Clinical and Basic Research Papers – March 2011

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Clinical Studies and Drug Effects


Finite element analysis (FEA) of proximal femur QCT scans was applied to estimate hip strength and load-to-strength ratio during a simulated sideways fall in an age-stratified random sample of community-dwelling adults. Odds ratios for prevalent overall and osteoporotic fractures were similar for FEA measures and hip aBMD and vBMD obtained from QCT images. C-statistics (estimated areas under ROC curves) were also similar (e.g., 0.84-0.85 in women and 0.75-0.78 in men for osteoporotic fractures). In women and men, the association with prevalent osteoporotic fractures increased below an estimated hip strength threshold of ~3000 N. —DK

Boyer KA, Kiratli BJ, Andriacchi TP, Beaupre GS. Maintaining femoral bone density in adults: how many steps per day are enough? Osteoporos Int. 2011 Feb 12. [Epub ahead of print] [Abstract]

Walking is the most common weight-bearing activity in older adults. However, the amount and intensity of walking needed to maintain a healthy skeleton is unknown. This study examined the relationship between habitual walking and femoral BMD in 105 healthy individuals (49-64 years of age) using a quantitative theory for bone maintenance: an index of cumulative loading (bone density index, BDI) was examined as a predictor of BMD. In women, BDI was slightly correlated with BMD. For a woman of average body weight, walking at 1.00 m/s requires 4,892 steps/day to maintain a T-score of -1.0. Substantially more steps (18,568 steps/day) are required for a 20% lighter woman (or an increase in speed to 1.32 m/s at 10,000 steps/day) for the same effect. The study is an important step (no pun intended!) on the way towards individualized exercise programs aimed at mechanical stimulation at the hip, which must also take into account genetic predispositions to exercise and factors affecting walking ability. —DK


Detailed analysis of 160 women from the FREEDOM trial shows that denosumab suppresses CTX levels below premenopausal levels shortly after injection in 100% of...
subjects and that CTX remained maximally suppressed in 79% of patients at the end of the first 6-month dosing interval and in 51% of women at the end of the last (3-year) dosing interval. —SF


This database analysis of more than 4,000 women confirms that persistence with both weekly and monthly bisphosphonates at one and two years is low (29% and 13%, respectively) and is associated with a higher fracture incidence (12% vs. 15% at two years in compliant vs. non-compliant women). —SF


This observational study from a Canadian database over 6 years used diagnostic codes to identify subtrochanteric or femoral shaft fractures following initiation of bisphosphonate therapy in women 68 years or older, excluding diagnostic codes for fractures resulting from trauma, motor vehicle collisions, and falls from a height, as well as fractures treated exclusively with hip replacement surgery. 716 subtrochanteric or femoral shaft fractures and 9,723 women who sustained a typical osteoporotic fracture of the intertrochanteric region or femoral neck were found. By matching each case to 5 controls, the study found bisphosphonate use for more than 5 years, but not less, to be associated with a decreased risk of typical osteoporotic fracture but a nearly 3-fold increased risk of subtrochanteric/femoral shaft fracture. The incidence of these fractures, however, remained low (0.13%/yr.). —SF

Cancer and Bone


Dysregulation of microRNAs has been implicated in tumorigenesis and metastasis. Here, miR-34a was found to be underexpressed in CD44+ prostate cancer stem cells in both xenografts (LAPC4, LAPC9, DU145) and primary tumors (n = 18). Enforced expression of miR-34a in CD44+ Du145 and LAPC9 prostate cancer stem cells inhibited orthotopic tumor growth and metastasis in vivo. In addition, systemically delivered miR-34a reduced lung metastasis in animals bearing LAPC9 orthotopic tumors. To determine whether CD44 is a functionally important target of miR-34a for prostate cancer development, CD44 expression was silenced in LAPC4, PC3 and DU145 cells. CD44 silencing phenocopied miR-34a overexpression in inhibiting tumor growth and metastasis formation. Thus, miR-34a is a key negative regulator of CD44+ prostate cancer stem cells, suggesting it could be used as a therapeutic agent against prostate cancer metastases. —PC

