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Poster Presentations

Poster Session 1

P1001
Spatiotemporal Expression Of Tbx18 During Endochondral Bone Formation
Ryuma Haraguchi*, Riko Kitazawa, Sohei Kitazawa*
Division of Molecular Pathology, Ehime University Graduate School of Medicine, Toon, Ehime, Japan

The developmental steps of endochondral bone formation are tightly regulated by the spatial and sequential expression of several transcription factors. Here, we provide new insight into the involvement of Tbx18 gene in the regulation of bone formation. In this study, we characterized its spatial and temporal expression pattern in the limb skeltal region. Tbx18 expression was detected in the condensed mesenchymal cells at E10.5 limb bud, while it became undetectable at E11.5 and E12.5. From stage E13.5 to E18.5, Tbx18 expression reappeared in chondrocytes. Finally at the postnatal stage, Tbx18 expression was observed in epiphyseal chondrocytes and osteocytes within the lacunae of mature trabecular bone. To explain these periodic and spatial Tbx18 expression patterns, on the assumption that Tbx18 gene expression is epigenetically regulated during mouse limb development, we examined the methylation status of the CpG-island in the mouse Tbx18 gene by a the methylation-specific polymerase chain reaction. Hypermethylation of the Tbx18 gene promoter became evident at an early embryonic stages in Tbx18-negaitive cells, and disappeared at a late embryonic stage in Tbx18-positive cells. Based on these data, we speculate that Tbx18 is a key regulator of bone formation during the limbs development, and that an epigenetic mechanism is involved in regulating dynamic change of Tbx18 expression.

P1002
Effect Of Intermittent Administration Of Pth On Spinal Fusion In Rats With Glucocorticoid- Induced Osteoporosis
Tsuyoshi Sugiura*, Masafumi Kashii, Yohei Matsuo, Tokimitsu Morimoto, Hirotsugu Honda, Takashi Kaito, Motoki Iwasaki, Hideki Yoshikawa*
Orthopaedic, Osaka university Graduate School of Medicine, Suita, Osaka, Japan

Objective: To determine the effect of intermittent administration of human parathyroid hormone 1-34 (PTH) on spinal fusion in rats with glucocorticoid-induced osteoporosis (GIO).
Methods: 5mg/kg Methylprednisolone (MP) was administered to male 8-week-old SD rats for 12 weeks. After 6 weeks MP administration, 19 GIO rats underwent posterolateral spinal fusion (L4-5) with iliac crest autograft. After surgery, rats were started on 5 times per week regimen of saline (n=9) or 40μg/kg^{-1} PTH (n=10) for 6 weeks after surgery. Time-course microstructural analysis of fusion mass and adjacent vertebra (L6) were analyzed using *in vivo* microCT at 2, 4, 6 weeks postoperatively. Fusion assessment and histological examination were performed.
Results: In the PTH group, bone volume and other microstructural parameters at fusion mass increased and reached its peak at 4 weeks postoperatively, and these parameters at fusion mass and adjacent vertebra were significantly greater than the saline group at 4, 6 weeks postoperatively. Manual palpation revealed the PTH group had higher fusion rate than the saline group (PTH:90%, saline:56%).
Conclusions: In GIO model animals, intermittent administrations of PTH accelerate bone formation and enhance bone turnover at the graft site, leading to the acceleration of spinal fusion. Moreover, it has protective effects of adjacent vertebral fractures.

P1003
Long-Term Corticosteroid Therapy Systemic Survey
Kawtar Nassar*, Saadia Janani, Wafaa Rachidi, Noufissa Etaouil, Wafaa Mkinsi*
Rheumatology, Casablanca, Morocco

Corticosteroids is a common requirement in practice.
Materials and methods: We conducted a descriptive survey, with 125 patients in Rheumatology hospital university in Casablanca, receiving systemic corticosteroid therapy. The survey was conducted between December 2011 and May 2012.
Results: The mean age was 46.5 years ± 10. Majority recieved prednisone at a dose greater than or equal to 20 mg per day for at least 2 months of treatment. 60% patients recognize be informed about the treatment. During treatment, 44 patients experienced various neuropsychological symptoms (35.2%), to varying degrees, including: irritability (16.8%), insomnia (18.4%), somnolence (6.4%), depression (7.2%, n = 9) with two cases of attempted suicides, headache in four cases, tremor (2 cases), and delusions of persecution in a cas. weight gain (41.6%), 28.8% of complications cutanées.15 cases of osteoporosis have been reported, five cases of fractures, and two cases of osteonecrosis.
Conclusion: Specific support and regular, as well as patient education on long-term systemic corticosteroids should be allocated on a systemic.

P1004

The Role Of Focal Adhesion Kinase And Sonic Hedgehog On The Process Of Fracture Repair

Yuu Horikiri[1]*, Tsuyoshi Shimo*[1]*, Masahiro Iwamoto*[2]*, Naito Kurio*[1]*, Tatsuo Okui*[1]*, Akira Sasaki*[1]
[1]Department of Oral and Maxillofacial Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [2]Division of Orthopedic Surgery, The Children's Hospital of Philadelphia Research Institute, Philadelphia, PA, USA

Introduction: We have previously reported that focal adhesion kinase (FAK) and sonic hedgehog (SHH) have regulatory mechanisms of osteoblast and osteoclast differentiation under the pathological condition in cancer induced bone destruction. However, the relationship and the role of FAK and SHH on the process of normal fracture repair has not been clarified yet.
Methods and results: Immunohistochemical analysis revealed that pFAK Tyr397 and SHH was expressed in bone marrow cells, and pFAK Tyr397 was also detected in ALP positive osteoblasts near the TRAP positive osteoclasts in the rib of mice fractured site on day 3 and 5. pFAK Tyr397 and SHH was detectable in osteoblasts in the fractured site on day 14. SHH upregulated FAK mRNA and pFAK Tyr397 in time dependently in osteoblastic MC3T3-E1cells. 5 lentivirus encoding short hairpin FAK RNAs (shFAK) infected MC3T3-E1 groups have a rounded morphology and were decreased cells proliferation, adhesion, migration and differentiation. SHH stimulated proliferation of MC3T3-E1 cells and osteoclast formation in a co-culture system containing MC3T3-E1 and murine CD11b+ bone marrow cells, but did not effect on the shFAK infected MC3T3-E1 co-culture group.
Conclusion: These findings suggest that SHH derived from bone marrow cells or osteoblasts stimulate proliferation and support of osteoclast formation through pFAK Tyr397 activation in osteoblast in the process of bone fracture repair.

P1005

Deferoxamine Alleviates Unloading-induced Delay Of Bone Defect Repair

Takeshi Matsumoto*, Syota Sato, Daichi Goto*
Osaka University Graduate School of Engineering Science, Toyonaka, Japan

We tested the hypothesis that deferoxamine (DFO), known to stabilize hypoxia inducible factor-1α expression, reduces the unloading-induced delay of bone repair via improving angiogenesis. Rats (♀, 12 wk) were given a drill-hole surgery on a tibial diaphysis and, soon after surgery, subjected to no treatment (C), hindlimb unloading (HU), or HU with the alternate-day administration of 3-μg DFO to the defect site (HU-DFO). On postoperative day 5 (DAY5: C, n=10; HU, n=13; HU-DFO, n=11) or 10 (DAY10: C, n=10; HU, n=11; HU-DFO, n=11), each rat was perfused with a zirconia vascular casting agent from the abdominal aorta, euthanized, and immersed in cold water to solidify the agent. The defect site was scanned by synchrotron lights (SPring-8, Harima, Japan), and taking advantage of the zirconium k-edge, vascular and bone images were obtained by subtraction CT with 2.74-μm voxel resolution. At DAY5, bone volume fraction (%BVf) was higher in HU-DFO (1.5±0.4) than in HU (0.3±0.1) (p<0.05), and these values were lower than 3.6±0.8 in C (p<0.05). Vascular volume fraction (%VVf) tended to be lower in HU (4.5±1.3) than in both C (7.4±2.0) and HU-DFO (8.3±1.2). At DAY10, %BVf was similar between HU-DFO (40±1) and C (44±2) but lower in HU (26±2) than in C (p<0.05). %VVf did not differ between HU-DFO (6.2±0.8) and HU (5.0±0.7), and these values were lower than 8.1±0.9 in C (p<0.05). These results suggest that the delay of bone defect repair under HU is alleviated by DFO-promoted angiogenesis.

P1006

Topological Expression Of Sfrp4 During Fetal Bone Formation

Ryosuke Tachibana[1]*, Ryuma Haraguchi*[1]*, Yuri Kameoka*[1]*, Kanazu Ariyasu*[1]*, Kiyoshi Mori*[2]*, Sohei Kitazawa*[1]*, Riko Kitazawa*[1]
[1]Division of Molecular Pathology, Ehime University Graduate School of Medicine, Toon, Ehime, Japan; [2]Division of Diagnostic Molecular Pathology, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan

Secreted Frizzled-related protein 4 (Sfrp4) is a member of the secreted Wnt antagonist family gene that interacts directly with Wnt-ligand, and antagonizes both canonical and non-canonical Wnt-β-catenin signaling. In adult mice, Sfrp4 expression is observed mainly in the periosteum, and, reflecting its antagonistic effect against β-catenin, its overexpression in osteoblasts suppresses osteoblast proliferation *in vivo*. In this study, we engineered an Sfrp4-LacZ mouse strain in which the bulk of the coding region of the Sfrp4 gene was replaced by the β-galactosidase (LacZ) reporter gene. The generation of Sfrp4-LacZ mouse provides the advantage, for *in vivo* studies of Sfrp4, of the highly sensitive and easy in situ detection of LacZ gene expression. We focused on spatial and temporal Sfrp4 expression patterns in the developing limb. Until stage E16.5, LacZ expression was not detected in the limb bud tissues; it became evident only in a restricted area of the trabecular bone region at E17.5. At the neonatal stage (P0), strong LacZ expression was observed on osteoblastic cells lining the trabecular bone surface of the long bone shaft; however, LacZ positive cells were not detected on periosteal osteoblastic cells or on cells in the articular cartilage. These characteristic spatiotemporal expression patterns of Sfrp4 suggest that Sfrp4 may play a role in the maintenance and remodeling phase of trabecular bone, but has little effect during fetal skeletal bone formation.

P1007

Predominant Expression Of Histone 3 Lysine 9 Methyltransferases In The Prehypertrophc Chondrocytes During The Growth Plate Chondrocyte Development

Hisashi Ideno[1,2]*, Akemi Shimada*[1]*, Taichi Kamiunten*[1]*, Kazuhiko Imaizumi*[2]*, Kazuhisa Nakashima*[1]*, Akira Nifuji*[1]
[1]Pharmacology, Tsurumi University, School of Dental Medicine, Yokohama, Japan; [2]Waseda University, Tokorozawa, Saitama, Japan

Epigenetic modifications, including methylation and/or acetylation of histone tails, play important roles in cell fate decision

and differentiation during development. Among them, Histone 3 Lysine 9 (H3K9) modifications regulate gene expression and chromatin structure. Mono-, di-, and tri- methylated states of H3K9 are regulated by H3K9 methyltransferases (H3K9MTases), such as G9a, GLP and SETDB1. Since G9a-, GLP-, and Sestb1-null mice all show embryonic lethality, their functions in organogenesis and later mouse embryogenesis are unclear. In this study, we examined distributions of H3K9MTases as well as H3K9me1, me2 and me3 during the growth plate chondrocytes development. Immunohistochemistry revealed that H3K9MTases as well as methylated H3K9 were scarcely detected in forelimb cartilage primordia at E12.5. G9a, GLP, and SETDB1 were detected at low levels in proliferating chondrocytes and at high levels in prehypertrophic and hypertrophic chondrocytes at E14.5 and 16.5. Distributions of H3K9me1 and me3 were over-lapped with those of H3K9 H3K9MTases. These results suggest that the H3K9MTases G9a, GLP, and SETDB1 are expressed predominantly in prehypertrophic and hypertrophic chondrocytes and that they could regulate gene expression and progression of chondrocyte differentiation through modification of H3K9 during the growth plate chondrocyte development.

P1008

Bruton Tyrosine Kinase Suppresses Osteoblastic Differentiation

***Shoichi Kaneshiro**, Chikahisa Higuchi, Kosuke Ebina, Kenrin Shi, Hideki Yoshikawa*
Orthopaedics, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan

Objectives: The Tec family of nonreceptor tyrosine kinases have been shown to play a key role in inflammation and bone destruction. Bruton tyrosine kinase (Btk) has been the most widely studied due to the critical role of this kinase in B-cell development and recent evidence showing that blocking Btk signaling is effective in ameliorating lymphoma progression and experimental arthritis. The role of Btk in osteoblastic differentiation has not been well elucidated. We report herein the role of Btk in osteoblast differentiation.

Methods: We investigated the effects of PCI-32765, a Btk inhibitor and knockdown of Btk on osteoblast differentiation in mouse preosteoblastic MC3T3-E1 cells and primary calvarial osteoblasts.

Results: Expression of Btk was detected in these two cells. Btk inhibitor stimulated alkaline phosphatase (ALP) activity and mRNA expression of osteoblastic markers. Mineralization of extracellular matrix was also promoted by treatment with Btk inhibitor. Knockdown of Btk caused increased ALP activity and mRNA expression of osteoblastic markers. In addition, Btk inhibitor suppressed phosphorylation of mitogen-activated protein kinase (MAPK) and Akt. Our results indicate that Btk might regulate osteoblast differentiation through MAPK and Akt. Btk inhibitor would be of potential use for the treatments of osteoporotic change in RA patients since it possess not only anti-inflammatory effect which has been reported but also pro-osteoblastic effect.

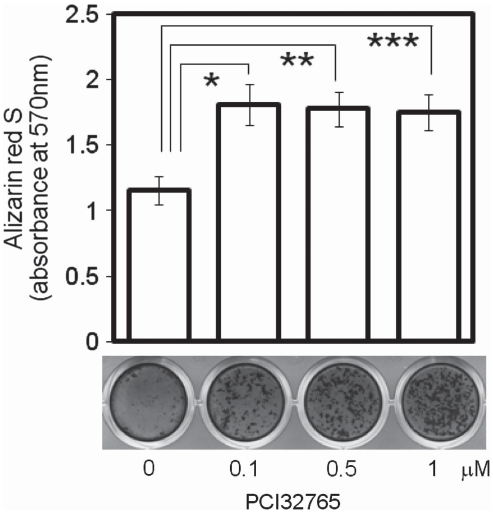


Figure 1 Effects of PCI32765(Btk inhibitor) on matrix mineralization in MC3T3-E1 cells.

P1009

Canonical Wnt Signaling Activates Mir-34 Expression During Osteoblastic Differentiation

***Masato Tamura**[1], Maki Uyama[1,2], Mari Sato[1], Masamitsu Kawanami[2]*
[1]Hokkaido University, Grad. Sch. Dent. Med, Biochem.Mol. Biol., Sapporo, Japan; [2]Hokkaido University, Grad. Sch. Dent. Med., Periodont. Endodont., Sapporo, Japan

Wnt signaling and bone morphogenetic protein (BMP)-2 signaling are critical roles in osteoblast differentiation. microRNAs (miR) represent a class of ~22 nucleotide noncoding RNAs that regulate gene expression by targeting mRNAs for cleavage or translational repression. Recent studies have revealed important roles for miRs in the regulation of gene expression on osteoblasts. In a previous study, we indicated that miR-206 of muscle-specific miRNA expression is down-regulated by BMP-2 at a post-transcriptional level in C2C12 cells. In this study, we performed microRNA profiling using Wnt3a-C2C12 cells and BMP-2-treated C2C12 cells. We identified miR34b and miR34c which are up-regulated by activation of canonical Wnt or BMP-2 signaling in C2C12 cells. Expression of mature miR-34 increased from lower levels at day 0 to maximum levels on day 28 of MC3T3-E1 cell differentiation. The miR34 expression also significantly increased after treatment with bortezomib, a 26S proteasome inhibitor dose-dependently. To examine the effects of these miRs on osteoblast differentiation, antisense inhibitor of miR was transfected to MC3T3-E1 cells and examined osteoblast-related gene expression. Knock-down of miR34 inhibited alkaline phosphatase mRNA expression and activities. These present studies indicated the possibility that miR-34 regulates gene expression by targeting negative regulators of osteogenic pathways and thereby contributes to osteoblast differentiation.

P1010

Impaired Bone Mineralization In Transgenic Medaka Fish Expressing Fra-1 In Osteoblasts

Tomohiro Matsumoto[1,5], *Yasunari Takada*[1], *Takashi Nakamura*[2], *Makoto Suematsu*[2], *Keiji Inohaya*[3], *Akira Kudo*[3], *Atsushi Shimizu*[4], *Koichi Matsuo*[1]

[1]Laboratory of Cell and Tissue Biology, School of Medicine, Keio university, Tokyo, Japan; [2]Department of Biochemistry, School of Medicine, Keio University, Tokyo, Japan; [3]Department of Biological Information, Tokyo Institute of Technology, Tokyo, Japan; [4]Department of Molecular Biology, School of Medicine, Keio University, Tokyo, Japan; [5]School of Medicine, Tokai University, Kanagawa, Japan

In vertebrates, bone mineralization is tightly regulated by osteoblasts to maintain mechanical properties of bone and calcium-phosphate metabolism. In mice, overexpression of the transcription factor Fra-1 in the whole body increases bone mass and decreases bone mineralization. However, the cell-autonomous function of Fra-1 in osteoblasts across vertebrate evolution remains unclear. Here, we expressed mouse Fra-1 in osteoblasts in medaka, which is largely transparent during development allowing live imaging of osteoblasts and vital staining of the skeleton. We first employed in silico analysis to predict that exogenous mouse Fra-1 could heterodimerize with medaka Jun proteins to form the transcription factor AP-1. We then generated transgenic medaka expressing mouse Fra-1 in osteoblasts to investigate a cell autonomous Fra-1 function *in vivo*. When we quantified expression of potential transcriptional targets of Fra-1 in embryos and larvae, we observed altered expression patterns indicative of perturbed bone mineralization. Fra-1 transgenic medaka also showed significantly lower fluorescence intensities than did wild-type controls when stained for alizarin complexone. Consistently, in micro-computed tomography, Fra-1 transgenic medaka showed lower bone mineral density than did wild-type controls (Figure). These data suggest that Fra-1 expression in osteoblasts impedes biomineralization, and that in vertebrates Fra-1/AP-1 activity in osteoblasts counteracts hypermineralization.

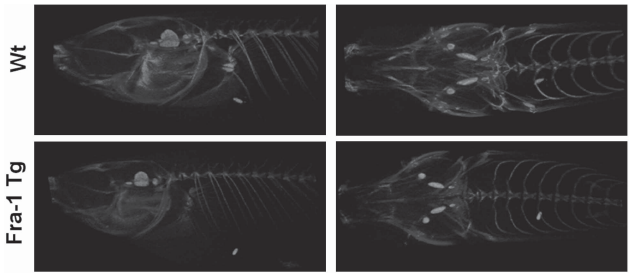


Figure 1 Micro CT images

P1012

Rac1 Suppresses Bmp-2-induced Osteoblastic Differentiation

Megumi Onishi[1,2], *Yuki Fujita*[1], *Hideki Yoshikawa*[2], *Toshihide Yamashita*[1]

[1]Department of Molecular Neuroscience, Graduate School of Medicine, Suita, Osaka, Japan; [2]Department of Orthopedic Surgery, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan

Small G proteins of the Rho family are pivotal regulators of several signaling networks. Ras homolog family (Rho) and one of its targets, Rho-associated protein kinase (ROCK), participate in a wide variety of biological processes, including bone formation. A previous study has demonstrated that the ROCK inhibits osteogenesis by recombinant human bone morphogenetic protein-2 (BMP-2) *in vivo* and *in vitro*. However, the effect of other Rho family members, such as Ras-related C3 botulinum toxin substrate 1 (Rac1) and cell division cycle 42 (Cdc42), on bone formation remains unknown. In this study, we investigated whether Rac1 also participates in BMP-2-induced osteogenesis. Expression of a dominant negative mutant of Rac1 enhanced BMP-2-induced osteoblastic differentiation in C2C12 cells, while a constitutively active mutant of Rac1 attenuated that effect. The Rac1 inhibitor NSC23766 enhanced BMP-2 induced osteoblastic differentiation in C2C12 cells. Knockdown of T lymphoma invasion and metastasis 1 (Tiam1), a Rac-specific guanine nucleotide exchange factor, enhanced BMP-2-induced alkaline phosphatase activity. Furthermore, we demonstrated that BMP-2 stimulated Rac1 activity. These results indicate that the activation of Rac1 attenuates osteoblastic differentiation in C2C12 cells.

P1013

The Study Of Adhesion Of Mouse Mesenchymal Cells On Titanium Plates With Various Surface Modifications

Michiko Nakatsuka[1], *Shunji Kumabe*[1], *Akihiro Hosoya*[2], *Yoshiya Hashimoto*[1], *Chizuko Inui-Yamamoto*[1], *Chunying An*[1], *Katsura Ueda*[1], *Aiko Morishita*[1], *Yasutomo Iwai*[1]

[1]Osaka Dental University, Hirakata, Osaka, Japan; [2]Matsumoto Dental University, Matsumoto, Japan

Objective: The present study was performed mainly on the Scanning Electron Microscopy (SEM) and immunochemical histology of initial osseointegration mimic the dental implant therapy.

Methods: We used the three types of titanium plates in this study (α-β type (Ti-6Al-4V) titanium (Ti) alloy subject to anodic oxidization (POI; JMM) or hydroxyapatite coating (HAC; JMM), and finely-blasted JIS type 4 (99%) Ti (SPI; THOMMEN/Morita)). Isolated immature mouse mesenchymal cells (MMSC) were seeded and cultured with the discs in conditioned

POWEREDBY 10 (GP Bio Sciences) in 12-well plates (ascorbic acid+β-glycerophosphate+dexamethazone, 100 000 cells per ml), thereby the cells proliferated and were induced to differentiate into mature osteoblast-like cells (Obs).
Results: In the 180-minutes specimens, we observed that the Obs proliferated and spread into flat and large polygonal proper cells. In addition, the immunohistochemistry of the attached Obs demonstrated the expression of F-actin and CD51 in 180-minutes specimens.
Conclusion: The results suggest that the MMSC attach on Ti discs with different microtextures within 180 minutes.

P1014
The Effects Of Dickkopf4 On The Proliferation, Differentiation, And Apoptosis Of Osteoblasts
***Shiro Hiramitsu**, Masakazu Terauchi, Toshiro Kubota*
Department of Comprehensive Reproductive Medicine, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan

Background: The Dickkopf family, comprising four members (Dkk1/2/3/4), is known to modulate Wnt/β-catenin signaling. Although the effects of Dkk1 on the Wnt/β-catenin signaling in osteoblasts are well studied, those of the other members, especially Dkk4, remain unclear.
Method: We examined the effects of Dkk4 on osteoblasts comparing normal MC3T3-E1 cells with their counterparts whose expression of Dkk4 was reduced by siRNA in the following experiments: proliferation (BrdU assay); osteoblast differentiation (CFU-ALP assay); expression of bone formation markers and osteoblast-specific transcription factors (qRT-PCR); apoptosis (TUNEL assay); transcriptional activity of TCF (luciferase assay); expression of Wnt-target genes (qRT-PCR). We also investigated the role of Dkk4 in pre- and post-menopausal women by assessing the correlation between the serum levels of Dkk family members and bone metabolism markers.
Result: The reduction of Dkk4 expression promoted osteoblasts proliferation and differentiation, while it inhibited their apoptosis. With decrease in Dkk4, the expression of Wnt-target genes was up-regulated through β-catenin/TCF pathway. In the clinical study, bone metabolism markers were positively correlated with the serum levels of Dkk4, but not with those of the other Dkk member including Dkk1.
Conclusion: Our findings suggest that Dkk4 inhibits bone formation through Wnt/β-catenin signaling and is related to the increased bone turnover in post-menopausal women.

P1015
Intracellular Ph Levels Regulate The Amount Of Available Voltage-Gated Proton Channels In The Plasma Membrane Of Murine Osteoclasts
***Miyuki Kuno**, Hiromu Sakai*
Physiology, Osaka City University, Osaka, Japan

Osteoclasts express voltage-gated proton channels (H+ channels) in the plasma membrane. Once activated, H+ channels could secrete massive amounts of protons into the extracellular space. The throughput is regulated primarily by transmembrane voltage- and pH- gradients which are essential for the gating and the driving force for H+ permeation. However, the current amplitudes fluctuate often even when the voltage- and pH gradients are constant. Probably the number of readily-available channels could change from time to time in response to cellular conditions. In this study, we examined whether the intracellular pH level (pHi) contributed to regulation of the amount of available H+ channels in RAW264-derived osteoclasts. Exposure to NH_4Cl (2-20 mM) increased pHi and accordingly decreased the H+ currents in dose-dependent manner. Washings of NH4Cl returned the pHi to the control level. However, the H+ conductance recovered only partially when the NH_4Cl exposure lasted for >5 min. Gating kinetics and the voltage-dependence for activation remained unchanged. It is likely that the number of available channels were reduced during the elevation of pHi. This reduction of the H+ conductance by the NH_4Cl exposure was accompanied by decreases in the cell surface area. Dynasore, a dynamin blocker, blocked the NH_4Cl-induced endocytosis. These data suggest that elevation of pHi decreased the number of available H+ channels and that membrane dynamics is involved in the process.

P1016
Antimicrobial Peptide Cramp Suppresses Osteoclast Formation Induced By Lps And Flagellin In Mouse Cocultures Of Osteoblasts And Hematopoietic Cells
***Kannji Horibe**[1], Yuko Nakamichi[2], Shunsuke Uehara[1], Midori Nakamura[1], Naoyuki Takahashi[2], Nobuyuki Udagawa[1,2]*
[1]Department of Biochemistry, Matsumoto Dental University, Shiojiri, Japan; [2]Institute for Oral Science, Matsumoto Dental University, Shiojiri, Japan

Toll-like receptors (TLR) and antimicrobial peptides contribute to the host defense. An antimicrobial peptide, cathelicidin-related antimicrobial peptide (CRAMP, encoded by the camp gene) not only kills bacteria but also binds to LPS and neutralizes its activities. CRAMP binds to formyl peptide receptor 2 (FPR2) and modulate a broad range of activities of neutrophils and monocytes. Here, we examined the role of CRAMP in murine osteoclastogenesis. FPR2 mRNA was expressed in bone marrow macrophages (BMMs, osteoclast precursors) but not in osteoblasts. The expression of FPR2 mRNA in BMMs was down-regulated by RANKL. Interestingly, osteoblasts enhanced the camp mRNA expression in response to LPS but not other osteotropic factors such as $1\alpha,25(OH)_2D_3$ and PTH. Osteoclastogenesis induced by LPS and flagellin in co-cultures of osteoblasts and BM cells was inhibited by CRAMP. However, CRAMP failed to inhibit osteoclastogenesis in the co-cultures induced by $1\alpha,25(OH)_2D_3$, PTH and other TLR ligands. CRAMP showed no effect on osteoclastogenesis in BMM cultures treated with RANKL and M-CSF. CRAMP showed the inhibitory effect on the survival of osteoclasts supported by LPS but not RANKL. CRAMP also inhibited TNFα production in macrophages treated with LPS and flagellin but not with other TLR ligands. These results suggest that CRAMP neutralizes LPS and flagellin probably through the direct binding, and that CRAMP induction by LPS in osteoblasts alleviates LPS-induced bone loss.

P1017

Denosumab And Odanacatib As Reference Inhibitors Of Osteoclast Differentiation And Activity In Human Osteoclast Cultures

***Katja Fagerlund**, Jukka Rissanen, Jussi Halleen*
Pharmatest Services Ltd, Turku, Finland

Human osteoclasts can be generated from bone marrow-derived CD34+ mesenchymal stem cells in the presence of M-CSF and RANKL. We have developed a human osteoclast culture system and studied the potential use of the RANKL inhibitor denosumab and the cathepsin K inhibitor odanacatib as reference inhibitors of osteoclast differentiation and activity, respectively. CD34+ human osteoclast precursor cells were cultured on bovine bone slices for 7 days. Different concentrations of denosumab were added in the cultures at day 0, and TRACP 5b activity was measured in the culture medium collected at day 7 as an index of the number of formed osteoclasts. Osteoclast activity was studied by allowing the formed mature osteoclasts to resorb bone during an additional 3-day culture period. Odanacatib was added into the cultures at day 7, and the amount of CTX-I was measured in the culture medium collected at day 10 to quantitate bone resorption during days 7-10. Denosumab and odanacatib showed strong concentration dependent inhibition of osteoclast differentiation and activity, respectively. Denosumab inhibited also osteoclast activity, suggesting that RANKL inhibition blocks both osteoclast maturation and function. We conclude that denosumab and odanacatib are useful reference compounds of human osteoclast differentiation and activity, respectively, and the used human osteoclast culture system is a reliable tool for identifying new osteoporosis drug candidates with anti-resorptive activity.

P1018

Bovine Lactoferrin Inhibits Differentiation Of Osteoclasts And Prevents Bone Loss In Ovariectomized Rats

***Tadashi Ninomiya**[1], Akihiro Hosoya[2], Toru Hiraga[2], Masanori Koide[1], Hiroaki Nakamura[2]*
[1]Institute for Oral Science, Matsumoto Dental University, Nagano, Japan; [2]Department of Histology and Cell Biology, Matsumoto Dental University, Nagano, Japan

Bovine lactoferrin (bLF), an iron-binding glycoprotein belongs to the transferring family has the inhibitory effects for not only inflammation and microbial activities, but for bone loss in osteoporosis. Whereas it is considered that bLF exert inhibition to bone resorption rather than promotion to bone formation in bone metabolism, the mechanism which bLF suppresses bone loss is remained to be elucidated. In this study, to clarify this, we examined the effects of bLF in osteoclast differentiation and function. The ovariectomized (OVX) rat with orally administration of bLF at 10 and 100 mg kg^{-1} were prevented bone loss, compared with OVX rats received saline. Osteoclast number was significantly decreased by bLF. Bone formation rate was slightly increased by bLF. *In vitro* studies using bone marrow cells showed that bLF reduced the mRNA expressions of cathepsin K, calcitonin receptor, and nfatc1, and inhibited osteoclast differentiation. In survival and pit assay, bLF down-regulated the survival of osteoclasts and reduced number of resorption pits on dentin slice. Lipoprotein receptor related protein 1 (LRP1) as a receptor of lactoferrin expressed in bone marrow macrophage with the stimulation of RANKL. This result indicates that bLF acts to osteoclast precursors with RANKL stimuli. These results collectively suggest that bLF inhibits differentiation of bone marrow macrophage into osteoclasts and function of mature osteoclasts. These roles of bLF may prevent bone loss in OVX rats.

P1019

Estrogen Is Involved In Osteoclast Activity But Neither In Osteoclastogenesis Nor In Osteoclast Apoptosis In Avian Medullary Bone

***Shinji Hiyama**[1], Yumiko Kadoyama[2], Takashi Uchida[1]*
[1]Department of Oral Biology, Hiroshima University Institute of Biomedical & Health Sciences, Hiroshima, Hiroshima, Japan; [2]Faculty of Dentistry, Hiroshima University, Hiroshima, Japan

Avian medullary bone (MB) is quickly being remodeled in the bone marrow cavity during oviposition cycle under the control of circulating estrogen. Briefly, MB is vigorously resorbed by osteoclasts at low levels of serum estrogen. When estrogen levels are elevated, bone formation rather than bone resorption is activated in MB. Furthermore, a single injection of estrogen (E2) to mail Japanese quails causes MB formation and subsequent MB resorption during a couple of days. Estrogen is known to promote apoptosis in mammalian osteoclasts, while it does not in avian MB osteoclasts. To address this discrepancy, we examined the effects of estrogen on MB osteoclasts in the male quail model. 17β-estradiol (17β) did not affect RANKL-induced osteoclast formation while it inhibited bone resorption *in vitro*. To further assess the effects of E2 on avian MB osteoclasts, MB with active osteoclasts were reexposed to E2 *in vivo*. In these quails, osteoclasts on the surface of MB gradually indicated a flattened morphology and Howship's lacunae were not present. The apoptotic response observed in bone marrow cells (BMCs), but not in these osteoclasts. Additionally, there was no difference in the number of RANK-positive cells, monocytes/macrophages and apoptotic BMCs after E2 reexposure. These results suggest that estrogen may be not involved in osteoclastogenesis and osteoclast apoptosis and rather suppress osteoclast activity in avian MB.

P1020

Arctigenin Inhibits Transcriptional Activity Of Nfatc1 By Its Nuclear Translocation-independent Mechanism

***Teruhito Yamashita**[1], Shunsuke Uehara[2], Nobuyuki Udagawa[2], Yasuhiro Kobayashi[1], Naoyuki Takahashi[1]*
[1]Institute for Oral Science, Matsumoto Dental University, Nagano, Japan; [2]Department of Biochemistry, Matsumoto Dental University, Nagano, Japan

NFATc1 induced by RANKL in osteoclast precursors is an essential transcription factor for osteoclastogenesis. The translocation of cytosolic NFATc1 into the nucleus is an important step to enhance its transcriptional activity, and is regulated by the Ca-dependent phosphatase calcineurin. Recently, we found

that a small compound arctigenin inhibited RANKL-induced osteoclast formation from bone marrow macrophages (BMMs) in culture. Here, we investigated the mechanism of the inhibitory action of arctigenin in osteoclastogenesis. Arctigenin induced nuclear translocation of NFATc1 in BMMs in the presence and absence of RANKL. However, the expression of osteoclast markers such as TRAP, cathepsin K, and OSCAR in BMMs was suppressed by adding arctigenin. ChIP assay revealed that arctigenin inhibited the recruitment of NFATc1 to the promoter of the Oscar gene, a target of NFATc1. Cyclosporin A (CsA), a calcineurin inhibitor, also inhibited osteoclast formation in BMM cultures treated with RANKL. The inhibitory effect of CsA on osteoclast formation was rescued by forced expression of a constitutively activated NFATc1 mutant (caNFATc1) in BMMs. In contrast, the forced expression of caNFATc1 failed to rescue the arctigenin-induced suppression of osteoclastogenesis. These results suggest that arctigenin does not inhibit nuclear translocation of NFATc1, but strongly inhibits the transcriptional activity of NFATc1, and that arctigenin is a new type of agent for preventing bone loss.

