MEETING REPORTS

Meeting Report from the 2nd Joint Meeting of the European Calcified Tissue Society and the International Bone and Mineral Society

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BONE FORMATION

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Genetic Factors

Throughout the symposium session, as well as the corresponding oral communications and posters concerned with bone formation, various genetic factors were discussed that are important in affecting bone mass and bone structure. Animal technology has provided a useful tool in gene discovery, utilizing knockouts and transgenic mouse models along with the application of cell and molecular biology techniques. Methodologies involving quantitative trait loci and microarray analyses have enabled some important discoveries, such as the role of lipo-oxygenase pathways in the control of bone mass, and the determination of how gender differences in skeletal size are directly mediated by sex steroids (1).

p53

p53, a tumor suppression factor, was shown to repress the expression of the transcription factor osterix, and to function as a negative regulator of bone formation (2). The p53 knockout mouse had an osteosclerotic phenotype manifested by an increase in bone mineral density. This was found to be mediated by an elevated expression of osterix, while Runx2, which acts upstream of osterix, was not affected.

Homeodomain Proteins

Evidence was presented for the roles of bone morphogenic proteins (BMPs), which have essential roles in early embryonic skeletal development, in signaling to Runx2-dependent transcription via a regulatory network of homeodomain proteins (3). The studies directly demonstrated that BMP2 induction of Runx2 gene expression involves a direct regulation by homeodomain proteins, which selectively bind to promoter elements of a Runx2 bone-specific gene at different stages of osteoblast differentiation.

Bone Anabolics That Upregulate BMP2

Ross Garrett described two classes of bone anabolic compounds that up-regulate BMP2 protein production, discovered via high throughput screening methods (4). The compounds were assessed using in vivo and in vitro models. The first class of compounds described were the statins, which act via the well-characterized mevalonate pathway, and were delivered...
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Topically to bypass clearance by the liver. The second class were proteosome inhibitors, which are also anabolic in vivo and in vitro. Velcade, used in the treatment of multiple myeloma, increased alkaline phosphatase, Runx2, Bsp and osteocalcin expression. The proteosome inhibitors activate the Gli family of transcription factors, which leads to an increase in BMP2.

BMP2 Activates β-catenin

Lim et al. (5) described how BMP2 stimulates osteoblast differentiation through activation of β-catenin signaling in osteoblasts. This activation was mediated through LRP5: BMP2 inhibition of Kremen-1 stimulated LRP5 mRNA expression in osteoblasts. On the other hand, β-catenin-deficient osteoblasts inhibited BMP2’s ability to stimulate osteoblast proliferation and mineralization. This work suggests that β-catenin plays an important role in BMP2-induced osteoblast differentiation. Evidence was also given (6) for β-catenin being a powerful enhancer of BMP2 gene expression in osteoblasts in vitro and in vivo. The BMP2 gene contains putative binding sites for TCF, a co-activator of the transcription factor for β-catenin. β-catenin is a pivotal signaling molecule that transduces Wnt signaling to target genes.

Dickkopf-1 Expression is Essential for Bone Matrix Mineralization

Evidence was presented that the down-regulation of Wnt signaling by the induction of Dickkopf-1 and -2 expression during osteoblast maturation is essential for bone matrix mineralization (7). Lithium chloride (LiCl), an intracellular activator of Wnt-signaling, inhibited the formation of mineralized bone nodules. Wnt3A inhibited formation of bone nodules only when it was added early in the differentiation process in an osteogenic cell line (KS483). Wnt3A induced β-catenin translocation to the nucleus in undifferentiated, but not in differentiated, KS483 cells. These data suggest that Wnt-signaling in maturing osteoblasts needs to be down-regulated to enable the formation of mineralized matrix.

In summary, these presentations give further evidence of novel genes and signaling pathways that determine bone formation. Further genetic studies in bone cells will be of considerable interest in understanding bone physiology and in the development of novel therapeutic approaches for osteopenia.

