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Oral Poster Presentations

Oral Poster Presentation 1

OP1001
The Role Of C-Type Natriuretic Peptide (CNP) In Mouse Fracture Healing

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Objective: C-type natriuretic peptide (CNP) and its receptor, guanylyl cyclase-B (GC-B) have been shown to play physiologically important roles in endochondral bone growth. The purpose of this study was to investigate the role of CNP in fracture healing, which remains unclear.
Methods: Open, stabilized femoral fractures were created in 8-week old CNP transgenic mice under the control of human serum amyloid P component promotor (*SAP-CNP-Tg* mice).
Results: By RT-PCR analysis of fracture calluses in wild-type (Wt) mice, the expression of CNP and GC-B peaked at post-fracture day (PFD) 10 and these high levels have continued to PFD 21. CNP and GC-B were immunohistochemically expressed in hypertrophic chondrocytes. Cartilagenous callus of *SAP-CNP-Tg* mice was more rapidly decreased from PFD 10 to 14 than that of Wt mice. At PFD 21, cartilagenous callus which was still observed in Wt mice was scarcely observed in *SAP-CNP-Tg* mice. *SAP-CNP-Tg* mice had significantly higher new bone volume analyzed by μ CT than Wt mice at PFD 14. Serum osetocalcin and TRAP5b levels in *SAP-CNP-Tg* mice at PFD 21 were both significantly higher than those in Wt mice. Callus strength of *SAP-CNP-Tg* mice at PFD 42 tended to be higher than Wt mice.
Conclusion: We demonstrated that CNP and GC-B were expressed in fracture callus. Fracture healing in *SAP-CNP-Tg* mice showed accerelated turnover of cartilaginous callus and bone remodeling. These results suggest that CNP may play important roles in fracture healing.

OP1002
Zinc Finger Protein 521 (Zfp521) Regulates Bmp-dependent Osteoblast Versus Adipocyte Lineage Commitment In Mesenchymal Progenitor Cells

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BMPs are multifunctional cytokines involved in cell proliferation and differentiation. BMP2, an osteoinductive factor, is clinically approved for use in bone regeneration and repair. Although multiple transcription factors and secreted proteins are known to mediate BMP signal intensity or duration, less is known about mechanisms determining lineage specificity in response to BMP. In this study, we examined the role of Zfp521 in BMP-induced osteoinduction. Deletion of Zfp521 in mesenchymal precursors inhibited heterotopic bone formation in response to calvarial injection of BMP2. Interestingly, marrow adiposity within BMP2-induced bone was markedly increased in Zfp521 deficient mice suggesting that precursor cells lacking Zfp521 differentiate preferentially into adipocytes instead of osteoblasts in response to BMP2. Consistent with a cell-autonomous role of Zfp521 in mesenchymal precursors, knockdown of Zfp521 in stromal cells prevented BMP2-induced osteoblast-marker expression and simultaneously enhanced expression of adipocyte-related genes. Importantly, Zfp521 is recruited to the PPARγ promoter and Zfp521 deficiency significantly increased expression of PPARγ, a cell-fate switch that favors adipocyte differentiation and inhibits osteoblast differentiation. Taken together, the data suggests that Zfp521 is critical for BMP-induced osteoblast commitment and identifies Zfp521 as the intrinsic repressor of PPARγ and hence adipocyte commitment during BMP osteoinduction.

OP1003
Induction Of Differentiation Of Mesenchymal Stem Cells To Osteoblasts Through Non-canonical Wnt5a / Ror2 Pathway Under Inflammation

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Backgrounds: The aim of this study was to assess the mechanisms of human mesenchymal stem cells (MSCs) differentiation into osteoblasts under inflammatory conditions.
Methods: hMSCs were cultured in commercial osteogenic medium in the presence of inflammatory cytokines *in vitro*.
Results: TNF-α, IL-1β and IL-6 enhanced RUNX2 expression, ALP activity and alizarin red S (ARS) staining in MSCs, whereas IL-4, IL-10 and IL-17 revealed minimal effects. TNF-α, IL-1β and IL-6 up-regulated the non-canonical Wnt5a / Ror2 signaling pathway. Among these cytokines, IL-1β most efficiently induced Wnt5a /Ror2 pathway and osteoblast differentiation from MSCs. Silencing of the Wnt5a or Ror2 by siRNA reduced RUNX2 expression, ALP activity and ARS staining of differentiated hMSCs enhanced by TNF-α, IL-1β and IL-6.

Conclusion: IL-1β effectively and rapidly induced human MSC differentiation into osteoblasts and mineralization, mainly through the non-canonical Wnt5a / Ror2 pathway, indicating that MSCs are involved in bone formation even under inflammatory conditions especially with IL-1β. Concurrently, MSCs are possibly involved in the homeostasis of bone metabolism under inflammatory condition.

OP1004

Osteocytes Produce Interferon (IFN)-β And Negatively Modulate Osteoclastogenesis

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Ifn-β deficiency causes osteoporosis due to excessive osteoclastogenesis, which has been thought to be owing to incapability of osteoclast precursors to produce IFN-β as a negative feedback factor of osteoclastogenesis in response to RANKL stimulation. Although osteocytes produce RANKL and OPG, other osteocyte-derived osteoclastogenesis-modulating factors remain to be determined. We found that osteocytic MLO-Y4 cells as well as isolated osteocytes of murine long bones express *Ifn*-β mRNA. The conditioned medium of MLO-Y4 cell cultures inhibited M-CSF/soluble RANKL-induced osteoclastogenesis from bone marrow (BM) cells due to reduction of c-Fos translation, of which inhibition was partially recovered by anti-IFN-β neutralizing antibody (αIFN-β Ab). To examine functions of osteocytes in more physiologically relevant conditions; that is, forming a 3-dimensional intercellular network in calcified matrix, we developed a culture system of osteocyte-purifed bone fragments (BFs) that are freed from non-osteocytic cells by collagenase/EDTA treatments and contain only osteocytes embedded in bone matrix. Osteocyte-purifed BFs expressed mRNAs of *Ifn*-β as well as osteocyte markers *Dmp1* and *Sost*, but not a osteoblast marker *Keratocan*. Osteocyte-purifed BFs prepared from *Opg* knockout or wild-type mice inhibited osteoclastogenesis when cocultured with BM cells and this inhibition was partially recovered by αIFN-β Ab. These data suggest that osteocytes produce IFN-β and inhibit osteoclastogenesis.

OP1005

CCN2 Enhances Osteoclastogenesis Via Its Direct Bindings To Rank And Opg

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CCN family 2/Connective tissue growth factor (CCN2/CTGF) is composed of four structural domains (IGFBP, TSP1, VWC, and CT) and is a multi-potent factor for mesenchymal cells. CCN2 is also known as a modulator of other cytokines and receptors via direct molecular interaction. We made use of a phage-display system to search additional factors binding to CCN2 and found receptor activator of NF-kappa B (RANK). RANK is expressed on osteoclasts and the signaling has a critical role in osteoclastogenesis. The binding of CCN2 to RANK with sufficient affinity was confirmed by using surface plasmon resonance (SPR) analysis. Functionally, CCN2 enhanced the RANK-mediated signaling such as NF-kappa B, ERK and JNK pathways in pre-osteoclast cell RAW264.7, whereas CCN2 did not influence to RANK-RANK ligand (RANKL) binding. Since osteoprotegerin (OPG) also binds to RANKL and inhibits the binding of RANKL to RANK, we examined the interaction of CCN2 with OPG. Then we found that CCN2 bound to OPG with high affinity comparable to that of RANKL. Solid phase binding assay with recombinant proteins indicated that both of RANK and OPG bound to the CT domain of CCN2. OPG markedly inhibited the binding of CCN2 and RANK, while CCN2 cancelled the inhibitory effect of OPG on osteoclast differentiation. These findings suggest CCN2 as a fourth factor in RANK/RANKL/OPG system regulating OPG and RANK via direct interaction.