Detection of disseminated tumor cells in bone marrow is an independent prognostic factor in primary breast cancer. Here, the authors conducted a proof-of-principle study to evaluate whether this tumor cell spread has already occurred in patients with ductal carcinoma in situ (DCIS). Thirty women with DCIS were included. Bone marrow aspirates were taken at the time of primary surgery. The authors found that hematogenous tumor cell dissemination into bone marrow was an early event in breast cancer development. —PC


The authors report that the Notch ligand Jagged1 is expressed by breast cancer cells and promotes osteolytic bone metastasis by activating the Notch pathway in bone cells. Jagged1 directly activates osteoclast differentiation and it stimulates IL-6 release from osteoblasts which, in turn, stimulates tumor growth. In addition, Jagged1 is an essential functional target of the cytokine TGF-β that is released during bone destruction. Importantly, γ-secretase inhibitor treatment reduces Jagged1-mediated bone metastasis by disrupting the Notch pathway in bone cells. Taken together, these findings provide the rationale for using γ-secretase inhibitors for the treatment of bone metastasis. —PC

Genetics


Menatetrenone (MK-4) is a vitamin K2 homologue that has been used as a therapeutic agent for osteoporosis. This study evaluated the influence of gamma-glutamyl carboxylase (GGCX) gene polymorphisms (rs699664, Arg325Gln) on the response of serum undercarboxylated osteocalcin (ucOC) and bone turnover markers 3 months after treatment with MK-4. The GGCX gene was chosen since its rare mutations cause defects in vitamin K-dependent proteins. In postmenopausal Thai women, there was a significant reduction of serum ucOC, beta-CTX and P1NP from baseline to 3 months, independent of genotype. Only in a small subgroup of women 65 years and older was there a significant difference in the response to vitamin K2 (reduction of ucOC). —DK


Growth and differentiation factor 5 (GDF5, also known as cartilage-derived morphogenetic protein-1) was previously found to be strongly associated with osteoarthritis (OA) and to play a role in height. The common SNP rs143383 in the 5'
untranslated region (UTR) of GDF5 influences GDF5 expression. The study by Williams et al. investigated whether this variant, beyond being an OA susceptibility locus, is associated with lumbar disc degeneration (LDD). Indeed, an association between LDD and rs143383 was identified in women from 5 European cohorts, with the same risk allele as in knee and hip OA (odds ratio 1.72 [95% CI: 1.15-2.57], P = 0.008).

The second study by Dodd et al. is the first report of deep sequencing of GDF5. Using the Sanger method, the authors sequenced GDF5 in 992 OA patients and 944 controls (from the UK, Spain and Greece; females and males; knee and hip OA cases). They covered the protein-coding region of two of five GDF5 exons, both UTRs, and ~ 100 bp of the proximal promoter, and detected 13 variants. Six were extremely rare, with minor allele frequencies (MAFs) of ~ 0.06%, and seven variants were common (MAFs of 2.5-39%). Three of the extremely rare variants are potentially functional; however, since they are extremely rare (unique), they do not explain the association signal marked by rs143383. Notably, there was a complete absence of variants with frequencies in-between the extremely rare and the common SNPs. —DK

Bone Modeling, Remodeling, and Repair


Two models of bisphosphonate-related ONJ have been published thus far, both using traumatic teeth extractions. This new rat study is based on the classical tooth ligature periodontitis model. Administration of zoledronate (ZOL) equivalent to the monthly human oncology dose (0.066 mg/kg), but repeated in these rats nine times over three weeks was associated with a reduction of alveolar bone resorption, necrotic bone in 47% and bone protruding into the oral cavity in 21% of ZOL-treated animals. A plausible model is proposed where bone injured by inflammation and infection (periodontitis) is not removed when bone remodeling is inhibited by bisphosphonates, leaving it exposed to even more injury and eventually the tissue dies. Of note, several models of experimental periodontitis showing the beneficial effects of bisphosphonates on alveolar bone loss have been published previously, without evidence of ONJ. Perhaps this means that we find what we look for...or is it the high cumulative dose of ZOL over a relatively short time that does it here? (For a review of recent clinical and preclinical advances in ONJ, see the Perspective by Drs. Allen and Ruggiero in the March 2011 issue of BoneKEy). —SF

