

# Invited Speaker Abstracts

## Keynote Lecture

### KL01

#### Mature Cells can be Reprogrammed to become Pluripotent

Shinya Yamanaka

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Professor Gurdon has received recognition for his landmark achievement in 1962, providing the first experimental evidence of reprogramming by the transplantation of amphibian somatic cell nuclei into enucleated oocytes. This breakthrough in technology introduced a new paradigm that nuclei of differentiated cells retain complete blueprint (information of whole body) and oocytes possess certain potential of reprogramming. Inspired by this paradigm shift and related research achievements, we identified four transcription factors that could induce pluripotency in somatic cells by their enforced expression, and successfully consolidated effective reprogramming methods in mouse (2006) and human (2007) cells. The established reprogrammed cells were named 'induced pluripotent stem (iPS) cells'.

These sequential studies lead to the discovery that mature cells can be reprogrammed to become pluripotent.

## Symposium 1- Osteoporosis: Anabolic

### IS01

#### Targeting Sclerostin in the Development of Therapeutics for Bone Disorders

Hua Zhu (David) Ke

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Wnt/ $\beta$ -catenin signaling plays a key role in bone formation. Sclerostin inhibits wnt/ $\beta$ -catenin signaling, leading to decreased bone formation. Accordingly, sclerostin antibody (Scl-Ab) increased bone formation, decreased bone resorption, and increased BMD and bone strength in animal models of osteoporosis and fracture healing. Increased bone formation resulted from activating bone formation on quiescent surfaces (modeling-based) and prolonging the formation of new bone on existing remodeling surfaces. Scl-Ab decreased the osteoclastic potential of bone marrow collected from treated rats, and decreased osteoclast number and surface *in vivo* in OVX rats. Co-treatment with an antiresorptive did not block the anabolic effects of Scl-Ab, indicating that these anabolic actions are independent of resorption. In a 26-week study in OVX rats, BMD and bone strength continuously increased and endocortical bone formation remained significantly elevated while trabecular and periosteal bone formation temporally increased

and returned to control levels. In postmenopausal women, a Scl-Ab (romosozumab) concurrently increased bone formation markers and decreased bone resorption markers, maximizing BMD increases. The increase in bone formation markers was temporal, returning to baseline within 12 months while bone resorption markers remained decreased. These data support the continued study of Scl-Ab as a bone forming agent.

### IS02

#### Eldecalcitol For Osteoporosis

Toshio Matsumoto

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In addition to the negative balance in bone remodeling, elderly osteoporotic patients suffer from a large negative balance in calcium metabolism by a decrease in the intestinal calcium absorption. Thus, the anti-fracture effect of almost all the anti-resorptive and the anabolic agents has been demonstrated under the supplementation with native vitamin D and calcium. However, the effect of native vitamin D on fracture prevention in osteoporotic patients is small, if any. Vitamin D is activated to 1,25(OH)<sub>2</sub>D to exert its actions, and 1,25(OH)<sub>2</sub>D<sub>3</sub> and its prodrug, 1 $\alpha$ OHD<sub>3</sub>, has been widely prescribed in Japan for the treatment of osteoporosis in combination with anti-resorptive agents. Because ligands for nuclear receptors can have tissue-specific effects like SERMs, there is a possibility that there may be ligands for vitamin D receptor that have stronger effects in bone. Eldecalcitol was developed under such concept, although the mechanism of its action remains largely unknown. Eldecalcitol shows potent effects in increasing BMD and preventing vertebral fractures in osteoporotic patients in comparison with alfacalcidol. The effect of eldecalcitol on calcium metabolism, bone turnover, bone geometry and fracture prevention in patients with osteoporosis will be overviewed and its mechanism of action will be discussed.

### IS03

#### Potential Therapeutic Targets for Age-related Bone Loss

Sundeep Khosla

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Increasing understanding of age-related bone loss is leading to potential therapeutic targets for reversing this bone loss. Given the critical role of estrogen in regulating bone metabolism, modulating estrogen action on bone remains a viable option. Mouse and human studies have shown that estrogen

increases osteoclast apoptosis and reduces osteoclast differentiation. Estrogen also regulates the apoptosis of osteocytes and osteoblasts, as well as modulating levels of oxidative stress and NF- $\kappa$ B activity in osteoblasts, thereby maintaining bone formation.

Additional anabolic approaches for age-related bone loss include teriparatide and inhibition of sclerostin activity. Animal and human studies have demonstrated that teriparatide suppresses sclerostin production. Interestingly, studies in humans have shown that teriparatide inhibits BMP action in mesenchymal cells, which may explain the waning of the anabolic effects of PTH with prolonged therapy. Serum sclerostin levels also increase significantly with age, providing a further rationale for inhibiting sclerostin action in aging.