P1021

Rank Harbors The Specific Cytoplasmic Domain That Regulates Osteoclastogenic Signaling And Its Subcellular Localization

Yuu Taguchi*, Jun-ichiro Inoue*
Division of Cellular and Molecular Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Osteoclasts play a crucial role in bone homeostasis in concert with osteoblasts, and RANK signaling is essential for osteoclastogenesis. Recently, we identified a novel domain in the cytoplasmic tail of RANK, named HCR. HCR functions as a platform for formation of signal complex, and emanates sustained RANK signaling, which is essential for osteoclastogenesis. However, the molecular mechanism of the HCR-mediated signals remains to be elucidated. Here we report that HCR regulates subcellular localization of RANK. Our immune-fluorescence microscopic analysis revealed that RANK was localized on plasma membrane before RANKL-stimulation, and became localized on plasma membrane and peri-nuclear region within 24 hours after stimulation. However, RANK mutant lacking HCR was retained on plasma membrane even at 24 hours after stimulation, indicating that HCR changes subcellular localization of RANK from plasma membrane to peri-nuclear region. We also found that HCR similarly regulates subcellular localization of RANK when precursor cells were co-cultured with stroma cells. Because the co-culture system is thought to mimic physiological osteoclastogenesis, these results strongly suggest that HCR regulates subcellular localization of RANK *in vivo*. We are currently investigating whether the signal complex formation on HCR can occur at the peri-nuclear region. Novel mechanisms of RANK signaling that involves specific subcellular localization will be discussed.

P1023

Osteoblasts Migrate Into Collagen Gel And Differentiate To Osteocyte-Like Cells: A Novel System For Analyzing Osteoblastic Terminal Differentiation

Kazuyoshi Uchihashi*, Shigehisa Aoki, Aki Matsunobu, Mihoko Yamamoto, Shuji Toda*
Department of Pathology & Microbiology, Saga University, Saga, Japan

Osteoblasts are believed to differentiate into osteocytes, becoming embedded in bone, or to undergo apoptosis after the bone matrix synthesis. The regulation of this terminal differentiation seems to be critical for both bone homeostasis and development of bone-forming reagents. However the mechanism remains unclear and there is no assay system currently available to analyze this process. To address this issue, we developed a new model in which osteoblasts are cultured on a type I collagen gel layer with osteogenic supplements. Osteoblasts gradually migrated into the gel, produced collagen fibrils, and differentiated to osteocytic cells with bone lacune- and canaliculi-like mineralization. Osteocalcin, DMP-1 and SOST protein expression was mainly expressed in the migrated cells within the mid-layer of the gel. Osteoblastic and osteocytic mRNA expression was significantly increased compared with those of the cells cultured on plastic dishes alone. The number of TUNEL-positive apoptotic cells gradually increased. The cells were distributed at the surface and in the mid-layer of the gel at 7 days and after 14 days of culture, respectively. These data indicate that our model reproduces transition from osteoblasts to osteocytes, suggesting the following: 1) migration of osteoblasts into collagen gel may play a critical role in osteocytic differentiation; and 2) spatiotemporal gene expression and apoptosis may be involved in the terminal differentiation of osteoblasts.

P1024

Extracellular Inorganic Phosphate Up-regulates The Expression Of Dentin Matrix Protein-1 Via Mek/erk Pathway

Jin Nishino*[1,3], Masanobu Kawai[1], Kazuaki Miyagawa[1,3], Miwa Yamazaki[1], Kanako Tachikawa[1], Mikihiko Kogo[3], Keiichi Ozono[2], Toshimi Michigami[1]*
[1]Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan; [2]Osaka University Graduate School of Medicine, Suita, Japan; [3]Osaka University Graduate School of Dentistry, Suita, Japan

Dentin matrix protein-1 (Dmp1) is an extracellular matrix protein of SIBLING family, and is expressed mainly in osteocytes. In an attempt to examine the effects of extracellular inorganic phosphate (Pi) on the gene expression of osteocytes, we found that an increase in extracellular Pi markedly elevated the expression of Dmp1 in osteocytes isolated from mouse bones. In the current study, we further investigated the mechanism

by which Pi regulates the expression of Dmp1. We treated a murine osteoblastic cell line MC3T3-E1 cells with various concentrations (1-10 mM) of extracellular Pi for 48h, and found that expression of Dmp1 and phosphorylation of ERK1/2 were induced by the increased extracellular Pi in a dose-dependent manner. Then, we examined the time course of Dmp1 up-regulation and ERK1/2 phosphorylation by harvesting the cells at 0, 0.5, 1, 6, 12, 24 and 48 h after treatment with 10 mM Pi. The phosphorylation of ERK1/2 reached the maximum at 1 h, while the marked increase in Dmp1 expression was observed at 12 h and thereafter. Interestingly, treatment with U0126, a MEK inhibitor, abolished the up-regulation of Dmp1 expression by the increased extracellular Pi. As to the expression of *Fam20C* encoding a secreted kinase that phosphorylates Dmp1, it was not altered by an increase in extracellular Pi. Thus, signaling by extracellular Pi leads to increased expression of Dmp1 via MEK/ERK pathway, which might be involved in the transition of osteoblasts to osteocytes.

P1026

Osteoclast Differentiation And Activation From Bone Marrow Cells Through The Stimulation Of Osteocytes On Surface-charged Calcium Apatite

Miho Nakamura[1], *Teuvo Hentunen*[2], *Jukka Salonen*[2], *Ken-ichi Nakahama*[1], *Ikuo Morita*[1], *Kimihiro Yamashita*[1]
[1]Tokyo Medical and Dental University, Tokyo, Japan;
[2]University of Turku, Turku, Finland

Apatite biomaterials are used for clinical applications as bone graft to reconstruct bone tissue in defects. We designed the osteocyte-stimulating biomaterials to be free from addition of growth factors. The purpose of this study is to investigate the osteoclast induction through interaction with osteocyte. MLO-Y4 cells kindly gifted from Prof. Bonewald were cultured on three types of the synthesized apatite surfaces, neutral, negative and positive surfaces, and additionally bone slice as a control. After 1d culture, bone marrow cells (BMC) isolated from mouse femur and tibiae were added and cultured for 7 and 14d. The cells were fixed and stained for TRAP or fluorescence for actin and nuclei. After the removing the cells, the resorption pits were observed and quantified using laser microscope. The TRAP-positive giant cells with multiple nuclei were formed both on the apatite and bone specimens after co-culture of MLO-Y4 with BMC. The number of TRAP-positive multinuclear cells and actin rings were statistically higher on the positive surface compared to neutral and negative surfaces. The resorption pits on positive surface were deeper than that of neutral and negative apatite surfaces. The possible explanations of the enhanced differentiation and activation on the positive surface are the cell-to-cell interactions and secretion of differentiation factors from MLO-Y4 cells.

P1027

The Early Mouse 3D Osteocyte Network In The Presence And Absence Of Mechanical Loading

Yasuyo Sugawara, *Hiroshi Kamioka, Yoshihito Ishihara, Naoko Fujisawa, Noriaki Kawanabe, Takashi Yamashiro*
Department of Orthodontics, Okayama University Graduate School, Okayama, Japan

Osteocytes are considered to act as mechanosensory cells in bone. In this study, we characterized the three-dimensional (3D) formation of the osteocyte network during bone growth and mechanical loading. In order to evaluate the effect of mechanical

[P1026]

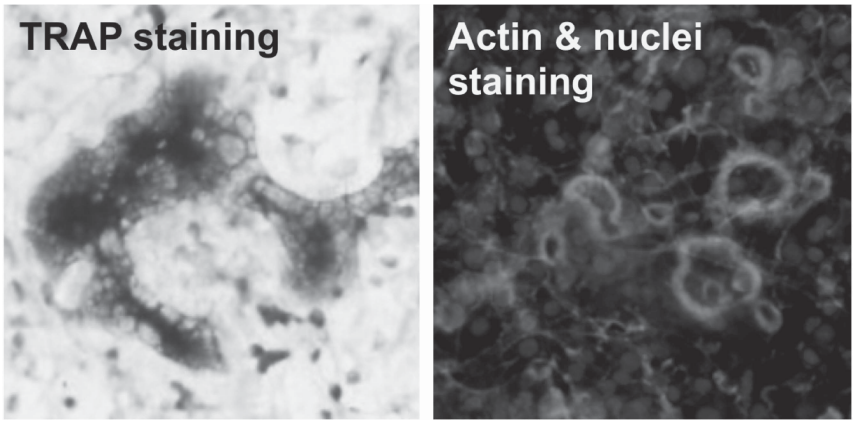


Figure 1 TRAP-positive and multinuclear cells with actin rings were formed on the synthesized apatite surface after co-culture of MLO-Y4 cells with bone marrow cells.

unloading, we subjected newborn mice to sciatic neurectomy in order to immobilize their left hind limb as an unloading model. The osteocyte network was visualized by staining osteocyte cell bodies and processes with fluorescently labeled phalloidin. We compared the osteocyte network in the femora of embryonic and 6-week-old mice in order to understand the morphological changes that occur with normal growth and mechanical loading. In embryonic mice, the osteocyte network in the femur cortical bone displayed a random cell body distribution, non-directional orientation of cell processes, and irregularly shaped cells. In 6-week-old mice, the 3D network contained spindle-shaped osteocytes, which were arranged parallel to the longitudinal axis of the femur. In addition, more and longer cell processes radiated from each osteocyte. The osteocyte network formation in both cortical bone and cancellous bonewas affected by mechanical loading. However, there were differences in the extent of network formation between cortical bone and cancellous bone in response to mechanical loading with regard to the orientation, nuclear shape and branch formation.

P1028

Changes In Sclerostin Localization In Alveolar Bones During Tooth Displacement

Ryuta Watanabe[1]*, Keita Aoki*[2]*, Atsushi Fujiwara*[2]*, Wataru Yano*[1]*, Kazuhiko Satoh*[1]*, Yasutoku Kogaya*[1]*, Noriyuki Kitai*[2]*, Sadakazu Ejiri*[1]
[1]Asahi University School of Dentistry, Department of Oral Anatomy, Mizuho, Gifu, Japan; [2]Asahi University School of Dentistry, Department of Orthodontics, Mizuho, Gifu, Japan

In order to elucidate the role of sclerostin in bone remodeling induced by mechanical stress, histological and immunochemical methods were used to assess rat alveolar bone receiving tooth displacement loading forces. 50 Wistar/ST rats were divided into control and experimental groups, with the latter receiving orthodontic upper molar displacement using the Waldo method, and sacrificed 1, 3, 5 and 7 days later. μCT images of their maxillae were analyzed using the finite element method (FEM), and decalcified paraffin sections were used in TRAP and immunohistochemical staining for sclerostin. In the controls, sclerostin-immunoreactive osteocytes were distributed throughout the alveolar bone, but FEM revealed that the reaction decreased in areas receiving strong mechanical stress. In the experimental subjects, sclerostin immunoreaction disappeared in the interradicular septum on the first day after loading, but was again detected on days 5 and 7, as the loading decreased. Sclerostin-immunoreactive osteocytes were not observed in either group near the resorption surface of the alveolar bone. Tooth displacement therefore seemed to result in an immediate suppression of sclerostin production, with a mechanical stress threshold value perhaps required for this suppression. At any rate, sclerostin seems not to play an active role during bone resorption.

P1029

CCN2 Is Up-regulated In Cultured Chondrocytes Treated With Low-Intensity Pulsed Ultrasound (Lipus)

Takashi Nishida[1]*, Satoshi Kubota*[1]*, Eriko Aoyama*[2]*, Nobuyasu Yamanaka*[3]*, Masaharu Takigawa*[1,2]
[1]Okayama University Graduate School, Okayama, Japan; [2]Okayana University Dental School, Okayama, Japan; [3]ITO co, LTD., Tokyo, Japan

We have reported that CCN family protein 2/ Connective tissue growth factor (CCN2/CTGF) promotes chondrocyte proliferation and differentiation and that it repairs cartilage in experimental animal osteoarthritis (OA) models. Therefore, we have thought that CCN2 is a target molecule of OA therapy. Recently, low-intensity pulsed ultrasound (LIPUS) is noted as a low invasive therapy to patients. The purpose of this study is to investigate whether LIPUS up-regulates the expression of CCN2 in cultured chondrocytes. LIPUS was applied using ST-SONIC (ITO co, LTD.) to human chondrocytic cell line HCS-2/8, rat chondrocytic cell line RCS, and rat primary articular cartilage (RAC) cells at frequency of 3.0 MHz and intensity of 60 mW cm^{-2} for 20 minutes. After 30 minutes and 5 hours of LIPUS treatment, quantitative Real-time PCR and Western blotting were performed, respectively. As a result, both gene expression and protein production of CCN2 was stimulated in HCS-2/8, RCS, and RAC cells treated with LIPUS. In addition, F-actin polymerization promoted immediately after LIPUS stimulation, and phospho-ERK1/2 was also activated at 10 minutes after LIPUS stimulation. These findings suggest that CCN2 is up-regulated through promotion of F-actin polymerization and activation of phosho-ERK1/2 in cultured chondrocytes treated with LIPUS. Based on these results, we may be able to use LIPUS on a daily bases as a useful therapy for OA.

P1030

Identification And Characterization Of Chondrocytic Fibroblast Growth Factor 1 As A Molecular Counterpart Of CCN Family Member 2

Tarek Abd El Kader*, Satoshi Kubota, Takashi Nishida, Takako Hattori, Eriko Aoyama, Takayuki Furumatsu, Takuo Kuboki, Masaharu Takigawa*
Okayama University, Okayama, Japan

Objective: CCN family member 2 (CCN2) regulates endochondral ossification and regenerates articular cartilage under the interaction with multiple cofactors. By protein array screening to find out novel CCN2 cofactors, we obtained several candidates including fibroblast growth factor 1 (FGF1). Here, we characterized the molecular action and possible role of FGF1 in cartilage in relation to CCN2.
Methods: Filgen Protoarray was screened by a CCN2 N-terminal modular probe. Interaction of FGF1 with CCN2 was

evaluated by a solid phase binding assay and surface plasmon resonance (SPR) analysis. Biological effects were estimated by the addition of these proteins to human chondrocytic HCS-2/8 cell culture. Gene expression in cell lines and human chondrocytes from osteoarthritis (OA) patients was analyzed by the real-time RT-PCR analysis.

Results: Among >9000 proteins screened, FGF1 showed significant signals for the binding to CCN2. Specific interaction between full-length FGF1 and CCN2 was confirmed by a solid phase binding assay. SPR analysis revealed a high affinity of FGF1 to CCN2. FGF1 gene expression was detected in HCS-2/8 that produces CCN2. Notably, elevated FGF1 gene expression was observed in OA chondrocytes.

Conclusion: Strong expression of FGF2 in OA chondrocytes that also produce CCN2 suggests a role of FGF1 in OA. Functional characterization of FGF1 in the presence or absence of CCN2 is currently underway.

P1031

Nuclear Factor-κB Activation By Type II Collagen Peptide Is Inhibited By Hyaluronan Via CD44 And ICAM-1 In Chondrocytes

Tadashi Yasuda

Department of Sports Medicine, Tenri University, Tenri, Japan

Degradation products of cartilage matrix are known to contribute to cartilage degradation in diseased joints like osteoarthritis (OA). We have recently found that the synthetic peptide of type II collagen named CB12-II can stimulate type II collagen cleavage with MMP-13 in chondrocytes. While hyaluronan (HA) of high molecular weight is used in the treatment of OA by intra-articular injection, little is known of HA effect on actions of CB12-II. This study was aimed to examine activation of nuclear factor (NF)-κB in association with MMP-13 production by CB12-II and its inhibition by HA via its receptors in chondrocytes. When cartilage explants from OA knee joints or isolated OA chondrocytes in monolayer were incubated with CB12-II, the peptide activated NF-κB with enhanced MMP-13 production. BAY11-7085 inhibited the CB12-II-induced MMP-13. HA of 2700 kDA inhibited NF-κB activation by CB12-II, leading to a decrease in MMP-13. Preincubation with the individual antibody to CD44 or intercellular adhesion molecule-1 (ICAM-1) partially reversed HA effect on CB12-II action. A combination of both antibodies completely blocked the HA effect. In contrast, non-specific IgG had no effect. Thus, the present study clearly demonstrated that HA suppressed CB12-II-activated NF-κB via CD44 and ICAM-1 in OA chondrocytes. HA could down-regulate the catabolic action of type II collagen fragments in OA joints through the mechanism demonstrated in this study.

P1032

Expression Of Nox Family In Mouse Femur During Endochondral Ossification

Kimiharu Ambe[1], *Yoshiaki Kashiwabara*[1], *Shinya Takahashi*[2], *Toshihiro Nakagawa*[1], *Hiroki Watanabe*[1]

[1]Division of Oral Histology, Department of Morphological Biology, OHU University School of Dentistry, Koriyama, Fukushima, Japan; [2]Department of Oral and Maxillofacial Surgery, Ohu University School of Dentistry, Koriyama, Fukushima, Japan

Recently it has been reported that reactive oxygen plays an important role in several physiological processes. Reactive oxygen is generated by reactive oxygen-synthesizing enzymes (Nox). Different homologs are expressed depending on the organs, tissues, and cells, and investigation of the types and functions of enzymes expressed in various tissues is underway. We immunohistochemically investigated expression and localization of the Nox family in endochondral ossification using a normal mouse femur. Weakly positive reactions with Nox1, Noxa1, and Noxo1 were observed in the zones of proliferative and pre-hypertrophic chondrocytes at 3 weeks of age. Nox4 was widely positive from the resting over the hypertrophic cell zone. At 18 weeks of age, none of the Nox types was expressed in chondrocytes as the zones disappeared. On the other hand, positive reactions with Nox1, Noxa1, Noxo1, and Nox4 were observed in osteoblasts in the zone of ossification at 3 weeks of age, and each Nox was also positive in osteoblasts arranged on the bone marrow side in the epiphyseal plate at 18 weeks of age. In addition, a reactive oxygen-eliminating enzyme, Mn-SOD, was observed only in pre-hypertrophic chondrocytes at 3 weeks of age, and not detected in osteoblasts. It was suggested that the Nox family is closely associated with endochondral ossification of the mouse femur, and Nox1 and Nox4 are closely involved in the chondrocyte maturation process and bone matrix formation.

P1034

Down-Regulation Of IFT88 Augments TGF-β1 Actions On The Proliferation And Differentiation Of Chondrogenic Cell Line ATDC5

Makiri Kawasaki[1,2], *Tetsuya Nakamoto*[2], *Takuya Notomi*[2], *Tadayoshi Hayata*[1], *Yoichi Ezura*[1], *Masaki Noda*[1,2]

[1]Department of Molecular Pharmacology, Tokyo Medical & Dental University, Tokyo, Tokyo, Japan; [2]Global Center of Excellence Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo Medical & Dental University, Tokyo, Tokyo, Japan

Primary cilium is a hair-like projection from the cell surface and exists in most of the mammalian cells including chondrocytes.

Primary cilia possess a transporting system called IFT (Intraflagellar transport), by which proteins required for the formation and functions of primary cilia are transported inside the cilia. Ift88 is one of the IFT constituents and known to be required for the formation of primary cilia and thus, in hedgehog signaling. The mutant mice lacking Ift88 specifically in Prx-1 expressing cells were reported to exhibit aberrant endochondral ossification and attenuated hedgehog signaling in the growth plate. However, the Ift88 mutant mice didn't completely reproduce the phenotype of Ihh null mice suggesting the involvement of Ift88 in other signal cascades. In this study, we showed that TGF-β1 treatment in ATDC5 decreased Ift88 expression, while increased Col2a1 and decreased Col10a1 expression. When Ift88 was depleted in ATDC5 by RNAi, the action of TGF-β1 on the expression of Col2a1 and Col10a1 was augmented. Moreover, TGF-β1 treatment promoted the proliferation of ATDC5 lacking Ift88 compared to the control. The number of ciliated cells in ATDC5 did not decrease regardless of the TGF-β1 treatment. We concluded that the down-regulation of Ift88 in ATDC5 changed the responsiveness to the TGF-β1 treatment, although we are yet to know if the down-regulation of Ift88 and TGF-β1 independently affects the differentiation and proliferation of ATDC5.

P1035

Crucial Role Of CCN2 In Energy Metabolism In Chondrocytes

Aya Maeda[1], *Satoshi Kubota*[1], *Yoshiaki Miyake*[1], *Kazumi Kawata*[1], *Takako Hattori*[1], *Takashi Nishida*[1], *Norifumi Moritani*[1], *Harumi Kawaki*[1], *Karen Lyons*[2], *Seiji Iida*[1], *Masaharu Takigawa*[1]

[1]Okayama university Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama city, Japan; [2]David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

CCN family protein 2 (CCN2) plays a critical role in the development and regeneration of bone and cartilage. Indeed, deficiency in both extracellular matrix (ECM) production and proliferation is observed in osteoblasts and chondrocytes from CCN2-null mice. These findings indicate that CCN2 is essential for the proper osteochondral metabolism. Here, we first confirmed that ECM production by chondrocytes was lower in CCN2-null mice than WT mice by staining the cartilage sections; then comparative metabolomic analysis was performed with chondrocytes from these mice. Absolute quantification of >100 metabolites revealed that the intracellular levels of a number of metabolites were affected by CCN2 deletion. Among them, substantial and stable decrease in ATP, CTP, GTP and UTP contents in chondrocytes was observed in CCN2-null mice. These results were also confirmed by ATP bioluminescence assay with chondrocytes from other individuals. In order to find out possible molecules that mediate the observed role of CCN2, we analyzed the gene expression profile in the CCN2-null mice. The result of DNA microarray analysis showed that the lack of CCN2 remarkably decreased ATP synthase subunit γ gene expression. Moreover, even temporary knocking down of CCN2 mRNA suppressed the expression of ATP synthase subunit γ gene in a human chondrocytic HCS-2/8 cells. Our results suggest that CCN2 plays an important role in energy metabolism in chondrocytes by supporting ATP production.

P1036

Expression Of CCN2 And Its Possible Roles In Odontogenic Myxofibroma

Tsuyoshi Shimo[1], *Yuu Horikiri*[1], *Tatsuo Okui*[1], *Naito Kurio*[1], *Shoko Yoshida*[1], *Yuichiro Takebe*[1], *Kanako Nakatsuma*[1], *Eiki Koyama*[2], *Akira Sasaki*[1]

[1]Department of Oral and Maxillofacial Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [2]Division of Orthopaedic Surgery, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

Introduction: Connective tissue growth factor (CTGF/CCN2) is expressed in several human tumors and is implicated as one of the important players in cell proliferation and extracellular matrix production during tumorigenesis. However, no studies have investigated the possible roles of CCN2 in odontogenic myxofibroma, a benign tumor of odontogenic mesenchymal origin. We report herein a case of odontogenic myxofibroma and clarify possible roles of CCN2 in the development of myxofibromas.

Methods and results: Immunohistochemical analysis revealed that CCN2 was detectable in MIB-1-positive proliferating mesenchymal cells adjacent to microvessels and in the endothelial cells in myxofibroma. The CCN2 expression level in proliferating odontogenic mesenchymal cells isolated from the early bell stage of developing tooth germs was 3 times higher than that in the confluent less-proliferating cells. In addition, recombinant CCN2 significantly increased proliferation and expression of collagen type I in odontogenic mesenchymal cells.

Conclusion: We clarify the effects of CCN2 on proliferation and ECM production in odontogenic mesenchymal cells and attempt to reconcile the possible roles of CCN2 in the growth of odontogenic myxofibroma.

P1037

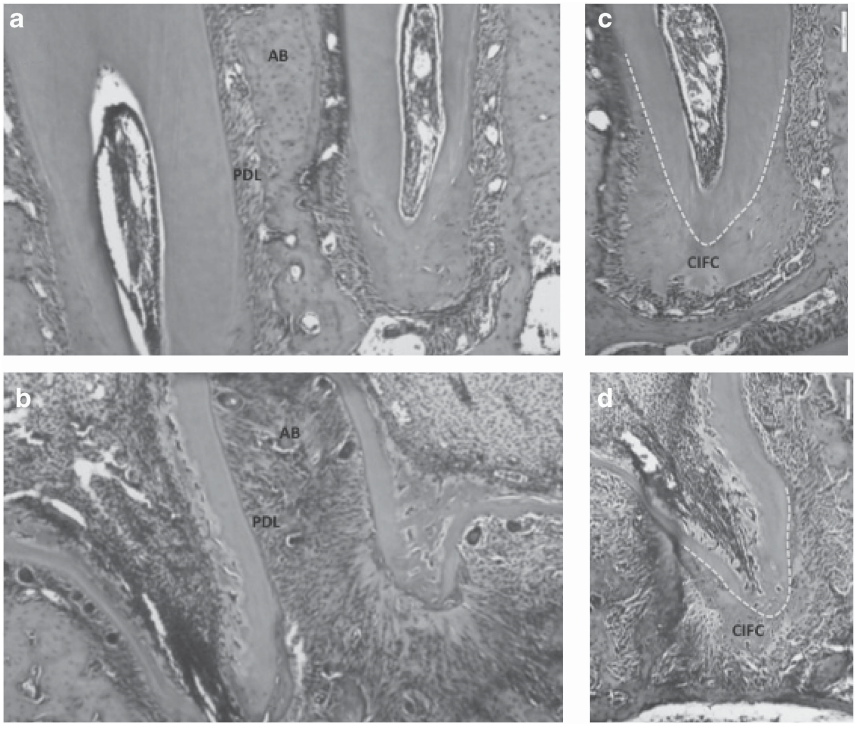
Bmp2 Gene Is Critical For Development Of The Periodontium

Audrey Rakian[1], *Jelica Gluhak-Heinrich*[1], *Marie Harris*[1], *Cui Yong*[1], *Jian Feng*[2], *Stephen Harris*[1]

[1]The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; [2]Baylor College of Dentistry, Texas A&M System, Dallas, TX, USA

Formation of the periodontium begins following the onset of tooth-root formation in a coordinated manner with root and alveolar bone development. Dental follicle progenitor cells can differentiate into cementoblasts, alveolar osteoblasts and periodontal ligament (PDL) fibroblasts. The genes that control this complex differentiation pathway to these 3 cell types of the periodontium are unknown. Recombinant Bmp2 has been shown to stimulate dental follicle cells to differentiate toward a cementoblast/osteoblast like phenotype *in vitro*. We now present data that shows Bmp2 gene is critical for proper formation

[P1037]



H & E stain of Control WT (A and C) and Bmp2-cKO$^{Osterix\text{-}Cre\text{-}EGFP}$ (B and D) mice mandible at 6-weeks. Yellow line indicates dentin-cementum junction. AB, alveolar bone, PD, periodontal ligament, CC, cellular cementum.

of these components of the periodontium. We conditionally removed the Bmp2 gene using the Osterix gene driving Cre recombinase (Bmp2-cKOOsterix-Cre-EGFP model), from a subset of follicle cells in the periodontium. With the use of histology, X-ray analysis, acid etch SEM and immunohistochemistry, we characterized the periodontium phenotype of mice molar teeth. The alveolar bone ridge was reduced and less alveolar bone was formed in the Bmp2-cKOOsterix-Cre-EGFP model. There was also a dramatic reduction in cementum formation near the root. In addition, the protein expression of cementum and alveolar bone markers such as DMP1, BSP and OPN, as well as the PDL marker Periostin was reduced. These results demonstrate that Bmp2 gene is a critical component of the network of genes that control periodontium development, including cellular cementum, alveolar bone and connecting PDLs.

P1038

Formation Of Bone-like Tissues By Dental Pulp Cells After Tooth Transplantation

Akihiro Hosoya*, Akira Yukita, Hiroaki Nakamura*
Department of Oral Histology, Matsumoto Dental University, Shiojiri, Nagano, Japan

While dental pulp is involved in the formation of bone-like tissue following various external stimuli, the origin of osteoblast-like cells and the process of their differentiation are still unclear. In this study, to clarify the source of these osteoblast-like cells, we transplanted green fluorescent protein (GFP)-labeled rat molars into the hypodermis of normal host rats. At 5 days after the transplantation, the upper region of the pulp was necrotic; however, cell-rich hard tissue was found on the surface of the dentin at the root apex. At 10 days, woven bone-like tissue also formed apart from the dentin in the upper pulp. After 20 days, these hard tissues expanded within the pulp cavity and became histologically similar to bone. GFP immunoreactivity was confirmed in the osteoblast-like cells within the root apex as well as in the upper pulp. Furthermore, immunohistochemical observation of alpha-smooth muscle actin, a marker for undifferentiated cells, showed a positive reaction in cells surrounding this bone-like tissue within the upper pulp, but not in those within the root apex. Immunoreactivities of Smad4, Runx2, and Osterix were detected in the hard tissue-forming cells within both areas. These results suggest that bone-like tissues induced by tooth transplantation have originated from 2 different types of dental pulp cells, such as progenitor cells having hard tissue-forming ability and undifferentiated cells possessing osteogenic potential.

P1039

3-Dimensional Evaluation Of Calcarb(Biocoral) And DFDBA Along With PRPIN The Treatment of Periodontal Osseous Defects

Ragini Sethi
Sahara Hospital, Lucknow, Uttar Pradesh, India

Background: Demineralized freeze dried bone allograft (DFDBA) have been utilized with varying success for the

[P1039]

Table 1 Postoperatively Bone gain Voluime(mm3) at 9 months - BIO-CORAL+PRP

S.No.	length(mm) Preoperative	Popstoperative	Width(mm) Preoperative	Postoperative	Depth(mm) Preoperative	Postoperative	Volume(cu.mm) Preoperative	Postoperative
1	4	3.5	3	3	5	4.5	30	23.625
2	4	3	4	4	4	4	32	24
3	3	3	3	3	3	3	13.5	13.5
4	3	3	3	3	4	3	18	13.5
5	3	3	3	3	5	5	22.5	22.5
6	3	2	3	3	5	4	22.5	12
7	4	3	3	3	6	5	22.5	22.5
8	3	3	3	3	6	6	27	22.5
9	4	3	3	3	4	3	24	13.5
10	2.3	2	2	2	4	3	9.2	6

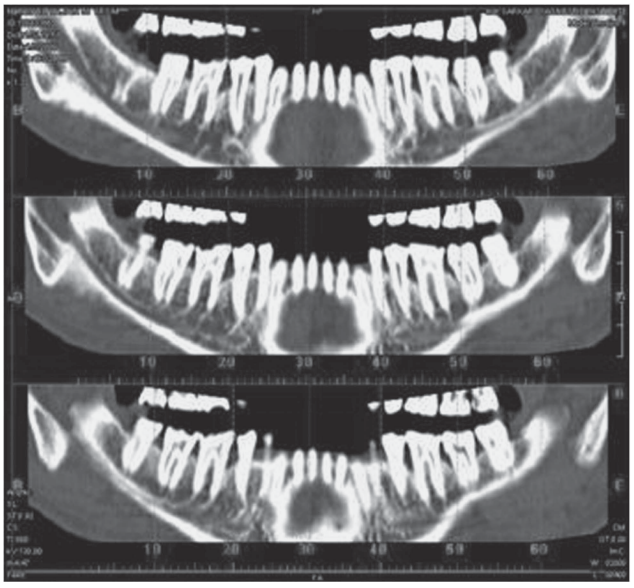


Figure 1 Preoperative Dentascan view of the Intrabony defects

regeneration of periodontium.This study was carried out to compare the effectiveness (qualitatively and quantitatively) of DFDBA with PRP, andcoralline calcium carbonate (Biocoral) (CalCarb) with PRP in the treatment of periodontal osseous defects.

Methods: 40 infrabony defects in forty patients were treated randomly with CalCarb with PRP (Group A), DFDBA with PRP (GroupB), CalCarb (Group C) and DFDBA (Group D). Clinical parameters such as Plaque Index, Gingival Index, Recession, Probing depth, ClinicalAttachment level were recorded at baseline, 3 & 9months postoperatively. Preoperative & postoperative hard tissue measurements (bone defect volume, percent bone fill, bone density) were recorded using Dentascan.

Results: Probing pocket depth reduction were significant at 9 months from baseline for all groups demonstrating 4.10 ± 1.1738mm (Group A),4.20 ± 0.89mm (Group B), 2.75 ± 1.09mm (Group C), 4.20 ± 0.92mm (Group D), respectively. Clinical attachment level gain were significantly improved for all groups A, B, C, D with 3.35 ± 1.2921mm, 3.80 ± 1.09mm, 2.75 ± 1.09mm, 3.70 ± 1.16mm. Maximum bone gain% was observed in Group A and minimum in Group C. Bone density was maximum in Group B and min in Group C.

CONCLUSION: Group A has significantly better bone gain % as compared to Group B.

P1040

Osteocyte-Derived OPG Contributes To Prevention Of Alveolar Bone Loss

Masanori Koide[1], *Yasuhiro Kobayashi*[1], *Tadashi Ninomiya*[1], *Midori Nakamura*[2], *Hisataka Yasuda*[3], *Naoyuki Takahashi*[1], *Nobuyuki Udagawa*[1,2]

[1]Oral Science, Matsumoto Dental University, Shiojiri, Japan; [2]Department of Biochemistry, Matsumoto Dental University, Shiojiri, Japan; [3]Planning & Development, Bioindustry Division, Oriental Yeast Co., Ltd, Tokyo, Japan

Periodontitis, an inflammatory disease of periodontal tissues, is characterized by the excessive loss of alveolar bone (AB). An increase in the RANKL/OPG ratio is thought to reflect the severity of periodontitis. However, it is not clear what kinds of changes in the expression and localization of RANKL and OPG contribute to the AB loss in periodontitis. Here, we measured the loss of AB in OPG-deficient ($OPG^{-/-}$) mice and RANKL over-expressing transgenic (RANKL-Tg) mice. At 12 weeks of age, the AB loss in $OPG^{-/-}$ mice was significantly greater than that in RANKL-Tg mice. The increased number of osteoclasts was observed in cortical areas of AB in $OPG^{-/-}$ but not in RANKL-Tg mice. Immunohistochemical analysis showed that the OPG-positive signal in osteocytes was higher than that in osteoblasts, and OPG-positive osteocytes were abundantly detected in the cortical areas of AB and long bones in both wild-type and RANKL-Tg mice. Anti-RANKL antibodies as well as OPG have been shown to block the RANKL-RANK interaction. We then examined whether the loss of cortical areas in AB in $OPG^{-/-}$ mice was protected by the administration of an anti-mouse RANKL antibody (clone OYC1 from Oriental Yeast). Treatment of $OPG^{-/-}$ mice with the anti-RANKL antibody effectively decreased the number of osteoclasts and suppressed the increased bone resorption in the cortical area of AB and long bones. Taken together, osteocyte-derived OPG mainly contributes to the prevention of the loss of AB.

