Clinical and Basic Research Papers – February 2011

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Clinical Studies and Drug Effects


This study assessed whether calcium-vitamin D3 fortified milk could enhance the effects of exercise on bone strength, structure, and BMD in men, in an 18-month factorial design randomized controlled trial. 180 men aged 50-79 years were randomized to the 4 arms: exercise+fortified milk; exercise-only; fortified milk-only; and controls. Exercise led to an average 2.1% net gain in femoral neck section modulus, an ~1.9% gain in aBMD, and a 2.2% gain in lumbar spine trabecular BMD. There were no main effects of the fortified milk at any skeletal site. Moreover, there were no combined effects or interactions between exercise/fortified milk at any skeletal site.
—DK


Spine surgeons have been concerned that bisphosphonates interfere with spinal fusion; studies in this regard were often based on very high doses of bisphosphonates. This randomized controlled trial allocated 20 patients with osteoporosis requiring single-level posterior lumbar interbody fusion to receive alendronate 35 mg/week or vitamin D. 95% of alendronate-treated patients had a solid fusion at 1 year compared to 65% of controls. No vertebral fractures were observed in the alendronate group, whereas 24% of patients in the control group showed subsequent vertebral fractures.
—DGL


Finding a drug that increases bone formation and reduces bone resorption at the cellular and tissue levels is an unmet need. The initial findings with this sclerostin antibody (AMG 785) are encouraging. 72 healthy subjects received AMG 785 or placebo (3:1) subcutaneously (0.1, 0.3, 1, 3, 5, or 10 mg/kg) or intravenously (1 or 5 mg/kg). Dose-related increases in P1NP, BAP, and osteocalcin were observed, with a
dose-related decrease in sCTx and increases in BMD (5.3% at the spine, 2.8% at the total hip compared with placebo), on day 85. Six subjects in the higher-dose groups developed anti-AMG 785 antibodies, 2 of which were neutralizing.


In this interesting work, CT-based high-resolution cortical thickness mapping of the hip in EUROFORS subjects shows discrete areas of cortical thickening with teriparatide. The distribution of effects suggests a possible synergistic effect of habitual load and PTH in the human proximal femur, since peak effects are seen at sites that are highly stressed by walking. —SF


It is a case report, but the very typical features of an atypical femoral fracture, including cortical thickening, seen here – in the absence of bisphosphonates – further support the hypothesis that susceptibility to atypical fractures in bisphosphonate users might be carried by gene mutations (cathepsin K in this case) causing alterations in bone turnover and/or strength. —SF

Cancer and Bone


Lysyl oxidase-related enzyme LOXL2 expression is correlated with metastasis and decreased survival in patients with aggressive breast cancer. In immunocompromised or immunocompetent orthotopic and transgenic breast cancer models, targeting LOXL2 is highly effective against spontaneous lung, liver and bone metastases. —PC


Human bone marrow-derived mesenchymal stem cells (hBMSCs) from a tissue-engineered bone subcutaneous implant home to orthotopic human breast tumors, enhancing their growth and increasing the frequency of skeletal metastases. IL-17B produced by hBMSCs promotes this metastatic process. —PC


The interaction between BMSCs and SUM159 breast cancer cells leads to the production of cytokines (IL-6 and CXCL-7) that regulate the in vitro expansion of breast cancer stem cells. In NOD/SCID mice, BMSCs traffic from the bone marrow to primary breast tumor sites in the mammary fat pad where they increase the breast cancer stem cell population. —PC

cAMP-dependent protein kinase type I, α regulatory subunit (Prkar1a) is a bone tumor suppressor gene, the loss of which induces osteosarcoma development in mice and RANKL overexpression in these tumors. In addition, RANKL was overexpressed in human osteosarcomas expressing low PRKAR1A levels. —PC


The authors identified miR-203 as being progressively lost in advanced metastatic prostate cancer. To assess the biological significance of miR-203, miR-203 was reintroduced in metastatic PC3 human prostate cancer cells. Re-expression of miR-203 suppressed metastasis-relevant traits in vitro (inhibition of cancer cell migration and invasion, reduction of proliferation), induced mesenchymal-to-epithelial transition (MET), and suppressed prostate cancer metastasis in vivo. —PC