Recent human studies have also demonstrated that sympathetic outflow, as measured directly, increases with age and, in postmenopausal women, correlates negatively with bone microstructure and serum bone formation markers. Thus, appropriate modulation of beta-adrenergic action on bone remains a viable approach for reversing age-related bone loss.

## Symposium 2- Osteoporosis: anti-resorptives

### IS04

#### Japanese Experiences in the use of Bisphosphonates

*Hiroshi Hagino*

Tottori University, Yonago, Japan

Since bisphosphonates (BP) can reduce the incidence of fragility fractures including hip fractures in patients with osteoporosis, they have become the first-line drugs for the treatment of osteoporosis. Numerous clinical studies have shown that most oral BPs that were originally developed for once daily administration demonstrate equivalent, or non-inferior efficacy and tolerability with weekly and/or monthly dosing regimens. Minodronate, a third-generation BP with an imidazopyridine ring side chain, is currently marketed in Japan for the treatment of osteoporosis. Preclinical studies have shown that minodronate is at least 10 times more potent than alendronate in inhibiting bone resorption, and possesses intermediate mineral-binding affinity. Monthly administration of minodronate is now available at 50 mg, 50 times the daily dosage. In a recent study risedronate taken once a month showed non-inferior efficacy with established dosage regimens in the increase of bone mineral density in Japanese patients with osteoporosis. Monthly BP offers patients with osteoporosis a new dosage option that may improve convenience as well as treatment adherence in those who are having difficulty complying with the currently approved daily and weekly dosage regimens. Monthly administration of alendronate 900  $\mu$ g intravenously is also available in Japan; in a head-to-head double blind clinical trial it proved a non-inferior efficacy with weekly oral alendronate administration.

### IS05

#### Denosumab for Osteoporosis

*Richard Eastell*

Department of Human Metabolism, University of Sheffield, Sheffield, United Kingdom

Denosumab is a fully humanised monoclonal antibody that is licenced for use in postmenopausal osteoporosis. The antibody is directed against the receptor activator of nuclear factor-kappaB ligand (RANKL), a key regulator of bone resorption. The administration of denosumab prevents RANKL binding to its receptor, RANK and this results in decreased bone resorption. The FREEDOM Trial included 7868 women between the ages of 60 and 90 years who had a bone mineral density T score of less than  $-2.5$  but not less than  $-4.0$  at the lumbar spine or total hip. Subjects were randomly assigned to receive either 60 mg of denosumab or placebo subcutaneously every 6 months for 36 months. As compared with placebo, denosumab reduced the risk of new radiographic vertebral fracture by 68%, hip fracture by 40% and nonvertebral fracture by 20%. In active comparator studies, denosumab had greater effect on bone turnover (decrease) and bone mineral density (increase) than the bisphosphonate alendronate. The FREEDOM study reported an excess of eczema and skin infections. It has been extended to 10 years. Denosumab differs from bisphosphonates in that it is not stored in bone and so once the treatment is stopped then there is a rebound in bone turnover and accelerated bone loss. Denosumab has been found to be effective in male osteoporosis including men taking androgen deprivation therapy for prostate cancer and for women taking aromatase inhibitors for breast cancer.

### IS06

#### Odanacatib Treatment for Osteoporosis

*Henry Bone<sup>1,2</sup>*

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Negative bone remodeling balance can result in osteoporosis. Strategies to increase bone mass depend on altering the balance to favor formation. Osteoclasts concentrate acid and cathepsin K under the ruffled border to dissolve the mineral phase and digest the matrix of bone. Products of bone resorption and other products of osteoclast activity ('clastokines') appear to be important in stimulating bone formation. Conventional antiresorptive medications for osteoporosis suppress osteoclast activity generally and secondarily decrease bone formation by osteoblasts. In principle, selective cathepsin K inhibition might reduce bone resorption with relative preservation of bone formation.

Odanacatib is a selective cathepsin K inhibitor with pharmacokinetic and pharmacodynamic properties that are suitable for once-weekly oral administration. Phase 2 clinical trial results support a dosage of 50 mg weekly. The study has been extended and 5-year data demonstrate significant decreases

in NTx and CTx, despite sTRAP5b levels that were not suppressed. Markers of bone formation decreased transiently, and then increased toward baseline levels. BMD increased progressively at the lumbar spine and femoral sites.

