

NOT TO BE MISSED

Clinical and Basic Research Papers – January 2008 Selections

Serge Ferrari, Editor-in-Chief
David G. Little, Associate Editor
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Bone Modeling, Remodeling and Repair

◆Hsiao EC, Boudignon BM, Chang WC, Bencsik M, Peng J, Nguyen TD, Manalac C, Halloran BP, Conklin BR, Nissenson RA. Osteoblast expression of an engineered Gs-coupled receptor dramatically increases bone mass. *Proc Natl Acad Sci U S A*. 2008 Jan 29;105(4):1209-14. [\[Abstract\]](#) [\[Full Text\]](#)

Osteoblast-selective expression of a mutated serotonin receptor with constitutive cAMP production led to an extremely high bone mass phenotype (osteosclerosis) in young transgenic mice. In contrast, transgene activation (by the Tet-O system) past 6 weeks of age did not result in similar effects, indicating that G_sα signaling in osteoblasts is most crucial for anabolism during early growth (bone modeling). —SF

◆Krum SA, Miranda-Carboni GA, Hauschka PV, Carroll JS, Lane TF, Freedman LP, Brown M. Estrogen protects bone by inducing Fas ligand in osteoblasts to regulate osteoclast survival. *EMBO J*. 2008 Feb 6;27(3):535-45. [\[Abstract\]](#)

*Recent data have shown that estrogen prevents bone loss by inducing (pre-)osteoclast apoptosis via a FasL pathway in these cells (see [Commentary](#) by Sundeep Khosla, *IBMS BoneKEy*. 2007 October;4(10):267-272). This new study restores the role of the osteoblast in this process by showing that estrogen, and SERMS alike, stimulate FasL in osteoblasts in vitro and on endosteal surfaces in vivo. Co-culture experiments further demonstrated that osteoclast apoptosis by FasL is not cell autonomous but is mediated by osteoblasts. —SF*

◆Onishi E, Fujibayashi S, Takemoto M, Neo M, Maruyama T, Kokubo T, Nakamura T. Enhancement of bone-bonding ability of bioactive titanium by prostaglandin E2 receptor selective agonist. *Biomaterials*. 2008 Mar;29(7):877-83. [\[Abstract\]](#)

Previous work has shown that an EP4 agonist increased strength in distraction osteogenesis. This study looked at the use of systemic EP4 agonist on the bonding strength of a titanium plate in rabbit tibiae. Either 10 or 100 µg/kg body weight ONO-AE2-724 or saline was given post-operatively and specimens harvested at 4, 8 and 16 weeks. Increased contact area between the plate and the bone was noted in the EP4 agonist groups. Mechanical detaching tests showed significantly more rapid gain of strength at 4 weeks and better maintenance of this improvement at 16 weeks in the high dose group. As this anabolic increases stromal cell differentiation as well as bone production, it may be useful in orthopedic medicine. —DGL

◆Robling AG, Niziolek PJ, Baldrige LA, Condon KW, Allen MJ, Alam I, Mantila SM, Gluhak-Heinrich J, Bellido TM, Harris SE, Turner CH. Mechanical stimulation of bone in vivo reduces osteocyte expression of Sost/sclerostin. *J Biol Chem*. 2007 Dec 17; [E-pub ahead of print]

Sost transcripts and sclerostin protein are dramatically reduced by mechanical ulnar loading, and a greater strain stimulus is associated with a greater reduction in *Sost* staining and sclerostin-positive osteocytes. In contrast, hindlimb unloading increases *Sost* expression in unloaded tibiae. These results demonstrate that regulation of sclerostin levels serves as a fine-tuning mechanism for osteocytes, via negatively controlling *Wnt/Lrp5* signaling for the regulation of bone formation in response to mechanical stimulation. These data provide the first evidence for the role of an osteocyte-derived regulator of *Wnt* signaling on bone formation in response to mechanical stress, and suggest sclerostin as an attractive therapeutic target for improving bone mass. —TM

- ◆ Shiraki M, Kuroda T, Tanaka S, Saito M, Fukunaga M, Nakamura T. Nonenzymatic collagen cross-links induced by glycoxidation (pentosidine) predicts vertebral fractures. *J Bone Miner Metab.* 2008;26(1):93-100. [\[Abstract\]](#)