OP1006

V-ATPase V0 Domain Subunits E1 And E2 In Bone Homeostasis

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V-ATPases deliver protons across the ruffled border of osteoclasts (OC). While important in bone resorption, additional roles in OC function and formation remains obscure. We have previously demonstrated the accessory subunit Ac45 regulates multiple facets of V-ATPase-mediated acidification, endocytosis and bone resorption, extending to non-canonical roles in OC formation and maturation *in vitro*. However, the complement of proteins by which Ac45 exerts its effects remains unclear. Protein-protein interactions using BRET identified novel V-ATPase V0 subunits e1 and e2 as binding partners for Ac45. Sustained suppression of e1/e2 by siRNA increased TRAP+ve OC (>3 nuclei) that exhibit reduced cell spread area. Bone resorption was also found to be markedly reduced. Further insights into their functional relevance in bone homeostasis were examined using OC-specific conditional knockouts (cKO) mice by Ctsk K- (mature OC), LyzM-, and RANK- (both OC precursors) Cre-LoxP system. Ctsk-e1 and -e2 cKO (ΔOC) mice exhibit severe osteopetrosis, not surviving beyond 8 weeks. Long bones of e1ΔOC had total occlusion

of marrow space by bone with 1° spongiosa containing only mineralized woven bone with bone density of 2° spongiosa comparable to that of cortical bone. In contrast, long bones of e2ΔOC exhibit unresorbed calcified cartilage surrounded by unremodeled primitive woven bone in both 1° and 2° spongiosa. In conclusion, V-ATPase e subunits are novel unrecognized regulators of OC function.

OP1007

Parathyroid Hormone-Related Peptide (PTHrP) Inhibits Chondrocyte Hypertrophy By Inducing Dephosphorylation And Subsequent Nuclear Translocation Of Histone Deacetylase (HDAC) 4

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PTHrP and HDAC4 delay chondrocyte hypertrophy but the molecular mechanisms have not been clarified. Here we report *in vivo* genetic and molecular evidences that PTHrP regulates HDAC4 to accomplish this delay. Our genetic tests support the hypothesis that PTHrP and HDAC4 work in a common pathway: 1) The HDAC4 knockout (KO) mouse shows a phenotype similar to that of the PTHrP KO; 2) Even though the PTHrP heterozygous (HET) and the HDAC4 HET have normal growth plate at birth, their double HET exhibits accelerated chondrocyte hypertrophy; and 3) The complete suppression of chondrocyte hypertrophy in the PTHrP transgenic (Tg) mouse is blocked by knocking out the HDAC4 gene in this mouse. HDAC4 has binding sites for the chaperon protein 14-3-3 and myocyte enhancer factor 2 (Mef2). HDAC4 translocates into nucleus through dephosphorylation at the 14-3-3 binding sites, and then represses the action of Mef2C, the master transcriptional regulator of chondrocyte hypertrophy. We examined if PTHrP regulates HDAC4 dephosphorylation and nuclear translocation *in vivo*, as the Lassar group showed *in vitro* (Kozhemyakina, MCB 2009). Immunochemical examination of the PTHrP Tg and KO mice, using confocal fluorescent microscopy showed HDAC4 dephosphorylation at the 14-3-3 binding site and HDAC4 nuclear translocation in response to PTHrP. These results are consistent with the idea that PTHrP inhibits chondrocyte hypertrophy by inducing HDAC4 dephosphorylation and subsequent nuclear translocation.

OP1008

Osseointegration Of Titanium Implants Is Not Impaired In Osteoporotic Bone

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Osseointegration of dental implants has made great strides towards implant durability and patient comfort, but the kinetics of osseointegration can be further improved. In this study, we investigated the kinetics of osseointegration of implants with machined (mach) and sandblasted, acid etched (SLA) surfaces in osteoporotic bone. Titanium pins were inserted bicortically into the submethaphyseal cancellous bone of the proximal tibia in adult rats. Osseointegration was followed for up to 12w histologically and by MicroCT. In intact animals, formation of a bone implant contact (BIC) area was accelerated for SLA implants as compared to mach. Two and 4w after implantation, BIC was 30.9%±18.2% and 36.8%±14.6% for mach and 53.3%±14% and 59.9%±15% for SLA (P<0.05). After 8w, the difference between the surfaces was lost. No differences were detected in relative bone volume (BV/TV) close (0µm-50µm) or removed (50µm-250µm) from the implant. In osteoporotic bone, SLA induced an acceleration in BIC formation when compared to mach (46.6% ± 6.5% vs 25.5% ± 3.8% after 1w, P<0.05), but OVX and sham animals did not differ. Four and more weeks after implantation, BV/TV in the periimplant bone was significantly reduced in osteoporotic bones. The data confirms the accelerated osseointegration of SLA implants. Furthermore, osseointegration is not affected in osteoporotic bone and the observed difference in bone density is due to structural and not metabolic changes in osteoporosis.

OP1009

The Novel Roles Of CCL2 And CCL3 On Osteoclast Differentiation And Alveolar Bone Resorption During Occlusal Traumatism

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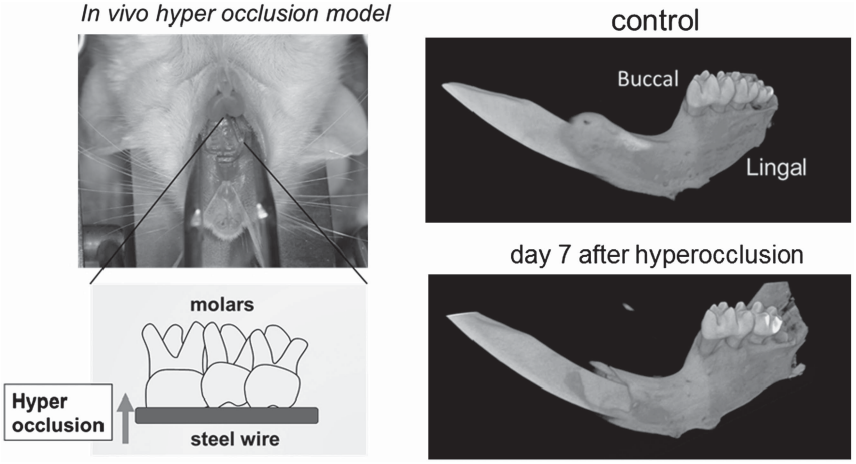
Excessive mechanical stress (MS) during hyperocclusion is known to result in disappearance of the alveolar hard line, enlargement of the periodontal ligament (PDL) space, and destruction of alveolar bone, leading to occlusal traumatism. We have recently reported that MS induces predominantly C-C chemokine ligand (CCL) 2 expression in PDL tissues, leading, via C-C chemokine receptor (CCR) 2, to MS-dependent osteoclastogenesis in alveolar bone. Thus, we hypothesize that ablation of the CCL2/CCR2 signaling pathway should suppress MS-induced osteoclastogenesis-associated chemokines and alleviate occlusal traumatism. We examined the effect of MS on chemokine expression and osteoclastogenesis using *in vivo* (image 1) and *in vitro* hyperocclusion models with CCL2-deficient (CCL2(–/–)) and CCR2-deficient (CCR2(–/–)) mice. Compared with that in wild type mice, expression of CCL3 in PDL cells and TRAP-positive cells in alveolar bone from CCL2(–/–) and CCR2(–/–) mice was up-regulated, even in the absence of MS. Furthermore, the expression of CCL3 and TRAP-positive cells were significantly increased after both four and seven days of hyperocclusal MS loading in CCL2(–/–) and CCR2(–/–) mice. Hyperocclusion induced compensatory CCL3 expression and promoted osteoclastogenesis to counterbalance deficient CCL2/CCR2 signaling, suggesting that co-expression of CCL3 with CCL2 may precipitate synergistic, MS-dependent alveolar bone destruction during occlusal traumatism.

