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MEETING REPORTS

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

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In the recent past, a number of new functions besides bone formation have been attributed to osteoblast lineage cells ranging from mesenchymal progenitors to osteocytes and lining cells, posing numerous questions for the bone field. During the recent IBMS Davos Workshop: Bone Biology & Therapeutics, some of these topics were addressed and will be discussed in this summary of osteoblast presentations that took place during the meeting.

The first steps of skeletal development are patterning and growth. In early development, the patterns of limb elements are determined by a system of signaling molecules including, among others, the family of *Hox* genes. In his talk, Stefan Mundlos (Charité, Berlin, Germany) gave an overview of the particular role of Hoxd13 in limb development and growth. *Hox* genes are considered as regulators of early patterning events and several *Hox* genes are associated with human limb malformations. For example, an extension of a poly-ala stretch in Hoxd13 leads to polysyndactyly, a phenotype that is also present in the *Spdh* mouse strain (1). *Spdh/Spdh* mice are characterized by (i) multiple digits with fusions; (ii) chondrocytes within the anlagen that do not differentiate; and (iii) delayed bone formation. The phenotype in the mice is caused by the occurrence of chondrocytes in the interdigital space (as assessed by the expression of Sox9) due to low local levels of retinoic acid (RA), an inhibitor of chondrogenesis. Indeed, the enzyme retinaldehyde dehydrogenase 2 (Raldh2), which converts retinol to RA, is a Hoxd13 target gene and its expression in the interdigital web is reduced in *Spdh/Spdh* mice. As a consequence, polysyndactyly represents a problem of growth/differentiation rather than a problem of patterning. The next question to be addressed was the determination of bone shape. Again the *Spdh* mouse proved to be helpful in giving at least partial answers to this question. Comparing long bones and carpal bones, a major difference consists in the distribution of joint surfaces with long bones forming joint surfaces at the ends, while carpal bones are covered with joint surfaces. In *Spdh* mice, metacarpal bones, like carpals, are covered all over with joint surfaces as characterized by the expression of joint markers such as Gdf5 and Sfz2. This may be caused by the failure of the mutated Hoxd13 to induce *Runx2* expression and as a consequence a failure to induce development of the perichondrium. In the *Spdh* mouse, the lack of a perichondrium results not only in a deficiency in forming cortical bone, but rather in the formation of joint surfaces. The mutation of *Hoxd13* therefore leads to a homeotic transformation of bone, one “kind” of bone being substituted by another “kind” of bone, the replacement of a metacarpal bone by a carpal bone.

Later in development and in the adult organism, remodeling is the major process occurring in bone. It has become evident that bone remodeling is regulated not only by hormonal and paracrine/autocrine factors, but also by neuronal cues and more specifically by sympathetic nerves. Florent Elefteriou (Vanderbilt University, Nashville, TN) summarized recent data on the regulation of bone remodeling by hypothalamic signals. Thus, the destruction of ventromedial hypothalamic (VMH) neurons increases bone mass (analogous to *ob/ob* mice). On the other hand, the loss of the leptin receptor ObRb specifically in VMH

neurons does not affect bone mass (2). Bone formation was found to be inhibited by afferent hypothalamic signals through activation of the β2-adrenergic receptor (β2AR) expressed by osteoblasts. Signaling through β2AR favors RANKL expression in osteoblast lineage cells by inducing the phosphorylation and activation of the transcription factor ATF4 (3). Therefore, factors regulating β2AR signaling pre- and post-synaptically may affect bone metabolism by either downregulating bone formation, which is *Clock* gene dependent (4), or by stimulating bone resorption through ATF4/RANKL. Sympathetic stimuli in stress-induced depression are also eliciting bone loss (5). The stress hormone dexamethasone stimulates the upregulation of β2AR in osteoblasts, rendering these cells more sensitive to sympathetic agonists. In the last part of his presentation, Dr. Elefteriou focused on the role of sympathetic stimulation in the development of bone metastases. Intravenous injections of MDA-231-GFP mammary cancer cells into mice, with a concomitant treatment of the animals with the sympathetic agonist isoproterenol, leads to an increase in lytic areas and the number of lytic lesions as well as an increase in the formation of metastases in bone within 7 days. Isoproterenol was found to increase the expression of RANKL in BMSCs and osteoblast lineage cells. In separate experiments, the migration of MDA-231 cells was found to be stimulated by RANKL (inhibited by OPG). In conclusion, it was hypothesized that sympathetic activation of osteoblasts induces the release of RANKL, which in turn attracts tumor cells to bone. Eventually this cycle might be broken by the application of β-blockers.