P1041

Three Histone 3 Lysine 9 Methyltransferases, G9a, GLP, And SETDB1, Are Expresssed During Mouse Tooth Development

Taichi Kamiunten[1], *Akemi Shimada*[1], *Hisashi Ideno*[1,2], *Kazuhisa Nakashima*[1], *Yoshiki Nakamura*[1], *Akira Nifuji*[1]
[1]Tsurumi University, Yokohama, Japan; [2]Waseda University, Tokorozawa, Japan

Mammalian teeth develop through epithelial-mesenchymal interactions that are regulated by genetic and epigenetic mechanisms. Histone 3 (H3) lysine 9 (K9) methylation is a crucial epigenetic modification that regulates gene expression and development. So far, functions of histone methyltransferases, which are responsible for H3K9 methylation, in tooth development have been poorly understood. In this study, we investigated localization of the H3K9 methyltransferases(H3K 9MTases) G9a, GLP, and SETDB1, as well as that of H3K9 mono- (me1), di- (me2), and tri-methylation (me3) during mouse tooth development. We performed immunohistochemistry on sections of mouse molar at embryonic days (E) 12.5, 14.5, and 16.5. At the cap stage (E14.5), G9a and Glp were weakly detected in the epithelium of tooth bud. At the bell stage (E16.5), G9a Glp, and SETDB1 as well as H3K9me1 and me3 were detected in epithelium of tooth germ. Distribution of H3K9me1 and me3 was overlapped with that of H3K9MTase in those tissues. By western blotting using epithelium and mesenchyme of E16.5 tooth germ, we confirmed that G9a and Glp proteins are enriched in the epithelium compartment. These results suggest that the H3K9MTases are enriched in the epithelium and their distributions are co-localized with H3K9me1 and me3 in tooth germ. Thus the H3K9MTases may regulate gene expression by affecting the methylation states of H3K9 during mouse tooth development.

P1042

Human Gingival Fibroblasts Cultured On Chitosan Film Crosslinked By Glutaraldehyde Could Inhibit Bacterial Invasion And Il-8 Relative Inflammatory Responses

Jhe-Yu Lin, Cheng-Nan Chen, Min-Fang Yang, ***Hsin-I Chang***
Department of Biochemical Science and Technology, Chiayi, Taiwan

Chitosan, which is a nature polymer, has been fabricated into various forms such as micro/nano particles or reservoir devices for drug delivery and membranes or matrices for tissue engineering. However, there is no research indicating chitosan anti-bacterial abilities after cell culture. In this study, we have fabricated chitosan solutions, partices and films by glutaraldehyde crosslinking reaction to study their effect on bacterial killing and the prevention of bacterial invasion on human gingival fibroblasts (HGF). The results show that all chitosan forms have anti-bacterial abilities. In comparison with chitosan particles, chitosan films and solutions demonstrated higher antibacterial ability and no bacterial invasion into HGF. After bacterial invasion, HGF cultured on chitosan films could inhibit bacterial invasion and gene expressions of inflammation markers such as IL-8, ICAM and VCAM but not COX-2 gene. Based on these results, we conclude that chitosan films could not suppress inflammation responses on COX-2 pathway but could prevent cell adhesion and gathering in immune system through the inhibition of IL-8, ICAM and VCAM expressions. Chitosan films crosslinked by glutaraldehyde showed good cell attachment and proliferation and can inhibit bacterial invasion and further immune responses. Therefore, chitosan films crosslinked by glutaraldehyde could be potential in tooth and bone biomaterial applications.

P1043

PPARγ Counteracts Lrp1-induced Vascular Calcification By Inhibiting A Wnt5a Signaling Pathway

Philippe Boucher
Philippe Boucher, Philippe Boucher, Illkirch, France

Vascular calcification is a hallmark of advanced atherosclerosis. Here we show that deletion of the nuclear receptor PPARγ in vascular smooth muscle cells of low density lipoprotein receptor (LDLr)-deficient mice fed an atherogenic diet high in cholesterol accelerates vascular calcification with chondrogenic metaplasia within the lesions. Vascular calcification in the absence of PPARγ requires expression of the transmembrane receptor LDLr-related protein-1 in vascular smooth muscle cells. LDLr-related protein-1 promotes a previously unknown Wnt5a-dependent prochondrogenic pathway. We show that PPARγ protects against vascular calcification by inducing the expression of secreted frizzled-related protein-2, which functions as a Wnt5a antagonist. Targeting this signalling pathway may have clinical implications in the context of common complications of atherosclerosis, including coronary artery calcification and valvular sclerosis.

P1044

A Case Of Neurogenic Myositis Ossificans, Medullary Origin

Kawtar Nassar*, Saadia Janani, Wafaa Rachidi, Noufissa Etaouil, Ouafaa Mkinsi*
Rheumatology, Casablanca, Morocco

The neurogenic myositis ossificans or para-osteoarthropathy is a particular form of heterotopic ossification in patients who achieved the consequences of spinal cord injury or brain. It is situated in the large joints. The differential diagnosis can be obtained by imaging. Treatment is primarily medical and includes non-steroidal anti-inflammatory. We report a case of neurogenic osteoarthropathy occurring six months after a staged spondylodiscitis with spinal cord damage in a patient 25.

P1045

Electrical Stimulation Of Denervated Rat Skeletal Muscle Slows Trabecular Bone Loss In Early Stages Of Disuse Atrophy

Hiroyuki Tamaki[1], *Kounosuke Tomori*[2], *Kengo Yotani*[3], *Futoshi Ogita*[3], *Hideaki Takahashi*[1], *Hikari Kirimoto*[1], *Hideaki Onishi*[1], *Noriaki Yamamoto*[1,4], *Norikatsu Kasuga*[5]
[1]Niigata University of Health and Welfare, Niigata, Niigata, Japan; [2]Kanagawa University of Human Services, Yokosuka, Kanagawa, Japan; [3]National Institute of Fitness and Sports, Kanoya, Kagoshima, Japan; [4]Niigata Rehabilitation Hospital, Niigata, Niigata, Japan; [5]Aichi University of Education, Kariya, Aichi, Japan

We determined the effects of direct electrical stimulation (ES) on the histological profiles in atrophied skeletal muscle fibers and bone tissues after denervation caused by nerve freezing. Direct ES was performed on the tibialis anterior (TA) muscle after denervation in 7-week-old rats divided into groups as follows: control (CON); denervation (DN); and denervation with direct ES, subdivided into a 4-mA (ES-4), an 8-mA (ES-8) or a 16-mA stimulus (ES-16). ES was performed at a frequency of 10 Hz for 30 min/day, 6 days/week, for 1 or 3 weeks. Muscle tension-time integrals induced by ES were greatest under the ES-16 condition. Marked trabecular bone loss in tibiae and muscle weight loss in TA were mainly observed at 7 days and at 1-3 days after denervation, respectively. At 3 weeks after denervation, ES-8 and ES-16 slowed muscle atrophy, while ES-4 did not. Expression of IGF-1 mRNA in denervated muscle increased significantly following ES-16. Higher trabecular bone area and osteoid thickness were observed in ES-16 as compared to DN rats at 1 week after denervation, but the ES groups showed no differences with DN at 3 weeks after denervation. The beneficial effects of ES on slowing bone and skeletal muscle atrophy may thus be associated with time-course, tissue-specific differences, and/or stimulation intensity.

P1046

Chair Rising Time Is Longer In Postmenopausal Women With History Of Nonvertebral Fracture

Jun Iwamoto, *Tsuyoshi Takeda, Hideo Matsumoto*
Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan

The objective of the present study was to clarify the relationships between physical function, bone mass, biochemical markers, renal function and history of nonvertebral fracture in postmenopausal women with osteoporosis. In total, 143 postmenopausal women with osteoporosis (mean age: 71.2 years), who were at osteoporosis treatment-naïve status, were recruited. Twenty-seven women had a history of nonvertebral fracture (nonvertebral fracture group), and 116 women did not (control group). Bone mass, biochemical markers, eGFR, unipedal standing time (body balance), and five-repetition chair rising time (muscle power) were compared between the two groups. Age, body mass index, bone mass, serum calcium, alkaline phosphatase, creatinine, albumin, urinary NTX, eGFR, and unipedal standing time did not differ significantly between the two groups. However, chair rising time was significantly longer (12.3 s vs. 9.6 s) and serum phosphorus level was significantly lower (3.1 mg dl^{-1} vs. 3.4 mg dl^{-1}) in the nonvertebral fracture group than in the control group. The odds ratio (95% confidence interval) for nonvertebral fracture with chair rising time >10.1 sec (mean) and serum phosphorus <3.38 mg dl^{-1} (mean) was 4.28 (1.78, 10.30) and 2.92 (1.18, 7.21), respectively. These results suggest that impairment of muscle power and low serum phosphorus level appeared to be associated with increased the risk for falls and nonvertebral fractures in postmenopausal women with osteoporosis.

P1047

Sarcopenia Is Significantly Associated With Bone Mineral Density In Korean Elderly Men

Yong Jun Choi[1], *Kwang-Min Kim*[2], *Yunhwan Lee*[3], ***Yoon-Sok Chung***[1]
[1]Endocrinology and Metabolism, Ajou University School of Medicine, Suwon, Republic of Korea; [2]Department of Family Medicine, Ajou University School of Medicine, Suwon, Republic of Korea; [3]Department of Preventive Medicine, Ajou University School of Medicine, Suwon, Republic of Korea

Background: This study is conducted to investigate the relationships among muscle mass, muscle strength, physical performance and bone mineral density (BMD) in Korean elderly men.
Methods: A total of 161 elderly males aged ≥ 50 years were enrolled from Ajou University Health Promotion Center. Grip strength, gait speed, weight, and height were measured. BMD and appendicular skeletal muscle mass (ASM) were also measured using dual energy X-ray absorptiometry (DXA). We used the height-adjusted ASM (ASM/height2) for muscle mass and the cutoff values of sarcopenia as 6.58 kg/m^{-2} according to the result from our previous study.
Results: The prevalence of sarcopenia and osteopenia in this study was 23% and 48%, respectively. Among sarcopenic subjects, 67.5% were osteopenic. Osteopenic subjects were more sarcopenic compared to normal subjects (32.5% vs. 14.5%). ASM/height2 was significantly associated with BMDs of spine, total hip, and femur neck. Gait speed was associated with BMDs of total hip and femur neck. Grip strength was associated with only femur neck BMD. BMDs of spine and hip were significantly associated with ASM/height2 after adjustment for age. Femur neck BMD was also associated with ASM/height2 even after adjustments for age and weight. Gait speed showed independent associations with BMDs of total hip and femur neck even after adjustments for age, weight, and height.
Conclusion: Sarcopenia and osteopenia are closely related in Korean elderly men.

P1048

Risk Factors For Multiple Falls In Japanese Men And Women: The Road Study

Shigeyuki Muraki[1], *Toru Akune*[1], *Sakae Tanaka*[2], *Hiroshi Kawaguchi*[2], *Kozo Nakamura*[3], *Hiroyuki Oka*[4], *Noriko Yoshimura*[4]

[1]Clinical Motor System Medicine, 22nd Century Medical and Research Center, The University of Tokyo, Tokyo, Japan; [2]Orthopaedics, The University of Tokyo, Tokyo, Japan; [3]Rehabilitation Services Bureau, National Rehabilitation Center for Persons with Disabilities, Saitama, Japan; [4]Joint Disease Research, 22nd Century Medical and Research Center, The University of Tokyo, Tokyo, Japan

Aim: To determine the risk factors for multiple falls.
Methods: Out of the 1690 subjects in the mountainous and seaside cohorts of the ROAD study, 1348 (452 men and 896 women; mean age, 63.9 years), who participated in the 3-year follow-up study, were analyzed. These subjects filled questionnaires regarding their falls between the baseline study and the follow-up study and questionnaires regarding their knee and lower back pain in the baseline study. Physical ability of the participants was estimated by measuring the grip strength and their 6-m walking time. Knee osteoarthritis (OA) was assigned a Kellgren Lawrence grade ≥3. A cutoff score of <24 using a Mini-Mental State Examination was used to select participants with cognitive impairment.
Results: During the 3-year follow-up, 54 (11.9%) men and 111 (12.4%) women reported multiple falls. After adjusting for age and BMI, multinomial logistic regression analysis in men showed that a longer walking time (OR, 1.11; 95% CI, 1.01-1.23) was a risk factor for multiple falls, but grip strength, bone and joint diseases and cognitive impairment were not. In women, whereas, a longer walking time (1.11; 1.02-1.20), cognitive impairment (4.95; 1.50-16.08) and knee pain (1.60; 1.00-2.54) were shown as risk factors for multiple falls, but radiographic knee OA was not.
Conclusion: The present longitudinal analysis using a large population revealed gender-based differences in risk factors for falls.

P1049

Eldecalcitol (ELD): Effect On Muscle In An Orchidectomized Rat Model

Varela Aurore[1], *Elisabeth Lesage*[1], *Solomon Haile*[1], *Joseph Arezzo*[2], *Hitoshi Saito*[3], *Susan Smith*[1]

[1]Charles River Laboratories, Senneville, Quebec, Canada; [2]Albert Einstein College of Medicine, New York, NY, USA; [3]Chugai Pharmaceutical Co. Ltd, Gotemba, Shizuoka, Japan

ELD is a Vitamin D3 analogue. A positive association between Vitamin D status and muscle function has been established. The objective of this study was to determine the effect of ELD on muscle/nerve function and muscle volume in aged Sprague-Dawley (SD) ORX rats when administered vehicle (Sham and ORX) or ELD (ORX) at 7.5 ng per kg per day by daily oral gavage for 13 weeks. Fat/lean and bone mass was measured by pQCT at 0, 6 and 13 weeks post-ORX at the proximal tibia. Muscle weights were recorded at necropsy. Nerve conduction velocity (NCV) was recorded in the mixed caudal nerve, the sensory digital nerve, and the distal motor branches of the tibial nerve innervating plantar muscles of the foot at 0, 6 and 12 weeks. Treatment of SD ORX rats with ELD at 7.5 ng/kg/day resulted in the partial preservation of cortical and cancellous bone mass as compared to an observed loss in ORX controls. At Week 6, muscle mass was maintained at sham levels as compared to a muscle loss in ORX controls. At Week 13, a partial preservation of bone mass and an increase in muscle weight was also noted. This was not associated with slowing of either sensory or motor NCV. These data suggest that ELD could have a potential benefit to preserve muscle mass.

P1050

Lower Chance Of Metabolic Syndrome But Lower Bone Mass In Korean Elderly Women With Elevated Osteocalcin

Kyoung Min Kim[1,2], *Eun Young Lee*[1,2], *Ju Mi Lee*[3], *Young Mi Yun*[4], *Yoosik Youm*[5], *Hyeon Chang Kim*[3], *Chang Oh Kim*[1], *Yumie Rhee*[1,2]

[1]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea; [2]Endocrine Research Institute, Yonsei University College of Medicine, Seoul, Republic of Korea; [3]Department of Preventive Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea; [4]Medical Research Center, Yonsei University College of Medicine, Seoul, Republic of Korea; [5]Department of Sociology, Yonsei University, Seoul, Republic of Korea

Recent animal studies have suggested a new regulatory circuit between bone and energy metabolism via osteocalcin (OCN). The aim of this study was to examine the association between serum OCN and metabolic profiles in women aged 64 and over. This is a community-based, cross-sectional study and 616 healthy ambulatory elderly women were enrolled. Serum OCN levels had significant negative correlations with fasting plasma glucose (FPG), HbA1c, insulin and HOMA-IR after adjusting for age and BMI (r=-0.186, -0.162, -0.147 and -0.166. p<0.001 respectively). After dividing the subjects into 3 tertiles by OCN levels, FPG, HbA1c, insulin and HOMA-IR varied inversely with the OCN tertiles (p<0.05, respectively). The prevalence of metabolic syndrome was significantly lower in the women with highest OCN with OCN tertiles (p<0.05 analyzed with linear by linear association). In contrast, those in the higher tertiles of OCN had lower bone mass in the lumbar spine and hip (p<0.05, respectively). In a multiple logistic regression analysis, the OCN level was inversely associated with the development of diabetes, but positively related with risk of osteoporosis after adjusting for the confounding factors (OR, 0.70; 95% CI, 0.54-0.86, p=0.028 for diabetes, OR, 1.28; 95% CI 1.19-1.37, p=0.01 for osteoporosis). Increased OCN level is associated with improved glucose tolerance, but decreased bone strength, which supports a potential link between bone and energy metabolism in elderly Korean women.

[P1050]

Table 1 Stepwise multiple logistic regression of OCN for diabetes and osteoporosis

	Diabetes			Osteoporosis		
	OR per 1SD increase of OCN	95% CI	p-value	OR per 1SD increase of OCN	95% CI	p-value
Crude	0.57	(0.44-0.69)	<0.001	1.31	(1.23-1.40)	<0.001
Model 1	0.57	(0.45-0.70)	<0.001	1.33	(1.24-1.42)	<0.001
Model 2	0.58	(0.45-0.71)	<0.001	1.30	(1.21-1.39)	<0.001
Model 3	0.70	(0.54-0.86)	0.028	1.29	(1.19-1.38)	0.01
Model 4	.	.	.	1.28	(1.19-1.37)	0.01

Model 1: age adjusted Model 2: as Model 1 and BMI adjusted Model 3: as Model 2 and FPG adjusted for diabetes, 25-hydroxyvitamin D adjusted for osteoporosis Model 4: as Model 3 and parathyroid hormone adjusted OCN, osteocalcin; CI, confidence interval; BMI, body mass index; FPG, fastic plasma glucose

P1051

Short-Term Changes In Bone Turnover Markers By Anabolic And Anti-Catabolic Treatment In The Rat Ovariectomy Model

***Jussi Halleen**, Zhiqi Peng, Katja Fagerlund, Mari Suominen, Jukka Rissanen, Jukka Morko*
Pharmatest Services Ltd, Turku, Finland

Short-term effects on bone turnover markers predict long-term effects on bone mineral density in clinical studies. We have studied short-term effects of anti-catabolic and anabolic treatments on markers of bone resorption and formation in the rat ovariectomy (OVX) model. Study groups included sham- and OVX-operated control groups, and OVX-operated groups receiving 17β-estradiol (E2), alendronate (ALN), zoledronate (ZOL) and human (1-34) parathyroid hormone (PTH). Each group contained 12 animals that were 3 months of age at the time of the operations. Dosing was started the next day after OVX and continued for 2 weeks. PINP, osteocalcin, CTX-I, and TRACP 5b were determined in serum before the start of treatment and at 2 weeks. OVX increased PINP, osteocalcin and CTX-I values and decreased TRACP 5b values. PINP values increased by PTH and decreased by E2, but were not affected by ALN or ZOL. Osteocalcin values increased by PTH and decreased by E2, ALN and ZOL. CTX-I values decreased by E2 but did not change by the other treatments. TRACP 5b values decreased by PTH, ALN and ZOL, but did not change by E2. We conclude that all markers showed the expected effects caused by OVX and treatment by E2. TRACP 5b, but not CTX-I, decreased by ALN and ZOL, similarly as in clinical studies. Osteocalcin was the only marker that was affected by all tested therapies, indicating that it may reflect partly bone formation and partly resorption.

P1052

BA058, An Human Pthrp Analog: Effects On Bone Density And Strength In An Osteopenic Rat Model

***Varela Aurore**[1], Elisabeth Lesage[1], Susan Smith[1], Gary Hattersley[2]*
[1]Charles River Laboratories, Senneville, Quebec, Canada;
[2]Radius Health, Cambridge, MA, USA

BA058 is a synthetic analog of hPTHrP (1-34) currently in currently in Phase 3 of clinical development for post-menopausal osteoporosis treatment. The objectives of this study were to determine the effects of BA058 on bone mass and strength in OVX rats. Six-month old female rats (18/group) were randomly assigned to 5 groups. Four groups were OVX and one underwent Sham surgery. Animals received daily subcutaneous injections of vehicle (Sham and OVX controls) or BA058 at 1, 5 or 25 μg kg^{-1} per day for 1 year, starting 3 months post-surgery. After 1 year, marked increases in bone mass and geometry measured by DXA and pQCT were observed (up to 4 fold for trabecular BMD at the tibia metaphysis. Increases in bone mass were associated with dose-related increases of approximately 90%, 44% and 172% in bone strength parameters at the femur diaphysis, femoral neck and lumbar vertebrae, respectively, when comparing the 25 μg/kg/day dose group to the OVX controls for peak load. Increases in biomarkers of bone turnover (P1NP, CTx, OC, DPD) were also observed as compared to OVX controls, consistent with the increases in bone mass. BA058 potentially offers a number of important advantages as a new treatment for post-menopausal osteoporosis, including the ability to build new bone rapidly and significantly improve bone strength.

P1053

Alendronate Does Not Prevent Deterioration Of Cortical Bone Material Properties In Underuse Model Rats

***Kouji Naruse**[1], Mitsutoshi Suto[1], Kentaro Uchida[1], Masashi Takaso[1], Ken Urabe[1], Yuko Mikuni-Takagaki[2]*
[1]Department of Orthopedic Surgery, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan;
[2]Department of Functional Biology, Kanagawa Dental College, Yokosuka, Kanagawa, Japan

Bone is affected by age, hormonal status, mechanical environment, and diseases. Lack of estrogen and sedentary lifestyle play the two most important roles in the pathogenesis of osteoporosis in elderly women. To evaluate these two factors independently, we introduced an animal model that prevents rats from standing by keeping them in cages of limited space. Half of the rats were ovariectomized in order to deprive them of estrogen. A combination of activity, cage control or sedentary, and alendronate (ALN), given or not given was examined, and the results of ovariectomized rats and of sham-operated rats were analyzed separately. The 8-week experimental period began 5 weeks after ovariectomy or the sham surgery. Parameters of bones in the lower extremities of rats were determined using three-point bending test, pQCT, microCT, and confocal laser Raman microspectroscopy. In the sitting rats, ALN did not prevent deterioration of breaking force and ultimate force of femur regardless of the ovariectomy, despite

the fact that trabecular BMD was increased by alendronate even in the sitting rats. Given ALN, their cortical bone resulted in the significantly decreased thickness as well as the inferior quality. Alendronate significantly decreased toughness of the sedentary ovariectomized rat femur. Taken together, bisphosphonates such as alendronate may not be the drug of choice for restoring osteoporotic bone for individuals of limited daily activity such as standing and walking.

P1054

Effect Of Ovariectomy On The Mechanical Properties Of Isolated Primary Bone Cells

***Padmalosini Muthukumaran**, Chwee Teck Lim, Taeyong Lee*
Bioengineering, National University of Singapore, Singapore, Singapore

Post menopausal osteoporosis primarily occurs due to estrogen deficiency. The influence of estrogen on mechanical aspects of osteoblasts cells has not been studied yet. This study aims to investigate the effect of ovariectomy induced osteoporosis on the structural and mechanical properties of isolated primary bone cells. 10-12 weeks old female Sprague Dawley rats were divided into 2 groups viz, SH (n=4) and OV (n=4) and were subjected to sham and ovariectomy surgery, respectively. After 4 weeks, the rats were euthanized. The long bones were used harvested and used for primary bone cell culture. Primary bone cells isolated from all rats showed positive ALP activity and mineralization ability. The isolated cells were grown in the presence and absence of estradiol and they were subjected to AFM indentation to determine the apparent elastic modulus (E*) of the cell. Primary bone cells from OV group showed significantly higher E* as compared to that from the SH group. TRITC-Phalloidin stained bone cells showed that the altered mechanical properties were associated with changes in the density of the f-actin filaments. Following estradiol treatment, the cells were also tested for mineralization, collagen expression and proliferation. The OV bone cells were found to be more responsive to estradiol than the SH bone cells. The results suggest that the pathogenesis of osteoporosis is associated with changes in the elasticity of bone cells that arise due to changes in f-actin network.

P1055

Does Low Bone Volume Associate With A Reduction In Osteocytes Density In The Human Lumbar Spine? (Preliminary Data)

***Mohammad Zarrinkalam**, Adnan Mulaibrahimovic, Ai-quoc Nguyen, Robert Fraser, Robert Moore*
Surgical pathology, SA Pathology, Adelaide, South Australia, Australia

There is limited information about the role and significance of osteocytes in the development of osteoporosis. In this study we investigate the relationship between osteocyte density and trabecular bone volume. Twenty three fresh samples of bone were collected from the base of the L4 lumbar spinous process in females (60 to 80 years old) undergoing decompressive laminectomy. All relevant available clinical information from the patients were also collected. The bone specimens were scanned by microCT and then processed for decalcified histology to estimate the total density of lacunae, as well as the density of empty lacunae. The relationship between the morphological parameters and the density of lacunae were tested. The average BV/TV of this cohort was 21.6%. Based on this value the specimens were divided into two groups a) high bone volume b) low bone volume. There was a significant difference between the BV/TV of the two groups, but the density of total lacunae and of empty lacunae were not significantly different. In general, the higher BV/TV was as a result of higher Tb.N. There was no clear relationship between the density of lacunae and the other morphological parameters of bone. In conclusion, there was enough variation between the BV/TV to divide them into osteoporotic and non-osteoporotic groups. Power analysis indicates that a minimum 34 samples per group is needed to test the relationship between the morphological parameters and the density of lacunae.

P1057

Associations Of The Presence Of Bone Deformity In The Lower Extremities With The Decreased Serum Creatinine Levels In Infants With Vitamin D Deficient Rickets

***Shinji Takeyari**[1], Takehisa Yamamoto[1], Katsusuke Yamamoto[1], Takuo Kubota[2], Taichi Kitaoka[2], Yoko Santo[3], Kenichi Satomura[3], Kazuo Hiroshima[4], Keiichi Ozono[2]*
[1]Minoh Hospital, Minoh, Osaka, Japan; [2]Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [3]Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Osaka, Japan; [4]Osaka Developmental Rehabilitation Center, Osaka, Osaka, Japan

Background: Type II muscle fibers were decreased in vitamin D deficiency. Also, the administration of active vitamin D normalized bone deformities in patients with vitamin D deficient rickets. However, it is unclear whether this improvement is associated with a recovered skeletal muscle mass. Thus, we investigated the association of the bone deformity with serum creatinine levels, a surrogate marker of skeletal muscle mass, in infants with vitamin D deficiency.
Subjects and Methods: We investigated 22 infants (male 7, female15) with the mean age of 1.89 +/-0.74(SD). Vitamin D deficiency was diagnosed on the serum 25OHD levels of <30ng ml^{-1}. We measured serum intact PTH, 25OHD, 1,25(OH)2D and other routine markers for bone metabolism. We measured metaphyseal-diaphyseal angle (MDA) in lower extremities XP and set the cut-off value of more than 12 degrees to be abnormal. The serum Cr levels were shown as %Cr which was calculated from the Japanese standard values for each age. We treated all patients with active vitamin D metabolites.
Results: Prior to the therapy, univariate analyses revealed that the only %Cr values were significantly associated with the presence of bone deformity in the lower extremities (p=0.022, n=21). The therapy normalized %Cr values significantly from 0.59 (95%CI 0.40-0.78) to 1.09 (0.88-1.30) (p=0.0029, n=5).
Conclusion: Reduced skeletal bone muscle was suggested to be associated with the bone deformity in Japanese infants with vitamin D deficiency.

P1058

Serum NT-proCNP Levels In Patients With Achondroplasia/Hypoplasia May Predict Response To Therapy With Growth Hormone

Takuo Kubota[1,2]*, Koji Miura*[1]*, Wei Wang*[1]*, Noriyuki Namba*[1]*, Taichi Kitaoka*[1]*, Yasuhisa Ohata*[1]*, Makoto Fujiwara*[1]*, Keiko Yamamoto*[1]*, Makiko Tachibana*[1]*, Yoko Miyoshi*[1]*, Keiichi Ozono*[1]

[1]Department of Pediatrics, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; [2]First Department of Oral and Maxillofacial Surgery, Osaka University Graduate School of Dentistry, Suita, Osaka, Japan

C-type natriuretic peptide (CNP) plays a critical role in growth. The amino-terminal propeptide (NT-proCNP) of CNP is an equimolar product of CNP biosynthesis and is easily measured in serum. Thus, serum NT-proCNP levels might be a useful marker of growth in short stature patients. In this study, we studied serum NT-proCNP levels in untreated patients with achondroplasia (ACH)/hypochondroplasia (HCH) (*n*=17) with mutated fibroblast growth factor receptor 3, and compared with those in growth hormone deficiency (GHD) (*n*=10) and idiopathic short stature (ISS) (*n*=32). Serum NT-proCNP levels inversely correlated with ages of patients with ISS in infancy and childhood. There were no obvious differences in serum NT-proCNP levels among patients with ACH/HCH, GHD and ISS at the ages from 3 to 5. Serum NT-proCNP levels in patients with ACH/HCH were increased 3 months following GH treatment, while a significant response of serum NT-proCNP levels was not observed in patients with GHD. When the patients with ACH/HCH were divided into two groups: Group A with more than 120% increase of serum NT-proCNP levels and Group B with less than 120%, increase in height and in delta height SD score was significantly larger at the year after GH treatment in Group A than in Group B. These results indicate that GH increases height by stimulating the production of CNP and that serum NT-proCNP levels can be a useful marker to predict the growth response to GH treatment in patients with ACH/HCH.

P1060

An Infant Of Fibrodysplasia Ossificans Progressiva With Multiple Anomalies And Noncompaction Of Ventricular Myocardium

Kosei Hasegawa[1]*, Miho Yamashita*[1]*, Hiroyuki Tanaka*[2]

[1]Department of Pediatrics, Okayama University Hospital, Okayama, Japan; [2]Okayama Saiseikai General Hospital, Okayama, Japan

Fibrodysplasia ossificans progressive (FOP) is characterized by congenital malformation of the great toes and progressive heterotopic ossification (HO). Typical FOP patients with R206H mutation in ACVR1 don't accompany malformation of hand and HO develops after 1 year old. (Case) 9 months old female infant was referred because of congenital multiple anomalies and recurrent tumorous lesion in her back and occipital lesion from 7 months. After her birth, noncompaction of ventricular myocardium was identified. Her multiple anomalies consist of syndactyly of hand and foot, auricular anomaly and hypoplastic mandible. Ossified left sternocleidomastoid muscle was found by CT examination. Heterozygous R258G mutation was identified in ACVR1. (Functional analysis) Wild and mutated (R206H, Q207D, R258G) ACVR1 expression vector was transfected to C2C12 cell. Expression of Runx2 mRNA was wild<R206H<R258G<Q207D. pSmad 1/5/8 was wild<R258G<R206H<Q207D and pP38 was R206H<Wild=R258G=Q207D. SB203580, inhibitor of p38, decreased the expression of Runx2 in a dose dependent manner in R258G, although this decrease was weaker in R206H. (Conclusion) R258G mutation constitutively activates BMP signaling not only via canonical Smad pathway as R206H, but also via non-canonical MAPK pathway. This difference might contribute to the skeletal malformation that was not found in typical FOP and to earlier induction of HO. (Reference) Fukuda et al. BBRC,2008, JBC, 2009

P1061

Establishment Of Morquio A Tissue Repository Bank

Shunji Tomatsu, *Eriko Yasuda, Kristen Ruhnke, Carol Barone, Diana Corao, William Mackenzie*

Biomedical Research and Orthopedics, Nemours/Alfred I. duPont Hospital for Children, Wilmington, DE, USA

Mucopolysaccharidosis IVA (Morquio A) is an autosomal recessive disorder caused by deficiency of the lysosomal N-acetylgalactosamine 6-sulfate sulfatase (GALNS). GALNS catalyzes degradation of keratan sulfate (KS) and chondroitin-6-sulfate (C6S). Most patients are identified by three years of age due to short trunked dwarfism, odontoid hypoplasia, pectus carinatum, kyphosis, genu valgum, and hypermobile joints. To assess tissue remnants obtained during surgery (bone, cartilage, ligament, connective tissues surrounding the bone, muscle) from Morquio A patients will lead to better understanding the pathogenesis of the disease. Histological analyses from 10 patients were performed on cartilage tissue from cervical vertebrae, femur, and the knee joint. Clinical assessments including X-rays, MRI and CT were performed. In pathology there was chronic proliferative synovitis and chronic inflammation of the fibro-adipose tissue, especially around the vessels in femur heads. The inflammatory infiltrate is mainly composed of small lymphocytes and macrophages. An autopsied case of 20 year-old male case showed marked narrowing airway and osteomalacia of trachea. In pathology tracheal cartilage contained a large amount of vacuolar cells. Pathogenesis of MPS IVA resulted from accumulation of GAG, leading to the chronic inflammation in cartilage, synovium and capsule. All patients with MPS IVA present major anesthetic risks and death can result if appropriate precautions are not taken.

P1062

International Morquio A Registry: Natural Course And Clinical Manifestation

Shunji Tomatsu[1]*, Eriko Yasuda*[1]*, Kristen Ruhnke*[1]*, Mary Smith*[2]

[1]Biomedical Research and Orthopedics, Nemours/Alfred I. duPont Hospital for Children, Wilmington, DE, USA; [2]Carol Ann Foundation, Phoenix, AZ, USA

The largest worldwide documentation of Morquio A patients is comprised by Registry Database. To study variables affecting the clinical outcome of the disease, patients are asked to fill out a questionnaire about family history, diagnosis, signs and symptoms, surgical history, physical activity and general complaints. Summary of complied data contributes to disease awareness and understanding of the variability, progression and natural history. We summarized collective data from a total of 401patients (210 males, 52.4%; 191 females, 47.6%) from 42 countries. Questionnaire age was 17.3 years on average. Onset age and diagnosis age were 2.2 years and 4.9 years on average. The mean age of patients enrolled was 14.6 years for males and 18.3 years for females, with a range of 0.5 to 73 years. Forty-seven percent of Morquio A patients are below 18 years of age compared to 53% of patients 18 years of age and older. Initial symptoms were recognized between 1 and 3 years of age (mean age, 2.1 years) and mean age at diagnosis for the patients was 4.7 years. Fifty percent of patients underwent surgical operations. The most frequent surgical sites include neck (51%), ear (33%), leg (26%), and hip (25%). The final adult height for affected males and females was 122.5 ± 22.5 cm and 116.5 ± 20.5 cm, respectively. Patients with a severe phenotype accounted for 72.3% of the total Morquio A patients registered while 20.4% of the patients had an attenuated phenotype.

