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MEETING REPORTS

Osteoclasts – Meeting Report from the IBMS Davos Workshop: Bone Biology & Therapeutics

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Anna Teti

Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy

The field of osteoclast biology has recently been the subject of intense investigation and many reports from the IBMS Davos Workshop: Bone Biology & Therapeutics were focused on new knowledge concerning osteoclast function, diseases and treatments. Naoyuki Takahashi (Matsumoto Dental University, Nagano, Japan) showed data (abstract 4) indicating that the place where osteoclast precursors are located determines the site at which osteoclastogenesis occurs. Dr. Takahashi's group has also identified post-mitotic quiescent osteoclast precursors, which have a long lifespan and differentiate into osteoclasts without any further progression in the cell-cycle (1). These precursors express RANK and c-Fms and require both M-CSF and RANKL to evolve towards the full osteoclast phenotype. Using an ectopic bone model, they demonstrated that late osteoclast precursors reach bone through the circulation and are captured by osteoblasts, which represent a sort of "osteoclast niche" in which bone-forming cells play a crucial role in keeping osteoclast precursors glued to the site of resorption. It was also shown by Toshihide Mizoguchi (Matsumoto Dental University, Nagano, Japan) *et al*. (abstract 150) that in the bone marrow there are two populations of circulating quiescent osteoclast precursors: $RANK^{high}$/c-Fms^{low} and $RANK^{low}$/c-Fms^{high}. Both could differentiate into mature osteoclasts by canonical osteoclastogenic treatment. However, only the $RANK^{high}$/c-Fms^{low} population was cell-cycle arrested and could originate osteoclasts even in the presence of the inhibitor of DNA replication, hydroxyurea.

We also learned at the Davos meeting that M-CSF and RANKL are no longer indispensable for osteoclast formation. In his talk, Dr. Takahashi reported that IL-34, highly expressed in the spleen, interacts with c-Fms, inducing the signal transduction pathway essential for M-CSF-mediated osteoclastogenesis. Therefore, IL-34 can replace M-CSF with similar efficiency. Dominique Heymann (INSERM UM957, University of Nantes, Faculty of Medicine, Nantes, France) *et al*. (abstract 146) showed that the level of IL-34 is high in human osteoclastomas, with uppermost expression in osteoclast-like cells and lower expression in stromal cells, which suggests a role for IL-34 in the pathogenesis of this giant cell tumor of bone (2). IL-34 has been tested in various models of osteoclastogenesis and it is now clear that it can entirely substitute for M-CSF in RANKL-induced osteoclast generation. In another study, Anna Rufo (University of L'Aquila, L'Aquila, Italy) *et al*. (abstract 191) showed increased osteoclast formation in Duchenne muscular dystrophy, in which reduced bone mass appears to be associated not only with reduced muscular traction but also with an imbalance of cytokine content and with osteoblast/osteoclast uncoupling. The most surprising result was the reduced RANKL/OPG ratio observed in the circulation of patients and in osteoblasts challenged with patients' sera, which was not consistent with the increased osteoclastogenesis observed in both patients and cell cultures. However, high IL-6 was a hallmark of patients and dystrophic MDX mice, which recapitulated the bone phenotype observed in the patients. The study showed that, in the presence of M-CSF, IL-6 alone was able to induce

osteoclastogenesis with efficiency similar to that of RANKL treatment. From this data, we should conclude that M-CSF and RANKL have lost their “nobility” and can no longer be considered irreplaceable pro-osteoclastogenic factors.

Masaru Ishii (Osaka University, Osaka, Japan) showed beautiful *in vivo* bone imaging (abstract 5) in which osteoclast migration could be analyzed in intact bone (3). He focused on the trafficking of osteoclast precursors to and from the bone surface, where these cells undergo fusion to form mature osteoclasts. Dr. Ishii's group used two-photon microscopy whereby osteoclasts can be visualized *in situ*. The movement of osteoclasts between the circulation and the endosteal surface was found to be controlled by the lipid mediator sphingosine-1-phosphate (S1P). With this elegant technique it was shown that osteoclasts express the S1P receptor and are sensitive to an S1P agonist that stimulates their mobilization *in vivo*. The S1P concentration is higher in blood, thus facilitating the return of osteoclast precursors into the circulation (4).

