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MEETING REPORTS

Molecular Signaling in Bone Remodeling: Asia Pacific Conference 2007

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The dynamic nature of bone as an organ has been long recognized, but real appreciation of its beauty and complexity has come with understanding of the cellular communication processes in bone remodeling. A number of dramatic new insights have been provided in the last few years by discoveries in mouse and human genetics, and several of these are being applied in efforts towards new therapeutic developments. An international conference on the topic took place in November, 2007 in Sydney. It brought together a group of scientists from Australasia, Japan, the US and Europe to discuss recent advances in understanding of the molecular and cellular control events in bone remodeling.

As pointed out by Ego Seeman (Melbourne), the loads imposed upon bone are a key factor in determining its structure. This is achieved through mechanisms of cellular communication in modeling and remodeling that adapt the bone composition and structure at all levels to cope with prevailing loads (1). Bone remodeling takes place in discrete "packages" throughout the skeleton, known as bone remodeling units (BMUs), in which a certain amount of bone is resorbed by osteoclasts and the same amount replaced sequentially by osteoblasts. The peak of the response to loading occurs during growth and in the very active life of the young, but in adulthood a number of changes take place. First, there is a major decline in periosteal bone formation, and second, the volume of bone formed in each BMU decreases, and although the volume resorbed in each BMU decreases, this is less than that formed – thus a negative BMU balance. Finally, in women in midlife, the rate of bone remodeling increases and the BMU balance becomes more negative, resulting in bone loss and structural decay each time a remodeling event occurs. In earlier adulthood, bone is removed on the endocortical, intracortical and trabecular components. The consequences of this are much less pronounced than after the menopause where the increased remodeling rate and greater negative BMU balance and decline in periosteal bone formation result in thinning and porosity of the cortex and loss of connectedness of the thinned trabeculae. In considering how these ideas might dictate therapeutic needs, a suggestion for the ideal drug for bone loss is one that increases periosteal apposition, and increases the bone formation and decreases the bone resorption in the BMU. Of course, nothing does that yet, and in the case of the only anabolic drug so far, PTH, it was pointed out that its use in human subjects is associated with increased cortical porosity.

Ideas of these age-related changes in bone substance and structure that are responsible for bone fragility have developed in the last few years, in parallel with a considerable increase in information on the control of formation of the cells of bone as well as on how they communicate with each other. The controls come from circulating hormones, local growth factors and cytokines produced either by the bone cells themselves or by immune cells. Concepts arising from mouse and human genetics continue to inform us in ways that we aim to translate to understanding mechanisms of human bone loss and how to control it.

One of the major recent advances in bone biology is the realization of the importance of the osteocyte, where new discoveries have

thrown much light on the vague idea that the osteocyte probably senses changes in pressure in bone to send signals to surface cells. Lynda Bonewald (Kansas City) spoke of this, pointing out that osteocytes that make up over 90% of all bone cells are viable for decades in bone and their long dendritic processes ideally equip them to communicate with cells on the surface (2;3). During the differentiation of osteoblasts to osteocytes, the cell reduces in size as the dendritic processes form. A number of markers of osteocytes have been developed recently, including MEPE, Phex, E11, dentin matrix protein 1 (DMP1), and sclerostin, the product of the *SOST* gene. E11 is an early osteocyte marker that inhibits mineralization and bone formation whereas DMP1 is essential for normal matrix mineralization. FGF23 is an osteocyte-derived hormone promoting phosphaturia and whose production is regulated by DMP1, Phex and MEPE. FGF23 promotes renal tubular reabsorption of phosphate and increases blood phosphorus, favoring mineralization. Genetic deficiency of FGF23 is responsible for the phosphate abnormality of hyperphosphatemic rickets, and null mutations for the syndrome of tumor-induced osteomalacia. The osteocyte is now recognized as having a central place in the regulation of bone remodeling and modeling, as new information comes of its role in cell communication processes in bone.