P1063

Relaxin Affects Differentiation And Mineralization Of MC3T3-E1 Cells Through Rxfp2

Carolina Duarte[1,2]*, Yukiho Kobayashi*[1,3]*, Tatsuo Kawamoto*[1,2]*, Keiji Moriyama*[1,2]

[1]Maxillofacial Orthognatics, Department of maxillofacial Reconstruction and Function, Graduate School of Medical and Dental Sciences, Tokyo medical and dental university, Tokyo, Tokyo, Japan; [2]Global center of excellence program, International research center for molecular science in tooth and bone diseases, Graduate School of Medical and Dental Sciences, Tokyo medical and Dental University, Tokyo, Tokyo, Japan; [3]Hard Tissue Genome Research Center, Tokyo medical and Dental University, Tokyo, Tokyo, Japan

Relaxin is a peptide hormone that binds to Rxfp1 and Rxfp2. Reduced bone mineral density in hypogonadism is related to the Rxfp2 mutation T222P, still, no effect has been reported of relaxin on bone formation. We examined relaxin's effect on osteoblastic cells as well as that of Rxfp1 and Rxfp2 individually.

Method: We established stable MC3T3-E1 clones lacking Rxfp1 (MCsiRxfp1) and Rxfp2 (MCsiRxfp2), and cultured them in differentiation medium with 20ngml^{-1} relaxin. We examined cell proliferation by MTT assay, osteoblast differentiation by ALP activity, matrix mineralization by Alizarin Red S staining, MMP proteolytic activity by zymography and cell signalling by Western blot.

Results: Administration of 20ngml^{-1} relaxin did not significantly inhibit proliferation, while increasing osteoblast mineralization and matrix degradation of MC3T3-E1 cells. Relaxin application increased Erk1/2 phosphorylation on all cell clones but more markedly on MCsiRxfp1 cells. MCsiRxfp1 cells showed increased cell proliferation and enhanced osteoblast differentiation and mineralization. In contrast, MCsiRxfp2 showed increased cell proliferation and enhanced matrix degradation.

Conclusion: Relaxin can affect mineralization and matrix degradation of MC3T3-E1 cells. Individual expression of Rxfp1 and 2 can alter the proliferation and biological function of MC3T3-E1 cells. Rxfp1 seems to relate to extracellular matrix metabolism while Rxfp2 relates to osteoblast differentiation and mineralization.

P1064

L51p, A Novel Bmp-2 Antagonist Inhibitor, Enhances Bone Formation Potential Of Bmp-2

Hany Khattab[1]*, Mitsu Ono*[1]*, Wataru Sonoyama*[1]*, Yasutaka Oida*[1]*, Shigehiko Shinkawa*[1]*, Kazushige Sugama*[2]*, Walter Sebald*[3]*, Takuo Kuboki*[1]

[1]Department of Oral Rehabilitation and Regenerative Medicine, Okayama University, Okayama, Japan; [2]Osteopharma Inc., Osaka, Japan; [3]Physiological Chemistry II, Theodor-Boveri-Institute for Biocenter of Würzburg University, Würzburg, Germany

Objectives: Bone morphogenetic protein 2 is a well known osteoinductive protein. The high BMP dosage in clinical use may induce adverse effects, such as ectopic bone formation. One of the possible ways to improve BMP-2 efficacy is to suppress activities of BMP antagonists. Our group generated the BMP-2 mutant (L51P) lacking in BMP receptor binding site and previously reported that L51P can bind to noggin and interfere with its binding to BMP-2. In our study, we examined the biological functions of L51P *in vitro* and *in vivo*.

Methods: C2C12 cells stably expressing the BMP-responsive mouse Id promoter were cultured with BMP-2 and/or L51P, and luciferase activity was measured. Osteogenic differentiation of MC3T3E1 cells was assessed by Alizarin red staining and quantitative RT-PCR for Alp and Ocn mRNAs. The levels of the p-Smad 1/5/8 was evaluated by western blots. *In vivo*, the effect of L51P on bone formation was examined. The BMP-2 and/or L51P-containing gelatin-hydrogels were implanted in the full-thickness calvarial defects of rats. After 4 weeks, bone formation was evaluated.

Results: Addition of L51P to BMP-2 induced the p-Smad 1/5/8, and accelerated the luciferase activity (3.1 fold) and mRNA levels of Alp (1.6 fold) and Ocn (2.6 fold) compared to BMP-2 alone. The radiographic examination of calvarial bone defect showed enhanced bone formation in combination of L51P and BMP-2.
Conclusion: Addition of L51P to BMP-2 enhances BMP-2 osteoinductive effect *in vitro* and *in vivo*.

P1065
EphrinB2 Signaling In Osteoblasts Is Required For Normal And PTH-Induced Bone Formation
Christina Vrahnas[1,3]*, Farzin Takyar*[1,2]*, Stephen Tonna*[1,2]*, T. John Martin*[1,2]*, Natalie Sims*[1,2]
[1]St. Vincent's Institute of Medical Research, Melbourne, Victoria, Australia; [2]The University of Melbourne, Melbourne, Victoria, Australia; [3]La Trobe University, Melbourne, Victoria, Australia

EphrinB2 expression by osteoblasts is stimulated by parathyroid hormone (PTH), and its interaction with EphB4 regulates osteoblast and osteoclast differentiation through both ephrinB2 and EphB4 signaling. This study examined the role of ephrinB2 (reverse) signaling in PTH anabolic action *in vivo*. 8-week-old male mice with ephrinB2 deleted in osteoblasts (Osx1Cre.efnB2fl/fl) and controls were treated with PTH (30μgkg^{-1}; 5/week) or vehicle for 4 weeks. Femora were analyzed by microCT, qPCR and reference point indentation, and tibiae by dynamic histomorphometry. Osteoblast number was 30% greater (p<0.01), and collagen1α1 and runx2 levels were significantly increased in efnB2 null mice compared to controls. Despite this, efnB2 null mice showed reduced mineral apposition rate (MAR) (p<0.05), cortical thickness (p<0.01), cortical tissue mineral density (p<0.05), bone material stiffness (p<0.05), sclerostin and osteocalcin mRNA levels compared to controls, suggesting impaired osteoblast function in the absence of efnB2. PTH treatment of control mice increased osteoblast surface, trabecular and periosteal MAR and decreased sclerostin levels. In efnB2 null mice, despite a significant PTH-induced increase in osteoblast surface, neither trabecular nor periosteal MAR were increased compared to vehicle. These results demonstrate that ephrinB2 signalling within the osteoblast lineage is required for normal and PTH-induced bone formation in both cortical and trabecular bone.

P1066
Dynamic Changes In Chromatin Accessibility During Early Osteoclastogenesis
Kazuki Inoue*, Yuuki Imai*
IMCB, Lab of Epigenetic Skeletal Diseases, The University of Tokyo, Tokyo, Japan

Osteoclastgenesis is strictly controlled by several transcription factors (TFs) and dynamic changes of chromatin states in response to various environmental signals. Chromatin accessibility is altered at cis-regulatory regions by the de novo binding of sequence-specific DNA binding TFs, resulting in increased hypersensitivity of local chromatin to DNase I attack. Therefore, delineation of DNase I hypersensitivity sites (DHS) by using a deep sequence approach (DNase-seq) is a powerful strategy to identify regulatory regions important for osteoclastogenesis. To identify cis-regulatory elements important for early stage of osteoclastogenesis, we performed *in vivo* DNase-seq by using RANKL-stimulated Raw264 cells. Analysis of DNase-seq data from RANKL-stimulated Raw264 cells revealed 13545 DHS regions in the whole genome, and 5411 regions of the whole DHS were unique in RANKL-stimulated Raw264 cells. Some of these RANKL-stimulation specific DHS were nearly located at the osteoclastgenic genes. 23.2% of these DHS are mapped to promoter regions, and DNase footprints by binding transcription factors were detected within 0.5 kb from transcription start site. We searched the putative TFs bound to each footprint using SeqPos, and identified motifs of oteoclastogenic TFs. These results provide a global view of the dynamic changes of chromatin structure during osteoclastogenesis, and we will find novel TFs regulating cell fate by DNase-seq.

P1067
Role Of Lectin-Like Oxidized Low-Density Of Lipoprotein Receptor-1 In Regulating Osteoclastogenesis And Inflammatory Bone Destruction
Mai Nakayachi[1,2]*, Junta Ito*[1]*, Mari Okayasu*[3]*, Chiyomi Hayashida*[1]*, Takuya Sato*[1]*, Naoto Suda*[2]*, Tatsuya Sawamura*[4]*, Yoshiyuki Hakeda*[1]
[1]Meikai University School of Dentistry, Div. of Oral Anatomy, Sakado, Japan; [2]Meikai University School of Dentistry, Div. of Orthodontics, Sakado, Japan; [3]The University of Tokyo Hospital, Tokyo, Japan; [4]National Cerebral and Cardiovascular Center, Suita, Japan

We have demonstrated that osteoclastogenesis is associated with the extracellular level of low-density lipoprotein (LDL). Here, we used scavenger receptor class A (SRA) and lectin-like oxidized LDL recptor-1 (LOX-1) knockout (KO) mice to elucidate the role of oxidized LDL in regulating osteoclastogenesis and inflammatory bone destruction. Although RANKL-induced osteoclast formation from SRA KO osteoclast precursors was equivalent to that from wild-type cells, the osteoclastogenesis from LOX-1 KO precursors was increased and the multinucleated cells formed in culture contained more nuclei than wild-type cells. However, RANKL-induced expression of osteoclastogenesis-related proteins such as NFATc1 and TRAP did not alter between LOX-1 KO and wild-type osteoclasts. In contrast, RANKL-induced Akt phosphorylation in LOX-1 KO osteoclast precursors was greater than that in wild-type cells, suggesting a pro-apoptotic receptor of LOX-1. When inflammation was induced by subperiosteal injection of lipopolysaccharide on calvaria *in vivo*, gene expressions of TRAP and RANKL in the injected region were elevated equivalently in both WT and SRA KO mice. In contrast, the elevation in LOX-1 KO mice was reduced compared to WT mice. In conclusion, although LOX-1 is potentially a negative regulator in osteoclastogenesis, LOX-1 may contribute to the inflammatory bone destruction.

P1068

Quantification Of Cell Networks: Computer-Assisted Method For 3D Mapping Of Osteocyte Populations In The Ageing Human Femur

Philippa Garner[1], *Ruth Wilcox*[1], *Lesley Hordon*[2,1], *Jean Aaron*[1]
[1]University of Leeds, Leeds, West Yorkshire, United Kingdom; [2]Dewsbury District Hospital, Dewsbury, West Yorkshire, United Kingdom

Osteocytes create a pervasive syncitium of processes and no part of the bone matrix is more than a few microns from a cell. A role in mechanotransduction has been proposed for the network whereby it directs remodelling and repair. To assess regional variability and the morphological influence of stress input, a novel method has been developed that combines undecalcified histology, confocal microscopy (CLSM) and image analysis software to enable reliable and convenient 3D quantitative characterisation with special reference to cancellous bone. Ageing femoral heads were used to compare the network in traditionally low stress (osteoporosis, OP) and high stress (osteoarthritis, OA) conditions. Segments were en-bloc stained in calcein fluorochrome before embedding in resin. Slices, 300μm thick, were examined by CLSM. Individual 2D Tiff images were imported into software (ScanIP, Simpleware, UK) that generated complementary 3D binary masks specifically representing cell body and process components. Corresponding in-house code (Matlab, Mathworks, USA) was written to quantitate the complementary paired masked aspects including the number of cells, their length, and interconnection. In OP, cells were sparse with fewer processes, forming a poorer interconnected syncitium than that found in OA. This novel method apparently enables topographic appraisal of the syncitium, and the prospect of a more precise evaluation of its potential for biomechanical exchange in ageing and disease.

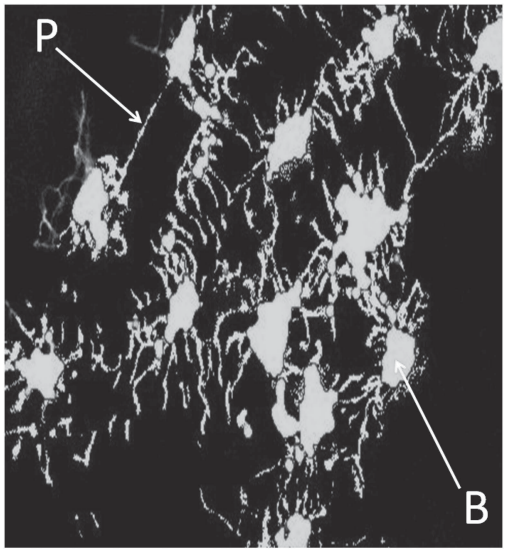


Figure 1 A 2D TIFF image of an osteocyte syncitium, from within osteoarthritic bone, showing the application of two binary masks for the quantification of the network, one representing the osteocyte cell body elements (B, blue), and the other the cytoplasmic processes (P, green).

P1069

The Development Of Culture Method For Chondrogenic Differentiation Of Human Ips Cells

Noriaki Koyama[1,2], *Masako Miura*[1], *Kazumasa Nakao*[2], *Eri Kondo*[1], *Toshihito Fujii*[1], *Naotetsu Kanamoto*[1], *Akihiro Yasoda*[1], *Hiroshi Arai*[1], *Kazuhisa Bessho*[2], *Kazuwa Nakao*[1]
[1]Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Department of Oral and Maxillofacial Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan

Human induced pluripotent stem cells (hiPSCs) form cartilaginous tissues in teratomas *in vivo*. However, no reliable system for *in vitro* chondrogenic differentiation of hiPSCs has yet been reported. Here, we examined the chondrogenic differentiation capability of hiPSCs using a multistep culture method consisting of embryoid body (EB) formation, cell outgrowth from EBs, monolayer culture of sprouted cells from EBs, and three-dimensional pellet culture. Monolayer-cultured cells expressed markers for mesenchymal stem cells. After two to three weeks of pellet culture, cells in pellets exhibited a spherical morphology typical of chondrocytes and were surrounded by extracellular matrix that contained acidic proteoglycans. The expression of type II collagen and aggrecan in pellets progressively increased. Histological analysis revealed that over 70% of pellets successfully underwent chondrogenic differentiation. However, immunohistochemistry (IHC) could not detect the expression of type II collagen. Then, we examined the effect of BMP2 and c-type natriuretic peptide (CNP) on chondrogenic differentiation by addition to pellet culture medium. The expression of type II collagen was partially detected both in BMP2- and CNP-treated hiPSC pellets by IHC. Our study demonstrates that hiPSCs can be efficiently differentiated into the chondrogenic lineage *in vitro* via generation of mesenchymal progenitor cells and that BMP2 and CNP can promote the chondrogenic differentiation of hiPSCs.

P1070

Suppressive Effect Of Mesenchymal Stem Cells With Nano-fiber Scaffold In Collagen-Induced Arthritis

Zhang Xiangmei[1], *Kunihiro Yamaoka*[1], *Koshiro Sonomoto*[1], *Hiroaki Kaneko*[2], *Makoto Satake*[2], *Yuka Yamamoto*[2], *Kondo Masahiro*[1], *Jidong Zhao*[1], *Shunsuke Fukuyo*[1], *Yosuke Okada*[1], *Yoshiya Tanaka*[1]
[1]The First Department of Internal Medicine, University of Occupational and Environmental Health, Japan, Kitakyushu, Fukuoka, Japan; [2]Integrative Technology Research Institute, Teijin Limited, Tokyo, Tokyo, Japan

Objectives: Mesenchymal stem cells (MSCs) possess pluripotency and anti-inflammatory activity *in vivo* and a potential as a treatment tool for bone destructive inflammatory disease. A nano-fiber sheet made by poly-lactic-co-glycolic acid is used as a scaffold for cellular therapy with bio-compatibility and flexibility.
Methods: MSCs was administered into rats with collagen-induced arthritis (CIA), intra-articularly (IA), intra-peritoneally (IP) or seeded on the nano-fiber sheet (nano-MSCs) and cultured for 24 hours *in vitro* and implanted into ankles (IMP).

Results: Treatment with nano-MSCs via IMP significantly suppressed the severity of arthritis evaluated by arthritis score, hind paw thickness and body weight compared to non-treated CIA, IA and IP. Radiological examination revealed suppressed bone destruction and histological analysis showed less inflammatory cell infiltration and bone erosion in the IMP group compared to IA and IP. Enlargement of the draining lymph nodes (LNs) and spleen in IMP were significantly decreased compared to non-treated CIA animals. Furthermore, nano-MSCs via IMP treatment increased production of TGF-beta and subsequently reduced chronic inflammation and germinal center formation.
Conclusion: Implantation of MSCs with nano-fiber scaffold efficiently suppressed arthritis, bone destruction and immune response, suggesting that a novel delivery system of MSC using nano-fiber appears a potential tool for joint repair in RA.

P1071

Mapping Early Bone Formation Gene Networks Allows Targeted Osteoinduction Of Human Periosteal Progenitors *In Vitro* And *In Vivo*

Scott Roberts[1], *Jeroen Eyckmans*[1,2], *Johanna Bolander*[1], *Jan Schrooten*[1], *Christopher Chen*[2], *Frank Luyten*[1]
[1]Department of Development and Regeneration, KU Leuven, Leuven, Vlaams Brabant, Belgium; [2]University of Pennsylvania, Philadelphia, PA, USA

Although human periosteum derived cells (hPDCs) seeded in calcium phosphate (CaP) rich matrices (CPRM) spontaneously undergo bone formation when implanted in mice, the molecular mechanisms that mediate this biological cascade are poorly understood. To assess the signalling pathways which are activated in hPDCs during CaP mediated bone formation, microarray based gene network discovery was employed. 946 genes were significantly regulated and differentially expressed in CPRM versus CaP depleted matrices (CPDM). Gene topology indicated that implantation 'activates' hPDCs independently of the matrix, however, gene expression dynamics progressed faster in CPRM than in CPDM, and was associated with differential activation of pathways related to inflammation (TNF alpha, NFkB, and IL6) and development (TGFβ, βcatenin, BMP, EGF, and ERK signalling). Growth factor mediated activation of these pathways *in vitro* promoted hPDC proliferation and induced expression of 10 bone markers within 11 days of stimulation. Moreover, *in vitro* pre-treatment of hPDC-laden CPRM with these factors enhanced osteogenesis *in vivo*. These data suggest that CaP is required to support osteoinductive processes in hPDCs that are initiated by the inflammatory response of the host following implantation. Additionally, our study shows that the identification and *in vitro* stimulation of tissue regulatory gene networks is a strategy that may allow enhanced target tissue formation by progenitor populations.

P1072

The Use Of Microrna- And Gene-Assisted Manipulation Of Mesenchymal Stem Cell (hMSCS) Derived Osteoblasts, Optimally Propagated And Differentiated In TiO2 Scaffolds

Jan Gordeladze
Department of Biochemistry, Institute of Basic Medical Science, Oslo, Oslo, Norway

MicroRNAs are small RNAs, 21-25 nt in length, encoded in the genome, and exert important regulatory roles. BMP2-induced osteoblast differentiation involves miR-135 and miR-133, which target Smad5, a mediator of the BMP-2 signaling, as

[P1072]

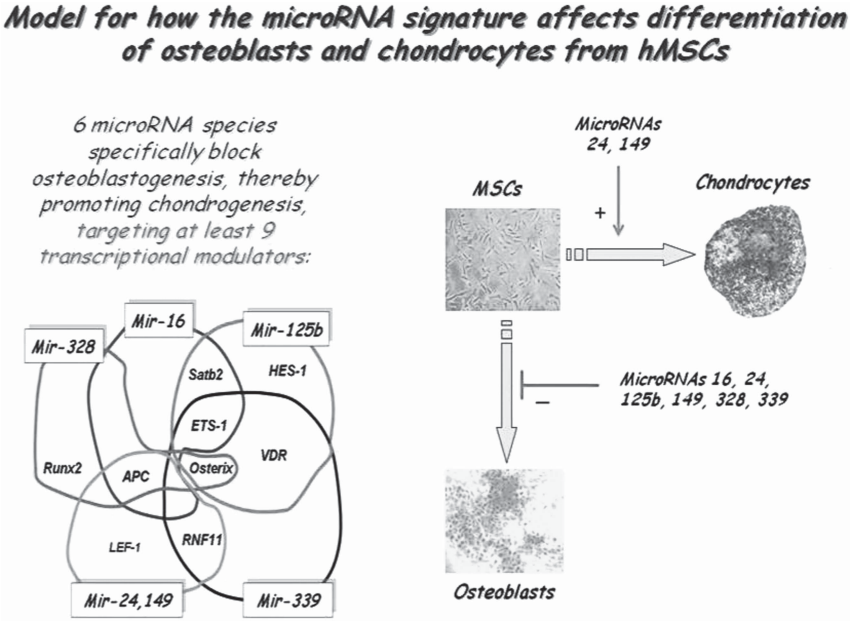


Figure 1

well as Runx2. Several other microRNAs (16, 24, 125b, 149, 328, and 339) have been shown to constitute an osteochondral 'signature' (Figure 1).
These microRNA species maintain important cellular functions constituting the osteochondral phenotypes, and/or serve as phenotype switches determining the fate of these cells differentiated from hMSCs. Recently, TiO2 was shown to be a promising scaffold material due to its microstructure, allowing for cell migration, vascularisation, and mimicking the pore size of trabecular bone. **Results:** TiO2 appeared superior to PLA and HA no matter which parameters were measured in osteoblasts derived from hMSCs: 1) osteoblast phenotypic markers, 2) osteochondral microRNA profiles, 3) osteoblast resilience towards phenotypic changes, 4) osteoblast resilience towards over-activation of osteoclasts and/or differentiation of osteoclasts from PBMCs, and 5) TiO2 seemed to enhance the ability of engineered osteoblasts to start the process of vascularisation.

P1073
Mineral Saturation Of Tibia When "Osteoapatite Ceramic" -015, Doped With Copper Is Implanted In Artificial Defect
Vladyslav Luzin, Andrey Ivchenko, ***Anton Yeryomin***
Luhansk state medical university, Luhansk, Ukraine

Aim: To study experimentally the mineral richness of the regenerate, which is formed by implantation of the "Osteoapatite ceramic" -015 (OK-015), doped with copper at concentrations of 0.25%.
Materials and methods: We carried out an experiment on 252 white male rats, animals were divided into four groups: first group - intact animals, second group - animals, which formed a cross-cutting bone defects on the border of the proximal metaphysis and diaphysis tibia. In the third group, the defect in the implanted hydroxyapatite blocks of biological origin with diameter 2.2 mm. In 4th groups the defect filled with OK-015 doped with copper at concentrations of 0.25%.
Results: Implantation of OK-015 in the defect region in comparison with the indicators of second group was accompanied by abnormalities in the dynamics of the chemical composition of the regenerate. From 7 to 30 days the water content in the emerging regenerate was lower than in the second group, respectively, 14.35%, 25.90% and 10.72%, while the share of the mineral component, however, prevailed in the same time - at 16.88%, 22.10% and 10.82%. The compact substance is characterized by a decrease of water content in diaphyses to 15 and 60 days of observation, an increase in organic matter content of 7 and 15 days, along with a decrease in its day to 30.
Conclusions: The results indicate an increase in speed of biological implant resorption in the presence of copper in its composition.

P1074
Synthesis And Evaluation Of Biomimetic Zinc Substituted Β-tricalcium Phosphate (Zn-tcp) Macrospheres For Bone Tissue Engineering
Joshua Chou[1,2]*, Mori Hiroe*[2]*, David Bishop*[1]*, Besim Ben-Nissan*[1]*, Makoto Otsuka*[2]
[1]School of Medical and Molecular Biosciences, University of Technology Sydney, Sydney, New South Wales, Australia; [2]Musashino University, Tokyo, Nishi-Tokyo, Japan

In the quest to develop suitable biomaterials in hope of stimulating localized bone formation, many strategies have been

[P1074]

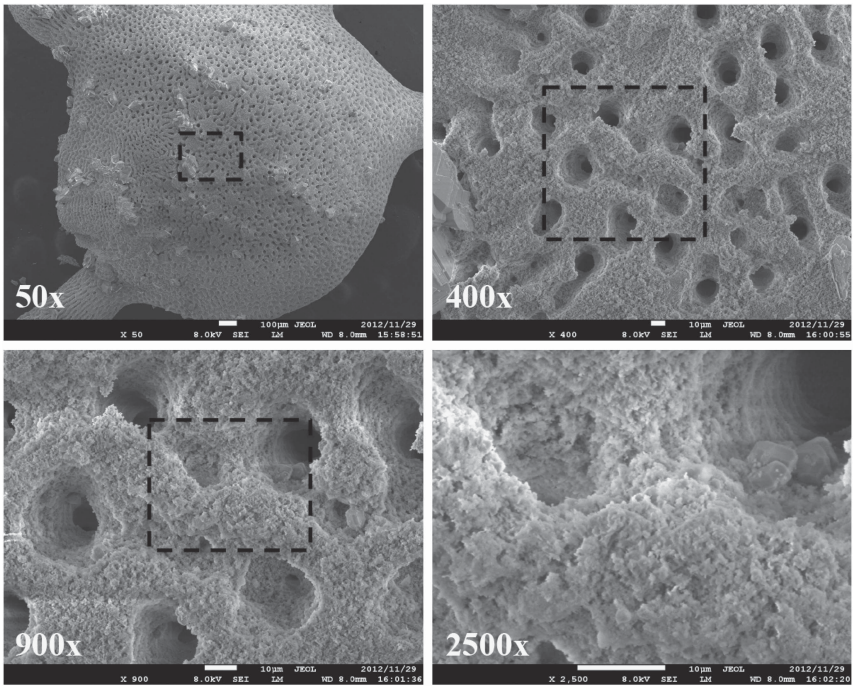


Figure 1 Scanning electron microscopy image showing at low magnification the hydrothermally converted beta-tricalcium phosphate material and at higher magnification detailing the uniform pore distribution.

imposed with some success. The incorporation of bioinorganics such as zinc, have shown in various studies to contribute to significant bone formation. Furthermore, clinical studies have connected the relationship of zinc deficiencies in patients with osteoporosis. In the present study, hydrothermally converted beta-tricalcium phosphate, from fossilized foraminifera exoskeletons were used as a precursor material for zinc ion substitution to produce zinc containing tricalcium phosphate. The material was characterized and evaluated for the amount of zinc substitution and its distribution, in-vitro release studies, in-vitro primary cell culture response and in-vivo evaluation in osteoporotic mice model. The samples were evaluated for 4 weeks in ovariectomized mice with weekly monitoring of bone mineral density and bone mineral content by X-ray CT analysis. At the end of the experimental period, the mechanical strength of the bone was also evaluated. This study shows the potential benefits of combining a biomimetic material with synthetic modification and the therapeutic efficacy in the treatment of osteoporosis.

P1075

The Role Of Endoplasmic Reticulum Stress In Bone Formation Suppressed By Advanced Glycation End Products

Ken-ichiro Tanaka[1]*, Toru Yamaguchi*[1]*, Hiroshi Kaji*[2]*, Ippei Kanazawa*[1]*, Toshitsugu Sugimoto*[1]
[1]Internal Medicine 1, Shimane University Faculty of Medicine, Izumo, Japan; [2]Department of Physiology and Regenerative Medicine, Kinki University Faculty of Medicine, Osaka-Sayama, Japan

Advanced glycation end products (AGEs) are involved in bone quality deterioration related to aging and diabetes mellitus. We previously showed that AGEs inhibited the osteoblastic differentiation of mouse stromal ST2 cells (CTI 2012). Although the quality management for synthesized proteins in endoplasmic reticulum (ER) is crucial for the maturation of osteoblasts, effects of AGEs on ER stress in osteoblast lineage are unknown. We therefore examined roles of ER stress in AGEs-suppressed osteoblastogenesis using AGE2 or AGE3. After reached confluence, AGEs administration inhibited the expression of Col1, Osterix, and OCN as well as the mineralization of ST2 cells. Moreover, AGEs induced apoptosis and decreased the proliferation of the cells. AGEs suppressed the levels of ER stress sensors such as IRE1α, ATF6, and OASIS, while they increased the levels of PERK and its downstream molecules, phospho-eIF2α, ATF4, and CHOP. A phospho-eIF2α inhibitor, salubrinal, did not affect the AGEs-suppressed expressions of Col1, Osterix, or OCN. In contrast, salubrinal partially recovered the apoptosis as well as the suppression of proliferation and mineralization induced by AGEs. These findings indicate that AGEs inhibit the differentiation of stromal cells into osteoblasts by suppressing IRE1α, ATF6, and OASIS. Moreover, AGEs induce apoptosis and decrease cell number by enhancing the PERK pathway. These processes, in combination, may accelerate AGEs-induced suppression of bone formation.

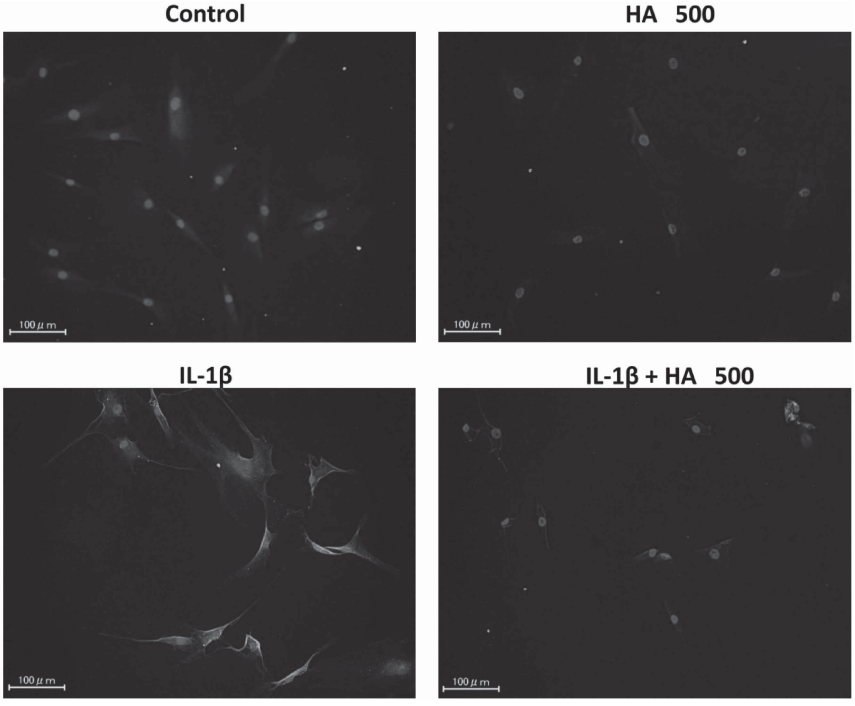
P1076

High Molecular Weight Hyaluronan Down-Regulates The Expression Of ADAMTS4 Via p38MAPK Signaling Pathway In Human Synoviocytes

Yoshihiro Kataoka[1]*, Wataru Ariyoshi*[2]*, Tatsuji Nishihara*[2]
[1]Oral and Maxillofacial Surgery, Kyushu Dental University, Kitakyushu, Japan; [2]Infections and Molecular Biology, Kyushu Dental University, Kitakyushu, Japan

Osteoarthritis (OA) is a destructive joint disorder with articular cartilage erosions and fibrillations, as well as clustering and

[P1076]



proliferation of articular chondrocytes. Recently, aggrecanases, aggecan-degrading metalloproteinases, are considered to play a key role in the progression of OA. ADAMTS4 (a disintegrin and metalloproteinase with thrombospondin motifs 4) is known to have aggrecanase activity. The purpose of this study was to investigate the mechanisms by which high molecular weight hyaluronan (HMW-HA) regulates ADAMTS4 expression induced by IL-1β in human fibroblast-like synoviocytes (HFLS) cells. We treated HFLS cells with IL-1β and HMW-HA. Expression of ADAMTS4 were monitored by real-time RT-PCR and Western blot analysis. The localization and secretion of ADAMTS4 protein were determined using immunofluorescence microscopy. HMW-HA suppressed both mRNA and protein expression of ADAMTS4 induced by IL-1β. HMW-HA also inhibited phosphorylation of p38 MAPK proteins induced by IL-1β. Inhibition of the p38 MAPK signaling pathway by chemical inhibitors also suppressed ADAMTS4 mRNA expression induced by IL-1β. Furthermore, CD44 function-blocking monoclonal antibody pretreatment effectively inhibited the down regulation of IL-1β-induced ADAMTS4 mRNA mediated by HMW-HA. These results suggest that HMW-HA may play an important role as a regulatory factor in synovial tissue inflammation.

Poster Session 2

P2001

Whole Body Vibration Can Attenuate Deterioration Of Trabecular Bone Micro-architecture In Rats With Spinal Cord Injury

***Akira Minematsu**, Yasue Nishii, Hidetaka Imagita, Tomoko Hanaoka, Susumu Sakata*
KIO University, Kitakatsuragi-gun, Nara, Japan

Aim: The aim of this study was to examine the effects of whole body vibration (WBV) on trabecular bone (TB) micro-architecture in rats with spinal cord injury (SCI).
Methods: Thirty-eight Wistar male rats (8 wk-old) were randomly divided into 3 groups; SCI, SCI+WBV (SW) and sham-operated (SO) groups. Lower thoracic nerve was cut in SCI and SW groups. WBV at 25 Hz for 20 min/day (5 days/wk during the period of 1 and 2 wks) was performed in SW group 1 wk after the operation. At the end of WBV, tibias were scanned by a micro-CT and the parameters of TB micro-architecture were obtained by analyzing micro-CT images.
Results: SCI caused the apparent deterioration of TB micro-architecture and thereby bone loss. After 1 wk-WBV, bone volume (BV), bone mass (BV/TV), trabecular number (Tb.N) and connectivity density (Conn.D) were significantly higher in SW group than in SCI group, while trabecular separation (Tb.Sp) and marrow space star volume (V*m) were significantly lower in SW group than in SCI group. Likewise, after 2 wk-WBV, BV, BV/TV, Conn.D and trabecular star volume (V*tr) were significantly higher in SW group than in SCI group, while trabecular bone pattern factor (TBPf) and V*m were significantly lower in SW group than in SCI group.
Conclusion: WBV for 1 or 2 wks could attenuate TB micro-architecture deterioration caused by SCI. This preservative effect of WBV is presented by the following parameters; BV, BV/TV, Tb.N, Tb.Sp, Conn.D, V*m and V*tr.