Osteoclast diseases were also addressed at the meeting. Fraser Coxon (University of Aberdeen, Aberdeen, UK) *et al*. (abstract 147) showed the involvement of autophagy in Paget’s disease of bone. This is a process whereby cells protect themselves from defunct cellular organelles and the accumulation of misfolded proteins. Dr. Coxon's group showed that autophagy is deregulated in Paget’s disease because cellular inclusions in pagetic osteoclasts appear to contain ubiquitin, proteasomal subunits and sequestosome-1 rather than viral proteins. In particular, sequestosome-1 appeared to interact with Autophagy-Linked FYVE-domain containing protein (ALFY). In studies of overexpression of the autophagy marker microtubule-associated protein 1A/1B-Light Chain 3 (LC3), they showed that the basal level of autophagy is higher in osteoclasts than in other cells, thus suggesting that this process is prominent and physiologically important for the normal function of bone-resorbing cells.

Kim Henriksen (Nordic Bioscience, Herlev, Denmark) presented an overview of antiresorptive treatments (abstract 16) (5) using ClC-7 deficiency, which characterizes a subset of patients affected by osteopetrosis, to demonstrate that osteoclasts are to be considered producers of osteoblast anabolics. It was suggested that it is not only factors released from the bone matrix during resorption, but also a variety of osteoclast cellular products that have pro-osteoblastic activity, and that conditioned media from non-resorbing osteoclasts are enriched of these factors. Although this work still appears preliminary and specific molecules have not yet been identified, the results point to new determinants that in the future may help determine therapies aimed at improving bone formation. Andrea Del Fattore (University of L'Aquila, L'Aquila, Italy) *et al*. (abstract 142) have instead investigated osteoclasts in ClC-7-dependent autosomal dominant osteopetrosis, for which a treatment with siRNA specific for the mutant allele is predicted to induce haplosufficiency, which for this disease is associated with a normal phenotype. The strategy is based on knowledge that heterozygous missense mutations in these patients cause the synthesis of dominant negative subunits of the dimeric ClC-7 antiporter. Thus, targeting the mutant allele by mutation-specific siRNA eliminates the mutant protein, leaving the normal allele intact and functional. Delivery of siRNA and organ targeting has been shown to be feasible *in vivo*, thus suggesting that the strategy is applicable in animal models of the disease.

In the area of osteoclast therapy, many reports further studied various bisphosphonates and denosumab in osteoporosis and cancer-induced bone disease. In addition, Nadia Rucci (University of L'Aquila, L'Aquila, Italy) *et al*. (abstract 151) have identified a new potential antiresorptive molecule, active *in vivo* in ovariectomy-induced osteoporosis (6). This is the extracellular matrix protein PRoline/arginine-rich End Leucine-rich repeat Protein (PRELP), in which the active anti-osteoclast domain is located in its N-terminus. The peptide targets late osteoclast

precursors, preventing their progression towards the mature phenotype. The mechanism of action is interesting as the peptide is an inhibitor of NF-κB transcriptional activity that acts in an osteoclast-specific manner because it must be internalized and transported to the nucleus through endocytic pathways requiring annexin-II and cell surface chondroitin sulfate proteoglycans. The peptide is promising because it appears to target and persist *in vivo* especially in bone, and does not affect other cell types, including osteoblasts and bone marrow mononuclear cells. Along with the newly-discovered odanacatib (7), described by Sevgi Rodan (University of Pennsylvania, Philadelphia, Pennsylvania, USA) to be a new, highly selective anti-cathepsin drug with favorable pharmacokinetics and improved metabolic stability, new avenues are being opened for better treatments for osteoclast diseases (abstract 17).

In conclusion, new determinants of osteoclast function are being intensely investigated and should soon lead to improved strategies to combat osteoclast diseases, either when osteoclast activity is enhanced (as in osteoporosis, bone metastases, and Paget's disease of bone) or impaired (as in osteopetrosis). This new knowledge is expected to have a strong translational impact, thus expanding the opportunities to cure skeletal derangements observed in many pathological conditions.

Conflict of Interest: None reported.

Note: Abstracts from the meeting have been published as a supplement to *Bone*. 2010 Mar;46(Suppl 1):S1-S90.

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