An international phase 3, placebo-controlled, fracture-endpoint trial was carried out, with more than 16000 participants. An interim analysis demonstrated robust anti-fracture efficacy. Accordingly, the core trial was completed and an extension is ongoing.

### **Symposium 3- Osteoporosis: pathophysiology and epidemiology**

#### **IS07**

#### **Advanced Glycation End-products (Ages) on the Risk of Fracture in Older Patients with Osteoporosis**

*Toshitaka Nakamura*

University of Occupational and Environmental Health, Japan, Kitakyushu, Japan

The prevalence of age-related diseases increases in patients with osteoporosis, such as hypertension, diabetes, chronic kidney disease and dementia. Recently, data are increasing on the effects of AGEs, whose formation is associated with the progress of these diseases and aging, on bone. AGEs are produced by non-enzymatic modification of amino-residues by glucose. AGEs, both of non-cross link (carboxyl methyllysine, etc) and cross-link (pentosidine (PEN), etc), are found in many tissues, blood and urine. AGEs are recognized by receptor for AGEs (RAGE) on different cells including bone cells. The AGE-RAGE interaction induces cytokines such as IL-6, reducing bone formation and increasing resorption. Accumulation of PEN cross-link in bone reduced the mechanical property in diabetic rats. Its bone content increased in the femoral neck of elderly patients with hip fracture. Serum PEN levels are associated with the presence of vertebral fracture in postmenopausal women with diabetes. Urinary PEN concentration was not an independent risk factor for fracture in healthy postmenopausal women, but it predicted the fracture risk and contributed the identification of subjects with a high risk of fracture in a hospital-based cohort. Increased accumulation of AGEs in common diseases and aging seems to be one of the causative factors of bone fragility in elderly patients with osteoporosis.

#### **IS08**

#### **Glucocorticoid-Induced Osteoporosis**

*Juliet Compston*

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The adverse effects of glucocorticoid excess on bone are well documented and result in significant morbidity and mortality. Rapid bone loss and increased fracture risk occur soon after the initiation of glucocorticoid therapy and are dose dependent. The increase in fracture risk is partly independent of bone mineral density, probably as a result of changes in bone material properties and increased risk of falling.

Early bone loss is associated with a transient increase in osteoclast number and activity, leading to an increased rate

of remodelling. Conversely, osteoblast number and activity are decreased, resulting in an uncoupling of resorption and formation at tissue level and hence further accelerating bone loss. In the later stages of glucocorticoid therapy, reduced osteoblast number and activity are the predominant changes. Increased production of RANKL plays an important role in the early increase in bone resorption, whilst reduced PPAR $\gamma$  and Wnt signalling have been implicated in the decrease in bone formation. Apoptosis of both osteoblasts and osteocytes is increased, whilst osteoclast apoptosis is reduced, at least in the early stages of therapy. Many conditions treated with glucocorticoids are associated with increased production of pro-inflammatory, pro-resorptive cytokines. Hypogonadism, increased renal and intestinal loss of calcium, vitamin D insufficiency and malnutrition may also play a role in bone loss.

#### **IS09**

#### **Pathogenesis and Treatment of Patients with Low BMD**

*Seiji Fukumoto*

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Patients with osteoporosis are basically diagnosed by low bone mineral density (BMD). However, BMD measured by dual-energy X-ray absorptiometry represents calcium content in bone. Therefore, patients with other diseases than osteoporosis can present with low BMD. Rickets and osteomalacia are diseases characterized by impaired bone mineralization and therefore low calcium content in bone. Of these, rickets is a disease in childhood before the closure of growth plates and osteomalacia develops in adults. It is theoretically necessary to exclude osteomalacia before the definite diagnosis of osteoporosis assessed by BMD. On the other hand, because the number of patients with osteoporosis is by far larger than that of osteomalacia, and also because there are no established diagnostic criteria for osteomalacia, it is likely that some patients with osteomalacia are actually diagnosed and treated as osteoporosis. While there are many causes of rickets and osteomalacia, chronic hypophosphatemia underlies most cases of rickets and osteomalacia. Vitamin D deficiency and a couple of diseases caused by excess activity of fibroblast growth factor 23 represent typical causes of osteomalacia. In this symposium, I would like to discuss about the pathogenesis, diagnosis and treatment of diseases with low BMD with special emphasis on osteomalacia.