P2002

Regional Differences In Microstructural And Mechanical Properties Of The Distal Femur

KwangKyoun Kim
Orthopedic surgery, Konyang University, Daejeon, Republic of Korea

Introduction: Aging and arthritic process raise different loads on various regions of the distal femur, and there would be changes in the microstructure and mechanical properties according to load differences. The purpose of this study is to analyze regional differences in the microstructural and mechanical properties of the distal femur depending on osteoarthritic changes using micro-images based on finite element analysis.
Materials and Methods: Distal femur specimens were obtained from ten donors with OA. As controls, the normal distal femur was sampled from ten age and gender matched donors. The areas of interest were six regions of the condyles of the femur. Structural parameter and Mechanical parameter were calculated.
Results: In control group, the lateral anterior region of the distal femur reflected subchondral trabecular remodeling, while in advanced OA group, the medial middle region showed prominent changes in the microstrucrural and mechanical properties. Trabecular bones from the distal femur in control and OA groups exhibited different microstructural and mechanical properties in the same region.
Conclusion: Changes of patello-femoral reaction force induced subchondral trabecular changes of the anterolateral region initially, and then progressed to the medial middle and posterior region in advanced OA.

P2003

Discontinuation Of Impact-loading Exercise Is Related To Reduction Of A Calcaneus Quantitative Ultrasound Parameter In Young Adult Japanese Females: A 3-year Follow-up Study

***Eri Nakazono**[1,2], Hitomi Miyazaki[1,2], Shimako Abe[2], Katsumi Imai[1,2], Takashi Masuda[2], Masako Iwamoto[1,2], Ririko Moriguti[1,2], Hiromi Ueno[2], Misaki Ono[1,2], Kayoko Yazumi[1,2], Kosei Moriyama[1,2], Shusi Nakano[1,2], Hiroko Tsuda[1,2]*
[1]Faculty of Nutrition Sciences, Nakamura Gakuen University, Fukuoka, Japan; [2]Health Promotion Center, Nakamura Gakuen University, Fukuoka, Japan

Purpose: The aim of this study is to determine the lifestyle factors that influence the maintenance of calcaneus osteo sono-assessment index (OSI) in young adult females after the attainment of peak bone mass.
Methods: Annual health check-ups including OSI measurements, anthropometrics, lifestyle analysis and blood examination were performed 4 times on 334 young Japanese females

enrolled in a university at the age of 18 years. According to the slope of OSI change during 3-year follow-up, the subjects were grouped into OSI loss (the lowest tertile) and OSI gain/stable (the second and third tertiles).
Results: At the baseline assessment, the OSI loss group had higher OSI and height and an earlier menarche age than the OSI gain/stable group. Performing impact-loading exercise in junior high and/or high school but discontinuing it at university was associated with increased risk of OSI loss, independent of OSI, height, and weight at baseline, age of menarche, energy-adjusted nutrient intake, and alcohol-drinking; the odds ratios were 4.6-5.9-fold greater compared with those performing impact-loading exercise at university. In particular, the amount and intensity of impact-loading exercise during high school (from 15-17 years of age) were associated with OSI loss.
Conclusion: Our study provides evidence that discontinuation of impact-loading exercises with high level activity performed during late adolescence is associated with increased risk of OSI loss in young adult females.

P2004
A Comprehensive Analysis Of Mechanical Stress-Regulated Gene Expression In Mouse Cranial Sutures
Mika Ikegame[1], *Mariko Kawai*[1], *Yoshiaki Tabuchi*[2], *Yukihiro Furusawa*[3], *Takashi Kondo*[3], *Masaki Nakano*[4], *Atsuhiko Hattori*[4], *Toshio Yamamoto*[1]
[1]Department of Oral Morphology, Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [2]Division of Molecular Genetics Research, Life Science Research Center, University of Toyama, Toyama, Japan; [3]Department of Radiological Sciences, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan; [4]Department of Biology, College of Liberal Arts and Sciences, Tokyo Medical and Dental University, Chiba, Japan

We have previously reported that tensile stress accelerated osteoblast differentiation and subsequent bone formation in mouse cranial sutures. During this process, gene expressions of BMP-4 and α-adaptin C, which is a component of the endocytosis machinery AP2, were up-regulated at 3 h after the loading. Other growth factors or cytokines, such as IGFs and WNTs, have also been reported to be up-regulated by mechanical stress. However, it is not clear which factor is more important in the process of mechanical stress-induced osteogenesis. Therefore, we performed a comprehensive analysis of tensile stress-regulated genes in the cranial sutures to elucidate the network of growth factors and cytokines which are responsible for mechanical stress. Calvariae from neonatal mice were cultured with or without tensile stress on the mid-sagittal sutures for 3 or 6 h. Afterwards, total RNA was isolated from the sutures and analyzed by GeneChip. Real-time qPCR was performed on the interested signaling molecules which expressions were increased by more than 1.5-fold in the GeneChip results. Expressions of BMP receptor type IB and some EGF-related factors were increased both at 3 and 6 h, and of some WNT-related factors were also changed at 6 h, but not distinct at 3 h. These results suggest that BMP and EGF signals contribute to the proliferation and differentiation of osteoblasts in cranial sutures at the early stage of mechanical stimulation.

P2005
Early Response Of Cranial Sutures To *In Vivo* Tensile Force Loading
Nobuo Takeshita, *Masakazu Hasegawa, Kiyo Sasaki, Daisuke Seki, Shunro Miyashita, Ikuko Takano, Teruko Takano-Yamamoto*
Division of Orthodontics and Dentofacial Orthopedics, Tohoku University Graduate School of Dentistry, Sendai, Miyagi, Japan

Sutures are fibroblastic tissues connecting bones in the craniofacial area. The growth of bones in the area occurs due to intramembranous ossification in sutures. Sutures respond to different types of mechanical stimuli, such as pressure from brain growth and muscle activity. However, the biological effects of mechanical stress on sutures are not well known. In this study, we examined the early response of sutures to tensile force and its molecular mechanism, focusing on mesenchymal stem cells (MSCs) and the expression of CTGF and VEGF. In our suture expansion model, 20 g tensile force was applied to the sagittal suture of murine cranium for 0, 3, and 12 hours. STRO-1, CD44, and CD73 were detected in the unloaded suture, and these expressions significantly decreased 12 hours after loading. Runx2 and osteocalcin expressions decreased in a time-dependent manner. On the other hand, expression of VEGF transiently increased 3 hours after loading, and endothelial cell markers simultaneously increased. CTGF showed the same expression pattern as VEGF, and a neutralizing antibody against CTGF inhibited the tension-induced VEGF expression. The administration of MEK and JNK inhibitors partly inhibited the tension-induced CTGF and VEGF expressions, respectively. These results suggest that tensile force induces CTGF and VEGF expressions partly via MAPK in the early response of sutures, and they may regulate differentiation of MSCs into endothelial cells followed by vascular formation.

P2006
The Tortuosity Influence To The Trabecular Bone Elasticity
Waldir Roque[1], *Angel Alberich-Bayarri*[2], *Katia Arcaro*[1]
[1]Mathematics Institute, Graduate Program in Applied Mathematics, Porto Alegre, Rio Grande do Sul, Brazil; [2]Quiron Valencia Hospital, Valencia, Valencia, Spain

Osteoporosis is characterized by remarkable bone mass loss and consequent increase in bone fragility. The bone mineral density is a highly important marker of bone quality, nevertheless it has been shown that it alone cannot successfully respond for the bone fragility. There are several other quantities that do also play a role in establishing the bone mechanical structural quality. The trabecular bone structure can be seen as a network of struts and theirs sinuosity seem to influence the structural rigidity.
This work aims to investigate the relationship between the tortuosity (τ) influence to the mechanical structure of the trabecular bone. For that the tortuosity has been estimated and then a study has been conducted to see its influence to the Young Modulus (YM). The former parameter, is estimated based on microtomographic images from ex-vivo distal radius

samples, the latter by simulation considering a linear relationship between the response force to the deformation imposed to the structure. The elasticity simulation is performed using Finite Element Methods and the results point out a prevalent strong linear correlation between τ and YM of -0.75; -0.54 and -0.79 in z, x and y directions, respectively. This result points out that in fact tortuosity does influence the structural elasticity of the trabecular bone.

P2007

Incidence Of Osteoporotic Fracture In Patients With High Plasma Homocysteine(Hcy) Level

Koji Nozaka[1], *Yoshiaki Kimura*[2], *Naohisa Miyakoshi*[1], *Shin Yamada*[1], *Michio Hongo*[1], *Takeshi Kashiwagura*[2], *Yuji Kasukawa*[1], *Tsutomu Sakuraba*[2], *Hidetomo Saito*[1], *Hiroaki Kijima*[1], *Yoichi Shimada*[1]
[1]Orthopedic Surgery, Akita University Graduate School of Medicine, Akita, Japan; [2]Akita City Hospital, Akita, Japan

Introduction: Recently, bone mass and quality affect bone strength independently. However, details on patients with high plasma Hcy levels and normal plasma Hcy levels undergoing clinical treatment for fractures remain unclear.
Objective: To retrospectively investigate patients with high plasma Hcy levels (High group) and normal plasma Hcy levels(Normal group) in order to compare bone quality markers, bone turnover markers, bone mineral density and the incidence of osteoporotic fractures.
Subjects and Methods: Subjects were 148 patients who underwent plasma Hcy measurement upon admission to our department from among the 2065 patients for whom bone mineral density was measured at our hospital.
(Results) Pentosidine (Pen) and ucOC was significantly higher in the High group (0.180μgmgCr and 9.68ngml^{-1}) than in the Normal group (0.042μgmgCr and 3.66ngml^{-1}). BAP, DPD, TRACP 5b and YAM were not significantly different between the High group and the Normal group. The incidence of fractures was 90.0%(27/30) in the High group, while the incidence of fractures was 49.2%(58/118) in the Normal group. The incidence of osteoporotic fracture (%) was significantly higher in the High group than in the Normal group.
Discussion: These results indicate that elevated bone quality marker which is plasma Hcy levels, urine Pen and ucOC in osteoporosis is useful marker for estimation of fracture risk independent of bone turnover and bone mineral density.

P2008

Effect Of Administration Frequency Of Teriparatide On Bone Metabolism In Rabbits

Hiroshi Yamane, *Aya Shimomura, Tomoya Tanaka, Katsuhiro Baba, Ryoko Takao-Kawabata, Yukihiro Isogai*
ASAHI KASEI PHARMA Co., Pharmaceuticals Research Center, Izunokuni-shi, Japan

Purpose: Teriparatide (TPTD) has shown the different effects on bone metabolism depending on administration frequency. To understand the pharmacological profile of TPTD on bone metabolism, we investigated the effect of dosing frequency in rabbits.
Methods: 6-month-old female New Zealand White rabbits were divided into 5 groups. Each group was injected subcutaneously with saline, 20 or 40 μgkg^{-1} of TPTD once a day, 140 or 280 μgkg^{-1} of TPTD once a week for 4 weeks. Bone metabolic markers in serum and urine were measured during administration period with several time points. After the period, lumbar vertebra, tibia and femur were collected to analyze BMD, bone structure and bone strength.
Results: After 4-week administration, both daily and weekly injection of TPTD increased the concentration of serum osteocalcin (OC) and the level of OC in daily groups was higher than that in weekly groups. Weekly groups sustained the higher level of OC until next administration. Daily injection of TPTD markedly increased the concentration of urine deoxypyridinoline (DPD) compared with weekly injection. After the autopsy, BMD of isolated bones was increased in TPTD-treated groups. Daily groups increased the rate of cortical porosity in tibial diaphysis, compared with weekly groups.
Conclusion: These results suggest that the higher frequency of TPTD may accerelate the bone turnover which causes the alteration of cortical bone structure in rabbit.

P2009

Vitamin D Deficiency And Insufficiency And Their Relevance To Bone Metabolism In Type 2 Diabetes

Hiroko Mori, *Yosuke Okada, Yoshiya Tanaka*
The First Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan

Objective: The aim of this study was the survey of vitamin D deficiency and sufficiency in type 2 diabetes, and the therapeutic effects of vitamin D on bone metabolism in these patients.
Method: Serum 25-hydroxyvitamin D (25(OH)D),1.25(OH)2D, BAP, OC, NTX, homocysteine, pentosidine and bone mineral density were measured. The primary outcome was the rate of satisfaction of 25(OH)D.
Results: 1) subjects: 270 patients, age 66, BM I24, HbA1c7.4, 25(OH)D 19.3 ng/ml.2) 92% of subjects: vitamin D insufficiency (<30): severe deficiency (<10) 3.7%, deficiency (10-19) 52.2%, insufficiency (20-29) 36.3%, sufficiency (30>) 7.8%. 3) Although there was significant negative correlation between 25 (OH)D and NTx, and BAP, there was no significant correlation between 25 (OH)D and bone quality. 4) There was significant positive correlation between 25 (OH)D and bone mineral density on radius. However, no correlation between 25 (OH)D and bone mineral density on L2-4 and femoral. 5) There was significant correlation between 25 (OH)D and intact-PTH. 6)Multivariate analysis revealed NTx and intact-PTH as the only significant determinant of 25(OH)D and the cut-off value of 25(OH)D was 19ng/ml from ROC analysis.
Conclusion: From these results vitamin D deficiency and insufficiency were common in type 2 diabetes and patients with a 25(OH)D concentration <19 ng/ml had a risk of osteoporosis,

indicating that the management of vitamin D is important for prevention of fractures in type 2 diabetes.

P2010

Microarray-Based Gene Expression Profiling Of Functional Parathyroid Adenomas

Sihoon Lee[1], *Hong Seok Choi*[1], *Seul Min Kim*[1], *Yoo Seung Chung*[1], *So Young Park*[2], *Eun Hye Ji*[3], *Jaesang Kim*[3]
[1]Gachon University School of Medicine, Incheon, Republic of Korea; [2]Kwandong University College of Medicine, Seoul, Republic of Korea; [3]Ewha Womans University, Seoul, Republic of Korea

Background: Knowledges about the genes involved in the organogenesis and the normal physiology of parathyroid gland has been gained from molecular pathophysiology of idiopathic hypoparathyroidism. Specifically, hypocalcemia due to inadequate PTH production is caused by mutations in the CaSR, GCMB and PTH gene itself. Goal of this study was to isolate potential key players among parathyroid-specific genes.
Methods: Gene expression profiles of 3 parathyroid adenoma samples were analyzed using the human genome wide illumina bead microarrays. For the control 2 standard universal human RNA preparations were used. Briefly, total RNA was extracted from the fresh frozen samples, and then amplified, purified and labeled with biotin-NTP. Labeled cRNA was hybridized to each bead array and array signal was detected and then scanned. The comparative analysis between samples and controls was carried out using fold-change.
Results: 47 differentially expressed genes (DEGs) emerged which showed 4 or greater fold increase of expression in parathyroid adenoma in all the 6 comparison combination sets. When DEGs were listed in the downward numerical order of expression, well-known players such as PTH, CaSR, MafB and GCMB were found at the top of the list, indicating this approach is sound and informative.
Conclusion: This data, although preliminary, suggest that there are novel potential candidate key players in this DEGs list. Further molecular genetic analyses are being pursued for these genes.

P2011

A Patient With Hypophosphatemic Osteomalacia Complicated By The Ossification Of Posterior Longitudinal Ligament

Shinji Takeyari[1], *Takehisa Yamamoto*[1], *Toshimi Michigami*[2], *Kosei Hasegawa*[3], *Hiroyuki Tanaka*[4], *Yasuo Yasuo Imanishi*[5], *Seiji Fukumoto*[6], *Taichi Kitaoka*[7], *Keiichi Ozono*[7]
[1]Minoh Hospital, Minoh, Osaka, Japan; [2]Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Osaka, Japan; [3]Okayama University Hospital, Okayama, Okayama, Japan; [4]Okayama Saiseikai General Hospital, Okayama, Okayama, Japan; [5]Osaka City Graduate School of Medicine, Osaka, Osaka, Japan; [6]The University of Tokyo Hospital, Bunkyoku, Tokyo, Japan; [7]Osaka University Graduate School of Medicine, Suita, Osaka, Japan

Background: Ossification of posterior longitudinal ligament (OPLL) was reported in genetic hypophosphatemic osteomalacia such as XLH, ADHR and ARHR. We experienced a case of hypophosphatemic osteomalacia complicated by OPLL, and investigated the responsible mutations.
Case: The patient was a 60-year-old male and from a cousin-to cousin marriage. He was pointed out to have short stature at the elementary school age. At 27 years old, hypophosphatemia was revealed by chance, and he was diagnosed as hypophosphatemic osteomalacia. However, tumors were neither detected by computerized tomography nor magnetic resonance imaging. He was treated with active vitamin D and phosphorus. OPLL was revealed at 35 years old. At 41 years old, we performed bone biopsy to find a periosteocytic hypomineralized lesion (HPL), a hallmark of XLH. Laboratory test at that time indicated a low serum 1,25(OH)2D level (21.1pgml^{-1}) despite a low level of serum phosphate (1.4mgdl^{-1}). %TRP and TmP/GF values were 52% and 1.2mgdl^{-1} respectively. Serum ALP level was normal (95 IUl^{-1}). Serum c-FGF23 value was elevated (500RUml^{-1}). Recently, we investigated PHEX, FGF23, DMP1 and ENPP1 genes, but the responsible mutations were not identified.
Discussion: The consanguinity in his family suggests that the patient was affected with hypophosphatemic osteomalacia of autosomal recessive inheritance. The presence of HPL suggests an abnormal function of PHEX due to mutations of unidentified gene.

P2013

Incorporation Of Recombinant Receptor-Associated Protein Into Parathyroid Cells-A Possible Role Of Megalin In Vitamin D Signaling In Hyperfunctioning Parathyroid Diseases

Yamagata Masayo[1,2], *Yasuo Imanishi*[1], *Nagata Tomoki*[1], *Ohara Masaya*[1], *Tsutsui Hidenobu*[2], *Toshimi Michigami*[3], *Masaaki Inaba*[1], *Yukimura Tokihito*[2]
[1]Osaka City University Graduate School of Medicine, Osaka, Osaka, Japan; [2]Osaka Ohtani University, Tondabayashi, Osaka, Japan; [3]Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Osaka, Japan

Context: Megalin is a multiligand endocytotic receptor involving in the reabsorption of 25-hydroxyvitamin D (25OHD) and vitamin D binding protein (DBP) in renal proximal tubules. Megalin expression decreased as well as vitamin D receptor in hyperfunctioning parathyroid tumors. Attenuated expressions of megalin will reduce 1,25-dihydroxyvitamin D production from 25OHD in parathyroid tumors, resulted in enhanced secretion of parathyroid hormone.
Objective: The aim of this study is to determine the role of megalin in incorporation of 25OHD and DBP in parathyroid cells.
Methods: Histidine-tagged recombinant protein for the soluble form of 39-kD receptor-associated protein (His-sRAP), which binds to the ligand-binding domain of megalin, was added to primary cultured parathyroid cells obtained by therapeutic parathyroidectomies. The incorporations of His-sRAP by megalin to the cells were determined by immunofluorescence using anti-His and anti-megalin antibodies.
Results: The expression of megalin was observed at the membrane region. The expression of His-sRAP was observed in the membrane region and cytosol 15 min after the addition of His-sRAP. The distribution of megalin overlaps with that of His-sRAP in the membrane region.
Conclusion: The incorporation of His-sRAP suggested megalin has a crucial role in uptake of 25OHD and DBP in

parathyroid cells, and the decrement of the megalin expression may contribute to the pathogenesis of hyperfunctioning parathyroid diseases.

P2014

Serum Magnesium Predicts Efficacy Of Daily Teriparatide On Lumber Bone Density In The Treatment Of Osteoporosis

***Yasuo Imanishi**, Yuki Nagata, Maki Yoda, Koichiro Yoda, Ohara Masaya, Shinsuke Yamada, Hitoshi Goto, Masaaki Inaba*
Department of Metabolism, Endocrinology & Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan

Context: Magnesium is a major component of bone as well as calcium. Daily teriparatide administration decreases serum magnesium, however, the importance of serum magnesium on bone metabolism is not well elucidated.
Objective: To determine the role of pre-treatment serum magnesium measurement on metabolic bone markers and lumber bone density.
Subject and Method: 25 patients with primary and glucose-induced osteoporosis (lumber BMD T-score -2.9 ± 1.4) were administered 20μg daily teriparatide.
Result: Significant increment of lumber BMD was observed in 48 week treatment. Serum magnesium significantly decreased on day 3, followed by the significant decrement of urinary magnesium excretion on day 30. Significant correlation was observed between pre-treatment serum magnesium and the increment of lumber BMD for 48 weeks (r= 0.404, P= 0.045). The increment of serum P1NP for 24 weeks, the well-known predictor of BMD increment by teriparatide treatment, significantly correlated to pre-treatment serum magnesium (r= 0.545, P= 0.009).
Conclusion: Pre-treatment serum magnesium can predict the efficacy of teriparatide therapy. Further study is necessary to characterize the roles of alimentary magnesium on osteoporosis treatment by dietary magnesium consumption.

P2015

Chromatin Dynamics During Bone Metastasis

***Woojin An**, Jinman Kim*
USC/Keck School of Medicine, Los Angeles, California, USA

Breast/prostate cancers frequently metastasize to bone, but the molecular mechanisms deriving their bone metastasis remain elusive. Osteoblasts produce M-CSF and RANKL and induce osteoclast differentiation and bone resorption. These cancer cells also secrete several growth factors such as TGF-β, VEGF and IGF to promote osteoclastogenesis. Transcriptional regulation of distinct sets of genes is fundamental to the normal phenotypic development of both osteoblasts and osteoclasts, so transcriptional deregulation in tumor cells may support bone metastasis. Posttranslational histone modifications and histone variants are key transcriptional regulators of genes that are critical for bone metastasis of cancer cells. Our long-term goal is to understand how various chromatin remodeling activities, via activation or repression of specific genes, contribute to metastatic bone tumors. To investigate these aspects, we established multiple protocols to interrogate the cellular function of histone modification and variant as epigenetic signals which would mediate bone metastatic processes. I will present some of our recent results supporting that histone variant macroH2A and histone acetylation/methylation are critical components in regulating osteoclast differentiation and bone metastasis. These results emphasize a new aspect of chromatin remodeling and illustrate the power of combined cellular and biochemical approaches for mechanistic studies.

P2016

The Role Of Insulin-Like Growth Factor-I And Focal Adhesion Kinase Signaling In Breast Cancer Induced Bone Metastasis

***Naito Kurio**[1], Tsuyoshi Shimo[1], Takuya Fukazawa[2], Hiromasa Kuroda[1], Yuu Horikiri[1], Tatsuo Okui[1], Shinji Hatakeyama[3], Munenori Takaoka[2], Yoshio Naomoto[2], Akira Sasaki[1]*
[1]Department of Oral and Maxillofacial Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [2]Department of General Surgery, Kawasaki Medical School, Okayama, Japan; [3]Novartis Institutes for BioMedical Research, Basel, Switzerland

Introduction: Insulin-like growth factors (IGFs), which are the most abundant growth factors stored in bone, have been implicated in progression and aggressiveness of many types of cancers. Recently we reported that a novel dual inhibitor for Focal adhesion kinase (FAK) and IGF-IR, TAE226, inhibit breast cancer bone metastasis, but the relation between FAK and IGF in tumor bone microenvironment has not been clarified yet. Using both *in vivo* and *in vitro* approaches, we investigated how IGF-I is involved in FAK in tumor progression in bone metastasis site.
Methods: A mouse model of bone metastasis was prepared by inoculating mice with tumor cell suspensions of breast cancer cell line MDA-MB-231 cells via the left cardiac ventricle. Oral administration of TAE226 was carried out at a dose of 30 mg/kg every day. The expression of IGF-I and FAK related signals were confirmed by Western blot analysis. To evaluate the effects of IGF-I *in vitro*, proliferation assay, migration assay, and angiogenesis assays were done.
Results: Treatment of mice with a TAE226 greatly decreased CD31 positive endothelial cells in bone metastasis site. IGF-I upregulated pFAK Tyr397 activity, and stimulated proliferation, migration and tube formation of endothelial cells *in vitro*, and these effects were inhibited in the presence of TAE226.
Discussion: IGF-I was critically involved in breast cancer progression in bone metastasis area through pFAK Tyr397 activation in endothelial cells.

P2017

An Acidic Milieu Confers The Resistance To Trail In Myeloma Cells Through The PI3K-Akt-mediated Epigenetic Down-Regulation Of The Trail Receptor DR4

Ryota Amachi[1,2]*, Masahiro Abe*[1]*, Keiichiro Watanabe*[1,2]*, Shiro Fujii*[1]*, Takeshi Harada*[1]*, Hirokazu Miki*[1]*, Shingen Nakamura*[1]*, Masahiro Hiasa*[1,3]*, Eiji Tanaka*[2]*, Toshio Matsumoto*[1]
[1]Dept. of Medicine and Bioregulatory Sciences, Tokushima Univ., Tokushima, Japan; [2]Dept. of Orthodontics and Dentofacial Orthopedics, Tokushima Univ., Tokushima, Japan; [3]Dept. of Biomaterials and Bioengineering, Tokushima Univ., Tokushima, Japan

TRAIL-mediated immunotherapy is an attractive anti-tumor maneuver because of its tumor-specific cytotoxic activity. In osteolytic lesions in myeloma (MM), MM cells and osteoclasts (OCs) create an acidic milieu by protons produced by OCs and lactate by MM cells, which confers drug resistance in MM cells. Here, we explored DR4 editing and the anti-MM activity of an anti-DR4 agonistic antibody in acidic conditions. DR4 expression was markedly reduced in MM cells cultured in media acidified by lactic acid or cocultured with acid-producing OCs. The acetylation of histones, H3 and H4, was suppressed along with the induction of Akt phosphorylation in MM cells at pH7.1 or lower. Treatment with the HDAC inhibitior valproate restored the DR4 expression in MM cells suppressed in the acidic conditions, suggesting HDAC-mediated suppression of DR4 in an acidic environment. Furthermore, the inhibition of the PI3K-Akt pathway by LY294002 or the Akt inhibitor Akt-in (Calbiochem) abrogated the suppression of histone acetylation and restored the DR4 expression in MM cells in acidic conditions. Although the anti-MM effects of the anti-DR4 agonistic antibody R1-B12 were blunted in acidic conditions, the Akt or HDAC inhibition sensitized MM cells to R1-B12. From these results, an acidic milieu in MM bone lesions is suggested to confer the resistance in MM cells against TRAIL-mediated immunotherapy through the PI3K-Akt-mediated epigenetic regulation of DR4 expression.

P2018

The Effects Of Zoledronate In Treatment Setting In A Nude Mouse Model Of Breast Cancer Bone Metastasis

Jussi Halleen*, Jukka Rissanen, Katja Fagerlund, Mari Suominen*
Pharmatest Services Ltd, Turku, Finland

Zoledronate (ZOL) is approved for adjuvant treatment of breast cancer bone metastases. We tested a clinically relevant dose of ZOL in treatment setting in a nude mouse model of breast cancer bone metastasis. GFP-transfected MDA-MB-231(SA) cells were inoculated into the left cardiac ventricle of nude mice. Bone lesions and growth of cancer cells were quantitated by radiography and fluorescence imaging at days 14 and 25. The mice were randomized to two groups (n=8/group) at day 14 according to body weight and osteolytic lesions. One group received vehicle and the other was administered with ZOL (100 μgkg^{-1} s.c.) once at day 15. Serum TRACP 5b and PINP were determined at days 1, 9, 17 and 24. Osteolytic foci were found in 31% of all mice at day 14. All animals in the control group and 75% of the mice in the ZOL group had lesions at sacrifice. Osteolytic lesion area was lower in the ZOL group at sacrifice, with only minimal increase from day 14. However, ZOL had no effect on tumor growth. TRACP 5b increased in the control group and was 251% from day 0 at day 24. ZOL decreased TRACP 5b at days 17 and 24. PINP decreased in the control group throughout the experiment. ZOL caused a rapid decrease in PINP that was significant at day 17 but not anymore at day 24. As a conclusion, a single clinically relevant dose of ZOL inhibited the increase of osteolytic area but was not able to inhibit tumor growth in the established disease in this aggressive model of breast cancer bone metastasis.

P2019

RankIs Synthesized By Both Oral Cancer Cells And Stromal Cells Participate In Osteoclastic Bone Resorption

Kiyoshi Sato[1,2]*, Ji-Won Lee*[2]*, Ken Omura*[1,3]*, Akira Yamaguchi*[2]
[1]Section of Oral and Maxillofacial Surgery, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan; [2]Section of Oral Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan; [3]Department of Advanced Molecular Diagnosis and Maxillofacial Surgery, Hard Tissue Genome Research Center, Tokyo Medical and Dental University, Tokyo, Japan

Bone destruction by invading oral cancer is associated with a poor prognosis, but the molecular mechanisms are not well understood. Several reports demonstrated that both stromal cells and tumor cells synthesize receptor activator of nuclear factor-κB ligand (RANKL) in various cancers, but the precise roles of RANKL produced by either cell type in cancer-associated bone resorption are unclear. Using immunohistochemistry, we demonstrated that RANKL-positive fibroblasts and cancer cells were located at sites of bone invasion in human oral cancers. HSC3 and HO-1-N-1, human oral cancer cell lines, expressed RANKL and stimulated Rankl expression in UAMS-32 murine osteoblastic cell line. We discriminated the roles of RANKL synthesized by stromal cells and cancer cells in cancer-associated bone resorption by using species-specific RANKL antibodies against murine RANKL and human RANKL, respectively. The present study revealed that RANKL produced by both stromal and cancer cells is involved in oral cancer-induced osteoclastic bone resorption. These results provide important information for understanding the cellular and molecular basis of cancer-associated bone destruction and the mechanism of action underlying RANKL antibody (denosumab) therapy.

P2020

Induction Of Bone Formation In Myeloma Osteolytic Lesions By Cathepsink Inhibition

Keiichiro Watanabe[1,2], *Masahiro Abe*[1], *Hiroshi Mori*[3], *Ryota Amachi*[1,4], *Masahiro Hiasa*[1,5], *Takeshi Harada*[1], *Shiro Fujii*[1], *Shingen Nakamura*[1], *Hirokazu Miki*[1], *Kumiko Kagawa*[1], *Itsuro Endo*[1], *Eiji Tanaka*[4], *Toshio Matsumoto*[1]
[1]Department of Medicine and Bioregulatory Sciences, The University of Tokushima Graduate School, Tokushima, Japan; [2]Department of Orthodontics, Tokushima University Hospital, Tokushima, Japan; [3]Discovery Research Laboratories, Minase Research Institute, Research Headquarters, Ono Pharmaceutical co., Ltd., Osaka, Japan; [4]Department of Orthodontics and Dentfacial Orthopedics, The University of Tokushima Graduate School, Tokushima, Japan; [5]Department of BIomaterials and Bioengineerings, The University of Tokushima Graduate School, Tokushima, Japan

Multiple myeloma (MM) enhances osteoclastogenesis while suppressing osteoblastogensis to develop devastating bone destruction. Unlike other anti-resorptive agents, cathepsin K inhibitors potently suppress bone resorption while sparing cytotoxic damage in osteoclasts (OCs). In the present study, we explored the effects of cathepsin K inhibition on bone destruction in MM. The cathepsin K inhibitor KK1-300-01 (KK1) potently suppressed pit formation enhanced in the cocultures of rabbit bone cells with MM cells. However, KK1 did not affect osteoclastogenesis, and allowed OCs to facilitate *in vitro* mineralized nodule formation by MC3T3-E1 cells, suggesting the preservation of OC-driven osteoblastogenesis by KK1. We next examined the *in vivo* effects of KK1 using human INA6 MM-bearing SCID-rab models, which exhibit tumor progression with osteolytic lesions in implanted rabbit bones. Oral dosing of KK1 prevented bone destruction with marked increase in bone trabecular size and BMD in the rabbit bones and tumor reduction within their bone marrow cavity. Histological analyses showed increased bone volume/total volume with a marginal change in OC numbers in the treated mice. Given OC-derived coupling, KK1 is suggested to spare the damage in OCs while inhibiting bone resorption to retain the coupling for bone formation together with reducing the release from bone of anti-anabolic factors such as TGF-beta, leading to robust bone formation and thereby MM contraction in bone.

P2021

Bone Malignant Melanoma Induces Angiogenesis With The Production Of Prostaglandin E2 By Host Stromal Cells

Kenta Watanabe, *Satoshi Yokoyama, Chiho Matsumoto, Michiko Hirata, Chisato Miyaura, Masaki Inada*
Tokyo University of Agriculture and Technology, Tokyo, Japan

Bone metastasis of malignant melanoma is accompanied by severe bone destruction with increased bone resorption. When B16 cells were subcutaneously implanted into both wild-type mice (Wt) and *mPges-1* null mice (*mPges1–/–*), *mPges1–/–* showed the suppression of B16 solid tumor formation. In the fluorescent imaging for *in vivo* analysis, the angiogenesis was detected by Angiosense as fluorescent signals, and dramatically suppressed in the group of *mPges1–/–*. By the intra-vein injection of B16 cells, the bone metastasis accompanied with angiogenesis was detected in the femur and tibia. In *mPges1–/–*, both metastasis and angiogenesis were perfectly suppressed compared with Wt. When dermal fibroblasts derived from Wt were co-cultured with fixed-B16 cells, PGE2 production was markedly increased in the culture medium. Dermal fibroblasts collected from *mPges1–/–* produced less amounts of PGE2. The increased production of vascular endothelial cell growth factor (VEGF)-A and basic fibroblast growth factor (bFGF) was detected in the dermal fibroblasts collected from Wt, but not from *mPges1–/–*. These results suggest that PGE2 produced by host stromal cells promotes VEGF-A and bFGF production, which leads the angiogenesis at the site of melanoma metastasis. The blockage of PGE2 signaling such as PGE receptor antagonist could be a possible candidate for the therapy of bone cancer associated with angiogenesis.

