569 postmenopausal women with low BMD were treated with either 20 µg subcutaneous teriparatide daily, 100, 200, 300, or 400 mg oral ronacaleret daily, 70 mg alendronate once weekly, or placebo. Ronacaleret increased spine BMD at 12 months (0.3-1.6%), less than teriparatide (9.1%) or alendronate (4.5%). Total hip, femoral neck, and trochanter BMD decreased with ronacaleret but increased with teriparatide and alendronate. Remodeling markers increased with ronacaleret and teriparatide and decreased with alendronate. These data seem to be ruining a good idea; endogenous PTH may be doing more harm than good when osteoclasts are around. —ES

The search for an effective calcilytic to treat osteoporosis is still ongoing. The compound investigated here holds new promise since oral administration to aged rats dose-dependently improved bone mass, trabecular and cortical structure at the proximal diaphysis, but increased serum calcium levels; it induced short serum PTH peaks in humans without post-dose hypercalcemia, similar to 20 µg subcutaneous teriparatide. —SF

These two separate studies investigated the effects of intact PTH administration in patients with hypoparathyroidism, in addition to standard vitamin D, calcium and
calcitriol treatment. In the bone biopsy study by Rubin et al., PTH(1-84) 100 µg subcutaneously every other day induced a marked increase in bone-forming and remodeling indices at 3 months, 1 and 2 years, with related changes in microstructure, namely increased trabecular number and cortical porosity. Consistent with these results, in the Sikjaer et al. study, twice-daily PTH(1-84) 100 µg markedly increased biochemical markers of bone turnover, decreased aBMD at the spine and hip, and maintained normal serum calcium levels while decreasing calcium and vitamin D dosing. The true clinical benefit of intact PTH in this setting remains to be established. —SF


This small, randomized controlled trial of weekly alendronate (ALN) + vitamin D (2800 IU) shows some intriguing results, namely that compared to placebo, ALN + low-dose vitamin D not only substantially increased 25OHD levels (+8.7 ng/ml) and fractional calcium absorption (+7% absolute increase) within one month, but also increased 1,25 dihydroxyvitamin D₃ and serum PTH levels by nearly 30%. Hence vitamin D supplements with concomitant anti-resorptive therapy leading to a secondary rise in serum PTH could synergize to increase 1,25 dihydroxyvitamin D₃ and calcium absorption. If that is true, ALN + D could be more than just a convenient combination, but also a true pharmacological principle. Too bad vitamin D-alone and ALN-alone groups were not included. —SF


Continued treatment with bazedoxifene (BZX) for 5 years (20 mg or 40 to 20 mg after 3 years) in 4,216 women with osteoporosis resulted in a continuous decrease in morphometric vertebral fracture incidence (-35 to -40% vs. placebo at 5 yrs.). Treating 927 subjects with BZX 20 mg in years 4 and 5 prevented new vertebral fractures in 6 of them compared to placebo. The incidence of non-vertebral and hip (<1%) fractures was similar in treated and placebo groups overall. —SF

Cancer and Bone


The estrogen receptor-related receptor alpha (ERRα) has been implicated in breast cancer and bone development. In this study, the authors have shown that ERRα plays a dual role in breast cancer progression: it upregulates the production of the angiogenic growth factor VEGF and the osteoclastogenesis inhibitor OPG by neoplastic cells, thereby promoting the vascularization of primary breast tumors, but decreasing metastatic growth of osteolytic lesions in bone. —SF

The aim of this study was to determine the association between survival and metastases detected by immunohistochemical staining of sentinel lymph nodes (SLNs) and bone marrow specimens from patients with early-stage breast cancer in a prospective observational study (median follow-up of 6.3 years). Of 3,326 SLN specimens examined by immunohistochemistry, 349 (10.5%) were positive for tumors. Of 3,413 bone marrow specimens examined by immunocytochemistry, 104 (3.0%) were positive for tumors. Immunohistochemical evidence of SLN metastasis was not associated with overall survival, whereas occult bone marrow metastasis was associated with a 94% increase in the risk of mortality (HR = 1.94; 95% CI, 1.02-3.67; P = .04). —PC