### **Symposium 4- Cancer and Bone: basic, translational and clinical**

#### **IS10**

#### **Epithelial-Mesenchymal Transition of Breast Cancer in Bone**

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Epithelial-mesenchymal transition (EMT) is a process in which the epithelial cancer cells change their cell shape to mesenchymal

morphology with increased mobility, invasiveness and metastasis under the influence of their surrounding environments. Since breast cancer preferentially spreads to bone, we hypothesized bone environments induced EMT in breast cancer. The MCF-7 human breast cancer cells were inoculated in the mammary fat pad (MF) and tibiae (TI) and examined for the expression of the mesenchymal marker Snail and epithelial marker E-cadherin. Real-Time PCR showed increased Snail and decreased E-cadherin expression in MCF-7 tumors developed in TI compared with MF. Snail changed cell shape from epithelial to mesenchymal with abolished E-cadherin. TGF $\beta$ , which is abundantly stored in bone, markedly promoted EMT. The bone-modifying agent ZOL profoundly inhibited EMT in breast cancer in bone. ZOL also inhibited TGF $\beta$ -induced EMT by reversing cell shape and decreasing Snail and increasing E-cadherin in culture. Etidronate and risedronate failed to decrease Snail expression. ZOL decreased Snail expression via proteasomal degradation. ZOL inhibited metastases of breast cancer inoculated in bone to lung. In conclusion, our results suggest bone environments promote EMT in breast cancer. ZOL inhibits the EMT via stimulation of proteasomal degradation of Snail, leading to inhibition of secondary metastases from bone.

#### IS11

##### Constituents of the Hematopoietic Stem Cell Niche

Paul Frenette

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The migration of hematopoietic stem cells (HSC) and their progeny from and into the bone marrow (BM) is regulated by autonomic nerves from the sympathetic nervous system (SNS) that release norepinephrine acting on adrenoceptors expressed on the niche cells and endothelial cells, thereby controlling the expression stem cell retention factors and homing receptors. Enhanced by circadian oscillation of endothelial selectins and VCAM-1, homing to the BM occurs predominantly during the active phase (night in mice) whereas egress is enhanced during the resting phase (day time in mice). Self-renewing perivascular mesenchymal stem cells (MSC), targeted by the SNS, represent a candidate niche cell that can be prospectively identified by transgenic expression of GFP under the Nestin promoter. Our recent studies suggest that subsets of Nestin+ cells exert distinct functions. In addition, BM CD169+ macrophages promote in Nestin+ cells the expression of factors that retain HSC in bone marrow. Thus, the SNS (inhibitory) and CD169+ macrophages (stimulatory) have opposite effects on HSC retention in the niche. Further recent studies suggest that CD169+ macrophages form erythroblastic islands that support erythropoiesis under steady state after stress. Interestingly, macrophage depletion normalized the erythroid compartment in a JAK2V617F-driven murine model of polycythemia vera (PV), suggesting that erythropoiesis in PV, unexpectedly, remains under the control of macrophages.

#### IS12

##### Cancer and Bone: Basic, Translational and Clinical

Robert Coleman

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Bone metastases result from the interactions between cancer cells in the bone marrow microenvironment, haematopoietic stem cells and normal bone cells. Bone-targeted treatments may modify the course of the disease via inhibitory effects on this 'vicious cycle' of growth factor and cytokine signalling between tumour and bone cells within the bone marrow micro-environment. Improvements in both disease free (DFS) and overall survival in women with early breast cancer have been demonstrated with oral clodronate and in several large randomised adjuvant trials of zoledronic acid. The evidence for this is particularly strong in patients with low levels of reproductive hormones including premenopausal women receiving ovarian suppression therapy and those who have passed through menopause at the time of diagnosis. A recent meta-analysis showed an 18% improvement in DFS (hazard ratio [HR]=0.82; 95%CI 0.74-0.92,  $2P<0.001$ ) The clinical implications of these findings will be discussed. Prostate cancer has the propensity to metastasize almost exclusively to bone and provides the ideal clinical setting for the evaluation of bone-targeted treatment to modify the course of the disease. In a study of men with castrate resistant prostate cancer, denosumab significantly increased bone metastasis-free survival by a median of 4.2 months over placebo (HR=0.85; 95%CI 0.73-0.98,  $P=0.028$ ), and delayed time to symptomatic first bone metastases. These exciting findings are changing clinical practice.

#### Symposium 5- Muscle and bone

#### IS13

##### Effects of Aging on Muscle and Bone

Steven Cummings

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No abstract provided.