Perhaps the most dramatic of recent discoveries of osteocyte function that has had a major impact on thinking in bone biology is its role in limiting bone formation (reviewed by Clemens Löwik (Leiden, Netherlands)). Mutations in the *SOST* gene were found responsible for the rare sclerosing bone dysplasias, sclerosteosis and van Buchem disease. Each is characterized by a greatly increased amount of bone. The *SOST* gene product, sclerostin, appears to be produced exclusively in bone by osteocytes, and was revealed as an osteocyte-specific negative regulator of bone formation (4;5). Although thought at first to be a bone morphogenic protein (BMP) inhibitor, its molecular action is to inhibit Wnt signaling through binding to the LRP5/6, thus preventing its participation in the receptor complex that activates the Wnt pathway and bone formation. When *SOST*(-/-) mice were generated by transgenic expression of *SOST* with the 52 kb deletion discovered in van Buchem disease, mice with a very high bone mass were generated, analogous with the human inactivating *SOST* mutation in van Buchem disease. The reverse syndrome, of severe bone loss, occurs in transgenic mice overexpressing sclerostin in osteocytes. Production of sclerostin by osteocytes is rapidly decreased by treatment with PTH or PTHrP, as well as by mechanical loading. In each of these cases, removal of sclerostin as a constitutive inhibitor of bone formation could at least partly explain the accompanying increased bone formation resulting from PTH treatment or mechanical loading. Physiologically, rapid changes in osteocyte production of sclerostin could signal to surface cells to limit the filling of remodeling spaces by osteoblasts, in addition to keeping lining cells in a quiescent state in non-remodeling bone surfaces. The human and mouse genetic evidence identified sclerostin as a compelling target for skeletal anabolic drug development, and progress has begun with preclinical studies indicating anabolic efficacy of neutralizing anti-sclerostin antibodies.

The first ideas that the cells of bone might communicate with each other came from the suggestion that osteoblasts control osteoclast formation, a hypothesis that was proved with the discovery of receptor activator of NF-κB ligand (RANKL) as the indispensable osteoblast-derived driver of osteoclast formation and activity. That process also requires local production of M-CSF, and is constrained by production of osteoprotegerin (OPG), the decoy receptor for RANKL. On the other hand, little has been known of the reverse possibility, of osteoclast products acting upon osteoblasts, even though this has been suspected in recent years. Some light was shed on this by Koichi Matsuo (Tokyo), who described genetic and pharmacological approaches that identified ephrinB2 as an osteoclast product interacting with its receptor, EphB4, on osteoblasts to promote osteoblast differentiation and bone formation (6). Most interestingly, reverse signaling took place between EphB4 and ephrinB2, leading to

inhibition of osteoclast formation through blockade of signaling by the transcription factors c-fos and NFAT1c. With both receptor and ligand as membrane-spanning molecules, these interactions are likely to be contact-dependent, and involvement of the ephrin/Eph signaling pathways in bone constitutes a previously unrecognized local control mechanism. Further discussion of cell communication processes came from Jack Martin (Melbourne, Australia), who took the local potential of the ephrin-Eph system further with evidence that PTH and PTHrP stimulate by up to ten-fold the production of ephrinB2 by osteoblasts *in vitro* and bone *in vivo* (7). It was reported that cells of the osteoblast lineage can themselves help regulate the filling of resorption spaces through intercellular communication via growth factors and cytokines. Although it has long been accepted that resorption of bone is needed to promote bone formation in the remodeling sequence, attention was brought to evidence that the active osteoclast itself might be the source of regulatory, bone-forming activity (8;9). This osteoclast-derived activity might also contribute to the anabolic effect of PTH and PTHrP, complementing the direct effect on committed pre-osteoblasts to enhance their differentiation and to inhibit apoptosis of mature osteoblasts and osteocytes.

Cytokines signaling through gp130 have long been known to influence bone cell function, particularly through promoting osteoclast formation. Natalie Sims (Melbourne) provided further analysis of gp130 mechanisms, beginning with studies of mice in which either of the two signaling pathways of gp130 were inactivated (10). This showed that inactivation of the SHP2/Ras/MAPK pathway yielded mice with increased bone turnover and bone loss. When these mice were crossed with IL-6-deficient mice, even more bone was lost, with no change in bone resorption but a decrease in bone formation, suggesting a coupling of bone formation that was IL-6-dependent and emanating from the osteoclast itself, rather than as a result of resorption. In further studies of bone effects, using mice mutated for cardiotropin-1 (CT-1), or for the receptors for IL-11 and for oncostatin M (OsM), each of IL-11, CT-1 and OsM was found to stimulate bone formation and inhibit adipogenesis *in vitro*. All three also rapidly activated C/EBPδ transcription and stimulated Runx2 activation of transcription, and both CT-1 and OsM were shown to promote bone formation *in vivo* when injected daily over the calvariae of mice. It was concluded that IL-11, CT-1 and OsM are all essential for normal bone metabolism, and have similar roles despite differences in details of interaction with the gp130 signaling pathway.