In this study, podoplanin (a type-I transmembrane sialomucin-like protein) was found to be highly expressed in metastatic osteosarcomas, suggesting it could be a candidate molecule for therapeutic targeting. —PC


1,960 patients with newly diagnosed multiple myeloma received zoledronate (n = 981) or clodronate (n = 979). The early use of zoledronate was better than clodronate for prevention of skeletal-related events (HR = 0.74; 95% CI, 0.62-0.87; P = 0.0004). —PC


The authors find that the expression of tenasin C (TNC) in breast cancer cells is associated with the aggressiveness of pulmonary and bone metastases. Cancer cell-derived TNC is essential for the outgrowth of micrometastases. This extracellular matrix component enhances WNT and NOTCH signaling activity in cancer cells, promoting cell survival. These findings highlight the importance of tumor-derived TNC in the formation of the metastatic niche. —PC

Genetics


Matthew Brown and colleagues report results of a GWAS of ankylosing spondylitis,
which is a common form of inflammatory arthritis predominantly affecting the spine and pelvis. They identified three new risk variants in the RUNX3, LTBR-TNFRSF1A, and IL12B regions, and four loci at PTGER4, TBKBP1, ANTXR2 and CARD9 that show suggestive association. Notably, PTGER4 – prostaglandin E receptor 4 – is a component of the “mechanostat” anabolic bone response to physical stress, found at the site of entheses (insertion of tendons and ligaments into bone). The paper also reports an interaction between the ERAP1 and HLA-B27 genes. —DK


Dupuytren's disease is a fibromatosis of the flexors of fingers that leads to flexion contractures. Very little is known about the heritability of this disease, despite some reports of familial aggregation. This paper reports a GWAS of 960 Dutch affected persons and 3,117 controls; 35 SNPs most strongly associated with Dupuytren's disease were further taken to replication in three additional case/control series from Germany, the United Kingdom, and the Netherlands. Six of the 9 identified loci contained genes known to be involved in the Wnt-signaling pathway, including WNT4, WNT2, WNT7B, and RSPO2. The latter gene encodes an R-spondin (Rspo), a member of the family interacting with frizzled receptors and LRP5/6 to induce β-catenin signaling; Rspo also competes with dickkopf (DKK) protein. Moreover, Rspo2 expression is required for Wnt11-mediated osteoblast maturation, thus making an interesting connection with bone biology. —DK


When the Russian czar Peter the Great advocated for smoking tobacco, he appealed to the benefit of decreasing appetite. He could not possibly know about the beneficial effect of nicotine on bone health. Indeed, Mineur et al. find that activation of hypothalamic α3β4 nicotinic acetylcholine receptors leads to activation of pro-opiomelanocortin neurons, which together with subsequent activation of melanocortin 4 receptors were critical for nicotinic-induced decreases in food intake in mice. Rothem et al. performed whole human genome gene expression microarray on RNA samples from osteoblast-like cells, MG-63, exposed to 100 µM nicotine, to identify nicotine-regulated genes. Quantitative real-time RT PCR analysis confirmed altered expression in 7 genes that promote osteoblast proliferation and/or anti-apoptosis processes, including cyclin D1, endothelin 1, BCL2-associated X protein (BAX), caveolin 1, and JUN. Furthermore, their results suggest that nicotinic stimulation of the same α3 nicotinic acetylcholine receptor in human osteoblasts is required to regulate those genes. —DK

Actinin-3 (ACTN3 gene) is expressed in muscle and its deficiency is detrimental to sprint and power performance in humans. Here it is shown that Actinin-3 is also expressed in osteoblasts; its deletion in mice leads to a low bone mass phenotype with decreased bone formation; and a stop polymorphism (R577X) in humans is associated with lower aBMD. A nice example of functional genetics from humans to mice and back to humans. —SF