P2022

Compressive Fatigue Life Of Subchondral Bone In The Equine Metacarpal Condyle

Sandra Martig[1], *Garry Anderson*[1], *Peter Lee*[2], *Robert Whitton*[1]
[1]Veterinary Hospital, University of Melbourne, Werribee, Victoria, Australia; [2]University of Melbourne, Parkville, Victoria, Australia

Racehorses suffer from fatigue induced subchondral bone injury (SCBI) and subsequent articular cartilage damage. We determined the fatigue life of equine metacarpal subchondral bone ex vivo and investigated factors influencing initial bone stiffness. Samples were loaded cyclically in compression [90 MPa (*n*=6), 78 MPa (*n*=5), 66 MPa (*n*=6), 54 MPa (*n*=6)] until fracture. The fatigue life curve was determined by linear regression from load and log transformed number of cycles to fracture. The influence of the following variables on initial stiffness was investigated: age (4 - 8 yrs), sample storage time (31 - 846 d), duration of current training period (6 - 32 weeks), leg, actual density (1.6873 - 1.8684 gcm^{-3}), SCBI grade (0 - 3), and cause of death (fatigue injury vs. other) using SPSS. Number of cycles to fracture was (median, range) 4001, 152 - 11568 at 90 MPa; 13204, 614 - 16425 at 78 MPa (n=3); 69908, 146 - 149855 at 66 MPa; and 223603, 78316 – 806792 at 54 MPa. The fatigue life curve was $\sigma = 112.2 – 9.6 \log10Nf$, $R2 = 0.52$, where Nf is number of cycles to fracture and σ is load. Removal of three horses with the highest SCBI grade resulted in: $\sigma = 134.2 - 14.1 \log10Nf$, $R2 = 0.72$. Mean initial stiffness was 2513 MPa (*n*=22). Actual density (ρ) was the only variable retained in the statistical model to describe initial stiffness (E): $E = (-8594.7) + 6110.4\rho$, $R2 = 0.37$, $P = 0.004$. These data can be used to model the development of SCBI and other fatigue related joint diseases.

P2023

Assessing Efficacy Of Osteoporosis Drugs: A Three-year Finite Element Analysis Study Of The Femoral Neck

D. Anitha, *Taeyong Lee*
Bioengineering, National University of Singapore, Clementi, Singapore

Osteoporosis remains a prevalent problem amongst the elderly and is known to vary from 10-20% in patients aged 50

[P2023]

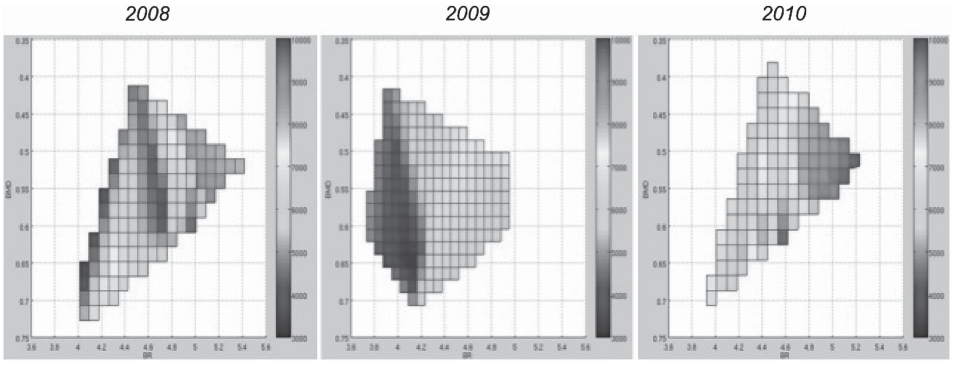


Figure 1 A triadic plot of patients from 2008 to 2010. X-axis: BR Y-axis: BMD Z-axis/ Colour scale: Fcr

years and older, depending on race and assessment methods. However, following osteoporosis therapy, increases in BMD can be deceptive. While the commercially available drugs seemingly counteract bone loss due to osteoporosis and consequently reduce fracture risk, the efficacy of these drugs in treating osteoporosis is still unclear. Therefore, we aim to compare drug influences, mainly ibandronate, risedronate and raloxifene, on BMD, peak fracture load (Fcr) and buckling ratio (BR) at the femoral neck. Fcr, derived from QCT-based finite element (FE) modeling and BR will provide insight into how each drug has uniquely asserted its therapeutic effects. This study used data from existing QCT in the period 2008-2010 of females who are 50 years of age or older and had been diagnosed with osteopenia or osteoporosis. The BMD was categorized into osteopenic, osteoporotic and normal groups. Geometric analysis is done by reslicing perpendicular to the femoral neck axis. The cortical thickness and radius is obtained by averaging all the profile ray values and BR is calculated. Structural analysis is performed by use of finite element analysis (FEA) software. With the use of appropriate boundary conditions, Fcr is obtained from force versus displacement curves. In our preliminary results, we have first analyzed ten patients from the risedronate group.

P2024

Reduced Radiation Sterilization Dose Of Bone Allografts Improves Bone Quality And Surgical Outcomes, Yet Retains Sterility Assurance Levels

Mark Forwood[1], *Huynh Nguyen*[1,2], *David Morgan*[4,2], *Evelyne Gineyts*[3]

[1]School of Medical Science, Griffith University, Gold Coast, Queensland, Australia; [2]Queensland Bone Bank, Brisbane, Queensland, Australia; [3]University of Lyon, INSERM U1033, Lyon, Rhone Alpes, France; [4]Brisbane Private Hospital, Brisbane, Queensland, Australia

Our objective was to determine an optimal radiation sterilization dose that achieves sterility assurance while minimizing deleterious effects on allograft bone quality. For sterility testing, we inoculated allograft bone with S. epidermidis and B. pumilus, then exposed them to gamma irradiation at 0, 5, 10, 15, 20 and 25 kGy. Mechanical and biological properties of cortical allografts and morsellised bone were determined following irradiation at these doses. A dose of 20-25 kGy eliminated both inoculated organisms at concentrations from 101-103, while 10-15 kGy sterilized bone samples to a bioburden concentration of 102. Irradiation did not generate pro-inflammatory bone surfaces, as evidenced by macrophage activation, nor did it affect attachment or proliferation of osteoblasts. At a dose of 15 kGy or greater, there was a significant decline in the energy absorption capacity of cortical and morsellised bone ($p < 0.05$); and the attachment and fusion of osteoclastic cells onto irradiated bone ($p < 0.05$). We observed no significant change in the content of bone collagen cross links, but there was a dose-response increase in denatured collagen in irradiated bones ($p < 0.05$). We conclude that 15 kGy is a threshold for radiation sterilization of bone allografts that provides an acceptable sterility assurance level, and above which allograft strength and biocompatibility declines significantly.

P2025

Effects Of Eldecalcitol On Cortical Bone Response To Mechanical Loading In Rats

Keita Nagira[1], ***Yusuke Yamasaki***[2], *Hiroshi Hagino*[2]

[1]Orthopedics Surgery, Tottori University Faculty of Medicine, Yonago, Japan; [2]School of Health Science Tottori University Faculty of Medicine, Yonago, Japan

Aims: We evaluated the effect of eldecalcitol on the cortical bone response to mechanical loading applied using a 4-point bending device.

Methods: 6-month old female Wistar rats were used and randomized into 4 groups (N=10/group): Vehicle administration (VEH), low dose eldecalcitol administration (ED-L, 0.025μgkg^{-1} BW), middle dose eldecalcitol administration (ED-M, 0.05μgkg^{-1} BW), and high-dose eldecalcitol administration (ED-H 0.1μgkg^{-1} BW). Eldecalcitol or vehicle was administered orally using the feeding needle at a dosage 3 times/week for 3weeks. Loads on the right tibia by 4-point bending on the same day. After calcein double labeling the rats were sacrificed and tibial cross sections were prepared from the region with maximal bending at the central diaphysis. Histomorphometry was performed at the entire periosteal and endocortical surface of the tibiae, dividing the periosteum into lateral and medial surfaces.

Results: The Formation surface (FS) and mineral appositional rate (MAR) were promoted significantly in ED-H at the medial and endocortical surface (p<0.01 vs. ED-L). The bone formation rate (BFR) was significantly promoted in ED-H at the

endocortical surface (p<0.01 vs. other 3 groups). All 3 parameters were significantly higher in the loaded tibiae than the non-loaded tibiae in the VEH group.
Conclusion: Eldecalcitol promoted the cortical bone response to mechanical loading at the high dose (0.1μgkg^{-1}) in rats.

P2026
Comparative Study Of Ultrastructure Of Biominerals Of Bone And Dentin Of The Rat Mandible
***Vladyslav Luzin**, Dmitri Astrakhantsev, Pavel Golubkov, Larisa Sokol, Ksenia Simrok, Marina Solyanaya*
Luhansk State Medical University, Luhansk, Ukraine

For the purposes of the study we used 252 rats with body weight of 135-145 grams. In the experiment we studied ultrastructure of mineral taken from ramus of mandible and dentin of incisor by means of X-ray scatter analysis. For X-ray analysis we used copper Kα radiation with wavelength of 0.1542 ηm; voltage and amperage of the tube were 30 kV and 10 mA respectively. Data used were parameters of elementary cells, crystallites dimensions and microtexture coefficient.
From the 7th to the 180th day of observation dimensions of elementary cells of bone mineral along α-axis increased from 9.38±0.001 10-10 M to 9.39±0,004 10-10 and along c-axis - from 6.84±0.003 10-10 M to 6.85±0.002 10-10 M. C/a ratio ranged within 72,94-73,04 102 M, which testifies for balance between crystallization and resorption of bone mineral. Crystallites dimensions increased from 40.77±0.64 ηm to 43.94±0.53 ηm and microtexture coefficient increased from 0.392±0.005 units to 0.432±0.001 units. In dentin mineral from the 7th to the 180th day of observation dimensions of elementary cells along α-axis increased from 9.36±0.004 10-10 M to 9.37±0.004 10-10 M and along c-axis - from 6.82±0.004 10-10 M to 6.83±0.005 10-10 M. C/a ratio ranged within 72.81-72.86 102 M. Crystallites dimensions increased from 28.86±0.36 ηm to 32.64±0.42 ηm microtexture coefficient increased from 0.563±0.010 units to 0.570±0.004 units. Our study showed that mineral of dentin is better organized than that of the bone tissue.

P2027
Differential Gene Expression In Growth Cartilage And Osteochondrosis
Babatunde Awodele, Eleanor Mackie, Charles Pagel, ***Michiko Mirams***
Faculty of Veterinary Science, University of Melbourne, Parkville, Victoria, Australia

Osteochondrosis (OC) is a developmental orthopaedic condition involving defective endochondral ossification and retention of cartilage in subchondral bone. We have recently shown expression of the hypertrophy-associated genes MMP13, COL10 and RUNX2 to be higher in equine OC cartilage than in control cartilage. The aim of this study was to identify genes associated with early OC, and examine whether they are regulated during normal chondrocyte terminal differentiation. From subtractive hybridisation of equine OC lesions and control cartilage, 79 putative differentially expressed genes were identified. In quantitative PCR (qPCR) studies, 9 genes were more highly expressed in OC lesions than in controls. Of these, osteopontin and integrin-binding sialoprotein are known to be upregulated with chondrocyte hypertrophy. An additional 23 genes poorly characterised in cartilage were examined in growth plate cartilage by qPCR. Expression of 13 genes (ATP6V0D2, BAK1, DDX5, GNB1, PIP5K2A, PP-1B, RAP1B, RP-S7, SRP20, SUB1 homolog, TMSB4, TPI-1, WSB) was increased and expression of three (CHM1, FOXA3, SERPINA1) was decreased in hypertrophic chondrocytes compared to cells in the resting and proliferative zones. The results provide further evidence that chondrocytes in OC lesions express hypertrophy-associated genes. Furthermore, we have identified a number of novel hypertrophy-associated genes; further studies will be required to determine whether they play functional roles in this process.

P2028
Shear Stress Inhibits Urokinase Plasminogen Activator Expression In Human Chondrocytes By Activation Of Amp Kinase
Chih-Chang Yeh[1], Hsin-I Chang[2], ***Cheng-Nan Chen[2]***
[1]Chiayi Branch, Taichung Veterans General Hospital, Chiayi City, Taiwan; [2]National Chiayi University, Chiayi City, Taiwan

Synovial macrophages, which can release proinflammatory factors, are responsible for the up-regulation of cartilage breakdown proteases, and play critical roles in cartilage degradation during the progression of osteoarthritis (OA). In addition, shear stress exerts multifunctional effects on chondrocytes by inducing the synthesis of catabolic or anabolic genes. We investigated the mechanisms underlying the modulation of human chondrocyte uPA expression by macrophages and shear stress. Real-time polymerase chain reaction was used to analyze uPA gene expression. Inhibitors and small interfering RNA were used to investigate the mechanism for the effects of PB-MCM and shear stress in chondrocytes. Stimulation of human chondrocytes with PB-MCM was found to induce uPA expression. We demonstrate that activation of the JNK and Akt pathways and NF-kB are critical for PB-MCM-induced uPA expression. PB-MCM-treated chondrocytes subjected to a lower level of shear stress showed inhibition of MCM-induced JNK and Akt phosphorylation, NF-κB activation and uPA expression. The PB-MCM-induced uPA expression was suppressed by AMP-activated protein kinase (AMPK) agonist. The inhibitor or siRNA for AMPK abolished the shear-mediated inhibition of uPA expression. These data support the hypothesis that uPA up-regulation stimulated by macrophages may play an active role in the onset of OA and in the shear stress protection against this induction.

P2029
Changing The Composition Of The Bone Tissue In The Ontogen
Vladyslav Luzin, Andrey Ivchenko, ***Anton Yeryomin***
Luhansk state medical university, Luhansk, Ukraine

Aim: To examine characteristics of macroelement composition of bones of albino rats in ontogenesis. In addition, we

have examined the effects of the bone defect in the bone on its macroelement composition while maintaining the functional load on it.
Materials and methods: We carried out an experiment on 252 white male rats, animals were divided into four groups: first group - intact animals, second group - animals, which formed a cross-cutting bone defects on the border of the proximal metaphysis and diaphysis tibia.
Results: Calcium content was less than control values, respectively on 6.24%, 8.87%, 9.57%, 7.59% and 6.92%. The phosphorus content in the same period increases, the ratio of calcium / phosphorus was less than a controlling on 10.42%, respectively, 14.58%, 14.70%, 11.04% and 10.17%. Under these conditions, sodium, potassium and magnesium in the humerus were bigger the rates of intact animals from 7 to 30 days of the experiment, respectively, on 13.91%, 13.03% and 12.51%, and 12.69%, 13.88% and 11.62% and 8.46%, 7.25% and 6.74%. We find an increase in the potassium content of bone ash (8.82%) at 180 day of experiment.
Conclusion: Thus, we can conclude about negative effect of defects on the bone mineral content.

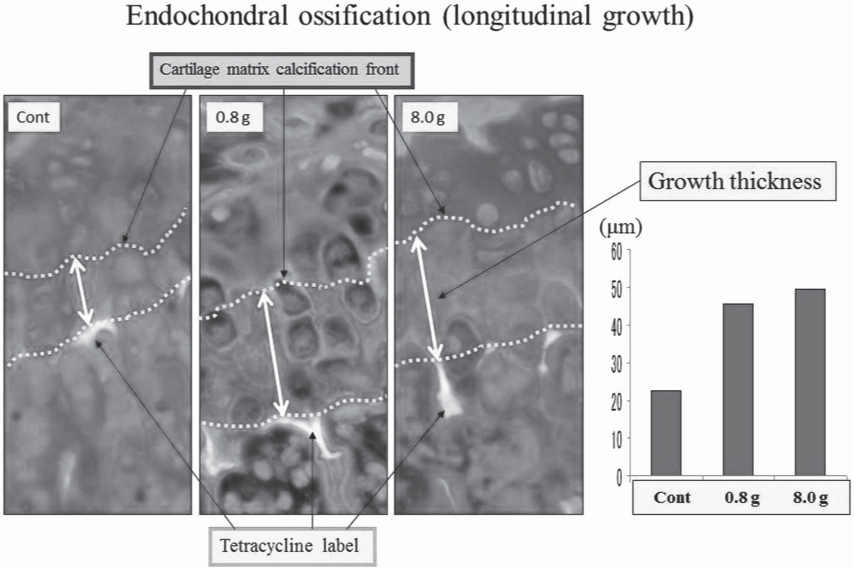
P2030
Organometric Parameters Of Mandible Molars In Mature Rats After Application Of Tibial Defect
Vitaly Morozov, ***Vladyslav Luzin****, Helen Morozova*
Luhansk State Medical University, Luhansk, Ukraine

Bone fractures are accompanied by not only a violation of their integrity, but also the development of the "fracture syndrome", which has a negative effect on the morphology and function of other organs of the skeletal system. The information concerning the characteristics of changes in the parameters of growth of molars of the mandible in the literature there are practically absent and contradictory. In the experiment, 84 mature male rats on days 7, 15, 30, 60, 90 and 180 days after the application through a defect in the proximal tibial shaft with calipers measure the length and height of the molar row of the mandible. The results were compared with the corresponding parameters of intact rats of the same age group. After statistical analysis of data in the program «STATISTICA 5.5» found, that the length of the molar row decreased from 30 to 90 day observation at 4.20%, 2.88%, 3.61%, and its height - only 90 days at 4.79% ($p<0,05$). Thus, in a simulation of the "fracture syndrome" has been a slowdown in the growth process of the molar row of the mandible, preferably from 30 to 90 day observation, which could be due to the mobilization of macro-and micronutrients of this organ in the area of reparative osteogenesis of the tibia, which caused disturbance of mineralization forming tissues of molars and, consequently, inhibition of their growth.

P2031
Effects Of Mechanical Load On Bone/Cartilage Development In Murine Long Bone Organ Culture Model
Satoshi Miyamoto*, Yasukazu Yonetani, Tatsuo Mae, Hideki Yoshikawa, Ken Nakata*
Department of Orthopaedic Surgery,Osaka University Graduate School of Medicine, Osaka-fu, Suita, Japan

Mechanical load plays a critical role in skeletal development. However, effects of mechanical stress on bone/cartilage formation are not well understood. We have developed a 3-D mechanical load culture system which applies an axial cyclic load to murine long bones. Effects of mechanical load on bone/cartilage development were examined by evaluating histological alteration at growth plate and endosteum of the diaphysis.
Forelimbs metatarsal bones of C57BL/6 strain of 3 week-old mouse were excised and processed organ culture. Metatarsals were erected in collagen sponge and were subjected to cyclic compressive load in the direction of the long axis at 0.5 Hz for 1 hour for 4 days at different magnitude of load (0.8 g and 8.0 g). After mechanical load, histological sections were examined.
Mineralized cartilage area in the growth plate of the loaded groups was significantly larger than that of the unloaded control. In endosteum, the number of osteoid osteocyte and

[P2031]



osteoid formation in the loaded group were significantly larger than those of unloaded group, which suggests that mechanical stimulation promoted the transition from osteoblast to osteocyte and osteoid formation. This study demonstrated that bone/cartilage development of 3 week-old murine long bone was influenced by mechanical load via endochondral and intramembranous ossification. This culture system could help to elucidate the mechanism in which mechanical stimulation has an effect on bone/cartilage development.

P2032

Morphometric Research Of The Thyroid Gland Against Application Of Glucocorticoids And Zoledronic Acid Zometa

***Vladyslav Luzin**, Ksenia Fomina, Anton Yeryomin*
Luhansk state medical university, Luhansk, Ukraine

Introduction: Bisphosphonate drugs are widely used to treat bone diseases. But their effects on other organ systems are not well understood. Therefore, our purpose is to examine histomorphometric features of the thyroid gland structure in an experiment on white laboratory rats of different ages with increased influence of glucocorticoids and zoledronic acid (Zometa). This work was carried out in accordance with the plan of research of SI "Lugansk State Medical University".
Materials and methods: To identify the morphofunctional features of the thyroid gland of control and experimental animals, the organ was studied on the cellular level: larger and smaller diameters of the follicles.
Results: The larger and smaller diameters of the follicles of the immature and mature rats after injection of hydrocortisone are higher than control values at 6.70% - 13.92%, in old animals - at 16.77% - 23.22 %. After exposure of dexamethasone deviations are less marked, above the 0.71% - 11.84%. In animals treated with Zometa, maximum and minimum diameters of the follicle are lower than the control values at 6.50% - 18.18% among all series.
Discussion: It was revealed that application of glucocorticoids leads to the changes in thyroid gland structure in rats of various age and these changes are noted at cellular level of its structural organization. Introducing of zoledronate into the rat organism decreases negative effect of glucocorticoids.

P2033

The Safety And Effects Of Eldecalcitol Treatment In Postmenopausal Women Undergoing Maintenance Hemodialysis

Naomi Sasaki
H.N.Medic, Sapporo, Japan

Backgroud and Purpose: Eldecalcitol(ELD) is a new active vitamin D3 analog developed in Japan which has attracted attention as an effective therapeutic drug for primary osteoporosis. However, as ELD leads to increased calcium absorption compared to conventional active vitamin D3, it has not been used in patients with severe renal insufficiency to date. We examined the safety and effects on bone metabolism of ELD treatment for 1 year in postmenopausal women undergoing maintenance hemodialysis.
Subjects and Methods: Forty postmenopausal women who had been receiving maintenance hemodialysis at our institution were enrolled. No patients with an average serum albumin-corrected calcium (Ca(Alb)) level of greater than 10.0mg/dL and not in stable control of hyperparathyroidism were included. ELD 0.5μg per day was added to existing active vitamin D treatment regimen and patients were followed for 1 year from the start of treatment.
Results: The changes of mean serum Ca(Alb), intact PTH, bone turnover markers and lumbar spine bone mineral density are shown in Table1.
Conclusion: Severe hypercalcemia can not be controlled did not develop in ELD treatment for 1 year. Lumbar spine bone density was significantly increased after 6 months ELD treatment, but the effects was not sustained until after 1 year.

Table 1

	Before	6 months after	1 year after
Serum Ca(Alb)	8.97±0.34	9.36±0.34	9.46±0.43
Serum intactPTH	109±66	84±54	79±43
Serum BAP	133±75	88±50	69±37
Serum TRACP-5b	688±361	491±283	450±235
Lumbar spine BMD	0.501	0.518*	0.509
Lumbar spine T score	–3.81	–3.57*	–3.71
Lumbar spine Z score	–0.69	–0.39*	–0.49

Mean±s.d., BAP: Bone specific ALP measured by PAGE method., *$P<0.05$ vs Before

P2034

Systematic Review And Meta-analysis Of The Effects Of Vitamin D Supplements On Bone Mineral Density

***Ian Reid**, Mark Bolland, Andrew Grey*
Medicine, University of Auckland, Auckland, New Zealand

Recent meta-analyses of vitamin D without calcium have not demonstrated fracture prevention. Bone mineral density (BMD) is able to detect biologically significant effects in much smaller cohorts. Therefore we have determined whether vitamin D supplementation influences BMD. Web of Science, Embase and Cochrane were searched for RCTs assessing vitamin D (D2 or D3) effects on BMD. 23 studies, mean duration 23.5 months, comprising 4082 participants, 92% women, average age 59 years, met the inclusion criteria. Nineteen studies were in predominantly Caucasian populations. Mean baseline serum 25-hydroxyvitamin D was <50 nmol/L in 8 studies (1791 participants). Twelve studies administered calcium supplements to all trial participants. Ten studies (2294 participants) used vitamin D doses <800 IU/day. BMD was measured at 1-5 sites in each study. There were 5 findings of significant benefit, 2 of significant detriment, and the rest were non-significant. Only one study found benefit at >1 site. Meta-analysis showed a small benefit at the femoral neck (0.8%, 95%CI 0.2, 1.4) with heterogeneity among trials. There was no effect at any other site, including the total hip. There was evidence of a bias toward positive results at the femoral neck and total hip. We conclude that vitamin D supplementation did not change BMD over an average duration of 2y, except at the femoral neck where there were small increases of uncertain clinical significance.

P2035

Hydroxyapatite Elevates Serum Calcium Less Than Calcium Salts But Suppresses Bone Turnover Comparably

Sarah Bristow, *Angela Stewart, Greg Gamble, Ian Reid*
Medicine, The University of Auckland, Auckland, New Zealand

High-normal serum calcium is associated with an increased risk of vascular disease, as are calcium supplements. The increase in serum calcium that occurs after the ingestion of supplements might account for their adverse cardiovascular effects. This study compared the calcaemic and bone turnover effects of preparations of hydroxyapatite with those of calcium salts. Ninety-seven postmenopausal women were randomized to calcium (1g per day) as citrate, carbonate or hydroxyapatite, or a placebo, for 3-months. Blood was collected prior to and for 8h after the first dose, and at 3-months. Eight participants randomized to citrate repeated the 8h session at 3-months. Serum calcium increased significantly for up to 8 hours after the ingestion of a calcium salt or hydroxyapatite, compared with control. This effect remained the same in the citrate group after 3-months. The increase in serum calcium and corresponding decrease in PTH were greater after ingestion of a calcium salt than hydroxyapatite. Despite this, bone turnover markers were suppressed similarly in both treatment groups at 3-months.We conclude that conventional calcium supplements raise serum calcium for at least eight hours following each ingestion. This calcaemic effect is not diminished after three months of continuous use. Hydroxyapatite preparations have a lesser calcaemic effect but suppress bone turnover comparably. Therefore, slowly absorbed calcium formulations may be safer and still retain comparable efficacy.

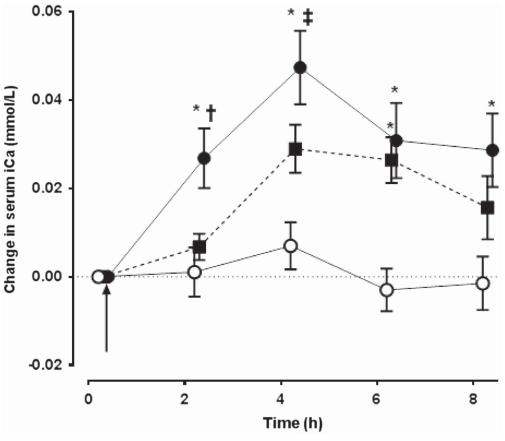


Figure 1 Changes in serum ionised calcium concentration in postmenopausal women following the ingestion of 1g of calcium as citrate or carbonate (●), 1g of calcium as hydroxyapatite (■), or a placebo containing no calcium (○). Values are means ± s.e.m. ↑ Supplement administration time. * Mean value significantly different from corresponding value for control, $p<0.05$; † mean value significantly different from corresponding value for hydroxyapatite, $p<0.05$; ‡ mean value significantly different from corresponding value for hydroxyapatite, $p=0.05$.

P2036

Genetic Screening Of Novel Negative Regulators In Osteoclast Differentiation

Fukutoshi Shirai[1], *Jin Gohda*[2], *Shinya Watanabe*[4], *Kentaro Senba*[3], *Yuu Taguchi*[1], *Jun-ichiro Inoue*[1]
[1]Division of Cellular and Molecular Biology, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [2]Research Center for Asian Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [3]Dept. of Life Science and Medical Bioscience, Waseda Univ., Tokyo, Japan; [4]Dept. of Clinical Genomics, Fukushima Medical University School of Medicine, Tokyo, Japan

Bone mass is controlled by the balance between osteoblastic and osteoclastic activities. Excess formation of osteoclast (OC) is closely related bone diseases. Formation of mature OCs from progenitor cells is promoted by M-CSF and RANK Ligand (RANKL) *in vivo*. Recent studies have reported that negative regulators of OC differentiation are expressed in progenitor cells to block spontaneous differentiation, and suppression of the negative regulators in response to RANKL stimulation is required for the proper activation of osteoclastogenic signals. It is also reported that RANKL-stimulation significantly suppresses expression of negative regulators. However, the negative regulation of OC differentiation remains to be elucidated. Therefore, we attempted to find novel negative regulators of OC differentiation.
To explore such negative regulators, we focused on genes that were down-regulated after RANKL-stimulation. To select down-regulated genes, we used multiple microarray data, which were obtained from rat bone marrow cells (BMC), mouse BMCs and RAW264.7 cells. Based on comparison of these data, we selected 14 genes that were consistently down-regulated in the data we used. Furthermore, to confirm the integrity of the screening, expression of the 14 genes in RANKL-stimulated mouse BMCs was also measured by real-time PCR. We then selected 13 genes as candidates of novel negative regulators. Effects of overexpression of candidates on OC differentiation are currently analyzed.

P2037

Identification And Analysis Of Function Of A Novel Splicing Variant Of Receptor Activator Of NF-κB

Riko Kitazawa[1,2], *Satomi Mukai*[2], *Yusa Nagai*[1], *Ryuma Haraguchi*[1], *Sohei Kitazawa*[1]
[1]Molecular Patholohy, Ehime University, Toon, Ehime, Japan; [2]Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan

Receptor activator of NF-κB (RANK) is a member of the tumor necrosis factor receptor (TNFR) family expressed in osteoclast precursors, and RANK-RANK ligand (RANKL) signaling is a key system for differentiation, activation and survival of osteoclasts. We have identified a novel alternative splicing variant of mouse RANK gene (vRANK) that contains a new intervening exon between exon 1 and exon 2 of full-length RANK (fRANK)

mRNA. Since this novel exon contains the stop codon, vRANK encodes truncated 45 amino acids that have a portion of the signal peptide of fRANK and an additional 19 amino acids that show no homology to previously reported domains. By transient transfection with vRANK-GFP and -Flag expressing constructs, vRANK was found localized mostly in the cytoplasm and partly in the cell membrane, but was not secreted into the culture supernatant. The expression of mouse vRANK mRNA was almost parallel to that of fRANK in RAW264 cells. Overexpression of vRANK in RAW cells decreased formation of TRACP-positive multinucleated giant cells and negated anti-apoptotic effect of sRANKL. Human RANK gene also contains intervening exon, which yields vRANK consisting of 38 amino acids. Vitamin D3 and TGF-β treatment induced vRANK mRNA expression in HL 60 cells through ERK and Sam68 dependent pathway. These results indicate that vRANK may be a novel osteoclast suppressor that reduces the number of RANKL-induced mature osteoclasts mainly by negating the anti-apoptotic effect of RANKL.

P2038

Inhibitory Effect Of Hyaluronic Acid On Osteoclastogenesis Via Rho Kinase

Wataru Ariyoshi*, Toshinori Okinaga, Tatsuji Nishihara*
Infections and Molecular Biology, Kyushu Dental University, Kitakyushu, Fukuoka, Japan

The objective of this study was to determine the effect of the high molecular weight of hyaluronic acid (HMW-HA) on the inductive activity of osteoclastogenesis in mouse stromal ST2 lineage cells. HA decreased the mRNA and protein expression of receptor activator of NF-κB ligand (RANKL). On the other hand, inhibition of HA synthesis by the HA synthase-2 inhibitor (4-MU) enhanced RANKL expression. To examine the role of CD44 as an HA receptor in down-regulation of RANKL, ST2 cells were pre-treated with CD44 function-blocking monoclonal antibody (mAb) prior to stimulate with HMW-HA. Inhibition of HA-CD44 binding by the CD44 mAb suppressed the HA-mediated inhibition of RANKL. Recent findings indicate that Rho kinase pathway has important roles in bone metabolism. Pull down assay revealed that HMW-HA transiently activated RhoA in ST2 cells. Pre-treatment with CD44 mAb inhibited the activation of RhoA protein mediated by HMW-HA. Moreover pre-treatment of Rho kinase pathway inhibitors also blocked the inhibition of RANKL by HA. To further clarify the role of the HMW-HA in osteoclastogenesis, we cocultured RAW264.7 cells, to serve as osteoclast precursors, with ST2 cells pre-stimulated with 1,25(OH)2D3. Culturing HMW-HA down-regulated the differentiation into osteoclast-like cells induced by 1,25(OH)2 D3-stimulated ST2 cells. These data indicated that HA-CD44 interactions down-regulate RANKL expression and osteoclastogenesis via the activation of the Rho kinase pathway.

P2039

Hair Follicle Cells Derived From Neural Crest Differentiate Into Osteoblasts And Support Osteoclast Differentiation

Eri Morisawa*[1,2], Masamichi Takami[1], Tetsuo Suzawa[1], Kazuyoshi Baba[2], Ryutaro Kamijo[1]*
[1]Department of Biochemistry,School of Dentistry,Showa University, Tokyo, Japan; [2]Department of prosthodontics, School of Dentistry, Showa University, Tokyo, Japan

Neural crest (NC) cells appear during embryogenesis, migrate in the embryo, and differentiate into various kinds of cell types such as neuron, bone, teeth, thymus, and hair. In the present study, we succeeded to induce osteoblast-like cells from NC-derived whisker follicle in mice. We collected and cultured NC-derived hair follicle cells labeled with EGFP in the P0-Cre/floxed-EGFP double transgenic mice,P0 is a specific marker of NC. The EGFP-positive hair follicle cells (NC-derived cells) continuously expressed Runx2 mRNA. Simulation of the cells with BMP-2 induced expressions of alkaline phosphatase, osteocalcin, and osterix mRNAs and produced mineralized matrices positively detected by alizarin red and von kossa staining. The EGFP-positive hair follicle cells also continuously produced M-CSF and OPG. Addition of active vitamin D_3 (10^{-8}M) to the cultures suppressed OPG expression and induced RANKL production in the cells. When bone marrow cells were cocultured with EGFP-positive cells in the presence of vitamin D_3, multinucleated osteoclasts appeared within 10 days. These results indicate that neural crest-derived hair follicle cells possess a capacity to differentiate into osteoblasts, which will be beneficial for the development of the method for bone regenerative medicine.