[OP1009]



OP1010

Chronic Kidney Disease Impairs The Strength Of Osseointegration

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Chronic kidney disease (CKD) has become a worldwide public health problem. However, its effect on osseointegration of dental implants is largely unknown. This study is to investigate whether CKD impairs the osseointegration of titanium implants. Uremia was induced in C57BL mice by 5/6 nephrectomy. Serum levels of BUN, FGF23, PTH and ALP were significantly increased, while those of calcium and phosphate were normal. Bone marrow stem cells (BMSCs) were obtained and cultured on titanium discs. There was no significant difference with respect to expression of Osx, Col-1, Ocn, and Opn, as quantified by qPCR. Meanwhile, Alizarin S Red staining showed comparable mineralized nodules formation in BMSCs culture obtained from both CKD and sham animals. For *in vivo* tests, the experimental titanium implants with SLA surface (1 mm in diameter, 2mm in length) were inserted in the distal end of femurs, and samples were collected after 2 weeks. Histomorphometrical analysis of bone-implant contact (BIC) ratios for CKD and sham groups were 60.23% and 68.08%, respectively. The P value (P=0.058) was very close to the point of significant difference, suggesting a trend of decreased BIC ratio for the CKD group. Moreover, the strength of bone-implant integration, as measured by a push-in method, was significantly lower for the CKD group (10.23±3.11) than that of sham group (14.09±1.69). This study demonstrated that CKD impaired the strength of osseointegration of titanium implants.

OP1011

Plasminogen Plays An Essential Role In Bone Repair

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Further development of bone regeneration is necessary to meet the pathological demand for bone reconstruction. Plasminogen (Plg) is a critical component of the tissue fibrinolytic system and it mediates tissue remodeling in the skin and liver. However, the role of tissue fibrinolytic system in bone regeneration remains unknown. Herein, we investigated bone repair and ectopic bone formation using Plg-deficient ($Plg^{-/-}$) mice. Bone repair after a femoral bone defect was delayed in $Plg^{-/-}$ mice, unlike that in the wild-type ($Plg^{+/+}$) mice. Chondrogenesis, osteoblastogenesis, angiogenesis associated with the increase in VEGF expression, and macrophage accumulation were decreased in $Plg^{-/-}$ mice. Moreover, Plg deficiency reduced the level of TGF-β in the MΦ derived from damaged femur. Conversely, heterotopic ossification was comparable between $Plg^{+/+}$ and $Plg^{-/-}$ mice, suggesting that the presence of bone marrow cells is essential for expressing primary role of Plg in bone repair. Our study using mouse primary osteoblasts and bone marrow cells indicated that osteoblasts and its precursors are not responsible for the effects of Plg deficiency on bone repair. In conclusion, our data provide novel evidence that Plg plays a crucial role in bone repair through enhancement of angiogenesis related to VEGF and TGF-β. Furthermore, the present study indicates that Plg contributes to the MΦ accumulation and their TGF-β expression, thereby leading to the enhancement of bone repair.

OP1012

Generation And Characterization Of C-Type Natriuretic Peptide (CNP) Knockout Rats Using Zinc Finger Nucleases

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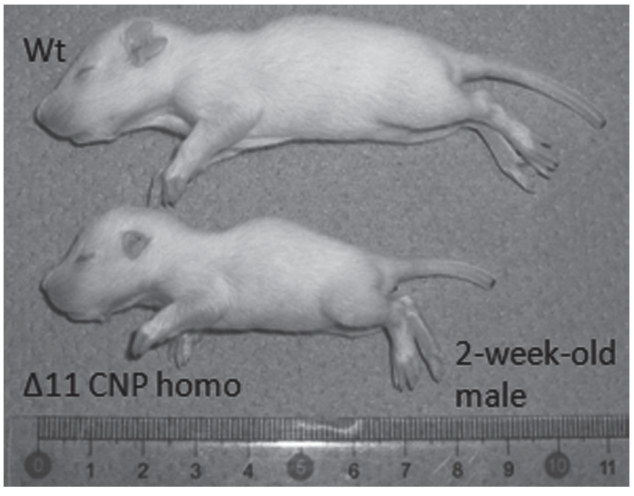
Previous studies using transgenic or knockout mice model have revealed that CNP is a positive regulator of endochondral bone growth via guanylyl cyclase-B (GC-B)/ cGMP pathway. Although the laboratory rat is a good model for physiological and pharmacological studies, the inability to utilize rat embryonic stem (ES) cells has prevented us from targeting a specific gene to elucidate its functions. Here we generated CNP knockout rats using zing-finger nucleases that can create a site-specific mutagenesis in a targeted gene, and then characterized the resultant phenotypes. The growth curves of naso-anal length revealed that one line of CNP knockout rat with 11 base pairs deletion of the CNP gene (Δ11 CNP) was significantly shorter than Wt rat both in male and in female (in 2-week-old male, Δ11 CNP homo: Wt = 83.2±1.3mm: 94.1±1.0mm, $P<0.01$). Radiographic analyses showed that long bones and vertebrae of Δ11 CNP homo rats were significantly shorter than those of Wt rats. In the histological analyses, the length of tibial growth plate of Δ11 CNP homo rat was significantly shorter than that of Wt rat. These skeletal phenotypes observed in Δ11 CNP homo rat were almost the same as those in another line of CNP knockout rat, which has 774 base pairs deletion in the CNP gene. These results demonstrate that CNP is a pivotal and physiological stimulator of endochodral bone growth not only in mice but also in rats.



Oral Poster Presentation 2

OP2001

Hypoxia-Inducible Factor-2α Regulates Pathogenesis Of Rheumatoid Arthritis

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Rheumatoid arthritis is a systemic autoimmune disorder that manifests as chronic inflammation and joint tissue destruction. Despite therapeutic advances, the etiology and pathogenesis of rheumatoid arthritis have not been entirely elucidated. Here, we explored the role of hypoxia-inducible factor-2α (encoded by EPAS1) in rheumatoid arthritis pathogenesis. Hypoxia-inducible factor-2α is overexpressed in rheumatoid arthritis synovium and its ectopic expression in the synovium triggered rheumatoid arthritis pathogenesis, whereas Epas1 knockdown in mice (Epas1+/-) inhibited experimental rheumatoid arthritis. Hypoxia-inducible factor-2α regulated rheumatoid arthritis pathogenesis by modulating angiogenesis and various functions of fibroblast-like synoviocytes, including proliferation; expression of cytokines, chemokines, and matrix-degrading enzymes; expression of RANKL (receptor activator of nuclear factor-κB ligand) and regulation of osteoclastogenesis. Additionally, hypoxia-inducible factor-2α-dependent up-regulation of interleukin-6 in fibroblast-like synoviocytes stimulated differentiation of TH17 cell, a crucial effector of rheumatoid arthritis pathogenesis. Thus, our results provide new insight into the mechanisms of rheumatoid arthritis pathogenesis.

OP2002

An Osteoprotegerin-like Peptide Inhibits Bone Loss In Collagen Type II-Induced Murine Arthritis

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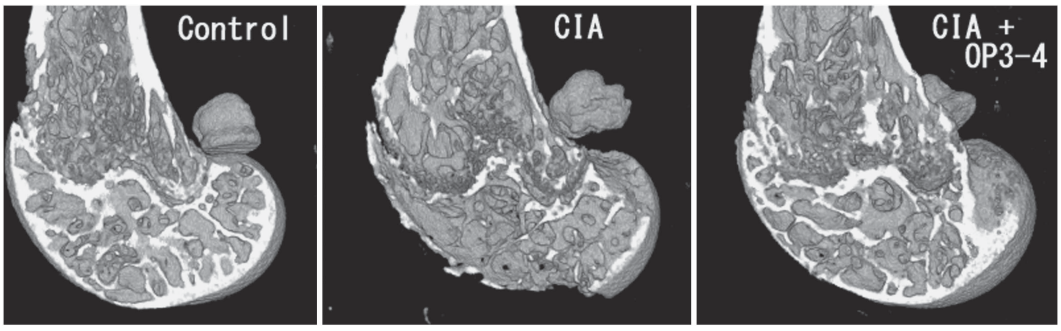
Objective: The cyclic peptide OP3-4 (YCEIEFCYLIR) was designed to mimic the most critical receptor activator of NF-κB ligand (RANKL) contact site on osteoprotegerin (OPG), and it prevents interactions of RANKL with its receptor RANK. Given the inhibitory effects of OPG on bone loss, but no effects on inflammation, the effects of OP3-4, OPG mimetic peptide, on inflammatory bone loss were evaluated.

