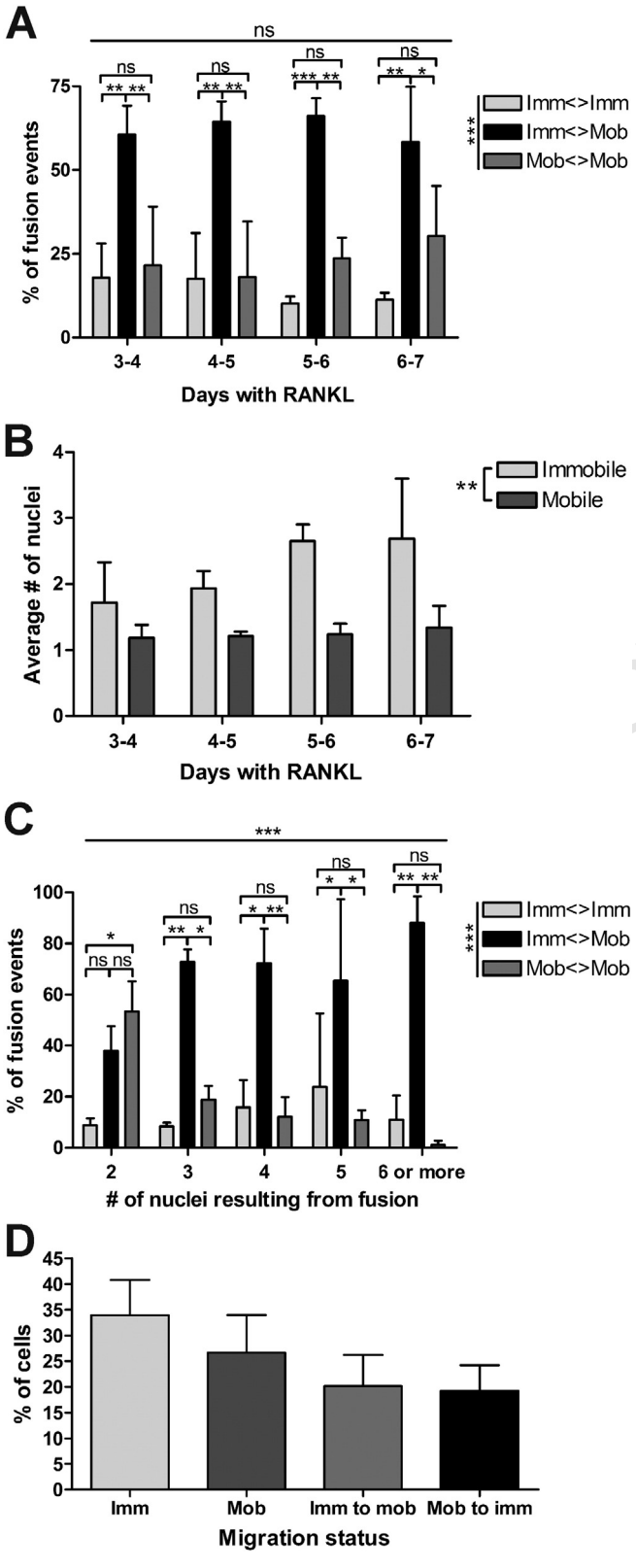

Fig. 1. The mobility of an OC or preOC plays a role in its choice of fusion partner. The video material of 3 independent OC cultures (please refer to Materials and methods for further detail) was thoroughly analyzed by two observers and all fusion pairs were categorized according to their mobility, number of nuclei and days of exposure to RANKL. A. Shows the frequency of fusions between two immobile (Imm<>Imm), an immobile and a mobile (Imm<>Mob) and two mobile (Mob<>Mob) fusion partners according to the total number of fusion events within each recording period. B. Shows the number of nuclei per cell engaged in fusion and stratified according to their mobility status for each recording period. C. Shows the frequency of fusions between Imm<>Imm, Imm<>Mob and Mob<>Mob fusion partners according to the number of nuclei in the resulting OC, for all four days combined. D. Shows the relative frequency of mono-nucleated cells remaining immobile, remaining mobile, or changing from one migration status to the other over the timeframe of 20 h (cultures from day 3–4 days with RANKL). The results shown in A, B, C and D represent the average of three independent experiments/donors +/− SD. Statistics: Two-way ANOVA was performed for comparisons between groups while Bonferroni post-tests were done for comparisons within groups. In C. the overall statistics reaching a p-value of less than 0.001 (***) reflects the degree of interaction between the mobility of fusion pairs and the nuclei-number of the resulting OC. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

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