#### IS14

##### Osteoporosis in Men

Eric Orwoll

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Fractures due to osteoporosis in men are a major health care challenge. Nevertheless, the rate of detection, prevention and treatment of osteoporosis in men lags well behind that in women. In part, that may be due to the previous absence of well-powered clinical trials that demonstrate the efficacy of anti-osteoporosis drugs in men. But Boonen *et al.* recently reported that zoledronate reduces the risk of vertebral fractures in men with low BMD (NEJM Nov1, 2012). Moreover, the responses

(biochemical markers, BMD change, fracture risk reduction) to most osteoporotic drugs in men are very similar to those in women. Thus, clinicians should feel very confident in their ability to use these treatments in men. Many factors affect bone mass and fracture risk, including genetics, lifestyle issues and medical diseases. Vitamin D insufficiency clearly affects bone loss and fracture risk in men. Sex steroids are also important in bone biology in men; serum levels of estradiol are more strongly associated with bone outcomes than testosterone. Moreover, measures of neither testosterone nor estradiol contribute little to the ability to predict fracture risk in men, raising very difficult issues about the use of testosterone therapy for fracture prevention in men. Interestingly, low vitamin D and low sex steroid levels commonly co-exist in older men, and men with both problems are at considerably higher risk than those with normal levels or those with abnormalities in one alone.

### IS15

#### **Towards the Elucidation of Androgen Signaling in Skeletal Muscles**

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Despite the use of androgenic steroids by athletes, their effects on athletic performances and physical functions remain poorly characterized. Whereas some clinical studies indicate that testosterone replacement in hypogonadal men, as well as in men with sarcopenia, increases skeletal muscle mass and strength, others suggest that androgens stimulate muscle mass, but not strength. In any event, the use of currently available androgens is limited, as they might promote prostate cancer.

Androgens mediate their effects predominantly through the androgen receptor (AR), a member of the ligand-dependent nuclear receptor superfamily. To characterize androgen signaling in skeletal muscles, we generated mice in which AR is selectively and efficiently ablated in myofibers. We have demonstrated that myofiber AR controls the mass of perineal but not limb skeletal muscles. Importantly, we have also shown that AR deficiency in limb myocytes impairs myofibrillar organization of sarcomeres, and decreases muscle strength, thus demonstrating that myocytic AR controls key pathways required for maximum force production. We are currently characterizing the underlying molecular mechanisms, to develop new screens to identify selective androgen receptor modulators (SARMs) that promote muscle strength but not prostatic epithelial cell proliferation, and identify new drug targets to limit muscle wasting.

### **Symposium 6- Tissue engineering**

#### IS16

#### **Chondrocyte Differentiation and Direct Conversion to Chondrogenic Cells**

*Noriyuki Tsumaki*

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Repair of cartilage injury with hyaline cartilage continues to be a challenging clinical problem. There is a significant need

to develop cell sources for chondrocytes in cell transplantation in regenerative medicine. One approach for preparation of chondrocytes is to induce chondrocytes directly from somatic cells. We found 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 cells expressed marker genes for chondrocytes but not fibroblasts. Induced cell lines generated stable homogenous hyaline cartilage-like tissue when subcutaneously injected into nude mice, suggesting that these cells are chondrogenic cells. Time-lapse observation using Nanog-GFP reporter transgenic cells showed that directly induced chondrogenic cells do not undergo pluripotent state during induction from fibroblasts. This result suggests that directly induced chondrogenic cells do not theoretically give rise to teratoma. This approach could lead to the preparation of hyaline cartilage directly from skin.

We found that Salt-inducible kinase 3 (SIK3) critically controls timing of chondrocyte hypertrophy in endochondral bone formation. SIK3 can be one of target molecules for maintenance of chondrocyte phenotype during chondrocyte differentiation process.

#### IS17

#### **Coordinated Regulation of Bone Remodeling and Fat Formation by the Zinc Finger Protein 521 and its Interactions with Ebf1**

*Roland Baron*

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Zfp521 is a transcriptional regulator that is expressed in hematopoietic and mesenchymal cells. To understand its function we deleted Zfp521 globally or conditionally in cells of the osteoblast (OB) or osteoclast (OC) lineages. Germline or deletion of Zfp521 in the OB lineage decrease bone formation and bone mass. Zfp521 represses Ebf1 transcriptional activity and the expression of early B cell factor 1 (Ebf1) target genes was markedly enhanced in Zfp521  $-/-$  OBs. By repressing Ebf1, Zfp521 favors OB maturation and bone formation while blocking OB-dependent and OC precursor cell-autonomous OC-genesis and bone resorption. Thus, through their coordinated and opposing actions within the OB and the OC lineages, Ebf1 and Zfp521 act as a rheostat to regulate bone homeostasis.