Conflict of Interest: The author reports that no conflict of interest exists.

LOCAL AND SYSTEMIC REGULATION

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This session began with a review of SOCS proteins by Warren Alexander (8). The eight SOCS proteins block signaling either by binding JAK kinase directly (SOCS1) or by binding cytokine receptors via phosphorylated tyrosines (e.g., SOCS3). Knockout mice for all eight SOCS genes have been generated. Remarkably, these mice typically have revealed that the physiologically relevant targets for each SOCS protein were not easily predicted from the previous cell studies, which usually involved overexpression of proteins. SOCS1 knockout mice die of widespread inflammation caused by exaggerated responses to interferon-γ. The mouse survives if it also has the interferon-γ gene knocked out. The SOCS3 knockout dies an early embryonic death through exaggerated placental responses to LIF.

Steve Goldring summarized his work and that of others exploring the ways that inflammatory cells in rheumatoid arthritis accelerate bone resorption (9). He stressed that RANKL, the key regulator of osteoclasts, was first discovered as TRANCE, a T-cell regulator of dendritic cell survival. Osteoprotegerin, thus, not only blocks the actions of osteoblastic RANKL to stimulate osteoclastogenesis and osteoclast activity, but it also interferes with T-cell activation of osteoclasts and T-cell communication with dendritic cells. Murine
models of inflammatory arthritis lacking osteoclasts due to the absence of RANKL display inflammation, but no erosion of bone. Dr. Goldring summarized the work from Dr. Takayanagi’s laboratory showing how some inflammatory signals, like interferon-γ and interferon-β, can interfere with osteoclast intracellular signaling. Thus, inflammatory cells regulate osteoclast-mediated bone erosions in an intricate way.

Bram van der Eerden previously showed that the TRPV5 transient receptor potential channel mediates renal tubular calcium reabsorption, and that mice lacking this channel exhibit hypercalcuria with high 1,25(OH)\(_2\)D\(_3\) levels and normal blood calcium and PTH levels. Here (10) he combined this knockout with that of the vitamin D 1α-hydroxylase gene to generate a model with low blood calcium, despite very high PTH levels. Thus, the high 1,25(OH)\(_2\)D\(_3\) in the TRPV5 knockout prevented hypocalcemia in the TRPV5 knockout mouse. The cause of the high 1,25(OH)\(_2\)D\(_3\) level in the TRPV5 knockout mouse remains unknown.

Petra Simic presented work exploring the possible role in bone of BMP6 administered intravenously (11). Previous work from this laboratory showed that estrogen increases the amount of BMP6 in bone matrix, and that oophorectomy has only modest effects on the low bone mass of BMP6 knockout mice. Here, BMP6 was administered systemically to mature, oophorectomized rats and oophorectomized BMP6 knockout mice. In each case, BMP6 administration reversed bone loss and caused no detected toxicity. Thus, BMP6 as a systemic therapy in the postmenopausal setting deserves further investigation.

Estelle Bianchi presented studies focusing on the role of β-arrestin2 in modulating the actions of PTH on primary osteoblast cultures (12). These investigators treated primary osteoblasts either with continuous exposure to PTH or to PTH administered for 6 of every 48 hours. PTH suppressed OPG mRNA transiently in the 6-hour, intermittent PTH model, and for a longer time in the continuous model. Long exposure to PTH was required for substantial increase of RANKL mRNA in cells from both wild-type and β-arrestin2 knockout mice. The cells from β-arrestin2 knockout mice did show higher levels of RANKL mRNA than wild-type cells, however, this was after 6 days of continuous exposure to PTH. Thus, β-arrestin2 regulates both OPG and RANKL responses to PTH.

Frank de Vries used the UK General Practice Research Database to determine the effect of intermittent high dose glucocorticoid use in patients with chronic lung disease on fracture risk (13). This group found that the modest increase in fracture did not differ between first time users and repeat users, as long as the repetition was not very frequent. Frequent pulses of glucocorticoids, often used to treat flares of chronic obstructive lung disease, were associated with an increased risk of fracture.