Advanced glycation end products (AGEs) crosslink collagen, making it like sugar candy and reducing the ability of bone to absorb energy by deforming; the material becomes too stiff. In 432 Japanese elderly women followed for 5.2 years, 97 incident vertebral fractures occurred in 72 subjects. Urinary pentosidine was a predictor of vertebral fracture (hazard ratio, 1.33; 95% CI, 1.01-1.76, P = 0.04). —ES

- ◆ Yamakawa K, Kamekura S, Kawamura N, Saegusa M, Kamei D, Murakami M, Kudo I, Uematsu S, Akira S, Chung UI, Nakamura K, Kawaguchi H. Association of microsomal prostaglandin E synthase 1 deficiency with impaired fracture healing, but not with bone loss or osteoarthritis, in mouse models of skeletal disorders. *Arthritis Rheum.* 2008 Jan;58(1):172-83. [\[Abstract\]](#)

Microsomal prostaglandin E synthase (PGES)-deficient mice were created and examined after ovariectomy; bone loss induced by hind limb unloading; osteoarthritis (OA) induced by instability in the knee joint; and bone fracture by osteotomy at the tibial midshaft. PGES-1(-/-) mice had normal skeletal phenotypes under normal physiologic conditions. Unloading and arthritis models showed no differences from wild type. However, in the fracture model, healing was impaired by mPGES-1 deficiency. Decreases in callus area and BMC accrual were found in the PGES-1(-/-) mice, and 50% of the mice failed to unite after 21 days. Normal fracture healing was restored by adenoviral reintroduction of mPGES-1. This paper is relevant as EP4 agonists enhance bone formation. Further, mPGES-1 inhibitors have recently been developed as possible replacements for NSAIDs and COX-2 inhibitors. Fracture healing issues will be important to address for this treatment approach. —DGL

- ◆ Yerramshetty JS, Akkus O. The associations between mineral crystallinity and the mechanical properties of human cortical bone. *Bone.* 2007 Dec 14; [Epub ahead of print] [\[Abstract\]](#)

The size and shape of carbonated apatite crystals affect the mechanical properties of bone. In 16 human cadaveric femurs, crystallinity explained 6.7% to 48.3% of the variation in monotonic mechanical properties. Tissue-level strength and stiffness increased with increasing crystallinity; ductility decreased. Crystallinity explained 11.3% to 63.5% of the variation in fatigue properties. —ES

Genetics

- ◆ Lisse TS, Thiele F, Fuchs H, Hans W, Przemecck GK, Abe K, Rathkolb B, Quintanilla-Martinez L, Hoelzlwimmer G, Helfrich M, Wolf E, Ralston SH, de Angelis MH. ER stress-mediated apoptosis in a new mouse model of osteogenesis imperfecta. *PLoS Genet.* 2008 Feb 1;4(2):e7; [Epub ahead of print] [\[Abstract\]](#)

A mutant mouse generated from an ENU (chemical) mutation program was characterized by skeletal features consistent with osteogenesis imperfecta. The causal mutation mapped to Col1A1 induced intracellular accumulation of unfolded procollagen chains, which in turn was responsible for a new mechanism of disease, namely the induction of an endoplasmic reticulum stress-specific response, ultimately leading to osteoblast apoptosis. —SF

- ◆Ridker PM, Chasman DI, Zee RY, Parker A, Rose L, Cook NR, Buring JE; for the Women's Genome Health Study Working Group. Rationale, design, and methodology of the Women's Genome Health Study: a genome-wide association study of more than 25 000 initially healthy American women. *Clin Chem*. 2008 Feb;54(2):249-55. [\[Abstract\]](#)

No results yet, but the design of this multi-million dollar study is worth mentioning because it exemplifies the future of genome-wide association (GWA) studies to investigate genetic susceptibility to common diseases, including osteoporosis, and potentially to identify common (pleiotropic) genetic risk factors. —SF

Treatment and Drug Effects

- ◆Allen MR, Gineyts E, Leeming DJ, Burr DB, Delmas PD. Bisphosphonates alter trabecular bone collagen cross-linking and isomerization in beagle dog vertebra. *Osteoporos Int*. 2008 Mar;19(3):329-37. [\[Abstract\]](#)

Bisphosphonates alter properties of bone collagen. RIS and ALN, but not raloxifene, increased PEN (+34-58%) and the ratio of PYD/DPD (+14-26%), and decreased the ratio of α/β CTX (-29-56%). Bone turnover rate correlated to PEN ($R = -0.664$), α/β CTX ($R = 0.586$), and PYD/DPD ($R = -0.470$). —ES