Methods: The human rheumatoid arthritis model, collagen type II-induced murine arthritis (CIA) was used in this study.

[OP2002]



Osmotic minipumps were implanted in the backs of all mice after the onset, day 7 after the second booster injections, and vehicle or OP3-4 (9 mg kg^{-1} per day or 18 mg kg^{-1} per day) was continuously infused for 3 weeks. Vehicle was also infused in naive mice (7-week-old male DBA/1J mice). The arthritis-score assessment had been performed until the mice were killed (day 49). Thereafter, radiographic analyses were performed.
RESULTS: The OP3-4 treatment did not inhibit CIA-induced increases in the arthritis score significantly. Micro CT images revealed that 18 mg kg^{-1} per day OP3-4 prevented CIA-induced bone loss at knee joints. OP3-4 also inhibited CIA-induced bone loss at diaphysis of femurs and tibiae. pQCT measurements of bone mineral density confirmed above observations.
CONCLUSION: The OPG mimetic peptide would be a useful template for the development of small molecular inhibitors to prevent both inflammatory bone destruction and systemic bone loss in rheumatoid arthritis.

OP2003

Age-Related Deterioration In The Osteocytic Canalicular Network Of Human Femoral Cortices: Implications For Altered Mechanosensitivity In Aged Individuals

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The lacunocanalicular network in bone represents a crucial domain ensuring the osteocytes' nutrition and oxygen supply as well as their mechanosensing function. Thus, a quantitative characterization of the canalicular connections in relation to age may represent a basis to obtain further insight into the process of age-specific bone remodeling and fracture. We aimed to unravel the number and distribution of canalicular connections in human femoral cortices considering the hypothesis that hampered mechanosensing in elderly individuals is ascribed, at least in part, to age-specific alterations of canalicular connections. The samples in this study comprised femoral mid-diaphyseal cross-sections taken from 16 cases at autopsy (young: *n*=7; aged: *n*=9). The bone samples were first embedded undecalcified in methylmethacrylate. Subsequently, following the polymerization process, all specimens were acid-etched and qualitatively assessed using high resolution scanning electron microscopy.
We found a significant decrease in canalicular connections within individual osteons and/or between osteons and interstitial bone in aged cases. This signifies hampered nutrition and oxygen supply to osteocytes as well as altered mechanosensing capabilities with aging. In contrast, young cases are equipped with wide-spread canalicular connections that ensure extensive cellular communication and adequate transduction of mechanical stimuli which represents a prerequisite for maintaining bone quality.

OP2004

Identification And Characterization Of A Zinc Finger Protein 521-NuRD Gene Silencing Complex: Potential Role In Mesenchymal Stem Cell Differentiation

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The differentiation and maintenance of cell populations are strictly controlled through gene regulation by specific transcription factors and epigenetic mechanisms. Several C2H2-type Zinc finger proteins are involved in cell lineage determination and we have shown that Zinc finger protein 521 (Zfp521) represses Runx2 or Ebf1 to regulate mesenchymal stem cell osteogenic vs adipose fate. In both cases, it is believed that Zfp521 associates with HDACs but the underlying molecular functions of Zfp521 are still largely unknown. To elucidate the molecular function of Zfp521, we analyzed complex formation by mass spectrometry (MS) after purification by immunoprecipitation and size fractionation of nuclear extracts in HEK-293A cells stably expressing Zfp521. MS analysis showed that Zfp521 forms a tight complex with all the core components of the Nucleosome Remodeling and Deacetylation (NuRD) complex. In addition, novel components were identified in the Zfp521-NuRD complex, including zinc finger protein 423 (Zfp423) a close ortholog of Zfp521. This finding was validated by purification of the Zfp423 complex. Complex formation was confirmed in untransfected C3H10T1/2 cells. The Zfp521-NuRD complex also has HDAC activity, confirming that it functions in gene silencing. Since Zfp521 represses Zfp423, preventing preadipose cell determination, our findings suggest that the interaction of Zfp521 and Zfp423 within this complex might act as a switch in mesenchymal stem cell determination.

OP2005

Severe Aortic Calcification In Men With Low Serum Levels Of DKK1 - The Strambo Study

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Preliminary data suggest a link between dickkopf-1 (DKK1) and cardiovascular pathology. We assessed the relation between serum DKK1 and abdominal aortic calcification (AAC) in 1146 community-dwelling men. Serum DKK1 level was measured by ELISA (BioMedica, Vienna, Austria). AAC was assessed from the lateral Vertebral Fracture Assessment scans (HOLOGIC Discovery A) using the semiquantitative score. Serum DKK1 was stable until the age of 55, then decreased (r=857, r= –0.16, p<0.001). In men aged >=55 prevalence of severe AAC (AAC score>5, n=139) increased with decreasing DKK1 levels (OR= 1.42 per 1SD decrease, 95%CI: 1.14-1.77, adjusted for age, weight, smoking, co-morbidities, season, and serum levels of parathyroid hormone and FGF23). Prevalence of severe AAC decreased with increasing DKK1 quartiles (24, 22, 11, and 9%, p<0.001). After adjustment for confounders, odds of AAC>5 was higher in men below the median of DKK1 (OR= 2.38, 95%CI: 1.49-3.78) vs men who had DKK1 levels above the median (>15.1 pM). Men who had DDK1<15.1 pM and hypertension had higher odds of AAC>5 (OR= 3.79, 95%CI: 1.96-7.35) vs men without these characteristics. Men who had DDK1<15.1 pM and and elevated FGF23 (fourth quartile) had higher odds of AAC>5 (OR= 4.35, 95%CI: 2.13-8.86) vs men without these characteristics. The results were similar for other AAC thresholds. In conclusion, in older men low DKK1 levels are associated with severe AAC independently of age and other potential confounders.

OP2006

Transcription Factor Nfat1 Regulates The Expression Of Multiple Catabolic And Anabolic Molecules In Articular Cartilage

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Osteoarthritis (OA) is the most common form of joint disease. No disease-modifying pharmacologic therapy is available largely because the pathogenetic mechanisms of OA remain unclear. Previous studies suggest that overexpression of matrix-degrading proteinases and cytokines may contribute to cartilage degradation. However, specific transcription factors that regulate the expression of catabolic molecules in articular cartilage remain to be identified. Our recent studies discovered that deletion of Nfat1, a member of the nuclear factor of activated T-cells (NFAT/Nfat) transcription factors, resulted in OA-like cartilage degradation with chondro-osteophyte formation and thickening of subchondral bone in adult mice. Overexpression of catabolic proteinases and proinflammatory cytokines with decreased expression of anabolic factors was observed in Nfat1–/– articular cartilage. Nfat1 binding sites were identified in the promoters of the genes for specific catabolic and anabolic factors, such as IL-1β, TNF-α, MMP13, ADMTS5, BMP7, and TGF-β1. These new findings suggest that Nfat1 regulates the expression of multiple catabolic and anabolic molecules in articular cartilage. Nfat1 deficiency causes OA mainly due to an imbalance between catabolic and anabolic activities of adult articular chondrocytes with dysfunction of peri-articular tissue cells. Thus, anti-OA agents that target Nfat1 could be more effective than drug candidates that target a single catabolic or anabolic molecule.