Bone Modeling, Remodeling, and Repair


This is the latest work implicating osteal macrophages (osteomacs) as important elements in the anabolic response to injury. When osteomacs were depleted in the Mafia transgenic model or via clodronate-containing liposomes, healing was adversely affected, whereas OPG treatment (inhibiting osteoclastogenesis but not affecting osteomacs) had no effect on intramembranous bone formation. —DGL


Mechano growth factor (MGF) is a splice variant of IGF apparently produced in situations of tissue damage. It has a unique C-terminal peptide (termed E peptide) that was active in MC3T3 proliferation. In vivo, a 5-mm segmental bone defect in rabbit radii treated with MGF-Ct24E by daily intralesion injection improved bone formation. Peptides are cheaper to produce than proteins; the current finding might enable a novel biologic treatment. —DGL


3 related papers from different groups. Each tests the combination of BMPs and bisphosphonates (BPs). In the fracture healing study by Doi et al., union was advanced and mechanical properties were best in the combined BMP/BP group. This was also the case in the bone chamber study by Belfrage et al., where the BP zoledronic acid was delivered locally. In the final study, in a model of pathologic fracture healing in NF1 heterozygous mice, combined BMP and zoledronic acid treatment resulted in better union than BMP alone. Combining anabolic and anti-catabolic agents is a powerful emerging strategy. —SF


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This is an Amgen group paper on the effects of antibody to Dkk1 that blocked binding to both LRP6 and Kremen2. Treatment with Dkk1-Ab in growing mice and rats increased BMD via increases in bone formation. However, unlike Scl-Ab, treatment of adult ovariectomized rats did not appreciably impact bone, an effect that was associated with decreased Dkk1 expression in the serum and bone of older rats. In adult bone Dkk1 does play a role in fracture healing, where it is again expressed in the adult animal. Callus density, strength and stiffness were improved with Dkk1 antibody, with no effect on the contralateral limb. Dkk1 is important in growing bones, the response to trauma and also in bone pathologies such as myeloma where it is expressed, but does not appear to be a potent factor in adult bone homeostasis based on these data. —DGL


In parabiotic experiments between GFP and WT mice, implants containing cells secreting stromal cell-derived factor-1 (SDF-1) or monocyte chemotactic protein-3 (MCP-3) increased homing from the GFP mouse circulation to the WT mouse. These factors may prove useful in tissue engineering. —DGL

Molecular and Cell Biology


Estrogen through its nuclear receptors (ERs) and a host of co-regulatory elements is the key regulator of the molecular programming of osteoclastogenesis. The first in vitro study reveals that estrogen response element binding protein (ERE-BP) acts as an antagonist of estrogen/ER binding to EREs in osteoblast progenitors, and thereby upregulates RANKL expression, while in osteoclasts it promotes RANK expression. The second study goes further by analyzing the role of ER signaling independent of its binding to EREs. Analysis of a knock-in mouse expressing an ER mutant that is defective in ERE activation (NERK1) showed that the skeletal phenotype of low aBMD, decreased cortical bone, but increased BV/TV was worse in NERK1 than ESR1 (ERα) KO mice. Indices of bone formation were further reduced in NERK1 mice, arguing for an inhibitory effect of non-classical estrogen effects on bone, that is, in the absence of ERE activation. —SF


3BP2 is encoded by the SH3-domain binding protein 2 (Sh3bp2) gene, and binds to Src family kinases. Gain-of-function mutation in the Sh3bp2 gene is associated with the majority of cherubism patients, causing cystic bone lesions with activated osteoclasts that lead to craniofacial abnormalities. The authors show that Sh3bp2(-/-) mice developed osteoporosis with reduced bone formation and impaired bone resorption. The tyrosine kinase Abl was not activated in Sh3bp2(-/-) osteoblasts, which failed to mature and form mineralized nodules in vitro. Src was not activated in Sh3bp2(-/-) osteoclasts, which spread poorly and were unable to resorb dentine matrix in vitro. Thus, 3BP2 is required for both bone resorption and formation, by acting as an adaptor protein for Abl in osteoblasts and Src in osteoclasts. —TM