- ◆Bolland MJ, Barber PA, Doughty RN, Mason B, Horne A, Ames R, Gamble GD, Grey A, Reid IR. Vascular events in healthy older women receiving calcium supplementation: randomised controlled trial. *BMJ*. 2008 Feb 2;336(7638):262-6. [\[Abstract\]](#) [\[Full Text\]](#)

This is a secondary analysis of an originally 5 year prospective, calcium citrate vs. PBO fracture trial in postmenopausal women (mean age 74), in which CV events were recorded as adverse events. A significantly higher number of myocardial infarctions and composite endpoint (MI, stroke or sudden death) events were observed among calcium users, despite a compliance rate of less than 60%. Actually, compliance was identified as an independent risk factor for CV events. These prospective data oppose previous results from observational studies and are reminiscent of the HRT saga. They suggest that the (small) benefits of calcium supplements in preventing fragility fractures should be weighed against the associated increase in CV risk. —SF

- ◆Ceibesoy O, Tutar E, Kose KC, Baltaci Y, Bagci C. Effect of strontium ranelate on fracture healing in rat tibia. *Joint Bone Spine*. 2007 Dec;74(6):590-3. [\[Abstract\]](#)

This is the first publication to look at strontium ranelate (SR) in fracture healing. Rats underwent closed tibial fractures externally held with a cast and received 450 mg/kg SR in their food. The tibiae were harvested and examined radiographically and by histology. The scoring system used produced almost identical results with all fractures maturing over time. More complete studies with controlled fixed fractures, and quantitative measures such as CT and mechanical testing, are required to further evaluate SR in fracture healing, but no major negative effects were seen in this model. —DGL

- ◆Kanis JA, Adams J, Borgström F, Cooper C, Jönsson B, Preedy D, Selby P, Compston J. The cost-effectiveness of alendronate in the management of osteoporosis. *Bone*. 2008 Jan;42(1):4-15. [[Abstract](#)]

The authors report that alendronate 70 mg weekly administered for 5 years was cost-effective for primary fracture prevention in women with osteoporosis irrespective of age, as was treatment of women with a prior fracture irrespective of BMD. NICE guidelines are misguided. —ES

- ◆Murkherjee S, Raje N, Schoonmaker JA, Liu JC, Hideshima T, Wein MN, Jones DC, Vallet S, Bouxsein ML, Pozzi S, Chhetri S, Seo YD, Aronson JP, Patel C, Fulcinti M, Purton LE, Glimcher LH, Lian JB, Stein G, Anderson KC, Scadden DT. Pharmacologic targeting of a stem/progenitor population in vivo is associated with enhanced bone regeneration in mice. *J Clin Invest*. 2008 Feb 1;118(2):491-504. [[Abstract](#)]

Bortezomib (Bzb), a proteasome inhibitor, has been shown to increase serum bone-specific ALP when used for the treatment of multiple myeloma. This paper demonstrates that Bzb induces mesenchymal stem cells (MSCs) to preferentially undergo osteoblastic differentiation. Bzb does not enhance the differentiation of osterix-positive osteoprogenitor cells to mature osteoblasts. The effect of Bzb was dependent on the presence of Runx2, and Bzb inhibited degradation of Runx2 and enhanced its transcriptional activity. Furthermore, Bzb increased bone formation and rescued bone loss in ovariectomized mice. The doses needed to cause these effects were one-fifth to one-third of those used for the treatment of multiple myeloma. These observations may lead to a new therapeutic approach for the treatment of disorders with deficient osteoblast function, including involuntal osteoporosis, by pharmacologically targeting MSCs toward the osteoblastic lineage. —TM