OP2007

PGE2 Regulate Breast Cancer Metastasis And Osteoblastic Rankl Production Through Its Receptor Subtype Of EP4

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Bone metastasis of breast cancer is accompanied by severe bone destruction. Here we examined the effects of EP4 antagonist, which block the binding of PGE to its receptor subtype EP4, on bone metastasized breast cancer cells. To examine the effects of breast cancer cells on bone resorption, mouse breast cancer cells (4T1) were co-cultured with bone marrow cells to evaluate the supporting capacities of osteoclast formation. Osteoblasts, cultured on the fixed-4T1 cell membrane, markedly induced RANKL expression and PGE2 production. Osteoclast formation was detected only in the co-cultures of bone marrow cells and osteoblasts with fixed-4T1 cell membrane, and EP4 antagonist completely inhibited the osteoclast formation. In organ cultures, 4T1 attachment to calvaria markedly induced the numerous osteoclasts and following bone resorption. EP4 antagonist clearly suppressed the 4T1-induced bone resorption, and 3D-micro CT analyses showed that the osteoclastic resorbing trail on bone surface was suppressed by adding EP4 antagonist. *In vivo* implantation of 4T1 into tibiae, severe bone destruction was seen in the control, and the administration of EP4 antagonist attenuated the bone destruction. These results suggest that the interaction between breast cancer cells and host osteoblasts induces PGE production and RANKL expression that leads osteoclast differentiation and following osteolysis. EP4 antagonist is a new candidate for the therapy of breast cancer with bone metastases.

OP2008

BA058, A Novel Human PRHrP Analog, Restores Bone Mass In The Osteopenic Monkey

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BA058 is a synthetic analog of hPTHrP (1-34) currently in Phase 3 of clinical development for the treatment of post-menopausal

osteoporosis. Aged female cynomolgus monkeys were assigned to 5 groups. Four groups were ovariectomized (OVX) and one group underwent Sham surgery. Treatment commenced after a 9-month bone depletion period (BD). Animals received a daily SC injection of vehicle or BA058 (0.2, 1 and 5 $\mu g\,kg^{-1}$ per day) for up to 12 months (Mo). Bone markers and DXA/pQCT were measured prior to OVX/sham surgery, at the end of BD and after 12 Mo of treatment. At the end of the BD period, increases in bone markers and decreases in BMD by DXA/pQCT were observed for OVX controls compared to pre-OVX or Sham controls. Densitometry values were comparable to or above pre-surgery levels at the spine and femoral neck (DXA), by 4 Mo of treatment, with 4 to 9% increases after 12 Mo of treatment relative to changes in OVX controls. Total tibia metaphysis BMD (pQCT) increased at all doses, 3 to 6 % from end of BD at 4 Mo and 8 to 11% after 12 Mo of treatment. Daily administration of BA058 for 12 Mo showed marked bone anabolic effects with complete reversal of OVX-induced osteopenia at all sites evaluated by DXA and pQCT, at all doses. Serum calcium levels remained within physiological ranges. BA058 potentially offers important advantages as a new treatment for post-menopausal osteoporosis, including the ability to build new bone rapidly.

OP2009

Ex Vivo Real-time Observation Of Ca2+ Signaling In Living Bone In Response To Shear Stress Applied On The Bone Surface

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Bone cells respond to mechanical stimuli by producing a variety of biological signals, and one of the earliest events is intracellular calcium ([Ca2+]i) mobilization. However, the behavior and connectivity of the [Ca2+]i signaling networks in mechanotransduction have not been investigated in intact bone. We herein introduce a novel fluid-flow platform for probing cellular signaling networks in live intact bone, which allows the application of capillary-driven flow just on the bone explant surface while performing real-time fluorogenic monitoring of the [Ca2+]i changes. In response to the flow, the percentage of responsive cells was increased in both osteoblasts and osteocytes, together with upregulation of c-fos expression in the explants. Treatment with 18 α-GA, a reversible inhibitor of gap junction, significantly blocked the [Ca2+]i responsiveness in osteocytes without exerting any major effect in osteoblasts. On the contrary, such treatment significantly decreased the flow-activated oscillatory response frequency in both osteoblasts and osteocytes. These findings indicated that flow-induced mechanical stimuli accompanied the activation of the autonomous [Ca2+]i oscillations in both osteoblasts and osteocytes via gap junction-mediated cell-cell communication. The present study suggests that cell-cell signaling via augmented gap junction-mediated [Ca2+]i mobilization could be involved as an early signaling event in mechanotransduction.

OP2010

Colles' Fracture As A Risk Factor For Subsequent Hip Fracture: A Population Based Study

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Background: The incidence of the hip fracture rises in worldwide. However, little evidence has shown the relationship between Colles' fracture and hip fracture in Asian population. We explored whether Colles' fracture increased the subsequent hip fracture in short-term period (within one year) in Taiwanese.

Patients and Methods: This study extracted newly diagnosed Colles' fracture patients in 2000-2006 as an exposed cohort (*N*=17 918). A comparison group (*N*=142 672) was randomly selected from patients without Colles' fracture by the ratio of 1:8 in the same year of exposed cohort. The subjects were followed up for one year since the recruited date. We compared the hip fracture incidence between groups and measured the hazard ratio of hip fracture for patients with Colles' fracture.

Results: The incidence of hip fracture within one year increased with age in both cohorts. It was 6 times (56.0 vs. 9.3 per 10 000 person-years) greater in 20 Colles' fracture cohort than in non-Colles' fracture cohort. The multivariate Cox proportional hazard regression analyses showed the hazard ratios (HR) of hip fracture in relation to Colles' fracture was 4.15 (95% confidence interval (CI) = 3.17-5.42). Among comorbidities, osteoporosis was also a significant factor associated with hip fracture (HR = 2.47, 95% CI = 1.83-3.35).

Conclusions: Patients with Colles' fracture and osteoporosis independently predict the subsequent hip fracture, especially in female and elderly people.

OP2011

Prevalence And Risk Factors For Osteopenia And Osteoporosis In Male Japanese HIV Patients

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Objectives: Bone mineral density (BMD) loss has emerged as a long-term complication among HIV patients. However, little is known about the prevalence of BMD loss in Asian HIV patients. In this study, we examined prevalence of BMD loss and clinical factors related to BMD loss in male Japanese HIV patients.

Methods: Forty Japanese male with HIV, aged 21 to 70, were enrolled from March 2010 to February 2012. BMD was

measured in the lumbar spines and the femoral necks by dual-energy X-ray absorptiometry (DXA) scan. A multiple regression analysis was conducted to determine independent variables related to BMD.

Results: According to WHO classification, the percentage of osteopenia and osteoporosis diagnosed in the lumbar spines were 5.1, 43.6, respectively, and were raised to 7.5, 47.5, respectively, in the femoral necks. The median T-scores of the femoral necks among the patients in their 40s and 50s were significantly lower than those in their 20s (P<0.0001). A multiple regression analysis revealed that age (P=0.0017), serum bone alkaline phosphatase (BAP, P=0.0234), estimated glomerular filtration rate (eGFR, P=0.0413) and urinary phosphate excretion (P=0.0110) were independent variables of BMD.

Conclusions: As the first report focused on Asian patients, 62.5% of Japanese male HIV patients were diagnosed either osteopenia or osteoporosis. DXA scan examination is recommended in patients over 40 or those with increased BAP, decreased eGFR or decreased urinary phosphate excretion.

Table 1 The prevalence of BMD loss

	The lumbar spines	The femoral necks
n	39	40
Median T-score(IQR)	–0.8(–1.5 to 0.1)	–1.2(–1.9 to –0.08)* , p=0.0461
Normal(%)	20(51.3)	18(45.0)
Osteopenia(%)	17(43.6)	19(47.5)
Osteoporosis(%)	2(5.1)	3(7.5)
BMD loss(%)	19(48.7)	22(55.0)

* indicates statistical significance with P<0.05. IQR, interquartile range; *n*, number of examined patients

OP2012

Human Beta-Defensin-3 Gene Therapy Improves Bone Healing With Staphylococcus Aureus Contamination In Rats