◆Agholme F, Isaksson H, Kuhstoss S, Aspenberg P. The effects of Dickkopf-1 antibody on metaphyseal bone and implant fixation under different loading conditions. Bone. 2011 Feb 15. [Epub ahead of print] [Abstract]

A Dkk1 neutralizing antibody was given in various modes of repair. In screw fixation after 28 days, the pull-out force was increased by over 100%. A 70% increase in bone formation in a bone chamber was also noted. Importantly, these studies model intramembranous bone formation and more complex endochondral systems remain to be fully evaluated with Dkk1 inhibition. —DGL


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Trabecular bone fragments were collected at the time of surgery for total hip arthroplasty after fragility hip fracture. Gene expression was analyzed at different time points. Inflammatory genes such as IL-6 and TNF-α were elevated early, and then declined. RANKL expression peaked between 4 and 7 days. SOST levels decreased over time. The authors conclude that promoting these effects might be beneficial.

—DGL


In this ingenious experiment, this group shows that the mechanical rules governing cell fate decisions in metaphyseal repair are the same as in diaphyseal repair, rebuking previous dogma. In a clever model of increasing motion in a semi-stable osteotomy, cartilage can be made to form between trabecular surfaces when strain is high. Metaphyseal bone tends to be more stable, but the rules governing its repair may be the same.—DGL


An innumerable number of studies have shown changes in bone microstructure with various osteoporosis treatments in animal models, but very few have studied the persistence of the effects upon drug withdrawal. In this OVX mouse model, PTH increased vertebral BV/TV and compression strength more than alendronate or raloxifene, but 4 months after treatment cessation, alendronate effects were maintained whereas PTH and raloxifene effects were lost. In contrast, PTH increased cortical bending strength at the tibia up to 4 months after withdrawal.—SF

Molecular and Cell Biology


The effects of interferon-γ on osteoclastogenesis and bone resorption are complex and somewhat controversial (see BoneKEy article by Ferrari-Lacraz S, Ferrari S. Is IFN-γ involved in bone loss or protection? Nothing is simple with cytokines. 2007 Feb;4(2):83-87). These two papers bring new evidence that IFN-γ has bone-sparing properties in vivo, both by inhibiting bone resorption and by stimulating bone formation.—SF


It has been shown recently that the hematopoietic stem cell (HSC) niche is characterized by the interplay between mesenchymal stem cells/osteoblasts and HSCs.
that reciprocally control their differentiation. This study now explores the role of osteoclasts and bone resorption in the HSC niche. Alendronate-mediated inhibition of osteoclastic bone resorption reduced the number of primitive HSCs, favoring the expansion of hematopoietic progenitors, and abolished the ability of PTH to increase the primitive HSC pool. —SF


Contrasting results have been published previously concerning the skeletal phenotype(s) of leptin-deficient mice (ob/ob, db/db), i.e., whether or not they have a high bone mass phenotype. This detailed analysis of db/db mice shows that in the absence of the leptin receptor, both trabecular and cortical bone volume are low, arguing for a bone-anabolic effect of leptin that overcomes its bone-inhibitory effects through the central nervous system. —SF


This study found that DKK1 protein levels were regulated via DKK1 3'UTR by miRNA control: miR-335-5p specifically targets DKK1 3'UTR. In vivo studies showed high expression levels of miR-335-5p in osteoblasts and hypertrophic chondrocytes of mouse embryos. The effects of endogenous miR-335-5p were reversed by anti-miR-335-5p treatment. When site-specific mutations were introduced into the sequence of the predicted binding site of miR-335-5p in the DKK1 3'UTR sequence, the inhibitory effect of miR-335-5p disappeared. As a result of down-regulation of DKK1 protein levels, Wnt signaling is significantly enhanced as indicated by elevated phosphorylation of GSK-3β and increased β-catenin transcriptional activity; expression levels of Wnt target genes (Runx2, BSP and OC) are significantly up-regulated, promoting osteogenic differentiation. —DK

Reviews, Perspectives and Editorials


Other Studies of Potential Interest


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 and Dr. Karasik report no conflicts of interest.