Zfp521 is also a key regulator of adipose commitment and differentiation. As in bone, Zfp521 binds and represses Ebf1. In adipocytes it also inhibits the expression of Zfp423, an enhancer of adipose determination that enhances PPAR $\gamma$ . Overexpression of Zfp521 inhibits adipogenesis, whereas knockdown or deletion of Zfp521 enhances adipocytic differentiation. Thus, Zfp521 acts as a brake to adipogenesis and OC-genesis while enhancing osteogenesis. In conclusion, Zfp521 exerts a coordinated and positive influence on bone homeostasis in large part due to the repression of Ebf1. Our results also suggest that Zfp521 acts as a critical switch in the commitment decision between the adipogenic and osteogenic lineages.

**IS18****Stem cell populations and signals that regulate bone regeneration**

Regis J. O'Keefe, MD, PhD, Chunlun Huang, PhD, Chao Xie, PhD, and Xinping Zhang, PhD

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While scar is a common aspect of the healing of many tissues, the unique mechanical properties of bone make it essential that regeneration is faithfully completed for the reconstitution of skeletal integrity. The primary event in the process of bone repair involves stem cell recruitment, proliferation, expansion, and accumulation at the fracture site. Potential sources of stem cell progenitors include bone marrow stem cells (BMSCs), periosteum-derived stem cells (PDSCs), systemic circulation-derived stem cells (CDSCs), Vascular endothelium-derived pericytes (VEDPs), and muscle-derived stem cells (MDSCs). While evidence suggests that stem cells from all of these sources contribute to repair, periosteal tissues appear to be the primary source of cells. COX-2/PGE-2 is a common signal involved in tissue regeneration, including bone. COX-2 expression is observed in early periosteum progenitors and in immature chondrocytes during fracture healing. Mice deficient in COX-2 have reduced proliferation of periosteum-derived stem cells, decreased accumulation of fracture callus, and a reduced rate of fracture healing and remodeling. Conditional gene deletion of COX-2 in mesenchyme/periosteum (Prx1-Cre;COX-2<sup>fl/fl</sup>) and in chondrocytes (Col2a1-Cre;COX-2<sup>fl/fl</sup>) demonstrates reduced fracture callus volume in both models, although the decrease was greatest in Prx1-Cre;COX-2<sup>fl/fl</sup> mice and intermediate in Col2a1-Cre;COX-2<sup>fl/fl</sup>. Experiments further show that as mice aged, gain of function of COX-2/PGE2 improved fracture healing compared to aged control mice. Periosteum-derived stem cells were isolated and placed in cell culture. Periosteal cells with COX-2 deletion have reduced potential to undergo osteoblast and chondrocyte differentiation *in vitro*. PGE2 binds to four different receptors, EP1, EP2, EP3, and EP4. PGE2 stimulates periosteal stem cell differentiation is mediated by the EP2 and EP4 receptors, which activate protein kinase A signaling. In contrast, both *in vitro* and *in vivo* experiments using EP1<sup>-/-</sup> cells and mice establish that signaling through the EP1 receptor maintains periosteal cells in the stem cell niche. Altogether the findings show that COX-2/PGE2 signaling targets stem cell populations involved in bone regeneration. Furthermore, our findings show that the balance of EP1 and EP2/EP4 signaling is involved in determining cell fate during the repair process.

**Symposium 7- Osteocyte****IS19****Osteocytes Acting like Osteoclasts and Osteoblasts**

Lynda Bonewald

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Recently, we have shown that osteocytes can emulate bone resorbing osteoclasts. Osteocytes can remove their perilacunar matrix in response to hormones such as parathyroid

hormone and parathyroid hormone related peptide. Genes thought to be specific for osteoclasts such as cathepsin K, tartrate resistant acid phosphatase, and components of the proton pump are activated in osteocytes deeply embedded in bone. We have also shown that these cells can generate protons and have the capacity to reduce the pH in their lacuno-canalicular network. Recently, we have found other genes expressed in osteocytes whose promoters are used to drive Cre recombinase expression to generate targeted deletion of genes in osteoclasts raising the question of specificity of these promoters. Another dogma being challenged is that therapeutics targeted to osteoclasts only directly affect osteoclast function. Our observations suggest that these therapeutics may also influence osteocyte function. Once the stimulus is removed, the osteocyte 'remembers' its former life as an osteoblast and can replace the perilacunar matrix. The surface area of the lacuna-canalicular system in bone is several of orders of magnitude greater than the bone surface. Control and regulation of this surface by the osteocyte appears to play an important role in bone metabolism and function.