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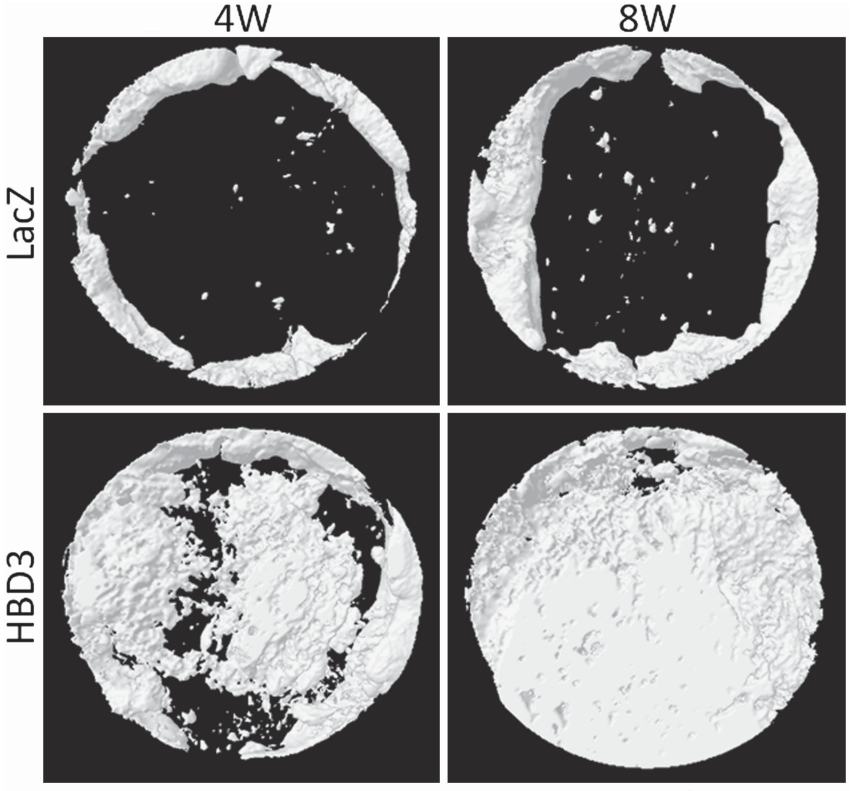
Background: Bacterial contamination during bone healing often compromise new bone formation. Human beta defensin-3 (HBD3), an antimicrobial peptide of innate immune system, is effective against *Staphylococcus aureus* (SA) which causes most bone infections. The purpose of this study was to explore the effect of HBD3 gene therapy on bone healing with SA contamination.

Materials and methods: *In vitro* cell culture and *in vivo* diffusion chamber models were used to verify the antimicrobial effect of HBD3 against SA. Collagen carriers with SA (to simulate SA contamination during bone healing) and rat bone marrow stromal cells (transduced with adenoviral vectors containing HBD3 or LacZ gene) were used to treat rat critical-sized calvarial defects. Bone healing was evaluated after 4 and 8 weeks by radiographic analysis, micro-CT measurement and histological analysis.

Results: The BMSC overexpressing HBD3 showed effective antimicrobial activity against SA both *in vitro* and *in vivo*. Results of bone healing assays indicated that HBD3 group formed more new bone than the LacZ group in rat calvarial bone defects contaminated with SA at 4 and 8 weeks. Furthermore, the pattern of new bone formation suggested that implanted

[OP2012]



Micro-CT reconstruction image of new bone formed in rat calvarial defect

BMSC in HBD3 group, but not in LacZ group, formed significant amount of bone in the SA-contaminated bone defect.
Conclusion: BMSC-mediated HBD3 gene therapy significantly improves bone healing in rat calvarial bone defects contaminated with *Staphylococcus aureus*.

Oral Poster Presentation 3

OP3001

Incomplete Atypical Femoral Fracture And Teriparatide

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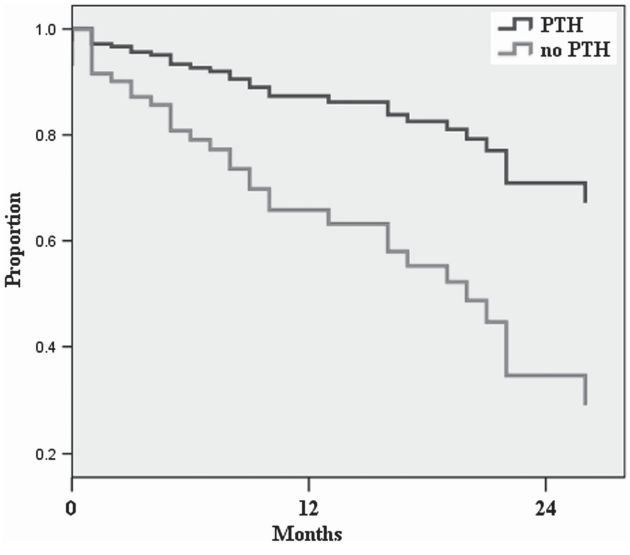
Background: Bisphosphonate-related atypical femoral fracture has been increasing concerns on osteoporosis treatment. Incomplete atypical femoral fracture can require surgical fixation, because it is at risk of progression to completed fracture. However, it is unclear which treatment can reduce a requirement of fixation in incomplete atypical femoral fracture. Our purposes are to (1) evaluate the clinical results of incomplete atypical femoral fracture, and (2) determine whether use of teriparatide can reduce a requirement of fixation in incomplete atypical femoral fractures.
Methods: We retrospectively reviewed 51 patients with 65 incomplete atypical femoral fractures. We compared the occurrence of complete fracture and requirement of fixation in teriparatide users and non-user. Minimum follow-up was 12 months (mean, 19.8 months; range, 12 to 82 months).
Results: Requirement of fixation in teriparatide users was smaller than those in non-user (9/30 vs. 12/18, *P*=0.018). In multivariate analysis, use of teriparatide showed a protective effect to requirement of fixation in incomplete atypical fractures. (Hazard ratio, 0.323; 95% confidence interval, 0.110-0.954) (Figure 1).
Conclusions: Use of teriparatide could be an alternative option during conservative treatment for incomplete atypical femoral fractures. Well-designed studies on teriparatide should be conducted to verify the efficacy for these conditions.



OP3002

Assessment Of Cervical Hip Fracture Risk From Radiograph-Based Texture Parameters: A Prospective Study

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Aims: Assessment of hip fracture risk is clinically based on bone mineral density (BMD) derived from DXA. Trabecular structure analysis of radiographic pictures has been suggested as a potential alternative and low-cost solution for risk assessment. In this prospective study we tested the potential of radiographic picture analysis to discriminate subjects with a cervical hip fracture.
Methods: Pelvic radiographs and femoral neck BMD measurements were taken from 618 women (79-82 years) in 2006. By the middle of the year 2012, 10 of the patients had suffered a cervical hip fracture. A Laplacian-based semi-automatic

Table 1 Distribution of neck bone mineral density (BMD), neck-shaft angle (NSA) and textural parameters of cervically fractured femurs versus control group.

	Cervical (n = 10)	Controls (n = 43)	p
BMD (g cm^{-2})	0.59 (0.08)	0.64 (0.09)	0.135
NSA (Deg)	129.5 (6.2)	125.2 (4.6)	0.017
Entropy	6.86 (0.16)	7.02 (0.17)	0.007
Homogeneity index	0.77 (0.01)	0.76 (0.01)	0.046
Contrast	0.66 (0.05)	0.71 (0.08)	0.046
Correlation	0.54 (0.08)	0.60 (0.06)	0.035

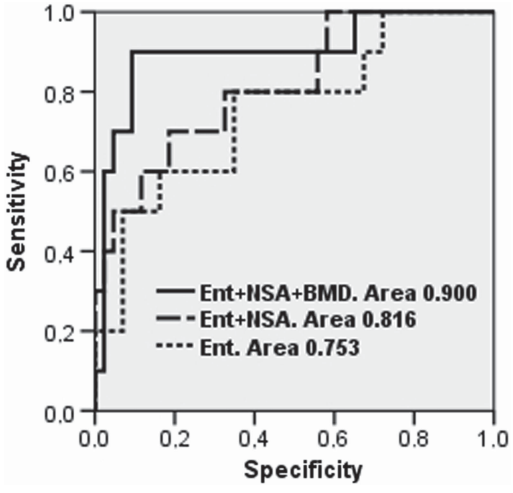

Figure 1 Receiver operating characteristic curves for predicting the fracture of femurs (10 bones versus 43 controls). *Ent* entropy, *NSA* neck-shaft angle and *BMD* neck bone mineral density.

custom algorithm was applied to the radiographs to calculate the texture parameters along the trabecular fibers in the lower neck area for these fracture cases and for 43 controls.
Results: The best predictor of hip fracture was the entropy for the textural parameters and the neck-shaft angle (NSA) for the geometrical measurements. The area under the curve in ROC analysis predicting the fracture was 0.753 for entropy, whereas it was 0.608 for BMD and 0.698 for NSA. The area increased to 0.816 and 0.900 while combining the entropy with NSA and then with BMD.
Conclusion: We present here a prospective study discriminating cervically fractured versus non-fractured groups. These preliminary results show the promising predictive ability of texture analysis from radiographs. Combination of the entropy parameter with NSA and BMD can further enhance the predictive accuracy.