On the topic of bone remodeling, the role of osteocytes in the homeostasis of bone is by now an established fact. Lynda Bonewald (University of Missouri, Kansas City, MO) presented evidence that the osteocyte's role is not only to function as the mechanostat but also that the osteocytic lacuno-canalicular system acts as an endocrine organ regulating mineral homeostasis. For this purpose, the osteocytic system is in intimate contact with the vascular system, which is demonstrated by the distribution of Procion Red within 10 minutes after injection into the tail vein. The role of osteocytes in mineral homeostasis has been supported by the following evidence: (i) mice deficient in Phex and Dmp1 show increased levels of FGF23 and are hypophosphatemic; (ii) osteoid-osteocytes take part in the regulation of mineralization (6); and (iii) canalicular width and lacunae are increased in lactating mice. This last observation is of particular interest, since osteocytes in lactating mice express the osteoclast marker enzyme TRAP and may be involved in active mineral dissolution, while osteocytes in bones from mice whose hind limbs were suspended for 4 weeks did not express TRAP and osteocyte lacunae were not increased. Ongoing studies are comparing gene expression in osteocytes from virgin and lactating mice. While no data are available yet, preliminary analysis revealed an upregulation of genes encoding proteins of bone turnover.

The role of osteocytes in the control of bone metabolism was also addressed in a talk by Michaela Kneissel (Novartis Institutes of Biomedical Research, Basel, Switzerland). Since osteocytes express both *Sost* and *PTHR1*, they are putative candidates to take part in the mediation of the anabolic effects of PTH on bone. Indeed, the effects of PTH were blunted in mice either overexpressing or deficient in *Sost* (7). While sclerostin was originally described to act as a BMP antagonist, it was subsequently found to interact with the Wnt pathway (8;9), specifically, with Lrp5 and Lrp4. This was supported by Piters *et al*. (poster #126), who described missense mutations in the *Lrp4* gene that resulted in a high BMD phenotype. The mutations are associated with polysyndactyly and hyperostosis of the skull. Lrp4 was suggested to facilitate the inhibition of bone formation mediated by sclerostin and the mutations are hypothesized to decrease Lrp4 binding to sclerostin.

Besides direct actions on osteoblast lineage cells, the anabolic effects of PTH have been suggested to be mediated by accessory non-bone cells. Roberto Pacifici (Emory University, Atlanta, GA) described the role of T-cells in PTH action. Previously, Dr.

Pacifici's group demonstrated that PTH fails to induce bone resorption in T-cell-deficient mice (10). Furthermore, daily injections of hPTH1-34 into *TCRβ*(-/-) mice deficient in T-cells caused a smaller increase in bone volume than in wild type animals. Furthermore, intermittent PTH does not increase bone volume in T-cell-deficient nude mice and in *Rag*(-/-) mice. Subsequent analysis revealed that $CD8^+$ T-cells release increased levels of Wnt10b in response to intermittent PTH and accordingly, Wnt10b levels were reduced in T-cell deficient animals. T-cell-mediated activation of Wnt signaling pathways in osteoblasts may play a key role in the increase of bone mass and strength induced by intermittent PTH.