**IS20****The Material and Structural Basis of the Earlier Gain and Later Loss of Bone's Material and Structural Strengths**

Ego Seeman

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Bone must serve paradoxical functions. It must be strong for loading, yet light for mobility. It must resist bending for leverage in a gravitational field, and yet be flexible; able to absorb energy by changing its dimensions without cracking - lengthening and narrowing in tension, shortening and widening in compression, and a combination of each in torsion.

These paradoxical properties are achieved by the material composition and structure of bone. Bone, the material, is a composite. Type 1 collagen is a triple helix conferring flexibility and tensile strength. The crystals of calcium hydroxyapatite confer rigidity and compressive strength. Paradoxically, they are the most brittle component of the matrix but are protected by protein molecules like osteopontin which contain 'hidden' length in their helical structure. Lengthening in tension by unfolding releases energy as non-covalent 'sacrificial' bonds are broken offsetting stress on crystal platelets.

Bone, the structure, is assembled in three-dimensions using differing proportions of mineralized bone matrix volume and void volume. At one extreme, bone is configured as an open-celled porous cancellous network of thin trabecular plates constituted as 30% mineralized matrix volume and 70% void volume formed by the medullary cavity producing a structure with a large surface area/bone matrix volume ratio. At the other extreme, bone is configured as a compact cortical structure comprising 70% mineralized bone matrix volume and 30% void volume formed by Haversian canals, Volkmann canals and the lacunar-canalicular system housing osteocytes and their processes.

The mineralized bone matrix is 'inside' the periosteal or outer surface, and 'outside' the endosteal or inner surface which consists of the endocortical, trabecular and intracortical surfaces. The intracortical surface is formed by myriads of

Haversian canals and Volkmann canals. It is useful to 'see' bone in this way because the changes that take place in its material composition and structure that determine its strength or compromise its strength do so by removal of bone from, and deposition of bone, upon each of these surfaces during growth, aging, disease and drug therapy (Figure).

Whatever the genetic and environmental factors contributing to the diversity in bone's material composition and structure, this diversity is produced through the final common pathway of bone modelling and remodelling. During growth, concurrent periosteal apposition and endocortical resorption vary at each point around a cross section in absolute and relative amounts producing the irregular external shape of a bone cross section, the varying cortical thicknesses at each point around a cross section and the differing distances a volume of cortex is displaced from the neutral axis establishing differences bone's bending strength. Orchestration of the absolute movements of these surfaces in space, and movements relative to each other, may be choreographed, in part, by the activity of geographically specific ensembles of osteocytes simultaneously regulating bone formation and resorption by the coordinated production or suppression of sclerostin, RANKL and other local factors.

Minimizing mass in larger skeletons is achieved by disproportionately greater endocortical resorption relative to periosteal apposition assembling the wider diaphyseal cross section. The net result is a thinner cortex relative to the size of the cross section - bigger bones are made with relatively less material, they have less bone within their periosteal envelope and so a lower apparent volumetric bone mineral density. They 'need' less material because depositing a volume of mineralized bone matrix further radially confers resistance to bending to the fourth power of this radius. Less material is needed to achieve a given resistance to bending. However, cortical area is preserved in larger bones because the relatively thinner cortex is distributed around the larger perimeter, so compressive strength is not compromised. In addition, wider bones are also assembled with higher porosity suggesting that the greater excavation of the medullary canal is accompanied by excavation of larger numbers of osteons, each with their central Haversian canal. Ironically, the energy advantage of minimizing mass may become a liability later in life when remodeling intensity increases and erodes a structure that is more liable to becoming fragile because it was constructed by minimizing mass.

While the diaphyseal cortex is determined by the relative degrees of periosteal bone formation and endocortical resorption, formation of the metaphyseal cortex is different. Appositional growth upon trabeculae arising from the growth plate result in their fusion; they are 'corticalized'. At the distal radius, a common site of fracture in children, trabecular corticalization is transiently delayed because it cannot 'keep up' with rapid distal radial growth prior puberty. Trabecular fusion is delayed producing transitory intracortical porosity and bone fragility. In addition, appendicular growth is more rapid than axial growth prior puberty. During puberty, appendicular growth decelerates and axial growth accelerates. The tempo - chronological age of onset, peak velocity, chronological age of offset, of these events vary by region, sex and race so that, unlike adulthood, morphological effects of exposure to a risk

factor and illness depend on the timing of exposure; the same illness has differing morphological effects according to age, site, sex, and race.