[P2038]

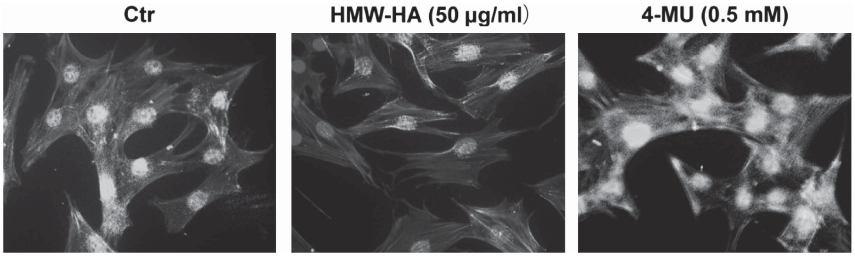


Figure 1 Effect of HA accumulation on RANKL expression in ST2 cells ST2 cells, grown on chamber slides, were incubated in the absence or presence of 50 μg/ml of HMW-HA or 0.5 mM of 4-MU for 24 h. After treatments, the cells were fixed and immunostained using a polyclonal antibody detected against RANKL and rhodamine isothiocyanate-phalloidin. Coverslips were mounted in medium containing DAPI, and cells were visualized using a BZ-9000. Baris in these images indicate 20 μm.

P2040

Regulation Of Human Osteoclastogenesis By Trem-1 Stimulation

Jong Dae Ji[1], *Tae-Hwan Kim*[2], *Bitnara Lee*[2], *Eunji Kwon*[2], *Sung Jae Choi*[1], *Young Ho Lee*[1], *Gwan Gyu Song*[1]
[1]Rheumatology, College of Medicine, Korea University, Seoul, Republic of Korea; [2]The Hanyang University Hospital for Rheumatic Diseases, Seoul, Republic of Korea

Triggering receptor expressed on myeloid cells (TREMs) are a family of cell surface receptors that play important roles in innate and adaptive immunity. Among them, TREM-2 has been extensively studied in the osteoclast differentiation and the essential role of TREM-2 in human osteoclastogenesis has been well established. However, much less has been known about the role of TREM-1 in human osteoclast differentiation. In this study, we investigated the role of TREM-1 in human osteoclast differentiation. Consistent with previous reports, TREM-2 expression was strongly increased during generation of human osteoclast precursors (pOCs). In contrast, TREM-1 expression was significantly decreased during generation of human pOCs. Stimulation of TREM-1 using agonistic TREM-1 antibody resulted in significant suppression of RANKL-induced osteoclastogenesis, as evidenced by diminished formation of TRAP+ multinucleated cells. In addition, TREM-1 stimulation strongly suppressed RANKL-induced expression of osteoclast-related genes such as cathepsin K, integrin β3 and NFATc1. TREM-1 stimulation also down-regulated gene expression and cell surface expression of M-CSF receptor that is essential for osteoclast differentiation and survival. In conclusion, we demonstrated that TREM-1 play a negative regulator in human osteoclast differentiation and our findings identify a new mechanism of negative regulation of osteoclastogenesis that play a role during inflammation.

P2041

Bone Sialoprotein Stimulates The Differentiation Of Dura-Derived Osteoprogenitor Cells Into Osteoblasts During Cranial Bone Repair

Jinxi Wang, *Qinghua Lu, John Yost, Andrew Miller, John Garlich*
University of Kansas Medical Center, Kansas City, KS, USA

Bone sialoprotein (BSP) is one of the extracellular matrix proteins in bone and tooth. Our previous studies revealed that BSP may enhance cranial bone repair in rodents. This study aimed to identify the tissue origins of BSP-responsive bone-forming cells in cranial defects. We examined the cellular response to BSP-collagen implants in mouse cranial bone defects and found proliferation and differentiation of osteoblastic cells in BSP-collagen implants, but not in collagen alone implants, near the dura and host bone. To identify whether bone-forming cells in the central region (distant from the host bone) of the defect were from the dura or host bone, a nitrocellulose membrane was inserted into the space between the dura and inner surface of the cranium. This membrane separated BSP implants from the dura and blocked the migration of dural cells into BSP implants (Figure1). No bone formation was observed in the central region of BSP-treated defects when a membrane was placed on the dura. qPCR analyses showed that expression levels of osteoblast marker genes were significantly lower in the BSP implants separated from the dura by a membrane than in the BSP implants with direct contact to the dura. These findings indicate that BSP stimulates the differentiation of dura-derived osteoprogenitor cells into osteoblasts during cranial bone repair. Bone-forming cells in the central region of BSP-treated cranial defects are primarily derived from the dura, not from the host bone.

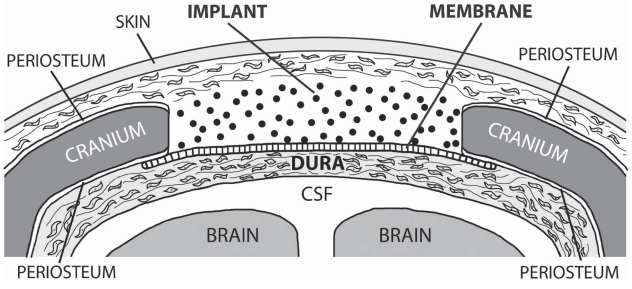


Figure 1 A diagram shows a nitrocellulose membrane that separates an implant from the dura.

P2042

Endocytic Incorporation Of Alendronate Promotes Bone Formation

Koichiro Komatsu[1], *Akemi Shimada*[1], *Tatsuya Shibata*[1], *Satoshi Wada*[1], *Hisashi Ideno*[1,2], *Kazuhisa Nakashima*[1], *Akira Nifuji*[1,2]
[1]Tsurumi University, Yokohama, Japan; [2]National Institute of Radiological Sciences, Chiba, Japan

Recent studies suggest that bisphosphonates (BPs) target bone-forming cells as well as bone-resorbing cells. We previously demonstrated that local application of a nitrogen-containing BP (N-BP), alendronate (ALN), for 5 min increased bone tissue in a tooth replantation model. In the present study, we extended that research to investigate mechanisms of bone formation by local application of ALN. Bone histomorphometry confirmed that bone volume and bone formation rate were increased. ALN increased proliferation of bone-forming cells on the bone surface, whereas it inhibited TRAP-positive osteoclasts. Moreover, ALN treatment induced more ALP-positive and osteocalcin-positive cells on the bone surface than PBS treatment. *In vitro* pulse treatment with ALN promoted osteocalcin expression. To track target cells of N-BPs, we applied fluorescence labeled ALN *in vivo* and *in vitro*. Fluorescence-labeled ALN was taken into bone-forming cells, in addition to bone-resorbing cells. This intracellular uptake was inhibited by endocytosis inhibitors. Furthermore, the inhibitor suppressed ALN-stimulated osteoblastic differentiation *in vitro*, and suppressed the increase in ALP-positive bone-forming cells and subsequent bone formation *in vivo*. These data suggest that local application of ALN promotes bone formation by stimulating proliferation and differentiation of bone-forming cells as well as inhibiting osteoclast function, and these effects occur through endocytic incorporation of ALN.

P2043

Co-operative Expression Of Sox11 And Sox4 Is Essential For Osteoblastogenesis

Jogeswar Gadi[1], *Ajita Jami*[1,2], *Min Jung Lee*[3], *Kalyani Ruthala*[4], *Han-Sung Jung*[3], *Sung-Kil Lim*[1,2]
[1]Division of Endocrinology and Endocrine Research Institute, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Seoul, Republic of Korea; [2]Brain Korea 21 Project for medical sciences, Yonsei University College of Medicine, Seoul, Seoul, Republic of Korea; [3]Division of Anatomy and Developmental Biology, Department of Oral Biology, Research Center for Orofacial Hard Tissue Regeneration, College of Dentistry, Yonsei University,, Seoul, Seoul, Republic of Korea; [4]Department of Anatomy, Embryology lab, Yonsei University College of Medicine, Seoul, Seoul, Republic of Korea

The high-mobility-group (HMG) domain containing Sox C transcription factors, Sox11 and Sox4 were found to be involved in neuronal, cardiac and skeletal development. However, the regulatory gene network mediated by Sox11 and Sox4 during skeletogenesis was poorly understood. Here we showed that, Sox11 and Sox4 expressed in bone cells during embryogenesis and postnatal developmental stages. *In vitro* differentiation of MC3T3-E1 osteoblast cells reduced the expression of Sox11 mRNA and protein during day 7, and the Sox4 expression was found to be increased as differentiation progressed until day 28. It was ascertain that Sox11 and Sox4 regulate the crucial transcription factors Runx2 and Osterix by binding to the regulatory sites on promoter and introns region. Furthermore, we found that Sox4 overexpression simulates the TCF/LEF reporter activity and influences the expression of Wnt signaling genes and stabilizes the β-catenin signal in primary calvaria cells. All together, we showed that both the Sox11 and Sox4 were essential in the early and late stages of osteoblast development and might work co-operatively.

P2044

Osteoblastic Cell Differentiation From Mouse Pluripotent Cell Line Regulated By Mechanical Stretching

Kosaku Kurata, *Takanobu Fukunaga, Yosuke Kasuga, Hiroshi Takamatsu*
Department of Mechanical Engineering, Kyushu University, Fukuoka, Japan

Mesenchymal stem cells have a potential to differentiate into various cell types, and are therefore regarded as an important cell source for tissue engineering. In addition to a number of chemical factors, mechanical stimulation is also known to act on the differentiation process, especially in osteoblasts because bone metabolism is greatly affected by external loading. The aim of this study was, therefore, to determine the threshold of mechanical stimulation that enables the pluripotent cells to differentiate into osteoblasts. Original elastic chamber with 10 independent culture wells was designed to apply 5 different strain magnitudes simultaneously to the cells. The pluripotent C3H10T1/2 cell line was suspended in collagen gel and cultured in the chamber. After 3 days, the cells were stimulated by both adipogenic and osteogenic induction factors. The cells were also respectively exposed to 2-hour cyclic stretching of 0.16, 0.30, 0.38, 0.44, and 0.50% at 1Hz. After the 4-day stimulation, ALP gene expression level was unregulated as the strain magnitude increased. On the contrary, PPARγ gene was suppressed in all stretched groups in a strain-dependent manner. Histological observation after 8 days also showed that the stretching at 0.44 and 0.50% strains increased ALP-positive spindle cell induction. In conclusion, the pluripotent cells cultured under mechanical stretching promoted osteoblastic cell differentiation, the threshold of which was approximately 0.4% strain.

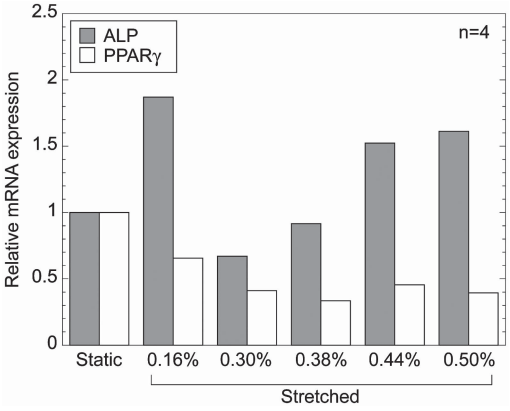


Figure 1 Relative mRNA expression of ALP and PPARγ

P2045

Errγ Inhibits Osteoblast Differentiation Through miR-433 Induction And Subsequent Suppression Of Runx2 Expression

Jeong-Tae Koh, *In-Hong Kang, Eun-Jung Kim, Won-Gu Jang*
Department of Pharmacology and Dental Therapeutics & Research Center for Biomineralization Disorders, School of Dentistry, Chonnam National University, Gwangju, Republic of Korea

MicroRNAs (miRNA) are involved in various biological processes including cellular differentiation. Previously, we reported that ERRγ inhibited osteoblast differentiation with decrease in Runx2 activity. Recently ERRγ is also reported to induce mir-433 in liver cells. This study was to investigate whether miR-433 can affect BMP2-induced osteoblast differentiation and that can explain the inhibitory mechanism of ERRγ. C3H10T1/2 mesenchymal lineage cells were used for the study, and miR-433 expression was detected by real-time PCR. For activation or inhibition of miR-433 expression, precursor form of miR-433 or anti-miR-433 was transfected. Functional activities of miR-433 and Runx2 were evaluated by luciferase assay. Osteoblastic differentiation was evaluated by analyzing alkaline phosphatase (ALP) activity and marker gene expression. BMP2 treatment decreased endogenous ERRγ and miR-433 expression, and overexpression of ERRγ or miR-433 inhibited

the expression of osteogenic genes such as Runx2 and ALP. A computer-based prediction algorithm led to the identification of three miR-433 binding sites on the 3′-UTR of Runx2 mRNA. MiR-433 directly targeted two of them, and decreased the level of Runx2 transcript. MiR-433 inhibited BMP2-induced 6×OSE-Luc activity. Anti-miR-433 recovered ERRγ-suppressed Runx2 expression and ALP activity. These results suggest that ERRγ inhibits osteoblast differentiation through miR-433 induction and subsequent suppression of Runx2 expression.

P2046

Novel Approach For The Differentiation Of Embryonic Stem Cells Into Osteoblasts Under A Chemically-defined Condition

Kosuke Kanke *[1,2], Hironori Hojo[2], Alexander Lichtler[4], Ung-il Chung[2,3], Shinsuke Ohba[2]*

[1]Department of Sensory and Motor System Medicine, University of Tokyo, Tokyo, Japan; [2]Division of Clinical Biotechnology, Center for Disease Biology and Integrative Medicine, The University of Tokyo, Tokyo, Japan; [3]Department of Bioengineering, The University of Tokyo, Tokyo, Japan; [4]Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA

Limited number of specific cell populations obtained from animals hampers extensive studies for protein interaction, transcriptional network, and epigenetics in osteoblast development. Embryonic stem cell (ESC)-based osteogenic differentiation may be an attractive model for the studies, given their capacity for long-term propagation and potential to generate all cell types of the adult organisms. Here, we report an efficient, mass-productive, and reproducible culture protocol directing mouse ESCs toward osteoblasts under a chemically-defined condition. The protocol is composed by three phases: ESC maintenance in serum-free 2i culture, mesoderm induction, and osteogenic induction. Mesoderm induction was achieved by serum-free media containing Wnt-signaling activator; cells were then cultured with Hh-signaling activator and helioxanthin-derivative for osteogenic induction. ESCs expressing green fluorescence protein under the control of 2.3-kb Col1a1 promoter fluoresced upon the induction. Consistent with this, osteoblast marker genes including Runx2, Sp7, Ibsp, and Bglap were strongly up-regulated by the induction. In contrast, other lineage markers did not alter with pluripotency genes being downregulated throughout induction. Thus, this system will be useful for *in vitro* mechanistic studies for osteoblast development in near-in-vivo settings, and the differentiation protocol can be applied to stem cell-based therapies for massive bone defects.

P2047

An *In Vitro* Osteoblast Culture Model Using Estrogen Responsive KS483 Mouse Osteoblast Precursor Cell Line

Katja Fagerlund *[1], Jukka Rissanen[1], Alan Chan[2], Jussi Halleen[1]*

[1]Pharmatest Services Ltd, Turku, Finland; [2]Percuros B.V., Leiden, Netherlands

The mouse clonal osteoblast progenitor cell line KS483 can be differentiated into bone forming osteoblasts in the presence of ascorbic acid and β-glycerophosphate. We have studied the effects of continuous exposure of 17β-estradiol (E2), selective estrogen receptor modulators (SERMs) raloxifene, bazedoxifene and lasofoxifene, and bone morphogenic proteins (BMPs) on osteoblast differentiation and activity using KS483 cells. Cultures for determining effects on osteoblast differentiation were stopped at day 8, and intracellular alkaline phosphatase activity measured in cell lysates was determined as a marker of osteoblast differentiation. Cultures for determining effects on osteoblast activity were stopped at day 13, and secreted PINP was determined as a marker of bone collagen synthesis at day 11, and calcium measurements as a marker of inorganic bone matrix synthesis at day 13. E2 and BMPs showed concentration-dependent stimulatory effects on osteoblast differentiation and activity. SERMs had no effects on osteoblast differentiation, but they showed concentration-dependent stimulation of osteoblast activity. We conclude that the KS483 cell line can be used for setting up reliable *in vitro* models for studying osteoblast differentiation and activity, and these models can be used for identifying novel compounds with anabolic and estrogen-like effects on bone formation.

P2048

Epigenetic Regulation Of Osteoclastogenesis By Histone Modification

Naohiro Izawa *[1,2], Takanori Fujita[2], Seitaro Nomura[2], Tetsuro Yasui[1], Yasunori Omata[1], Hiroyuki Aburatani[2], Sakae Tanaka[1]*

[1]Department of Orthopaedic Surgery, Faculty of Medicine, The University of Tokyo, Tokyo, Japan; [2]Genome Science Division, Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan

Recent studies have uncovered that epigenetic regulation, such as histone methylation and acetylation, plays a critical role in determining cell fate. In particular, the expression of key developmental genes tends to be regulated by tri-methylation

of histone H3 Lysine 4 (H3K4me3) and lysine 27 (H3K27me3). In addition, acetylation of histone H3 Lysine 27 (H3K27ac) is known as an enhancer which decides the timing of development. However, epigenetic regulation of the differentiation of osteoclasts, which derive from cells of monocyte-macrophage lineage, is still unclear. Using ChIP-seq technique for H3K4me3, H3K27me3 and H3K27ac and FAIRE-seq technique, we analysed modifications and structural changes of chromatin during osteoclstogenesis. With expression of genes from RNA-seq, some regulatory factors for development of osteoclasts were identified.

P2049

The R740s Mutation In The V-ATPase A3 Subunit Impairs *In Vitro* Osteoclastogenesis Via Inhibition Of NFATc1 Nuclear Translocation

Irina Voronov[1]*, Noelle Ochotny*[1]*, Valentin Jaumouille*[2]*, Celeste Owen*[3]*, Jane Aubin*[4]*, Morris Manolson*[1]

[1]Faculty of Dentistry Research Institute, University of Toronto, Toronto, Ontario, Canada; [2]Cell Biology Program, Hospital for Sick Children, Toronto, Ontario, Canada; [3]Centre for Modeling Human Disease, Mt Sinai Hospital, Toronto, Ontario, Canada; [4]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

Vacuolar H+-ATPases (V-ATPases) are multisubunit enzymes responsible for acidification of the resorption lacunae in osteoclasts (OCs). In OCs, V-ATPases containing the a3 subunit localize to the ruffled border in resorbing cells or to the lysosomes in non-resorbing cells. Heterozygous mice with an R740S mutation in a3 (+/R740S) have mild osteopetrosis due to decreased bone resorption. Furthermore, lysosomal pH is increased in +/R740S compared to +/+ OCs. *In vitro*, OC-genesis is impaired compared to +/+ cells. To investigate the connection between lysosomal pH and OC-genesis, cells were treated with lysosomal inhibitors ammonium chloride, chloroquine (CHQ) and V-ATPase inhibitor concanamycin A. All inhibitors decreased OC formation in +/+ cells, but not in +/R740S cells, confirming a link between lysosomal pH and OC-genesis. Next, nuclear translocation of a key transcription factor NFATc1 was assessed by immunofluorescence; it was decreased in +/R740S compared to +/+ cells suggesting a connection between lysosomal pH and NFATc1 activation. To clarify this connection, we examined the levels of regulator of calcineurin 1 (RCAN1), an endogenous inhibitor degraded via lysosomal pathway. Immunoblotting showed that +/+ OCs treated with CHQ had significantly higher levels of RCAN1 compared to controls thus providing explanation for decreased NFATc1 activation. Future experiments will determine whether higher lysosomal pH affects activation of other transcription factors in OCs.

P2050

Overexpression Of Stomatin Induces Osteoclast Differentiation And Decreases Bone Density In B6 Mice

Jia-Fwu Shyu[1]*, Chi-Hung Lin*[2]*, Jui-Hao Lee*[2]*, Hsin-Yu Wu*[1]*, Wen-Hui Chan*[1]*, Hung-Shu Ma*[1]*, Wen-Jei Yao*[1]

[1]Department of Biology and Anatomy, National Defense Medical Center, Taipei, Taiwan; [2]Institute of Microbiology and Immunology, National Yang Ming University, Taipei, Taiwan

Cell-cell fusion is a crucial step to function and physiology of osteoclasts. Stomatin is a 31-kDa integral protein, associate with lipid raft, and together with associated membrane proteins are suggested to play a role in cell fusion process. Our previous study indicated that stomatin over-expressed Chinese hamster ovary (CHO -K1) cells may induce the formation of multinucleated cells through the cell-cell fusion process. To study the function of stomatin in osteoclasts, we constructed stomatin with Enhanced Green Fluorecence Protein (EGFP) gene and transfected it into RAW 264.7 cells. Overexpression of stomatin induced multinuclear osteoclast formation as demonstrated by fluorescent microscopy and positive TRAP stain. Increase of calcitonin-induced cAMP production was found in these cells. Western blot and confocal analysis showed increase expression of DC-STAMP and CD9 and their colocalization with stomatin and lipid raft. In animal study, results of micro-Computed Tomography and histomorphometric analysis showed decrease of bone density and trabecular number and thickness in the stomatin-overexpression B6 mice. Interestingly, increase of bone resorption as well as formation which indicated high bone turnover in these mice was found. In conclusion, this study provided evidence indicates that stomatin may play a role in the process of the multinucleated osteoclast formation and bone remodeling.

P2051

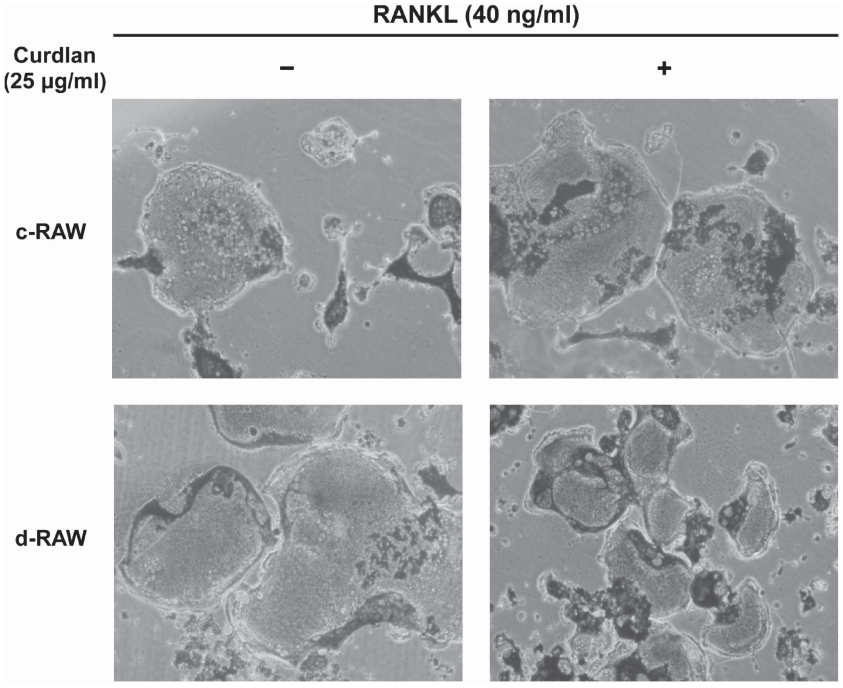
Curdlan Regulates Osteoclastic Differentiation And Function

Toru Yamasaki[1,2]*, Wataru Ariyoshi*[2]*, Ryuji Hosokawa*[1]*, Tatsuji Nishihara*[2]

[1]Oral Reconstruction and Rehabilitation, Kyushu Dental University, Kitakyushu, Japan; [2]Infections and Molecular Biology, Kyushu Dental University, Kitakyushu, Japan

Dectin-1 is a C-type lectin receptor that is primarily expressed by myeloid cells. Upon ligation of β-glucans to dectin-1, a number of cellular events follow. However, less attention has been paid to the effect of β-glucan on bone metabolism. In the present study, we examined the effect of curdlan, one of the β-glucans, on osteoclastogenesis induced by RANKL. RAW264.7

[P2051]



cells overexpressing dectin-1 (d-RAW) and carrying Neo gene as a control cells (c-RAW) were cultured in the presence or absence of RANKL and curdlan. Following tartrate resistant acid phosphatase (TRAP) staining, TRAP-positive multinucleated cells were counted. Curdlan suppressed mature TRAP positive multinucleated cell formation induced by RANKL in a dose-dependent manner. In addition, we found that curdlan down-regulated pit formation and actin ring formation induced by RANKL. Furthermore, we investigated the level of mRNA of nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 (NFATC1) and osteoclast stimulatory transmembrane protein (OC-STAMP) by real-time RT-PCR. Curdlan suppressed NFATC1 and OC-STAMP mRNA expression activated by RANKL. These results indicate that activation of dectin-1 signaling by curdlan regulates osteoclast differentiation and function.

P2052

PAK4 Kinase Inhibitor, PF-3758309 Inhibits Osteoclast Fusion Via Down-Regulating Cell Migration And Mrna Expression Of Osteoclast Maturation-related Molecules

Sik-Won Choi[1]*, Jeong-Tae Yeon*[2]*, Byung Jun Ryu*[1]*, Kwang Jin Kim*[2]*, Young-Jin Son*[2]*, Seong Hwan Kim*[1]
[1]Korea Research Institute of Chemical Technology, Daejeon, Republic of Korea; [2]Department of Pharmacy, Sunchon National University, Suncheon, Jeollanam-do, Republic of Korea

The prevention or therapeutic treatment of loss of bone mass is an important means of improving the quality of life for patients with disorders related to osteoclast-mediated bone loss. Multinucleated osteoclasts are formed by the fusion of mononuclear osteoclasts which is an essential for bone resorption. p21-activated kinases (PAKs) are involved in cytoskeleton organization that is also important for osteoclast fusion. Herein, we showed that PAK4 kinase inhibitor, PF-3758309 (being developed as anti-cancer drugs) did not affect the formation of pre-osteoclasts (pOCs), but interestingly, it significantly inhibited their fusion. Moreover, the mRNA expression of fusion-mediating molecules such as the d2 isoform of vacuolar ATPase V0 domain (Atp6v0d2), integrin αv and integrin β3 was strongly inhibited by PF-3758309 in the fusion process of pOCs. Furthermore, PF-3758309 repressed migration of pOCs that is required for their fusion. A lipopolysaccharide (LPS)-induced bone erosion was also preformed assess the effects of PF-3758309 *in vivo*. Our data indicate for the first time the potential of PF-3758309 to inhibit the cell-to-cell fusion of pOCs by regulating their migration and expression of fusion-related molecules. Therefore, PF-3758309 may be of use in the prevention of osteoclast-related bone loss.

P2053

A Synthetic Peptide Derived From TRAF1 Inhibits Osteoclastogenesis And Bone Erosion

Yuichi Mine[1]*, Seicho Makihira*[2]*, Takahiro Shuto*[2]*, Hiroki Nikawa*[1]
[1]Department of Oral Biology and Engineering, Integrated Health Sciences, Institute of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan; [2]Section of Fixed Prosthodontics, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan

There is a need for the development of a material with few side effects to treat diseases of osteolysis, such as a bisphosphonate-related osteonecrosis of the jaw. We previously showed that TRAF1 is a negative regulator of RANKL-dependent osteoclastogenesis. We thus hypothesized that synthetic peptides derived from TRAF1 could have a similar function in

pre-osteoclast cells. This study was carried out to examine the inhibitory effects of TRAF1-derived peptides on osteoclastogenesis. Two peptides derived from TRAF1 (T1 and T2), N-terminally conjugated with an eleven-arginine sequence (11R), were synthesized. 11R is well known as a membrane-permeable sequence. In the presence of T1, the mRNA expression of the osteoclast differentiation markers TRAP, cathepsin K, DC-STAMP and β3-integrin in BMMs exposed to RANKL under M-CSF stimulation, were significantly inhibited, as compared with those in BMMs exposed to M-CSF and RANKL without T1. T1 also decreased the number of TRAP-positive osteoclasts and hydroxyapatite resorption in BMMs stimulated with M-CSF and RANKL compared to those in M-CSF- and RANKL- stimulated cells without T1. Finally, we compared the ratios of bone volume to tissue volume (BV/TB) in the femur of LPS-injected mice with T1 or 11R. LPS significantly decreased the BV/TB ratio and T1 injection prevented LPS from decreasing the BV/TB ratio. These results suggest that a synthetic peptide derived from TRAF1 may be a candidate of therapeutic agent to block osteolysis.

P2054

Absence Of Nucleotide-Binding Oligomerization Domain (NOD) 2 Attenuates Osteoclast Differentiation

Hye-seon Choi, ***Ke Ke***
University of Ulsan, Ulsan, Republic of Korea

Nucleotide-binding oligomerization domain (NOD) 2 belongs to pattern recognition receptors for innate immune system. The detection of pathogen-associated molecular pattern (PAMP) by NOD activates multiple proinflammatory signaling pathways for effective antimicrobial response. The differentiation and function of osteoclast are affected directly or indirectly by proinflammatory cytokines such as TNF-α and IL-1. NOD2 is associated with TNF-α-induced osteoblast activation to enhance osteoclast formation. NOD2 interacts with serine-threonine kinase to induce activation of NF-κB and MAPK both of which are important for RANK/RANKL signaling, suggesting that NOD2 could also modulate bone metabolism via action in BMM. Lack of NOD2 decreased osteoclastogenesis by reducing expression of c-Fms and RANK on osteoclast precursors. Our data suggests the potential of NOD2 for amelioration of bone loss by affecting osteoclastogenesis. This work was supported by a KHIDI Grant (A111295), KRF Grants (BRL 2009-0087350; 2010-0002644) funded by the Korean government.

P2055

Knowledge On Osteoporosis In Guardians Of Hip Fracture Patients

Yong-Ki Min[6], ***Young-Kyun Lee[1],*** *Yong-Chan Ha[2], Deog-Yoon Kim[3], Dong-Won Byun[4], Jae Seok Chang[5], Kyung-Hoi Koo[1]*
[1]Seoul National University Bundang Hospital, Seongnam-si, Republic of Korea; [2]Chung-Ang University College of Medicine, Seoul, Republic of Korea; [3]Kyung Hee University Medical Center, Seoul, Republic of Korea; [4]Soonchunhyang University College of Medicine, Seoul, Republic of Korea; [5]Asan Medical Center, College of Medicine, University of Ulsan, Seoul, Republic of Korea; [6]Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea

Background: Treatment gap between the treatment guideline and real clinical practice of osteoporosis has been worldwide found. Although insufficient knowledge of guardians on osteoporosis might be one important obstacle to diagnose and treat osteoporotic patients, there was no study on the knowledge of guardians. We evaluated the guardians' knowledge on osteoporosis compared with the knowledge of orthopedic doctors, using a self-administered questionnaire, modified Facts on Osteoporosis Quiz (FOOQ).
Methods: In March and April 2012, the knowledge of osteoporosis was measured in 40 guardians of hip fracture patients and 40 orthopedic surgeons using, a modified FOOQ.
Results: In terms of treatment and prevention of osteoporosis, the modified FOOQ score of the guardians have inadequate knowledge and understanding about the osteoporosis, compared with orthopedic doctors ($p<0.001$).
Conclusion: The level of guardians' knowledge on osteoporosis should be considered and improved to achieve satisfactory osteoporosis treatment in hip fracture patients.

P2056

Incidence Of Fragility Fractures In Sakaiminato City, Japan

Tsukutani Yasuto*, Hiroshi Hagino*
Orthopedic Surgery, Tottori University, Yonago, Tottori, Japan

Purpose: To elucidate the incidence of hip, distal radius, proximal humerus, and vertebral fractures from 2010-2011 in Sakaiminato city, Japan.

Method: A survey of all fractures in patients ≥50 years old for hip, distal radius, proximal humerus, and spine (clinical vertebral fractures) was performed in Sakaiminato city, Tottori prefecture, Japan. This survey was done for the years 2010-2011. The age- and gender-specific incidence rates (per 100,000 person-years) were calculated based on the population of Sakaiminato city in each year. The incidence rates for hip, distal radius, and proximal humerus were compared with those from 1994-1995 in Tottori Prefecture.
Results: The age-adjusted incidence rates (per 100000 person-years, adjusted to the population structure of 2010 in all of Japan ≥50 years) of hip fracture for men and women were 96 and 354 in 1994 and 162 and 401 on average for 2010-2011. The rates for distal radius fracture for men and women were 69 and 328 in 1995 and 72 and 396 in 2010-2011. The rates for proximal humerus fracture for men and women were 27 and 93 in 1995 and 30 and 85 in 2010-2011. The rates for clinical vertebral fracture for men and women were 358 and 1012 in 2010-2011.
Conclusion: Significant increases were observed in the incidence of limb fractures with time . We suggest the reason for this increase is that a greater proportion of seniors with poor health are living longer during a time when their bones are considerably weakened.

P2057

A Simple Statistical Model To Evaluate The Risk Factors For The Fast Looser Of Bone And The Present Bmd Simultaneously In Postmenopausal Osteoporosis

Takehisa Yamamoto[1], *Takeshi Ibata*[2], *Takayuki Tsuda*[3], *Kazushige Adachi*[4], *Tetsuhiko Yoshizumi*[5], *Ryutaro Komuro*[2], *Sayomi Iida*[2], *Mariko Wada*[6], *Hiroshi Sudo*[7]
[1]Department of Pediatrics,Minoh City Hospital, Minoh, Japan; [2]Department of Internal Medicine,Minoh City Hospital, Minoh, Japan; [3]Department of Orthopedics,Minoh City Hospital, Minoh, Japan; [4]Department of Gynecology, Minoh City Hospital, Minoh, Japan; [5]Department of Radiology,Minoh City Hospital, Minoh, Japan; [6]Minoh Medical Health Center, Minoh, Japan; [7]Minoh City Medical Association, Minoh, Japan

Background: The presence of a fast looser of bone (FLB) is one of the major concerns for osteoporosis. However, no research showed the relationship between the risk factors for FLB and the present BMD at the same time by using data on the first visit.
Subjects and Methods: We investigated apparently normal 200 Japanese females for 8 years. The mean age was 64. We used a % YAM value, which was based on the mean BMD of normal Japanese female aged from 20 to 44. We adjusted it by the body height of each patient (max-YAM). After calculating the difference between the max-YAM to YAM on the first visit (dYAM), two figures were drawn by the dYAM values divided by the duration from the age of 45 or menopause to the age on the first visit. The mean of these values was used as an annual bone loss rate (BLR). BMD and BLR were classified into 4 categories by using quartiles. After selecting parameters by multiple regression and decision tree analysis, we used a mean covariance structure model.
Results: High BMD decreased BLR (P=0.012), while high BLR decreased BMD (P=0.005). BMD and BLR were positively associated with a short duration after menopause and a low or high u-NTX value respectively. Also, they were positively associated with a lean body mass and a body height respectively (P<0.001).
Conclusion: This simple statistical model for osteoporosis based on a single measurement of BMD might be useful for assessing the roles of several factors associated with osteoporosis.