Yuji Ito presented studies of the mechanisms of glucocorticoid suppression of osteoblast function (14). Using a promoter-reporter gene transfection strategy, the group identified a DNA sequence upstream of the IL11 gene responsible both for PTH stimulation of transcription and for the suppression of this stimulation by glucocorticoids. Glucocorticoids decreased the binding of AP1 to its DNA target sequence. Strikingly, the investigators showed that both neutralizing antibodies to IL11 and siRNA that suppressed IL11 levels diminished the ability of PTH to minimize osteoblast apoptosis caused by glucocorticoids. Thus, suppression of IL11 may contribute to the negative actions of glucocorticoids.

Conflict of Interest: The author reports that no conflict of interest exists.
In her plenary presentation, Miep Helfrich started the stimulating session on osteoclast physiology by describing recent developments in our understanding of the molecular pathology of osteoclast diseases, particularly Paget’s disease and osteopetrosis (15). Although duplicating mutations in the signal peptide of RANK and mutations in the ubiquitin-binding (UBA) domain of p62 are clearly associated with familial severe/early onset and late onset familial Paget’s disease, respectively (16–18), in her conclusion, Dr. Helfrich made the point that much remains to be learned regarding the exact effect of these mutations on signaling pathways in osteoclasts, and how this relates to the Pagetic osteoclast phenotype. She also stressed how the identification of mutations in animal models of osteopetrosis (such as oc/oc and grey lethal) are providing new insights into the molecular pathways regulating osteoclast function, particularly cell polarization, vesicular trafficking and ion regulation. In the workshop on vesicular trafficking in bone cells (a recurring theme throughout the meeting), Miguel Seabra (19) described the crucial role of Rab GTPases in this process and summarized recent studies demonstrating that inhibition of Rab prenylation inhibits osteoclast function (20). Likewise, it was also reported (21) that osteoclasts from gunmetal mice (that are deficient in Rab prenyl transferase activity) have abnormally low levels of prenylated Rab proteins and are defective in bone resorption in vitro. Although very little is known about the specific role of Rab GTPases in osteoclasts or other bone cells, with a few exceptions (22–24), the use of oligoarrays and gene-specific primers to profile the expression of Rabs in human osteoclasts was described (25). In particular, Rab13 and Rab32 appear to be highly induced in mature osteoclasts, suggesting an important role in bone resorption. The downstream effectors of Rab GTPases are also largely uncharacterized in osteoclasts. Surprisingly, Rac1 was identified as a binding partner for Rab7 in a bacterial two hybrid screen, and found to colocalize with Rab7 in the ruffled border of resorbing osteoclasts (26). This appears to be the first demonstration of a direct interaction between 2 small GTPases, although how this interaction might regulate osteoclast polarization remains to be clarified. Similarly, the exact function of the grey lethal protein remains unknown, although it was indicated (27) that this 338 amino acid protein in the vesicular compartment probably also plays a key role in membrane trafficking in osteoclasts. Finally, an exciting presentation (28) demonstrated the feasibility of in utero bone marrow transplantation from allogeneic mice as a means of rescuing the osteopetrotic phenotype in oc/oc mice that harbor a defect in the vacuolar H⁺-ATPase in osteoclasts. Such an approach would be a major step forward in the treatment and potential cure of infantile autosomal recessive osteopetrosis.

**Osteoclast Differentiation**

In the second plenary lecture, Hiroshi Takayanagi (29) described recent studies illustrating the crucial role of the NFATc1 transcription factor as a RANKL-activated master switch for the terminal differentiation of osteoclasts, and the costimulatory signals provided by ITAM-bearing FcRgamma and DAP12 adapter proteins (30;31). Whilst TRAP, cathepsin K, calcitonin receptor and OSCAR were described as targets of NFATc1, transactivation of the beta3 integrin gene in a reporter assay using RAW264 cells, as well as NFAT binding to 2 consensus sites in a conserved region of the beta3 promoter, was also demonstrated (32).

