Clinical and Basic Research Papers – July 2011

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Clinical Studies and Drug Effects


Analyzing the rate of subtrochanteric or diaphyseal femur fractures using health care utilization data, the authors found similar incidence rates among bisphosphonate (BP) and raloxifene/calcitonin users (1.46 and 1.43 per 1000 person-years, respectively). A non-significant trend for increased risk among BP users was noted with 5 years or more of utilization, with only 8 relevant fractures in total in this subgroup (BP, 6, raloxifene/calcitonin, 2). Although the study design does not allow the authors to specifically evaluate atypical fractures, it minimizes confounding by indication, which is progress with regard to previous observational studies. —SF


This meta-analysis of 13 RCTs of calcitonin effects on pain reduction in osteoporotic vertebral fractures finds a beneficial effect on acute (1-4 weeks), but not chronic (6 months) pain. However, it is striking to see upon how few observations (n=589) such a common practice relies. —SF


In the context of the growing controversy about calcium intake/supplements benefit (fracture reduction) vs. risk (cardiovascular), this extremely large epidemiological survey brings new, provocative data with regard to the optimal dose of calcium intake. Based on a 19-year prospective survey of the Swedish Mammography Cohort, comprising close to 1 million person-years at risk, the incidence of a first fracture (14,738 women in total) increased by up to 18%, and 29% for first hip fracture in the lowest calcium intake quintile (<750 mg/d) compared to the third quintile (882-996 mg/d), but did not further decrease – it actually increased again for hip fractures in the highest quintiles, i.e., there was a non-linear relationship. Moreover, fracture risks were highest in the low calcium intake-low vitamin D group. These results would argue for a

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benefit of moderate calcium intake with vitamin D, but against calcium intakes higher than 1200 mg/d, in agreement with the recent report from the Institute of Medicine in the U.S. (also read the editorial by P. Burckhardt in Osteoporosis Int. 2011 Jun;22(6):1645-7). —SF

Cancer and Bone


The bisphosphonate zoledronic acid (ZOL) blocks the mevalonate pathway, leading to intracellular accumulation of mevalonate metabolites (IPP/Apppl). IPP/Apppl accumulation in ZOL-treated cancer cells may be recognized by Vγ9Vδ2 T cells as tumor phosphoantigens in vitro. However, the significance of these findings in vivo remains largely unknown. In this study, marked differences were found in IPP/Apppl production among different human breast cancer cell lines post-ZOL treatment. In ZOL-treated mice bearing subcutaneous breast cancer xenografts, human Vγ9Vδ2 T cells infiltrated and inhibited growth of tumors that produced high IPP/Apppl levels, but not those expressing low IPP/Apppl levels. Moreover, IPP/Apppl not only accumulated in cancer cells but it was also secreted, promoting Vγ9Vδ2 T-cell chemotaxis to the tumor. Without Vγ9Vδ2 T-cell expansion, ZOL did not inhibit tumor growth. Thus, cancers producing high IPP/Apppl levels after ZOL treatment are most likely to benefit from immunotherapy. —SF


Analysis of the Austrian Breast and Colorectal Cancer Study Group trial-12 (ABCSG-12) at 48 months’ follow-up showed that the addition of ZOL to adjuvant endocrine therapy in premenopausal women with early breast cancer reduced by 36% the risk of disease progression (HR = 0.64; 95% CI, 0.46-0.91; P = 0.01; Gnant et al., NEJM, 2009). The long-term clinical efficacy of addition of ZOL to endocrine therapy has now been evaluated at a median follow-up of 62 months (i.e., more than 2 years after treatment completion). The effects of ZOL persisted at 62 months with a 32% reduction in the risk of disease progression (HR = 0.68; 95% CI, 0.51-0.91; P = 0.009), indicating a clinically meaningful effect. —PC


Induction of new bone formation is frequently seen in the bone lesions from prostate cancer. MDA-PCa-118b xenografts generated from prostate cancer bone metastases were found to induce strong ectopic bone formation in SCID mice and to express high levels of BMP-4. Additionally, treatment of SCID mice bearing MDA-PCa-118b tumors with LDN-193189, a small molecule BMP receptor 1 inhibitor, significantly reduced tumor growth. Thus, this study supports a role for BMP4-mediated osteogenesis in the progression of prostate cancer in bone. —PC

In this study, integrin-β7 expression in multiple myeloma was correlated with poor survival outcomes. Functionally, integrin-β7 silencing altered the homing of myeloma cells into the bone marrow in vivo, and reduced myeloma cell adhesion and migration in vitro. These findings pave the way for a novel therapeutic approach targeting this integrin. —PC