OP3003

Increasing Trend Of Hip Fracture Incidence In South Korea: A Prospective Cohort Study

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Background: Recent studies from developed countries have shown that the incidence of hip fractures is either decreasing or have reached a steady stable rate. However, there was no report of long-term trend of hip fracture incidence in developing countries. This prospective longitudinal cohort study was perfomed to estimate the ten-year hip fracture incidence trend of people over 50 years of age in South Korea.
Methods: The number and incidence of hip fractures was obtained information of patients over 50 years of age who sustained a hip fracture from the records of eight hospitals in Jeju Island between 2002 and 2011. Gender-specific incidence rate were calculated based on estimated for the population in the United States in 2008
Results: There were a 101% increase in the number of hip fracture from 151 in 2002 to 304 in 2011. The crude incidence of hip fracture increased from 126.6 /100 000 to 183.7/100 000. The Gender-specific incidence rate increased from 100.6/100 000 for men and 194.4/100 000 for women in 2002 to 114.2/100 000 for men and 278.4/100 000 for women in 2011.The annual increase in rate of hip fracture incidence was 4.3% (5.3% in woman and 2.2 in man).
Conclusion: The total number of hip fracture increase twofold and incidence rate of hip fracture steeply increase during ten-year study period. The increase in hip fractures is raising socioeconomic concern especially with the rapid aging of the population in a developing country like South Korea.

OP3004

Bone Turnover Marker Plot And Fracture Prediction

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Postmenopausal osteoporosis is characterised by an increase in bone turnover and an imbalance between bone resorption and bone formation. An approach has been described 'The Bone Marker Plot' that allows calculation of the rate of bone turnover and balance between bone formation and bone resorption from measurement of bone turnover markers. The aim of this study was to apply this approach to the prediction of fractures in older women. We studied 745 older women (55 to 80 years) and 213 younger women (20 to 39 years) from the Osteoporosis and Ultrasound Study (OPUS). We included women with bone turnover marker measurements who had no disease known to affect bone turnover, no anti-resorptive treatments close to baseline or during the study, and, if in the older group, were postmenopausal. We measured serum procollagen type I N-propeptide (PINP) and C-telopeptide of type I collagen (CTX) by automated immunoassay analyser (iSYS, IDS) on the samples taken at baseline.We collected information about non-vertebral fractures by questionnaire and vertebral fractures by spinal radiographs after an interval of 6 years. There were 115 women with incident fractures over 6 years (23 vertebral, 92 non-vertebral). The older women had higher rates of bone turnover and more negative balance than the younger women (Mahalanobis distance 0.83, $P<0.001$). The women with vertebral fractures had more negative balance than those without fractures (odds ratio 0.61, 95% CI 0.40 to 0.93, $P=0.020$) but not significantly different turnover. The women with non-vertebral fractures had more negative balance that those without fractures (odds ratio 0.65, 95% CI 0.45 to 0.94, $P=0.021$) but not significantly different turnover. This suggests that balance rather than turnover may be an important determinant of fracture risk in older women.

OP3005

Accumulated Uremic Toxins Deteriorates Bone Material Properties In Rats With Chronic Kidney Disease

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Background: The risk of hip fracture is significantly elevated in patients with chronic kidney disease (CKD). The contribution of bone mass to bone strength in uremic condition seems less than that in ordinary osteoporotic condition. However, little is known about bone material change in CKD. We investigated

the role of uremic toxins (UTx), which accumulates along with the development of CKD, focusing on bone material properties in experimental CKD rats.
Method: CKD rats without abnormal calcium metabolism were created by 5/6 nephrectomy + thyroparathyroidectomy + PTH supplementation. They were then divided into two groups; those administered oral charcoal absorbent that decreases the circulating levels of UTx (CKD-A), and those received vehicle (CKD-V).
Results: The non-uremic control (C) group, the CKD-A group and the CKD-V group shared comparable bone mineral density levels. Compared with the C group, bone elasticity was significantly deteriorated, mineral and collagen compositions including mineral matrix ratio and non-physiological collagen crosslinks pentosidine were increased, and bone biological apatite orientation was disrupted in the CKD-V group, respectively. These abnormalities were all partially cancelled in the CKD-A group.
Conclusion: Accumulated UTx are likely candidate that promote bone fragility by deteriorating bone elasticity through changing material properties in CKD.

OP3006
Odanacatib Treatment Reduces Remodeling And Stimulates Modeling-based Bone Formation In Central Femur And Lumbar Vertebra Of Adult OVX Monkeys
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Odanacatib (ODN), a selective and reversible Cathepsin K inhibitor has been demonstrated to reduce trabecular (Tb) bone turnover while preserving endocortical (Ec) and stimulating periosteal (Ps) bone formation (BF) in overiectomzied (OVX) monkeys. Here, we investigate the bone site specific mechanism of ODN on bone modeling (Mo) and remodeling (Re). OVX-monkeys (13-19 yrs, *n*=8-11/group) were treated with vehicle (Veh) or ODN (6 or 30mg kg^{-1}, q.d., p.o.) for 21-months. Calcein were given at 12-mo. of dosing. At the lumbar vertebrae (LV) Tb surface, ODN dose-dependently reduced the number of remodeling hemiosteons (Re.Ho.N) without changing wall thickness (Re.W.Th) vs. Veh, and reduced LV.Tb BFR and activation frequency (Tb.AcF). ODN did not change Mo.Ho.N/BS and Mo.W.Th. In the central femur (CF), the high dose of ODN tended to reduce Ec.Re.BFR/BS and AcF, without affecting Re.W.Th. Remarkably, ODN increased Ec.Mo.Ho.N/BS, Mo.AcF, Mo.W.Th and BFR/BS. At Ps surface, ODN dose-dependently increased all BF parameters. The results demonstrate that ODN reduces remodeling and stimulates modeling-based bone formation. While Tb contains predominantly Re hemiosteons, ODN increases the ratio of Mo/Re hemiosteons. The findings could explain the bone site specific actions of ODN on trabecular and cortical surfaces in the monkeys. This unique mechanism of ODN on bone formation clearly differentiates this agent from the standard anti-resorptives.

OP3007
Three-dimensional Morphological Analyses Revealed That Bone Volume Indicates Trabecular Length And Branching In CKD-MBD
Junichiro Kazama[1], *Koji Matsuo*[2], *Suguru Yamamoto*[2], *Kazuko Kawamura*[2], *Minako Wakasugi*[2], *Ichiei Narita*[2]
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Background: Cancellous bone volume (BV/TV) is a practical indicator of cancellous bone connectivity in patients with end-stage CKD. However, why the amount of BV/TV successfully indicates topological parameters remains unexplained. Thus, we attempted a study to reveal the morphological property of cancellous bone microstructure in patients with CKD-MBD, with a special attention to its association with BV/TV.
Methods: Serial computed tomographic images were generated from biopsied iliac bone samples obtained from 46 CKD5D patients (MD 10, OF 11, OM 5, MX 7, AB 13) by a micro-computed tomography system. Quantitative morphological analyses were performed in the reconstructed virtual 3-dimensional images.
Results: BV/TV demonstrated significantly positive correlations with trabecular number (P<0.0001, $r2$=0.789) and fractal dimension (P<0.0001, $r2$=0.692), significantly negative correlations with trabecular separation (P<0.001, $r2$=0.175), structure model index (P<0.0001, $r2$=0.251) and trabecular bone pattern factor (P<0.0001, $r2$=0.436), while did no correlation with trabecular thickness.
Conclusion: So-called trabecular thinning, which is considered to be a morphological characteristic in primary osteoporosis, was not found in patients with CKD-MBD. Therefore, BV/TV was tightly associated with trabecular length and trabecular branching, which could explain why BV/TV successfully indicates 3-dimansional cancellous bone connectivity in CKD patients.