Although the roles of BMPs in bone formation are well documented, two oral presentations described a role for BMPs in osteoclast differentiation. The first (33) described a severe osteopenic phenotype in Col1a1-BMP4 transgenic mice, with markedly increased numbers of osteoclasts, as well as an apparent lack of attachment of osteoblasts to bone matrix. Conversely, the second presentation (34) reported significantly reduced osteoclastic resorption in mice overexpressing the BMP antagonist noggin under the Col1a1 promoter. Together, these studies support a role for BMPs in regulating osteoclast differentiation/resorption, as well as osteoblast and chondrocyte differentiation and function. Similarly, a role for Wnt
signaling in the negative regulation of osteoclastogenesis, as well as in the regulation of the osteoblast lineage, was suggested, since activation of Wnt signaling by LiCl significantly inhibited osteoclast formation and resorption in the absence of osteoblasts in vitro. (35) Mature human osteoclasts and CD14+ progenitors were also found to express LRP6 but not LRP5, suggesting that LRP6 may mediate Wnt signaling in osteoclasts. The emergence of another new pathway regulating osteoclast formation and function was presented (36), where mice lacking the cannabinoid receptor CB1 were shown to have significantly higher BMD than littermate controls and were protected from OVX-induced bone loss. Furthermore, the endogenous CB1 agonist anandamide stimulated osteoclast formation in vitro. Thus, the CB1 receptor and the endocannabinoid system appear to play an important and hitherto unknown role in regulating osteoclast physiology and bone mass (37), although the exact signaling pathways involved remain to be elucidated.

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OSTEOPOROSIS ASSESSMENT AND TREATMENT

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Two clinically relevant topics were discussed in this session: first, the assessment of fracture risk by Chris de Laet of the Scientific Institute of Public Health in Brussels, Belgium (38) and second, current evidence of efficacy of combination therapies for osteoporosis by Joel Finkelstein of Massachusetts General Hospital in Boston, Massachusetts, USA (39).

Dr. de Laet reviewed the evidence of the relationship between bone mineral density (BMD), fracture risk and interventions and discussed current views on the identification and treatment of subjects at risk for fractures. Osteoporosis is operationally defined as a BMD value 2.5 standard deviations lower than the mean of healthy premenopausal women (T-score), and measurement of BMD by DXA is currently the gold standard for diagnosing the disease. In addition, BMD is a strong risk factor for fractures, and each standard deviation decrease is associated with a doubling of this risk. In clinical practice, patients are selected for BMD measurements by the presence of clinical risk factors, and treatment is subsequently offered to women with a BMD T-score below a certain BMD cut-off value; international guidelines generally recommend a T-score of -2.5. Thus, the BMD diagnostic threshold is often used in clinical practice as an intervention threshold. This high risk strategy ensures that the most severely affected patients will receive treatment, but a large number of women at high risk for fractures will not be offered therapy. For example, in the Rotterdam epidemiological study, investigators showed that the majority of osteoporotic fractures occur in women with BMD T-scores higher than -2.5. Similar results have been obtained in other studies, underscoring the weakness of this approach for patient care.