Genetics


The genetic disorder spondyloenchondrodysplasia (SPENCD) is characterized by vertebral and metaphyseal dysplasia, spasticity with cerebral calcifications, and a strong predisposition to autoimmune diseases. These two groups independently identified biallelic mutations in ACP5, encoding tartrate-resistant acid phosphatase (TRAP), on chromosome 19p13 with loss-of-function of TRAP in all the SPENCD patients studied. Lack of TRAP activity results in hyperphosphorylation and gain-of-function of osteopontin (OPN). Extracellular phospho-OPN causes the skeletal defects and may also cause the cerebral calcifications with neurologic symptoms. Intracellular phospho-OPN causes enhanced TLR9 signaling, which increases IFN-α production by dendritic cells. Elevated IFN-α leads to systemic autoimmunity by increasing antigen presentation. These findings reveal a novel link between TRAP activity, OPN and IFN-α signaling in the genesis of common autoimmune disorders. —TM


The etiology of osteoarthritis (OA) is multifactorial, with a strong heritable component. These 2 papers reported genome-wide association studies (GWAS) of OA. In the first paper, a meta-analysis of 4 studies was performed in 2,371 Caucasian cases of knee OA and 35,909 controls. Replication of the top hits was attempted with data from 10 additional replication datasets, including East Asian cases and controls. One genome-wide significant locus was identified on chromosome 7q22, which contains six genes: PRKAR2B, HPB1, COG5, GPR22, DUS4L, and BCAP29. COG5 (component of oligomeric golgi complex 5) was previously reported in a subsample of this meta-analysis. Expression of all 6 genes was confirmed in primary cells derived from joint tissues.

In the second paper, the authors performed a GWAS for knee and hip OA in 3,177 cases and 4,894 population-based controls from the UK. Replication of promising signals was carried out both in silico and de novo in independent samples of European descent. However, none of the association signals reached genome-wide significance levels. The authors believe they need even larger sample sizes and homogenous phenotypes to succeed. —DK


Many mutations causing monogenic bone disorders remain to be identified. This work broadens the spectrum of diseases associated with SOST mutations, in addition to sclerosteosis and Van Buchem disease, by identifying 2 mutations that prevent the secretion of sclerostin, resulting in the most severe form of osteosclerosis in 2 affected individuals. —SF

**Bone Modeling, Remodeling, and Repair**


In a rat model of unstable titanium plate osseointegration, OPG-Fc and high-dose alendronate, but not low-dose, inhibited bone resorption and/or osteoclastogenesis. This experiment provides the rationale to test RANKL inhibitors to prevent and/or treat prosthesis loosening. —SF


Nell-1 appears to be a novel growth factor with potent osteoinductive capacity, also inhibiting adipogenic differentiation. This study shows that Nell-1 protein in a demineralized bone matrix (DBM) carrier formed more bone than DBM alone in a critical defect model. It was not clear how mechanically competent the bone was. The
IBMS BoneKEy. 2011 February;8(2):65-73
http://www.bonekey-ibms.org/cgi/content/full/ibmske;8/2/65
doi: 10.1138/20110492

(authors state Nell-1 is downstream of Runx2. —DGL)


Given the concern regarding ONJ, it seems reasonable to also examine the effects of bisphosphonates on mandibular fracture healing. Diaphyseal fracture healing has been shown to be improved by zoledronic acid treatment. This group examined mandibular fracture healing in rabbits given a single dose of 0.1 mg/kg zoledronic acid. Biomechanical testing data showed that this treatment resulted in a significant increase in healed bone strength. This result was supported by radiologic, histologic, and histomorphometric findings. —DGL

Molecular and Cell Biology


Using an in vivo murine model, the authors show that muscle-derived stromal cells (MDSCs) harvested after 3 days of exposure to an adjacent fracture differentiate into osteoblasts and form bone nodules in vitro. The osteogenic potential of these cells exceeds that of adipose and skin-derived stromal cells and is equivalent to bone marrow stromal cells in their contribution to accelerated healing. The main factor responsible for this is TNF-α. At low concentrations, TNF-α promotes MDSC migration with an ensuing osteogenic differentiation; it is, however, inhibitory at high concentrations. —DK


The authors show that retroviral expression of two reprogramming factors (c-Myc and Klf4) and one chondrogenic factor (SOX9) induces polygonal chondrogenic cells directly from adult dermal fibroblast cultures. Induced chondrogenic cells expressed marker genes for chondrocytes and produced homogeneous hyaline cartilage-like tissue without type I collagen expression when subcutaneously injected into nude mice. Hyaline cartilage-like tissue remained for at least 16 weeks without tumor formation. This approach could lead to the preparation of patient-specific hyaline cartilage directly from skin, without generating induced pluripotent stem (iPS) cells. —TM