The discovery of bone resorption control by RANKL has identified a pathway that is clearly amenable to the development of drugs to prevent excessive bone resorption. Among the targets for these efforts are the complex signaling mechanisms accompanying RANKL regulation of osteoclasts that have been elucidated by Hiroshi Takayanagi (Tokyo) (11;12). In a study of RANKL-induced genes in hemopoietic cells, the transcription factor, NFAT1c, was found to be increased 20-fold, shown to be regulated by calcium signaling, and activated by calcineurin through phosphorylation, whereby it was translocated to the nucleus to activate transcription. Genetic experiments established the essential role of NFAT1c as an integrator of RANK signaling, with costimulatory signals also essential for activation of the pathway in osteoclastogenesis. The regulatory signals involved in these controls, as well as the responsible cytokines, illustrate clearly the links between the immune system and skeletal development and maintenance. No longer can the skeleton be regarded as an inert packaging structure for interesting cells and cytokines, with so many of the cytokines and control mechanisms emerging with crucial roles in the maintenance of a normal skeletal mass. There is a long and increasing list of cytokines whose genetic ablation or overexpression results in severe bone phenotypes, either osteopetrosis or osteoporosis, from inadequate or excessive osteoclast formation, respectively. From the evolutionary point of view, it identifies the importance for survival of the tightly regulated processes of bone resorption and formation in bone remodeling, so that

skeletal strength and form can be maintained.

Molecular mechanisms involved in estrogen prevention of bone loss have remained elusive, although the hormone clearly attenuates bone resorption. The consensus has been that estrogen withdrawal enhances responses to any or all of a number of bone-resorbing cytokines, including IL-6, IL-1, TNFα and RANKL, There is now evidence from mouse genetics for a critical role of osteoclastic ERα in mediating estrogen action on bone in females (Shigeaki Kato, Tokyo) (13). When ERα was selectively ablated in differentiated osteoclasts, the female, but not the male mice, exhibited clear trabecular bone loss, similar to the osteoporotic bone phenotype in post-menopausal women. Estrogen treatment did not prevent bone loss in mice with ERα-deficient osteoclasts that underwent ovariectomy. Osteoclastic apoptosis was induced by estrogen, occurring simultaneously with up-regulation of Fas ligand (FasL) expression in intact trabecular bones of normal mice, but not in mice with ERα-deficient osteoclasts. ERα was also required for similar effects of estrogen and tamoxifen in cultured osteoclasts. These findings suggest that the osteoprotective actions of estrogen and SERMS might be mediated at least in part through osteoclastic ERα in trabecular bone; and the life span of mature osteoclasts is regulated through activation of the Fas/FasL system.

Conclusion

Mechanisms of the skeletal response to cancer were also discussed, but the emphasis in these summarized presentations was on the molecular mechanisms by which bone is resorbed during the remodeling process, and the replacement of bone is tightly regulated with signals from the matrix to osteoblast precursors, stimulation of osteoblasts provided by growth factors released from the matrix during resorption, signaling among the osteoblasts themselves, and finally, from the osteoclast lineage to osteoblasts. The balance between volumes of bone resorbed and formed is established by contributions from many intercellular communication pathways. The recent advances reported at the conference draw attention to molecular pathways that are influential in regulating bone balance. With advancing age, abnormalities occur in the cellular machinery that compromise the integrity of the bone; a decline in the volume of bone formed by each BMU, continued resorption of a volume of bone by each BMU, an increase in remodeling rate in midlife in women and in both sexes late in life due to secondary hyperparathyroidism, and a decline in periosteal apposition. A better understanding of the cellular mechanisms of bone modeling and remodeling, and the effects of advancing age on these mechanisms, is likely to assist in identifying new targets for therapy that will assist in preventing and reversing bone fragility.

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Conflict of Interest: Dr. Seeman reports that he is an advisory committee member for Sanofi-Aventis, Eli Lilly, Merck Sharp & Dohme, Novartis, and Servier, and that he lectures occasionally at conference symposia for those companies. Dr. Martin reports no conflicts of interest.

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