In other chronic diseases, the estimation of the probability, or the absolute risk, of a clinical outcome is accepted for treatment decisions. In osteoporosis as well, it is being increasingly recognized that patients should be selected for treatment on the basis of a high fracture probability, rather than on BMD alone. In studies in the Netherlands and in Sweden, examination of the fracture probability in relation to age and BMD showed that for a given BMD T-score, the probability for fracture is higher with increasing age. Thus, age includes risk factors for fractures in addition to that of BMD. It is therefore important to identify factors that contribute to fracture risk independently of BMD. In addition, these factors should be easily assessed in clinical practice and should be applicable to both genders and to different populations. Such factors, recently identified by meta-analyses...
of large epidemiological databases worldwide, include prior fracture, parental history of fracture, glucocorticoid use, low body mass index, smoking and rheumatoid arthritis. The combination of age with such risk factors, with or without BMD, can lead to the development of fracture risk assessment algorithms. Such algorithms can lead, in turn, to definition of intervention thresholds, according to fracture probability, which are applicable to individual patients. There are two requirements for the implementation of such algorithms in clinical practice. The first is the definition of the period of calculation of the absolute risk, and the second is the intervention threshold. For the first, there is general agreement, consistent with results of intervention studies, that a 10-year fracture probability is an appropriate timeframe. The second is based mainly on economic considerations, namely the level of risk that is acceptable to society and health care providers, which will obviously vary among countries. Therefore, it has become clear that intervention thresholds in osteoporosis should differ from diagnostic thresholds and should be based on the fracture probability or the absolute risk of fracture.

Dr. Joel Finkelstein reviewed existing evidence of combination therapies for osteoporosis. Several studies have explored the effects of combining two inhibitors of bone turnover, for example, a bisphosphonate with hormone therapy (HT), or a bisphosphonate with raloxifene. Regardless of whether the two agents are started simultaneously or if a bisphosphonate is added to ongoing HT, additional increases in BMD with combination therapy are small, and it is not known whether these increments will reduce fracture risk more than a single agent alone. It is also theoretically possible that such combinations may suppress bone turnover excessively and may compromise bone strength. Moreover, compared to a single agent, combinations of two agents will increase the rate of adverse effects, will increase the costs of therapy, and will probably reduce patient compliance. These considerations, together with the uncertainty about an additional effect on fracture reduction, led Dr. Finkelstein to conclude that combination therapy with two inhibitors of bone turnover should not be recommended.

Much more attractive is the possibility of combining agents with different actions on bone remodeling, such as an inhibitor of bone turnover with a stimulator of bone formation. Such an approach is now feasible due to the availability of PTH(1-34), and several studies have been performed with either PTH(1-34) or PTH(1-84), which has not yet been approved. When PTH was added to ongoing HT, BMD increased substantially compared to HT alone, but it was not clear whether this increase in BMD was larger than with PTH monotherapy. Moreover, when PTH was given concomitantly with alendronate in different studies, it appeared that alendronate reduced the ability of PTH to stimulate osteoblastic activity and to increase BMD. Thus, based on available evidence, concomitant administration of PTH and alendronate is not a valid option for the management of patients with osteoporosis. The question of whether combination of PTH with a weaker inhibitor of bone turnover, such as raloxifene, will have a different effect was addressed by Krege, et al. (40). These investigators randomized 137 women with osteoporosis to treatment with either PTH(1-34) 20 µg/d and raloxifene 60 mg/d, or PTH(1-34) and placebo, for 6 months. The main finding of the study was the similar increase in bone formation (assessed by PINP) in the two treatment groups, while bone resorption (assessed by CTX) increased less with combination therapy compared to PTH monotherapy. These changes in biochemical markers of bone turnover were associated with a significantly higher increase in total hip BMD in the PTH/raloxifene group.

More interesting, however, is the possibility of giving these agents sequentially, for example, by administering an inhibitor of bone turnover followed by PTH, or PTH followed by an inhibitor of bone turnover. The former approach is relevant in clinical practice, particularly in patients with severe osteoporosis who continue fracturing while on treatment with an effective inhibitor of bone turnover. Initial data from such studies...
indicate that BMD responses in the short-term can be different depending on the agent used. For example, PTH following raloxifene treatment induces increases in BMD greater than PTH given after alendronate treatment. However, it appears that the BMD response in the latter group is delayed rather than impaired. Finally, starting therapy with PTH followed by an inhibitor of bone turnover appears to be the most effective combination, as the latter consolidates and sustains the effect of PTH. Black, et al. (41) presented the second year results of the PaTH study, a trial comparing one year of therapy with PTH(1-84) alone, alendronate alone or the two in combination. After one year, patients on PTH were randomized to either placebo or alendronate for another year. Following one year of PTH, there were significant gains in BMD in patients treated with alendronate, while there were losses in those on placebo. The differences between the two groups were striking, particularly for trabecular bone assessed by QCT. Although there are still several questions that need to be answered before an effective combination therapy for osteoporosis can be introduced to clinical practice, there are indications that sequential therapy with an anabolic agent followed by an inhibitor of bone turnover currently represents the most rational therapeutic combination.