The authors previously demonstrated that sphingosine-1-phosphate (S1P) controls the migration of osteoclast precursors (OPs) between the blood and bone via the S1P receptor 1 (S1PR1) that directs positive chemotaxis toward S1P. Here they show that OPs also express S1PR2, which mediates chemorepulsion under high S1P concentrations. S1PR2-deficient mice exhibit decreased bone resorption with moderate osteopetrosis, suggesting that S1PR2 contributes to chemorepulsion away from the blood where S1P levels are high. Inhibition of S1PR2 by the antagonist

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JTE013 changed the migratory behavior of OPs, and relieved osteoporosis by reducing the number of osteoclasts on the bone surface. The reciprocal regulatory axis of S1P-dependent OP chemotaxis by S1PR1 and S1PR2 may become a therapeutic target in diseases with enhanced bone resorption. —TM


CD40 ligand (CD40L)-CD40 interactions between T cells and antigen-presenting cells are a key co-stimulatory pathway for adaptive immune and inflammatory processes and a target for the development of biologic treatment of inflammatory disorders. Considering the role of T cells in osteoclast activation and bone loss, including in response to estrogen deprivation, it comes as no surprise that CD40L knockout mice and mice treated with CD40L neutralizing antibody were protected against ovariectomy-induced bone loss. Even more interestingly, an interaction between T cell-expressed CD40L and “stromal cells” controlled the expansion of stromal cells as well as osteoblast proliferation and differentiation, including their production of osteoclastogenic factors. —SF


Forkhead box (FoxOs) activate antioxidant scavenger proteins; FOXO1 is known to inhibit proliferation in a variety of cells. Studies suggested that FoxO activation in response to oxidative stress plays a major role in the adverse effects of aging on both osteoblast and osteoclast number. In this study, investigators examined the functional role of FOXO1 in a culture system in which pre-osteoblastic MC3T3-E1 cells undergo terminal differentiation in vitro. FOXO1 knockdown by RNAi reduced the number of mineralized nodules formed. However, FOXO1 overexpression in MC3T3-E1 cells reduced proliferation. These findings indicate that FOXO1 plays an important role in promoting osteoblast differentiation and suppressing proliferation in differentiating cells and confirm observations that oxidative stress seems to be one of the determinants of osteoporosis. —DK

Public Health


A second fracture in the year after a first is common and costly; three recent papers make this and other points. The notion of vertebral fractures as “the” osteoporosis fracture is flawed. Recognition of the burden of disease produced by nonvertebral fractures raises many issues, one being the few studies addressing anti-nonvertebral fracture efficacy of drugs. Song et al. report the 1-year medical costs associated with
second fracture(s) in patients with an initial hip, clinical vertebral or non-hip non-vertebral fracture. For privately insured patients, the 1-year second fracture rate was 8.0%, 5.1%, and 4.0%, and 1-year incremental costs were $47,351, $43,238, and $23,852, respectively; for Medicare patients, corresponding rates and costs were 8.8%, 9.2%, and 8.2%, and $18,645, $19,702, and $19,697. The nationally projected annual cost of second fracture was $834 million for patients with commercial insurance and $1.13 billion for Medicare patients.

In the paper by Pike et al., during the 2 years following an incident fracture, patients with a non-vertebral fracture (hip, femur, pelvis, lower leg, upper arm, forearm, rib, and multiple sites) were compared to controls with osteoporosis but without a fracture (N = 3,781). Comorbidity rates and resource use remained higher among non-vertebral fracture patients during the second year following a non-vertebral fracture. Patients with fractures of the pelvis, hip, and femur had the highest excess costs in the second year ($5,121, $3,930, and $3,828, respectively). Although hip fractures had the highest excess costs over both years, non-hip non-vertebral fracture patients made up a larger proportion of the sample and were more costly than controls.

Finally, in the study by Morin et al., 21,067 incident fractures in men were followed by 10,724 (50.1%) deaths, and 49,197 incident fractures in women were followed by 22,018 deaths (44.8%). 76% percent of the fractures were at sites other than the hip and vertebrae. The risk of death was increased in both sexes for hip, vertebral, humerus, wrist (in men only), and other fracture sites. Post-fracture mortality was higher in men than women. —ES

Reviews, Perspectives and Editorials

Marie PJ, Kassem M. Extrinsic mechanisms involved in age-related defective bone formation. J Clin Endocrinol Metab. 2011 Jan 5. [Epub ahead of print] [Abstract]


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.