Rankl blockade by soluble receptors is an effective strategy to prevent osteolytic lesions in animal models of osteosarcoma. Here, intra-tumor injections of Rankl-directed siRNAs protected animals bearing osteosarcomas from tumor-associated osteolysis, but had no effect on tumor growth. In contrast, Rankl inhibition mediated by RNA interference substantially improved the therapeutic response of primary osteosarcoma to ifosfamide. —PC


Blockade of osteoclast activity efficiently decreases tumor burden and bone destruction in immune-compromised animals bearing human osteolytic cancers. In this study, using genetic and pharmacologic models, T cell activation diminished skeletal metastasis while T cell depletion enhanced it, even in the presence of the antiresorptive bisphosphonate ZOL. Thus, these data suggest that T cells are critical regulators of tumor growth in bone. —PC

Genetics


This is a case report on a young patient with a Complex Chromosomal Rearrangement (CCR) at chromosome 20; the diagnosis was cross-confirmed by array Comparative Genomic Hybridization (array CGH) and Fluorescent In Situ Hybridization (FISH) techniques. The rearrangement showed a very unusual architecture, with segments deleted, duplicated, inverted, and shifted. Clinically, DXA BMD was extremely reduced (aBMD z-score -3.7; vBMD z-score -5.3); proximal hand phalanges QUS was severely reduced; and deformation of lumbar vertebral bodies with severe compression fracture was also evident. The affected region – 20p12 – contains few genes, one of which is BMP2, which might be responsible for bone symptoms. —DK

Acrodysostosis (OMIM 101800) is a rare form of skeletal dysplasia with severe brachydactyly, facial dysostosis, nasal hypoplasia, short stature, and multiple hormone resistance that resembles Albright's hereditary osteodystrophy (pseudohypoparathyroidism Type Ia), but without GNAS mutations. Studying 3 patients with this condition, the authors identified a nonsense mutation in the gene coding for cyclic AMP (cAMP)-dependent regulatory subunit of protein kinase A. —SF


Phenotypic correlations between traits, such as different bone measures, are rarely explored. With a sophisticated statistical approach, this group evaluated the power of bivariate analysis via simulation results. To further demonstrate the efficacy of their bivariate test, the authors applied the method to GWAS of lumbar spine and femoral neck BMD in a sample of men with extreme truncate design. Unrelated men from Network in Europe on Male Osteoporosis (NEMO) had either low (LS Z-scores ≤ -2) or high (LS and FN Z-scores > 0.5) BMD. Their bivariate GWAS found suggestive SNPs in 3 genomic regions (6q22.1, 15q14 and 22q11); these SNPs have not been reported by previous GWAS of BMD. Interestingly, two of them, SLC2A11 on 22q11 and RYR3 on 15q14-15, are located in genes expressed in skeletal muscle. —DK

**Bone Modeling, Remodeling, and Repair**


This randomised study of a single dose of zoledronic acid showed no acceleration of osteotomy healing by blinded external fixator removal date or DXA. There were no adverse effects on healing. —DGL


Strontium ranelate has been shown to reduce osteoprotegerin (OPG) expression by osteoblasts in vitro. In this study, 8 weeks of strontium administration to growing OPG-deficient mice did not reduce osteoclast number in vivo nor in primary bone marrow cell cultures, contrary to WT mice. Both control and strontium-treated animals also received calcium supplements. Intriguingly, bone-forming indices were also not increased in OPG KO mice compared to WT. In turn, trabecular number and BV/TV were significantly increased in WT, but not in OPG KO mice. —SF


Osteoporotic rats had a diminished angiogenic response and lower blood perfusion than shams. —DGL

Human embryonic stem cells differentiated before implantation were less effective than bone marrow-derived adult stem cells in these experiments. Given the disadvantages of using embryonic stem cells, they do not seem attractive candidates at this point for bone repair. —DGL

Molecular and Cell Biology


cAMP signaling through the PTH/PTHrP receptor induces expression of anti-apoptotic genes and osteoblast survival, but the downstream (intracellular) regulatory mechanisms remain unknown. This study demonstrates that β-arrestin, an intracellular signal regulatory protein that downregulates cAMP signaling, inhibits PTH-induced osteoblast survival, whereas connexin 43, by binding to β-arrestin, facilitates cAMP signaling and promotes survival. —SF


The authors demonstrate that pannexin 3 (Panx3) is induced during osteoblast differentiation, and promotes osteoblast differentiation. Panx3 forms hemichannels that allow release of ATP into the extracellular space. The released ATP binds to purinergic receptors and activates PI3K-Akt signaling. Akt then activates the unique Panx3 ER Ca\textsuperscript{2+} channel and causes an increase in intracellular Ca\textsuperscript{2+}. Calmodulin (CaM) is then activated by Ca\textsuperscript{2+}, leading to the activation of CaMKII and calcineurin to enhance osteoblast differentiation. Panx3 also forms gap junctions and propagates Ca\textsuperscript{2+} waves between adjacent cells for differentiation. Thus, this study clarifies the functions of Panx3 and its important role in the regulation of osteoblast differentiation. —TM