The pristine state of the material composition and structure of bone achieved during growth is maintained by bone remodeling during adulthood. Both the surface area upon which remodeling takes place and the volume of the mineralized matrix to be remodelled are important. To remodel bone, signals within matrix deep to a surface must traverse that matrix volume to reach a nearby point on one of the components of the internal (trabecular, endocortical, intracortical Haversian, Volkmann and perhaps lacunar) surfaces upon which matrix remodeling will then be initiated. If the signals are transmitted to the surface, a bone remodeling compartment (BRC) is created, the roof of which is formed by the flattened osteoblasts of the surface. Within each BRC, precursors from the marrow or blood are recruited to form osteoclasts and osteoblasts responsible for the excavation and replacement of bone respectively.

Trabeculae, as thin plates, have a low matrix volume and a large surface area so signals within matrix can readily traverse the matrix volume to find a surface to initiate trabecular remodeling. Drugs like the bisphosphonates can easily adsorb upon, and be distributed within, the small matrix volume so osteoclasts resorbing matrix encounter and engulf bisphosphonates, which then prevents further resorption and structural decay. By contrast, cortical bone has a large matrix volume and a small surface area, so signals deep within matrix may not find a surface as readily to initiate remodelling allowing accumulation of microdamage, particularly in interstitial bone (between osteons) as this bone is not well innervated by osteocytes. Bisphosphonates bind avidly to a surface and may fail to penetrate and be distributed within deeper matrix so osteoclasts initiating remodeling upon a Haversian canal surface may not encounter and engulf matrix containing drug and so continue resorbing bone; a factor that may partly explain the weak non-vertebral anti-fracture efficacy of most drugs.

Around midlife, the volume of bone formed and resorbed by each basic multicellular unit both decrease, but the former decreases more than the latter, resulting in a negative bone balance; the cause of bone loss. Every time bone is remodelled, less is deposited than was removed producing structural decay. If remodeling depth decreases with age, as most data suggest, this results in smaller osteons and hemiosteons so the absolute and relative amount of interstitial bone (which has fewer osteocytes, higher mineral density and higher pentosidine crosslinking of collagen) increases. The smaller osteons also have a larger central Haversian canal (porosity) because less of refilling and so fewer lamellae and perhaps fewer osteocytes. Worsening of the negative bone balance and accelerated remodelling intensity accelerates structural decay; more bone is removed from an ever decreasing volume of bone; trabeculae are rapidly lost because their surface/volume ratio is high, but as trabecular bone only 20% of the whole skeleton, the slower loss of cortical bone, 80% of the skeleton, results in similar net amounts of cortical and trabecular bone loss during and after menopause; an observation that is contrary to prevailing notions that trabecular bone loss dominates after menopause.

After 60 years of age, cortical bone loss dominates. Porosity increases focally as the volume of bone resorbed upon a Haversian canal is incompletely replaced. Canals coalesce focally producing giant pores (in cross-section), cortices thin and fragment ('trabecularize'). It is unclear whether resorption is initiated upon an osteocyte lacuna wall, if so, this may result in an increase in porosity by increasing pore numbers. If porosity increases by initiation of remodeling upon Haversian and Volkmann canals, then the increase in porosity will be the result of enlargement of existing pores. (Most porosity in bone is under 100 microns and represents cross sections of Haversian and Volkmann canals.) Whatever the case, the low cortical surface area/mineralized matrix volume ratio increases as more porosity creates more surface to receive matrix signals for remodeling to be initiated. Remodeling becomes self-perpetuating. Cortical bone loss accelerates with age. Across life, most bone loss is cortical, most occurs after 65 years. The porous structure loses its ability to resist crack propagation predisposing to fractures. About 80% of all fractures in the community are non-vertebral not vertebral. Understanding why and how bone's break requires the study of bone's material composition and structure; its 'qualities' and its 'strengths'. Bone densitometry was a beginning, but

leave it in the 20th century as a memory; its use ignores morphology and ensures no thought will occur.

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### Osteocytes and Bone Resorption

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RANKL is a key cytokine for osteoclast differentiation and function. The main source of RANKL in bone has been thought to be osteoblasts or bone marrow stromal cells. Using the *in vitro* osteocyte culture system, however, we identified RANKL to be among the mechanical responsive genes in osteocytes. We further analyzed RANKL expression in osteocytes by sorting EGFP-positive cells from calvarial cells derived from CAG-CAT EGFP transgenic mice crossed with DMP-1 Cre mice. RANKL expression in osteocytes was about ten times higher than that in osteoblasts. Osteocyte-specific RANKL knockout mice revealed the crucial role of osteocytes in supporting osteoclastogenesis in adult bone remodeling. This provided a molecular basis for osteocyte regulation of osteoclastic bone resorption.