Previous analysis of the bone phenotype of TRPV6 (the major intestinal calcium channel) KO mice showed that bone mineralization in these mice was only impaired on a low-calcium diet. Since TRPV6 is also weakly expressed in some bone cells, it remained unclear whether the latter observation was only due to poor intestinal calcium absorption and/or impaired mineralizing function in osteoblasts. The present study characterizes the expression of TRPV6 in osteoblasts and osteoclasts and demonstrates that mineralization in vitro and in vivo is not altered by TRPV6 expression. —SF


The authors utilized the mouse Col2α1-Cre promoter that is active not only in chondrocytes but also in osteoprogenitors to conditionally delete the Nf1 gene in osteochondroprogenitor cells. These mice recapitulated the skeletal abnormalities of NF1 patients, with progressive scoliosis and kyphosis, tibial bowing, and skull and anterior chest wall malformation. The Ras-ERK pathway was constitutively activated in BMSCs of these mice, and blockade of Ras activation via inhibition of Ras prenylation by lovastatin during embryonic development attenuated the increased cortical porosity in mutant mice. These data suggest that activation of the Ras-ERK pathway by Nf1 loss-of-function in osteochondroprogenitors is responsible for the vertebral and tibia lesions in NF1 patients. —TM


The authors crossed mice carrying a Dmp1 promoter-driven GFP with the Immortomouse expressing a thermolabile SV40 large T-antigen regulated by IFN-γ, and generated a clonal cell line, IDG-SW3, from long bone chips of these mice. These cells can be expanded at 33°C in the presence of IFN-γ and then differentiated at 37°C in the absence of IFN-γ. IDG-SW3 cells are Dmp1-GFP-positive and T-antigen-negative under osteogenic conditions. They differentiate into osteoblasts,
early osteocytes that express E11/gp38, Dmp1, MEPE, and Phex, and late osteocyes that develop a dendritic morphology and express SOST/sclerostin and FGF23. When 3D cultures are implanted in calvarial defects in vivo, they accelerate bone healing. This cell line should be of great use for studying osteoblast-to-osteocyte transition and osteocyte biology. —TM

Health Economics


A cross-sectional estimate of cost was made in a random sample of 12,700 Medicare recipients. Three cohorts aged 65 or over were defined: patients experiencing a fracture, patients with a diagnosis or self-report for osteoporosis or past hip fracture, and controls. Of 30.2 million Medicare recipients in 2002, 1.6 million (5%) were treated for a fracture and 7.2 million (24%) had osteoporosis without a fracture. The estimated effect of fractures on annual medical cost in the US was $14 billion. Half of the non-fracture osteoporosis patients received drug treatment, averaging $500 per treated patient or $2 billion nationwide. The annual cost of osteoporosis and fractures in the US elderly was estimated at $16 billion using a national 2002 population-based sample. —ES

Reviews, Perspectives and Editorials


Other Studies of Potential Interest

◈Bishop KA, Coy HM, Nerenz RD, Meyer MB, Pike JW. Mouse Rankl expression is regulated in T cells by c-Fos through a cluster of distal regulatory enhancers designated the T cell control region. J Biol Chem. 2011 Jun 10;286(23):20880-91. [Abstract] [Full Text]


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Conflict of Interest: Dr. Clézardin reports receiving research support from Novartis (Basel, Switzerland) and honoraria for advisory work and speaking engagements from Novartis and Amgen. Dr. Ferrari reports that he receives research support from Amgen and Merck Sharp & Dohme, and is an advisory committee member and lectures occasionally at conference symposia for the Alliance for Better Bone Health (sanofi-aventis/P&G), Amgen, Merck Sharp & Dohme, Eli Lilly, Servier, and Novartis. Dr. Little reports that he receives royalties, research funds and consultancy fees from Novartis Pharma, as well as research support from Stryker Biotech. 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. Matsumoto reports that he is a member of the advisory board for Eli Lilly, and receives consultancy fees from Chugai, Astellas, Teijin, JT, and Daiichi-Sankyo. Dr. Karasik reports no conflicts of interest.