Type XII collagen is expressed by osteoblasts and localizes to areas of bone formation. A transgenic mouse null for type XII collagen exhibits shorter, more slender long bones with decreased mechanical strength and altered vertebrae structure compared to WT mice. Col12a(-/-) osteoblasts have decreased bone matrix deposition with delayed terminal differentiation. Col12a(-/-) osteoblasts are disorganized and less polarized with disrupted cell-cell interactions, decreased connexin43 expression, and impaired gap junction function. These data demonstrate that extracellular type XII collagen plays important regulatory roles in determining osteoblast shape, organization and communication, and that these events are crucial for osteoblast differentiation and bone matrix formation. —TM

Macica C, Liang G, Nasiri A, Broadus AE. Genetic evidence that parathyroid hormone-related

*PTHrP* is a well-known regulator of chondrocyte differentiation at the growth plate. What this study demonstrates is that male mice with targeted deletion of *PTHrP* in articular chondrocytes develop degenerative features after destabilization of the medial meniscus. These observations provide novel insights into a possibly unique mechanism of osteoarthritis. —SF


It should not be forgotten that besides its major inhibitory effects on osteoclastogenesis, estrogen exerts important stimulatory effects on osteoblastic functions, that is, not only anti-apoptotic effects. This study shows that estradiol directly interacts with the Wnt pathway downstream of β-catenin to co-regulate gene expression and osteoblastic functions. —SF


This study demonstrates that hormone-sensitive lipase (HSL)-deficient mice are resistant to age-related bone loss. In the bone marrow of aged HSL(-/-) mice, the number and size of adipocytes are small. Runx2 and osteocalcin expression are higher in osteoblasts from HSL(-/-) mice with increased growth rates and higher osteogenic potential. Although the precise mechanism whereby HSL(-/-) enhances osteoblast differentiation and counteracts age-related bone loss remains unclear, this study provides interesting information about the fat-bone axis. —TM


Bone aging is a recognized model of an organism’s general aging. A naturally occurring flavonoid, resveratrol, a phytoestrogen and agonist for the longevity gene SIRT1, is found to promote spontaneous osteogenesis and prevent adipogenesis in human embryonic mesenchymal stem cell progenitors. In particular, resveratrol up-regulated the expression of the osteo-lineage genes RUNX2 and osteocalcin (OC), while suppressing the adipo-lineage genes PPARγ2 and leptin. The study found that a resveratrol-induced SIRT1/FOXO3A complex binds to a FOXO response element of the RUNX2 promoter in vivo. Taken together, these results again support a role of red wine components for bone rather than fat tissue benefit. —DK

**Muscle and Bone**


*These two studies investigated bones affected by dystrophin deficiency, which is a...*
basis for Duchenne Muscular Dystrophy (DMD). Both used mdx mice, which are a model of human DMD. Thus, the study by Novotny et al. showed that tibiae of mdx mice had up to 50% lower strength and stiffness compared to wild-type mice; they had reductions of 6-57% in cortical cross-sectional moment of inertia and cross-sectional area; and up to a 78% reduction in trabecular bone. Importantly, this compromised bone strength was more obvious in 7-week-old than in 24-month-old mice.

The second group investigated the changes that occur in the femur of mdx mice at 21 days of age. They also demonstrated lower strength, stiffness and energy absorption capacity in mdx femora. Mdx femora were shorter, had a smaller cortical area and thickness, as well as manifested changes in collagen organization in the extracellular matrix. Interestingly, at 3 weeks of age the muscle damage in mice is still not significant; thus the bone is affected even in the absence of significant muscle fiber degeneration. —DK

Epidemiology


Older patients, men, and those with heart failure or liver disease have an increased risk of death shortly after hip fracture. A nomogram was developed to evaluate the individual risk of death upon admission in hip fracture patients. —SF

Reviews, Perspectives and Editorials


Other Studies of Potential Interest


Garcia LA, King KK, Ferrini MG, Norris KC, Artaza JN. 1,25(OH)2Vitamin D3 stimulates myogenic differentiation by inhibiting cell proliferation and modulating the expression of promyogenic growth factors and myostatin in C2C12 skeletal muscle cells. *Endocrinology*. 2011 Jun 14. [Epub ahead of print] [Abstract]


**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 Daichi-Sankyo. Dr. Karasik reports no conflicts of interest.