OP3008
Effects Of Salvianolate On Bone Formation And Resorption In Glucocorticoid Treated Sle Mice
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This study was to investigate the effects of salvianolate (Sal), a total polyphenol known as an antioxidant, on bone tissue metabolism in a spontaneous SLE mice model. Fifteen weeks old MRL/lpr mice were treated with vehicle, GC (prednison 6mg/kg/d by oral gavage), salvianolate (60mg kg^{-1} per day by intraperitoneal injection) and GC plus salvianolate respectively for 12 weeks. MRL/lpr mice were very low bone mass with only 1.6% in trabecualr area (Tb.Ar) comparing to wildtype mice

which was with 10% in %Tb.Ar. These mice have damaged glomerulus accompanied with substantial expression of IL-6 in distal femur marrow. GC treatment were inhibited glomerulus inflammation, however, remarkably decreased bone mineral apposition rate (MAR) and bone formation rate (BFR/BV) and percent osteoblast surface, increased distal femur IL-6 expression and osteoclast number when compared to MRL/lpr mice. Salvianolate treatment alone in MRL/lpr mice increased bone mass to 3.1% accompanied with increased in bone formation rate (BFR/TV) and trabeculae osteoblast surface, decreased distal femur IL-6 expression and osteoclast number. Salvianolate treatment in GC treated MRL/lpr mice increased bone mass to 5.2% (increase of 123% in %Tb.Ar) accompanied with increased in bone formation rate and decreased femur IL-6 expression when compared to GC treated MRL/lpr mice.
Conclusion: Salvianolate prevents GC induced depression of bone formation and elevation of bone resorption in a spontaneous SLE mice model.

OP3009
Long Circulating Enzyme Replacement Therapy Rescues Bone Pathology
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Mucopolysaccharidosis (MPS) type VII is a lysosomal storage disease caused by deficiency of the lysosomal enzyme β-glucuronidase (GUS), leading to accumulation of glycosaminoglycans (GAGs). Enzyme replacement therapy (ERT) effectively clears GAG storage in the viscera. Recent studies showed that a chemically modified form of GUS (PerT-GUS) showed prolonged circulation, reduced CNS storage more effectively than native GUS. To evaluate the effectiveness of long-circulating PerT-GUS in reducing the skeletal pathology, we treated MPS VII mice for 12 weeks beginning at 5 weeks of age with PerT-GUS or native GUS and used micro-CT, radiographs, and quantitative histopathological analysis for assessment of bones. I. Micro-CT examination of bones in knee joints of MPS VII mice treated with PerT-GUS II. showed marked improvements compared with those of untreated MPS VII mice. Large clear vacuoles in knee joints are still observed in GUS treated mice, while vacuoles are markedly diminished in PerT-GUS treated mice. The growth plate region in PerT-GUS treated mice showed the following: a) substantial improvement of architecture by reduction of thickened cartilage layer and irregular surface, and b) reduced cell area in the proliferative zone (P = 0.022 native GUS vs. PerT-GUS). These pathological improvements correlated with marked improvements in the clinical bone deformity and radiographs. ERT using long circulating PerT-GUS is highly effective at clearing CNS storage of GAGs.

OP3010
Oliogopeptide Based Sclerostin Inhibitor Abrogated Wnt/b-catenin Signaling Inhibited By Sclerostin In Both MC3T3E1 And INS-1 Cells
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Wnt/b-catenin signaling is involved in pancreas development, islet function, and insulin secretion. SOSTDC1 is expressed in pancreas as well as in bone at early embryonic period. SOSTDC1 has moderate homology with Sclerostin. Both SOSTDC1 and Sclerostin inhibit Wnt/b-catenin signaling by binding to YWTD propeller 1 and 2 of LRP5 or LRP6. Recent report was shown that Sclerostin-neutralizing monoclonal antibodies inhibit binding of Sclerostin to the LRP5 receptor, resulting in the activation of canonical Wnt signaling pathway. In this study, we investigated the inhibitory potency of SOSTDC1, the abrogation capacity of Sclerostin inhibitors on Wnt/b-catenin signaling, and insulin secretion. The effects of known SOST inhibitors were tested with LEF-TCF reporter DNA in MC3T3E1 and INS-1 cells. In SPR analysis, Sclerostin and SOSTDC1 revealed a comparable binding activity to E1 domain of LRP5. Furthermore both Sclerostin and SOSTDC1 could inhibit Wnt3a induced Wnt/b-catenin signaling with comparable potency. Interestingly, a linear peptide did not shows any abrogating activity, meanwhile a cyclized peptide abrogated Wnt/b-catenin signaling inhibited by Sclerostin but not by SOSTDC1, which indicated that the binding site of SOSTDC1 on E1 domain of LRP5 might be little different from that of Sclerostin.

OP3011
Evidence Of Early Bone Resorption In A Sheep Model Of Burn Injury. Implications For The Calcium Sensing Receptor
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Burned children develop hypocalcemic hypoparathyroidism and lose 7% lumbar spine BMD by 6wk post-burn. By 2 wk they have adynamic bone, yet IV bisphosphonates within 10d block bone loss, suggesting early post-burn resorption. High inflammatory cytokines and endogenous glucocorticoids can explain early resorption. Our aim was to assess bone for histologic and biomechanical evidence of early resorption using an established sheep model of burn injury. 4 sheep were burned (B) 40% body surface area under anesthesia; 3 sheep were sham controls (C). 1B & 3C sheep were sacrificed 2d post-burn; 3B sheep were sacrificed 5d post-burn. Iliac crests underwent backscatter

scanning electron microscopy, quantitative histomorphometry, biomechanical compression. 3/3 sheep killed 5d post-burn had bone surface scalloping, an effect of resorption. 0/4 killed at 2d had scalloping, *P*=0.029.1/3 of 5d sheep had quantitative doubling of eroded surface and halving of bone volume vs C.Young's modulus was 1/3 lower in 5d sheep vs C, *P*=0.08, suggesting weaker bone. Data support early post-burn resorption in a sheep model also used to show parathyroid calcium receptor (CaR) up-regulation in the same time frame. Both resorption and CaR up-regulation are mediated by cytokines. We postulate that CaR up-regulation in burns is an adaptive response to prevent hypercalcemia secondary to bone resorption.

OP3012

Soluble Klotho Does Not Rescue But Rather Exaggerates Skeletal Defects In Klotho-Deficient Mice

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Klotho-deficient mice suffer from a syndrome resembling accelerated human aging, including skeletal impairments and chronic renal failure. Klotho is a type I transmembrane protein and expressed in restricted tissues (for example, proximal tubule), while its truncated form in circulation (soluble Klotho, sKL) may be involved in several biological functions. Membrane Klotho forms a complex with FGF receptor and FGF23, a phosphaturic hormone secreted from bone, in kidney to promote FGF23-dependent phosphate wasting. We then assessed whether recruitment of sKL rescues renal and skeletal defects in klotho-deficient mice. Chronic administration of sKL to young male klotho-deficient mice did not alleviate the symptoms of hyperphosphatemia, hypercalcemia and hypervitaminosis D. Also, there was no improvement in the expression of the FGF23 target genes in kidney. However, sKL exaggerated klotho-deficient skeletal phenotypes, such as a reduction in growth plate width and mineral apposition rate. Serum FGF23 was kept at high levels in klotho-deficient mice with or without sKL, and sKL-FGFR-FGF23 complex formation was observed in bone but not in kidney of klotho-deficient mice treated with sKL. sKL decreased Phex gene expression, which was abrogated by anti-FGF23 antibody in an ex vivo model of bone from klotho-deficient mice. Thus, recruitment of sKL exaggerates skeletal defects in klotho-deficient mice. These results suggest that sKL and FGF23 may act cooperatively in bone but not in kidney.