Other Studies of Potential Interest

- ◆Bai S, Kopan R, Zou W, Hilton MJ, Ong CT, Long F, Ross FP, Teitelbaum SL. Notch1 regulates osteoclastogenesis directly in osteoclast precursors and indirectly via osteoblast lineage cells. *J Biol Chem*. 2007 Dec 22; [Epub ahead of print]
- ◆Bradley EW, Ruan MM, Oursler MJ. Novel pro-survival functions of the kruppel-like transcription factor EGR2 in promotion of M-CSF-mediated osteoclast survival downstream of the MEK/ERK pathway. *J Biol Chem*. 2008 Jan 15; [Epub ahead of print]
- ◆Hadfield KD, Farrington Rock C, Inkson CA, Dallas SL, Sudre L, Wallis GA, Boot-Handford RP, Canfield AE. HTRA1 inhibits mineral deposition by osteoblasts: Requirement for the protease and PDZ domains. *J Biol Chem*. 2007 Dec 22; [Epub ahead of print]
- ◆Ichikawa-Shindo Y, Sakurai T, Kamiyoshi A, Kawate H, Iinuma N, Yoshizawa T, Koyama T, Fukuchi J, Iimuro S, Moriyama N, Kawakami H, Murata T, Kangawa K, Nagai R, Shindo T. The GPCR modulator protein RAMP2 is essential for angiogenesis and vascular integrity. *J Clin Invest*. 2008 Jan;118(1):29-39. [[Abstract](#)]
- ◆Kirkbride KC, Townsend TA, Bruinsma MW, Barnett JV, Blobe GC. Bone morphogenetic proteins signal through the transforming growth factor-beta type III receptor. *J Biol Chem*. 2008 Jan 9; [Epub ahead of print]
- ◆Mansergh FC, Wells T, Elford C, Evans SL, Perry MJ, Evans MJ, Evans BA. Osteopenia in Sparc (osteonectin)-deficient mice: characterization of phenotypic determinants of femoral

strength and changes in gene expression. *Physiol Genomics*. 2007 Dec 19;32(1):64-73. [\[Abstract\]](#)

◆ Moan J, Porojnicu AC, Dahlback A, Setlow RB. Addressing the health benefits and risks, involving vitamin D or skin cancer, of increased sun exposure. *Proc Natl Acad Sci U S A*. 2008 Jan 15;105(2):668-73. [\[Abstract\]](#) [\[Full Text\]](#)

◆ Onodera S, Oshima S, Nishihira J, Yasuda K, Tohyama H, Irie K, Koyama Y. Active immunization against macrophage migration inhibitory factor using a novel DNA vaccine prevents ovariectomy-induced bone loss in mice. *Vaccine*. 2008 Feb 6;26(6):829-36. [\[Abstract\]](#)

◆ Palmer SC, McGregor DO, Macaskill P, Craig JC, Elder GJ, Strippoli GF. Meta-analysis: vitamin D compounds in chronic kidney disease. *Ann Intern Med*. 2007 Dec 18;147(12):840-53. [\[Abstract\]](#)

◆ Stolk L, van Meurs JB, Arp PP, Hofman A, Pols HA, Uitterlinden AG. The RIZ Pro704 insertion-deletion polymorphism, bone mineral density and fracture risk: The Rotterdam study. *Bone*. 2008 Feb;42(2):286-93. [\[Abstract\]](#)

◆ Surmann-Schmitt C, Dietz U, Kireva T, Adam N, Park J, Tagariello A, Onnerfjord P, Heinegård D, Schlötzer-Schrehardt U, Deutzmann R, von der Mark K, Stock M. UCMA, a novel secreted cartilage-specific protein with implications in osteogenesis. *J Biol Chem*. 2007 Dec 21; [Epub ahead of print]

◆ Wan C, Gilbert SR, Wang Y, Cao X, Shen X, Ramaswamy G, Jacobsen KA, Alaql ZS, Eberhardt AW, Gerstenfeld LC, Einhorn TA, Deng L, Clemens TL. Activation of the hypoxia-inducible factor-1alpha pathway accelerates bone regeneration. *Proc Natl Acad Sci U S A*. 2008 Jan 15;105(2):686-91. [\[Abstract\]](#) [\[Full Text\]](#)

◆ Zhu ED, Demay MB, Gori F. Wdr5 is essential for osteoblast differentiation. *J Biol Chem*. 2008 Jan 16; [Epub ahead of print]

◆ Zirngibl RA, Chan JS, Aubin JE. Estrogen receptor-related receptor alpha (ERRalpha) regulates osteopontin expression through a non-canonical ERRalpha response element in a cell context-dependent manner. *J Mol Endocrinol*. 2008 Feb;40(2):61-73. [\[Abstract\]](#)

Conflict of Interest: Dr. Ferrari reports that he receives research support from Amgen and consultancy/speaker's fees from Merck Sharp & Dohme, Eli Lilly, and Amgen. 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 no conflict of interest.