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HOT TOPICS OF SPECIAL INTEREST

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The final session of the meeting consisted of presentations of abstracts that had been selected from the entire program as hot topics of special interest. These abstracts were selected from both the basic and clinical components of the meeting. In the past, this final session has been a highlight of these meetings, and this year was no exception.

An interesting variety of abstracts were selected. An abstract on the phenotype of mice with null mutations in the cannabinoid receptor 1 was presented (36). These mice have increased bone mass, along with greater bone mineral density, than corresponding wild-type controls. They did not develop ovariectomy-induced bone loss. A small molecular weight inhibitor of the receptor (called AM251) inhibited bone resorption in vitro. The phenotype of these mice suggests that such inhibitors, if efficacious, safe and effective, may have utility in diseases of increased bone resorption.

An abstract was presented on different Dickkopf (Dkk) proteins, including Dkk1, Dkk2 and Dkk4 (42). Several of these proteins, which are important soluble antagonists of Wnt signaling, have been shown to block osteoblast differentiation in vitro and to enhance adipocyte differentiation. The null mutant mice are embryonic lethals, but the heterozygotes have increased bone mass and increased bone formation rates. The authors suggested that drugs that block Dkk1/LRP5 interactions should be anabolic for bone.

The next presentation examined lithium as an anabolic agent (43). Lithium has been used extensively in the past as a treatment for manic-depressive disorder. It has multiple actions on calcium homeostasis and bone, including the alteration of the set point for PTH to cause increased PTH secretion, and also affects the Wnt signaling pathway. Consequently, there could very well be effects on the regulation of bone formation in patients who are taking lithium in pharmacologic doses. A retrospective, observational study was performed in over 100,000 Dutch patients who were taking lithium, and it appeared that there may be a dose-response effect on decreasing fracture risk in these patients.

Another presentation focused on the potential for allogeneic bone marrow transplantation to rescue the bone phenotype in the oc/oc murine model of malignant osteopetrosis (28). Infantile malignant osteopetrosis in humans is a fatal disorder of mixed genetic nature with severe...
osteoclast dysfunction and a paucity of effective treatments. In this presentation, an approach was described using in utero bone marrow transplantation in the oc/oc mouse model of the disease. The goal is to avoid some of the severe bone abnormalities arising in fetal life, as well as graft versus host disease. The results seemed to be quite promising, with dramatic beneficial effects in some mice. This approach provides the promise of avoiding some of the problems associated with bone marrow transplantation in humans, postnatally.

The next presentation focused on the potential of interspecies synergy to identify genes responsible for increased bone mineral density (44). QTL mapping in inbred mice pointed to chromosome X, and association mapping of the corresponding region of the human genome led to identification of a number of SNPs and to a focus on the PIRIN gene, which contained several SNPs. PIRIN encodes a nuclear protein of unknown function.

Finally, there were two presentations on the potential for Src inhibitors in the treatment of diseases associated with increased bone resorption (45;46). In the first, a Src inhibitor was utilized in a model of breast cancer metastasis to bone. Breast cancer cells transfected with c-Src behaved more aggressively and caused enhanced bone metastasis in vivo. A c-Src inhibitor reduced cachexia and lethality in these tumor-bearing mice, and inhibited bone resorption in vitro. In the second study, a different small molecular weight inhibitor of c-Src and c-Abl, administered to healthy adult male volunteers, reduced markers of bone resorption while treatment was continued.

Conflict of Interest: The author reports that no conflict of interest exists.

References


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