Osteoblast lineage cells are controlled and regulated by a large number of intrinsic and extrinsic factors, cytokines, components of the ECM, receptors, and signalling molecules, just to name a few. Basu Roy *et al*. (poster #120) described the necessity for *Sox2*, an FGF-regulated gene, for osteoblast survival. *Sox2*(-/-) animals express a low bone mass phenotype and do not proliferate *in vitro*. Only reconstitution of Sox2 expression rescues the phenotype, since the protein inhibits differentiation of osteoblast lineage cells. Another inhibitor of osteoblast differentiation was presented with Wnt3a. This member of the Wnt family of growth factors prevented the expression of *Osterix* in MC3T3-E1 cells, thus inhibiting differentiation and stimulating proliferation. As shown by Thouverey and Caverzasio (poster #130), the Wnt3a signal is mediated through the PDGF receptor. Hamidouche *et al*. (poster #122) demonstrated the role of autocrine FGF18 in the osteogenic commitment of MSCs to the osteogenic lineage. Dexamethasone was found to induce *Fgf18* expression. Lentiviral overexpression of *Fgf18* or silencing of FGF receptors abrogated the FGF18-induced expression of *Runx2*. Another example of the plasticity of phenotypes and the regulation of bone cell differentiation by the local environment was provided by Liu *et al*. (poster #118) who demonstrated that muscle lineage cells contribute significantly to bone healing after fracture. Vascularization and bone formation are critically linked in processes like bone repair and VEGF is frequently proposed for use as a therapeutic agent to stimulate the process. Bartunkova *et al*. (poster #119) investigated the effects of different VEGF isoforms in bone healing using gain-of-function models. They suggested that the isoforms may exert different effects on vascularization and bone healing and that high doses of the growth factor result in aberrant vasculo- and osteogenesis.

Andersen *et al*. (poster #160) presented an interesting extension of the original studies of the bone resorptive compartment (BRC) (11). Careful observation of the BRC on tissue sections revealed a separation of the remodeling area from the marrow cavity by a canopy of flattened osteoblast-like cells that are associated with the vasculature. In an extension of these studies, the absence of this structure in the BRC in the bones of patients with postmenopausal osteoporosis (PMO) was observed. The intactness of the BRC is critical for the length of the reversal and bone formation phases of the remodeling cycle. The extension of the transition between reversal cells and osteoblast differentiation in PMO bone observed in this study may be caused by the anatomical changes of the BRC and, as is suggested, may be a target for the treatment of PMO.

Finally, MacRae *et al*. (poster #188) investigated the role of phosphatases in the formation of matrix vesicles and mineralization. PHOSPHO1 is a bone-specific phosphatase implicated in inorganic phosphate generation. Previously it was found that deficiency in tissue non-specific alkaline phosphatase (TNALP) did not prevent mineralization. PHOSPHO1 deficiency, however, leads to scoliosis and osteomalacia (see also (12)). Deficiency in both TNALP and PHOSPHO1 caused a complete absence of mineralization of the skeleton. Most fascinating was a presentation by Capannolo *et al*. (poster #142) that proposed a potential therapy for osteopetrosis caused by mutations in CLC7 chloride channels. Approximately 70% of patients presenting with an osteopetrotic phenotype carry mutations in the *CLC7* gene. In this study, human osteoclasts from patients were treated with siRNA blocking

the dominant negative mutant CLC7 variant, resulting in the rescue of osteoclastic resorptive activity. Making use of a recently established model of osteosarcoma, *i.e.*, mice deficient in *p53* and *Rb* (13), Baker *et al*. (poster #96) presented findings on the epigenetic changes in osteosarcoma and differentiated osteoblasts. All osteosarcoma cell lines established from the animal model overexpressed members of the polycomb repressive complex (for further information see (14)). The study highlights the need for the elucidation of epigenetic signatures of osteosarcoma cells since they may identify underlying causes of the disease.

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

Conflict of Interest: None reported.

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