P2058

Increased Bone Remodeling Associated With Lactation Induced-osteoporosis Effectively Reversed By Teriparatide In Korean Premenopausal Women

Jo Eun Kim, *Sujin Lee, Sung-Kil Lim, Yumie Rhee*
Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea

Major changes in maternal bone occur during lactation. Pregnancy and lactation-associated osteoporosis (PLO) is very rare, but it can cause severe vertebral compression fractures. However, knowledge of the characteristics and mechanisms of maternal bone metabolism after lactation are still rather limited especially in Korean. (1) We compared the parameters related to bone metabolism both in 21 PLO patients (31.2±2.3 y.o.) and 5 women with idiopathic osteoporosis (34.2±4.7 y.o.). (2) We have analyzed the effect of human recombinant parathyroid hormone ((1-34), teriparatide, TPTD) or calcium and vitamin D in 6 women with PLO who had completed full 1-year treatment. There were no specific differences in general risk factors of osteoporosis in both groups except all PLO patients having vertebral fractures vs. 20% in premenopausal osteoporosis women without any differences between BMD of any site. All fractures occurred within 5 months of lactation in PLO patients. Most notably, serum calcium and phosphate and osteocalcin levels in PLO patients were all significantly higher (P<0.05, respectively). Follow up study done in 3 PLO with TPTD, and 3 with calcium and vitamin D showed different recovery of lumbar BMD (19.0% vs. –3.4%, P<0.05). In conclusion, rapid bone loss due to increased bone remodeling seemed to have a role in the development of fractures in PLO. Lumbar BMD significantly.

P2059

Novel Insights Into Mechanisms Of Bone Loss From Allergic Inflammation Using Experimental Mouse Model

Masahiro Ouchi[1,2], *Junko Hatakeyama*[1], *Naohisa Murata*[1,2], *Masao Yoshinari*[3], *Reona Aijima*[1], *Hideki Ioi*[2], *Ichiro Takahashi*[2], *Mizuho Kido*[1]
[1]Department of Molecular Cell Biology and Oral Anatomy, Graduate School of Dental Science, Kyushu University, Fukuoka, Japan; [2]Department of Orthodontics, Graduate School of Dental Science, Kyushu University, Fukuoka, Japan; [3]Division of Oral Implants Research, Oral Health Science Center, Tokyo Dental College, Tokyo, Japan

The dramatic increase in number of allergic disease over the last decade is of great concern worldwide. Asthma or atopic

dermatitis, with a typical relapsing and remitting clinical course, constitutes the majority of cases of allergies and are treated with or without anti-allergic medicine. Data on prevalence of low bone mineral density in allergic patients evaluated in relation to the corticosteroid therapy, did not clearly demonstrate the allergies contribute to bone loss. We sought to determine whether systemic administration of ovalbumin (OVA) in the presence or absence of an adjuvant was associated with osteopenia in mouse model.
Serum level of IgE was significantly more elevated in mice with OVA sensitization or OVA sensitization with aluminium hydroxide (Alum) as an adjuvant than in that of Alum or vehicle treatment. Marked infiltration of leukocyte was observed in OVA -challenged lung. Histomorphometric analysis revealed that the number of osteoclasts increased at 3rd and 5th weeks. mRNA expression levels of proinflammatory cytokines elevated quickly after the OVA or OVA with Alum stimulation and the elevation was maintained to 5th weeks then dropped. The expression of receptor activator of nuclear factor-kappa B ligand was elevated significantly after OVA with Alum stimulation through 1st to 5th weeks. In addition, the higher production of leukotriene was also observed. Our data suggest that the systemic allergy lead osteoclastgenesis and cause osteopenia.

P2060

Reduced Renal Function, Hyperglycemia And Liver Dysfunction Independently Affects Circulating Sclerostin Level

Se Hwa Kim[2], *Soo Young Yoon*[2], *Yumie Rhee*[1]
[1]Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea; [2]Department of Internal Medicine, Kwandong University Myongji Hospital, Ilsan, Republic of Korea

Circulating sclerostin, a Wnt inhibitor produced by osteocytes is known to be negatively associated with estrogen and parathyroid hormone. However, relation to kidney or liver function with sclerostin level is unclear. The aim of the study was to investigate interaction between sclerostin and renal, liver function in Koreans. A first set of 434 subjects with variable renal status (male 200, female 234) was analyzed. Additional 104 subjects with liver cirrhosis or not were recruited. Serum sclerostin level was analysed by ELISA (Biomedica Co.), and renal function by estimated glomerular filtration rate (eGFR). There was a strong relation between sclerostin level with eGFR (r=–0.619 and –0.574, respectively in men and women, p<0.001). The serum sclerostin level was significantly higher in patients with eGFR<30 ml/min compared to eGFR 30-59 or ⩾60 ml/min^{-1} (p<0.001). Age, serum creatinine (Cr), and presence of diabetes were independent determining factors for sclerostin level in women with eGFR ⩾30 ml/min^{-1}. However, only age and serum Cr were independent factors for sclerostin level in men. Furthermore, subjects with liver cirrhosis showed higher sclerostin levels compared to control group after adjusting for age, sex, BMI, and eGFR (67.3±5.1 vs. 32.4±4.6 pmol^{-1}). Our findings showed serum sclerostin is higher in subjects with eGFR under 30 min/min or liver cirrhosis. Upon analyzing circulating sclerostin level, renal or hepatic dysfunction should be taken into consideration.

P2061

Serum Uric Acid And Lumbar Spine Bone Mineral Density In Peri- And Postmenopausal Japanese Women: A Cross-sectional Analysis

Shinya Ishii[1], *Mariko Miyao*[2], *Mariko Tanaka-Ishikawa*[2], *Masahiro Akishita*[1], *Yasuyoshi Ouchi*[1]
[1]Department of Geriatric Medicine, University of Tokyo, Graduate School of Medicine, Bunkyo-ku, Tokyo, Japan; [2]Center for health check-up and preventive medicine, Kanto Central Hospital, Setagaya-ku, Tokyo, Japan

Serum uric acid (UA) is an endogenous antioxidant and considered to have protective effects on bone. However, evidence supporting this claim is still limited. To explore the association between serum UA and bone mineral density (BMD), we retrospectively analyzed data from 581 consecutive women aged older than 50 years presenting to a community hospital to have a health checkup. Lumbar spine BMD was measured by dual energy x-ray absorptiometry. Multiple linear regression model was used to adjust for age, body mass index, smoking and drinking habit, physical activity, presence of diabetes mellitus or hypertension, serum calcium, estimated glomerular filtration rate and C-reactive protein. Mean UA value in this population was 4.82 mg/dl^{-1} with a standard deviation of 0.97 mgdl^{-1}. Mean BMD levels were 1.01 g/cm^{-2} in the first quartile of UA (i.e. lowest UA levels), 1.03 g/cm^{-2} in the second quartile, 1.04 g/cm^{-2} in the third quartile and 1.08 g/cm^{-2} in the fourth quartile

Table 1 Unadjusted and adjusted associations of serum uric acid levels with lumbar spine bone mineral density

	Effect size (95% confidence interval)	p
Model 1	0.027 (0.013-0.042)	<.001
Model 2	0.021 (0.007-0.036)	0.003
Model 3	0.020 (0.006-0.035)	0.006
Model 4	0.020 (0.006-0.035)	0.006
Model 5	0.016 (0.001-0.031)	0.04

Model 1: unadjusted Model 2: adjusted for age, body mass index Model 3: adjusted for age, body mass index, smoking, drinking, physical activity Model 4: adjusted for age, body mass index, smoking, drinking, physical activity, presence of diabetes mellitus or hypertension Model 5: adjusted for age, body mass index, smoking, drinking, physical activity, presence of diabetes mellitus or hypertension, serum calcium, estimated glomerular filtration rate, log(C-reactive protein).

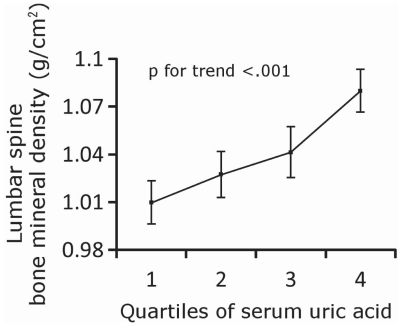


Figure 1 Mean values of lumbar spine bone mineral density across the quartiles of serum uric acid. Error bar represents the standard error.

and we observed a significant trend towards increasing BMD levels with higher UA levels (*p* for trend <.001). Multiple linear regression analysis showed that higher UA levels were significantly associated with higher BMD levels independent of covariates. We concluded that higher serum UA levels are beneficial for bone in peri- and postmenopausal Japanese women who have lower body weight than Caucasians. Further studies are warranted to elucidate the underlying mechanisms of the association between serum UA and BMD.

P2062

Primary Biliary Cirrhosis And Osteoporosis, A Case Report

***Kawtar Nassar**, Saadia Janani, Wafaa Rachidi, Noufissa Etaouil, Ouafaa Mkinsi*
Rheumatology, Casablanca, Morocco

Primary biliary cirrhosis is a chronic cholestatic. It occurs in 95% of cases in women, most frequently in peri-menopausal. Bone loss usually observed could be aggravated by liver disease, leading to an increased risk of fracture. Bone mineral density is reduced compared to the control population of the same age and sex. The objective of our work is to recall this association through our case. The pathophysiology of bone loss during the cirrose bilaire primitive is still widely misunderstood. We recall this association bilaire cirrhosis and primary osteoporosis through the case of a patient admitted to the rheumatology department. Most studies have found an increase in bone turnover with an imbalance in favor of resorption, increased after menopause, as evidenced by the case of our patient. Affected individuals are at high risk of bone loss and osteoporotic vertebral fractures. And it is likely that the severity of cirrhosis biliary primitif does not play a role in bone loss observed in these patients.

P2063

Survey 85 Specialists On The Perception Of The Discomfort Caused By The Adverse Effects Of Long-term Corticosteroid

***Kawtar Nassar**, Saadia Janani, Wafaa Rachidi, Noufissa Etaouil, Ouafaa Mkinsi*
Rheumatology, Casablanca, Morocco

Widely prescribed in practice by both specialists and generalist, the objective of our work is evaluated by specialists perception of the discomfort caused by the side effects of systemic drugs for long periods. **Materiels and methods:** Between December 2011 and May 2012, we conducted a descriptive survey with 85 medical specialists at the University Hospital of Casablanca.The limitation period was greater than one year for 62.3%. The initial prescribed dosage was > 20 mg per day for at least 2 months at 48.23%. The adverse reactions considered by practitioners most annoying was taking the weight gain for 54 (63.5%), diabetes in 50.6%, trophic skin disorders (41%), lipodystrophy (38.8%),desequiliber blood pressure for 32 practinians and epigastric pain for 30. The neuropsychiatric disorders for 23 physicians (27%), 32% of osteoporosis and osteonecrosis in 16 practitioners, myopathies or cramps at 13. **Conclusion:** Better care of patients under long-term corticosteroid requires control systemic side effects with regular support patients to optimize adherence.

P2064

Circulating Levels Of Sclerostin Are Increased In Patients With Ossification Of Posterior Longitudinal Ligament Of The Spine

***Masafumi Kashii**[1], Yukitaka Nagamoto[2], Takahito Fujimori[1], Youhei Matsuo[1], Tsuyoshi Sugiura[1], Hirotsugu Honda[1], Takashi Kaito[1], Motoki Iwasaki[1], Hideki Yoshikawa[1]*
[1]Orthopedic surgery, Osaka University Graduate school of Medicine, Suita, Osaka Pref, Japan; [2]National Hospital Organization Osaka National Hospital, Osaka, Japan

Backgrounds: The SOST gene encoding sclerostin is an osteocyte derived negative regulator of bone formation. There is no data regarding the relationships between sclerostin and ossification of posterior longitudinal ligament of the spine (OPLL).
Objective: This study aimed to evaluate serum sclerostin levels in OPLL patients and identify the relationship between serum sclerostin and bone turnover markers, OPLL type and numbers of ossified vertebra.
Methods: Seventy-eight OPLL patients were studied and compared with age and sex matched 39 controls with spinal canal stenosis without OPLL. Serum sclerostin levels were measured by ELISA.
Results: Serum sclerostin levels in OPLL patients is significant higher than controls (OPLL: mean 64.1 SD 39.3 $pmol^{-1}$, Control: mean 44.9 SD 17.7$pmol^{-1}$, p=0.005). In OPLL patients, the positive correlation between age and sclerostin levels was found in male OPLL patients (r= 0.43, p=0.002). There are no relationship between serum sclerostin levels and bone turnover markers, OPLL type and numbers of ossified vertebra.
Conclusion: The relationship between age and serum sclerostin levels strongly suggests that progressive ossification of spinal ligaments with advancing age promote systemic secretion of sclerostin by osteocytes, and there will be a negative feedback system to suppress ossification by sclerostin in OPLL patients.

P2065

Use Of Teriparatide Yield Successful Results In A Patient With Pregnancy And Lactation-Associated Osteoporosis: A Case Report

***Masafumi Kashii**, Takako Hattori, Motoki Iwasaki, Hideki Yoshikawa*
Osaka University Graduate school of Medicine, Suita, Osaka Pref, Japan

Introduction: Pregnancy and lactation-associated osteoporosis (PLO) is relatively rare disease that can cause multiple vertebral compression fractures (VCFs).
Case: We present a case of 39-year-old woman with PLO treated by teriparatide (TPTD), the human recombinant PTH1-34. She had a family history of osteoporosis and had prevalent L1 VCF. She (gravida 1 para1) complained low back pain one month after delivery, and multiple VCFs (T7, T12-L4)

with severe back pain appeared 3 months after delivery. The diagnosis was confirmed by DXA scan results (L1-4 T-score –5.5SD, total hip T-score –4.1SD). Due to the severity of osteoporosis, 20μg per day TPTD with calcium aspartate and vitamin K2 was started for a period of 24 months.
Results: After TPTD administration for 15 months, T-score (L1-4) had significantly improved at the lumber spine as well as at the hip (L1-4 T-score -3.5SD, total hip T-score -3.5SD). The relative increase of BMD at the spine and total hip was 55% and 17%, respectively. Bone turnover markers significantly increased. Now, she could care her child without any complication, and had a hope to have a second baby.
Discussion: Our report revealed the beneficial effect of TPTD for PLO with the significant increase of BMD and the improvement of clinical symptoms. Use of TPTD yield successful results in a patient with severe PLO patients.

P2066
Calcium Imbalance And Pervasive Bone Loss In Cecectomized Rats
Narattaphol Charoenphandhu[1,2]*, Prapaporn Jongwattanapisan*[1,2]*, Panan Suntornsaratoon*[1,2]*, Kannikar Wongdee*[2,3]*, Nitita Dorkkam*[1,2]*, Nateetip Krishnamra*[1,2]
[1]Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand; [2]Center of Calcium and Bone Research (COCAB), Faculty of Science, Mahidol University, Bangkok, Thailand; [3]Office of Academic Management, Faculty of Allied Health Sciences, Burapha University, Chonburi, Thailand

Physiological significance of the cecum in regard to body calcium metabolism is controversial since it has the highest calcium transport rate as compared to other intestinal segments but was reported to contribute less than 10% of the total calcium absorbed by the intestine. We therefore investigated changes in calcium absorption in all intestinal segments and bone mineral density in rats with cecum being surgically removed (cecectomy). In a 4-week calcium balance study, the cecectomized rats manifested an increase in fecal calcium loss with marked decreases in the fractional calcium absorption and urinary calcium excretion only in the fist week post-surgery, suggesting the presence of a compensatory mechanism that helped to restrict calcium wasting. This compensation resulted from the enhancement of cellular energy-dependent colonic calcium transport and calcium transporter gene expression, probably by the activation of the calcium-sensing receptors in the colonic epithelial cells. Surprisingly, even with a robust colonic compensation, the osteoclast-induced bone resorption and osteopenia still occurred in both cortical and trabecular sites, indicating that bone calcium release was also necessary to maintain normocalcemia. The presence of compensatory colonic calcium hyperabsorption and pervasive bone loss after cecectomy confirmed that the cecum was important for body calcium metabolism.

P2067
Characterization Of A Deletion In Tissue-nonspecific Alkaline Phosphatase (p.F327del) Found In Japanese Patients With Hypophosphatasia
Akiko Kiyama*, Tomohiro Matsumura, Hideo Orimo*
Biochemistry & Molecular Biology, Nippon Medical School, Bunkyo-ku, Tokyo, Japan

Hypophosphatasia is an inherited systemic bone disease caused by mutations of the tissue-nonspecific alkaline phosphatase (TNAP) gene. A total of 261 mutations in the TNAP gene have been reported to date. A deletion of p.F327 (p.F327del) has been reported in Japanese patients with severe form hypophosphatasia as compound heterozygotes with c.1559delT, a severe allele and the most frequent mutation in Japanese patients. We analyzed p.F327del in U_2OS human osteoblast-like cells, which express a trace level of TNAP. U_2OS cells were transfected with expression plasmids containing mutant TNAP. Enzymatic activity and Western analysis of the cell extracts were assessed at 48 h after the transfection. The remaining cells were cultured for additional 5 days with 10 mM β-glycerophosphate to estimate mineralization. The cells transfected with p.F327del showed 3.4 % of the wild type in enzymatic activity at 48 h, and 1.3 % in enzymatic activity and 15.8 % in mineralization after 5 days culture with β-glycerophosphate. These values were similar to that of c.1559delT. Western analysis revealed reduced expression and impaired glycosylation of the mutant protein. A 3D model of the mutant protein based on the structure of human placental alkaline phosphatase indicated that p.F327 is located in a core β-sheet and the deletion may cause deformity of the β-sheet. Those results suggest that p.F327del is a severe allele and associates with the severe phenotype of the patients.

P2069
A Two-year History Of Weight-bearing Physical Activity Attenuates Ethnic Differences In Bone Strength And Geometry In Pre/-Early Pubertal South African Children
Rebecca Meiring[1]*, Ingrid Avidon*[1]*, Shane Norris*[2]*, Joanne McVeigh*[1]
[1]Exercise Physiology Laboratory, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, Gauteng, South Africa; [2]MRC/WITS Developmental Pathways for Health Research Unit, Department of Pediatrics, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, Gauteng, South Africa

We examined the interplay between ethnicity and weight-bearing physical activity on the material and volumetric properties of bone in a pre- to early-pubertal South African Black and White population. Seventy six children reported on all their physical activities over the past two years in an interviewer administered physical activity questionnaire (PAQ). All

participants underwent a whole body and site-specific bone density scan using DXA and pQCT. Children were classified as being either high or low bone loaders based on the median peak bone strain score obtained from the PAQ. Compared to White low bone loaders, Black low bone loaders had greater site-specific bone mass as measured by DXA and greater bone mass and indices of strength at the radius and tibia as measured by pQCT. Ethnic differences were either attenuated or reversed in the high bone loading group in bone variables measured by DXA and pQCT. Ethnic differences were also observed in bone geometry at the radius and tibia of the low bone loading group but not in the high bone loading group. In conclusion, the present study shows that participation in weight-bearing physical activity results in increases in bone mass and structurally better bones as measured by DXA and pQCT in pre-/early pubertal South African children. Ethnic differences in bone health are attenuated when participation in weight-bearing activity is accounted for.

P2070

Tenocytes Regulate Cell Survical Of Osteoblasts *In Vitro*

Satoshi Wada[1], *Akemi Shimada*[1], *Hisashi Ideno*[1,2], *Yoshiki Nakamura*[1], *Kazuhisa Nakashima*[1], *Akira Nifuji*[1]
[1]Tsurumi University, Yokohama, Japan; [2]Waseda University, tokyo, Japan

At the insertion sites, bony outgrowth that extends from the skeleton into tendons or ligaments are often observed, which can be associated with their tensile forces. However, molecular mechanisms underlying the bony outgrowth at the insertion site is unclear. We hypothesize that direct interaction between tendons and bone may regulate bone growth. To this end, we investigated whether tenocytes affects cell functions of osteoblasts. At first we co-cultured primary tenocytes and osteoblastic MC3T3-E1 (MC) cells and examined the effects of tenocytes on osteoblastic phenotypes. The expression of osteocalcin (OCN) was significantly reduced in the co-culture compared with the single-culture of osteoblasts. Next we investigated whether soluble factors derived from tenocytes affect osteoblastic phenotypes in MC cells. We collected conditioned medium from tenocytes or MC cells (as controls) and added them on MC cells. When conditioned media from tenocytes was added, ALP activity and protein content were significantly reduced compared with conditioned media from osteoblasts. In addition, by DAPI and caspase3 staining, we observed increase in apoptotic nuclei and caspase3 staining, suggesting promotion of apoptosis in MC cells. These results suggest that soluble factors derived from tenocytes inhibit osteoblastic function and cell survival *in vitro*. Thus blunting of these negative effects of tenocytes may explain bony outgrowth at the insertion site.

P2071

Volumetric Bone Density, Geometry, And Strength In Male Runners; Road Cyclists; Mountain Bikers And Swimmers

Joanne McVeigh, *Alessandra Cimato, Ingrid Avidon*
Exercise Laboratory, School of Physiology, University of Witwatersrand, Johannesburg, Gauteng, South Africa

42 trained male (23.9±3.9yr) runners (RUN(n=13)), road cyclists (RC(n=10)), mountain bikers (MB(n=10)) and swimmers (SWIM(n=9)) were compared for radial and tibial bone geometry, area, content (BMC), density (BMD), and estimates of strength using DXA and pQCT. pQCT was used to assess volumetric BMC (vBMCtot, mg mm^{-3}), area (Ar.tot, mm^2), cortical bone area (Ar.ct, mm^2), and vBMC (vBMC.ct, mg/mm^{-3}), periosteal circumference (mm), bending strength (polar strength strain index (SSIp), mm3 and bone strength index (BSI)) at the proximal (66%) radius and tibia and distal (4%) radius. Variables were adjusted for body size differences. Serum BAP and CTX were measured. RC had significantly less Ulna BMC, BMD (–17%, –10%, p<0.05) and a smaller radius area and BMC (–8% and –17%, p<0.05) compared to MB. RC had significantly smaller radius area than SWIM (–10%, p<0.05) and RUN (–7%, p<0.05). RC had less spine BMD (–11%, p<0.05) than RUN, less vBMCtot and Ar.Ct than RUN (–17% and –16%, p<0.01) at the proximal tibia, and less vBMCtot,Ar.tot and Ar.ct (–12% to –17%, p<0.05) than MB and smaller periosteal circumferences than MB and SWIM (–8% and –6%, p<0.05) at the proximal radius. RC had smaller vBMCtot, Ar.Tot and BSI than MB and RUN (–10% to –21%, p<0.05) at the distal radius. CTX (ng ml^{-1}) was higher in SWIM compared to MB (+52%, p<0.05). MB had more favourable bone geometry at the radius, and RUN at the tibia when compared to RC. Greater bone strength in MB and RUN is attributable to greater bone area.

P2072

Distribution Of The Body Circumferences And Bony Mass In Young Male Dependently On The Somatotype And Physical Activity

Lubov Stklanina, *Alexander Tyurenkov, Vera Makarova, Vladyslav Luzin*
Luhansk State Medical University, Luhansk, Ukraine

In 222 young male (17-21 y.o.) the chest, waist and gluteal circumferences were measured. The bony mass was obtained from the calcaneal tuber by the DXA-densitometry. Somatotype division was done using the shoulder index (acromial distance/height). Male were subdivided taking account their physical activity into the physically active group (physical training 3 and more times per week) and passive (less than 3 workouts per week). Correlative relations were established between the bony mass and body circumferences. As a result the body circumferences are predicted by the physical activity. The difference of the chest, waist and gluteal circumferences

between the physically active and passive persons was significant ($p<0.05$) and takes 3.00-4.00 cm. In physically active persons this circumferences were strictly depend with the somatotype: the greatest (94.56cm) circumferences were found in brachymorphic somatotypes, lesser one (83.00cm) - in dolychomorphic, middle (90.07 cm) - in mesomorphic. For the physically passive males the greatest waist and gluteal circumferences were found in dolychomorphic persons, the smallest - in mesomorphic. The bony mass reveals the significant reverse correlation (rx/y -0.92) with the waist circumference only for the passive persons. So, the high waist circumference in dolychomorphic passive persons predicts the lower bony mass and could be the aware fact for the osteoporosis.

P2073

Osteogenesis And Osteoclast Inhibition In Rheumatoid Arthritis Patients Treated With Bisphosphonates Alone Or In Combination With Pitavastatin Over An 18-month Follow-up After More Than 4 Years Of Treatment With Bisphosphonates

Masakazu Nagashima
Tokyo Metropolitan Bokutoh Hospital, Tokyo, Tokyo, Japan

Objective: To investigate the effects of bisphosphonates (Bis) alone and in combination with statin, on the BMD and bone metabolism of rheumatoid arthritis (RA) patients.
Methods: Seventy-seven RA patients who had been receiving Bis for over 4 years were divided into two groups: Bis and Bis +statin (*n*=42 and 35; average age, 66.4 and 65.3 years; average disease duration, 24.9 and 20.8 years, respectively). Serum levels of NTX, TRACP-5b, PICP, and RANKL were measured over an 18-month period of treatment and follow-up. The BMD levels of the two groups at the radius, lumbar spine, and femoral neck were compared.
Results: A significant increase was only observed in the BMD of the lumbar spine at 18-months, but the BMDs of the radius and femoral neck decreased during the follow-up period in the Bis group. Among the markers of bone metabolism, serum NTX was up-regulated after 6 months in the Bis+statin group. Serum TRACP-5b was significantly increased during the follow-up period in the Bis+statin group, but only at 18 months in the Bis group. Serum PICP recovered to base line in the Bis+statin group, whereas that in the Bis group did not observably recover during the post-administration follow-up, but rather decreased.
Conclusion: Our results suggest that both bone resorption and bone formation were inhibited by long-term administrating of Bis alone, whereas combination therapy with Bis+statin may be associated with a less marked inhibition of bone metabolism.

P2074

The Application Of Curcumin-Loaded Liposomes On Osteoporosis Treatment

Yu-Han Su[1], Chih-Chang Yeh[1,2], Cheng-Nan Chen[1],
Hsin-I Chang[1]
[1]Department of Biochemical Science and Technology, Chiayi, Taiwan; [2]Orthopaedics department, Chiayi Branch, ThaichungVeterans Hospital, Chiayi, Taiwan

Curcumin is a non-water-soluble polyphenol compound that has anti-inflammatory potential. Recent studies found that curcumin can reduce the osteoclastogenic activity for preventing bone loss. To increase cellular uptake of curcumin, we used soybean phosphatidylcholine to encapsulate curcumin for liposome formation. In this study, curcumin-loaded liposomes have been characterized in particle size, encapsulation efficiency, liposome stability and cellular uptake. The results show that there are about 70% entrapment efficiency of curcumin in liposomes and particle sizes are stable after liposome formation. Curcumin-loaded liposomes can inhibit macrophage inflammation and differential activities. In comparison with curcumin only, curcumin-loaded liposomes have no significant cytotoxicity and can remain the osteoblast differential functions. With IL-1β stimulation, curcumin-loaded liposomes can successfully down-regulate the expression of inflammation markers on osteoblasts and showed high OPG/RANKL ratio to prevent osteoclastogenesis. In this study, we observed that curcumin can be encapsulated in liposomes successfully and which can reduce osteoclast activity and maintain osteoblast functions.

Drug	Particle size (nm)		Entrapment(%)	
	No extruder	Extruder	No extruder	Extruder
Empty	959.6± 204.3	99.7± 5	-	-
Curcumin(Cur)	1110.0±149.0	110.0±4.8	-	69.5±1.4

Values represent the mean ± SD for at least three experiments.

P2075

A 12 Month Consumption Of An Olive Polyphenolic Extract Improves Both Bone Formation In Postmenopausal Women With Osteopenia

Rafal Filip[1,2], *Sam Possemiers[3,4], Arne Heyerick[4,5], Grzegorz Raszewski[1], Marie-Jeanne Davicco[6], Veronique Coxam[6]*
[1]Osteoporosis Outpatient Department, Institute of Rural Health, Lublin, Poland; [2]Faculty of Human Nutrition and Consumption, Warsaw University of Life Sciences, Warsaw, Poland; [3]Laboratory of Microbial Ecology and Technology (LabMET), Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium; [4]BioActor BV, Maastricht, Netherlands; [5]Laboratory of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium; [6]INRA, UMR 1019, UNH, CRNH Auvergne, F-63000 Clermont-Ferrand, France, and Clermont Université, Université d'Auvergne, Unité de Nutrition Humaine, Clermont-Ferrand, France

Introduction: Several animal studies showed that olives, extra virgin olive oil and its main phenolic micronutrients oleuropein, hydroxytyrosol and tyrosol, exert a protective effect on both bone loss in ovariectomised rats with induced inflammation. Also, pre-clinical and clinical studies have proven that pro-inflammatory cytokines can accelerate bone loss. Moreover, estrogen deficiency after the menopause is also associated with increased production of pro-inflammatory cytokines. The aim of this study was to investigate the effect of intake of an olive polyphenol extract on bone turnover in postmenopausal women with osteopenia.
Methods: 64 osteopenic patients, with a mean T-score between 1.5 and 2.5 in the lumbar spine (BMD L2-L4) for bone

mineral density, were included in the double blind study and randomly assigned to a treatment or placebo condition. The treatment group received daily 250mg Olive Extract for 12 months and 1g calcium/day.
Results: Upon 12-month administration, osteocalcin (OC) levels increased significantly in the treatment as compared to placebo (p=0.04). Simultaneously, bone mineral density decreased in the placebo group (p=0.014) whereas no decrease was observed in the treatment group.
Conclusions: This study suggests the potential usefulness of a specific olive polyphenolic extract in osteopenic postmenopausal women. Olive polyphenolic extract induced beneficial changes in serum OC, however, the long-term effect needs to be further investigated.

Poster Session 3

P3001
Specialists Survey In Therapeutic Education Of Patients Treated For Knee And Hip
***Kawtar Nassar**, Saadia Janani, Wafaa Rachidi, Noufissa Etaouil, Ouafaa Mkinsi*
rheumatology, casablanca, anfa, Morocco

Osteoarthritis is degenerative joint disease,most common and disabling responsible for disability. Prevention and therapeutic education for this chronic disease is an international recommendation, including the EULAR 2012. purpose: Knowing the opinion of specialists on the role of therapeutic education in patients with osteoarthritis.Between January and July 2012, a medical specialists cross-sectional survey in the Hospital university of Casablanca, concerning therapeutic education of patients treated for knee and hip osteoarthritis.43 specialists answered the questionnaire. The average patients treated for knee and hip osteoarthritis per week were 11.25. therapeutic education is justified by the lack of knowledge of the disease osteoarthritis for all physicians, lack of treatment knowledge 83.7%, the frequency of erroneous beliefs disease for 74.4%,of treatment by 62.7%. It must be an integral part of any consultation for 81.39%. All physicians reported practicing therapeutic education by oral information.Therapeutic education is important for both patients treated for knee osteoarthritis for all physicians. However, the limitations are, lack of time on the part of doctors for 81.3%, the false beliefs of patients for 53.4%, comprehension difficulties patients for 60.4% , and lack of motivation to 53.4%. 72% of physicians believe that patients are interested.

P3002
A Case Of Scleroderma Destructive Arthropathy
***Kawtar Nassar**, Saadia Janani, Wafaa Rachidi, Noufissa Etaouil, Ouafaa Mkinsi*
Rheumatology, Casablanca, Morocco

Systemic sclerosis is an autoimmune disease generalized connective tissue, characterized by multiple organ damage and skin. It is unique among other connectivity by a pathological process original: Microvascular abnormalities, initially spastic, an autoimmune involvement and fibroblast activation. Articular manifestations are often present and may usher in the disease. However, the destructive arthropathy is rare. Its occurrence worsens the functional prognosis.The treatment of osteo-articular manifestations included analgesics, anti-inflammatory drugs, steroids low dose of colchicine associated with the rehabilitation of an orthotic posture. In the case of rheumatoid, evolution is rapidly destructive. We report a 47 years patient case, followed for 11 years for systemic sclerosis, with cutaneous, articular, made inflammatory polyarthralgia, pulmonary and heart. The radiological changes in joint damage showed significant destructive, erosion of the ends of two large bones, severe acro-osteolysis in the first three fingers. Knowledge of these manifestations superimposed can improve our therapeutic approach.

P3003
The Addition Acpa Value To Rheumatoid Factor In The Diagnosis Of Rheumatoid Arthritis
***Kawtar Nassar**, Saadia Janani, Wafaa Rachidi, Noufissa Etaouil, Ouafaa Mkinsi*
Rheumatology, Casablanca, Morocco

The diagnosis of recent rheumatoid arthritis(RA)need meeting clinical and paraclinical parameters, among whom we find the Ac anti CCP, which occupy a great interest in the diagnosis of RA refocused in the prognosis of the disease and in some differential diagnosis. Purpose: Evaluation value anti-CCP compared to rheumatoid factor(FR) in the diagnosis of early RA.
Materials and methods: Prospective study including 75 cases presented early rheumatoid,in the Rheumatology department,University Hospital of Casablanca.All patients beneficied a combination dosage rheumatoid factor and anti-Ac CCP. Patients whom the diagnosis of RA was confirmed, we emerged the characteristics of anti CCP relative factor arthritis (sensitivity and specificity).
Results: Among 75 patients included, the diagnosis of rheumatoid arthritis,was confirmed for 45 cases.38 had positive ACPA,32 patients had rheumatoid factor positif. 27 RA were rheumatoid factor and anti CCP hight value. The Ac antiCCP specificity in our patients was 29 (VVN). As for sensitivity, it is 38 antiCCP (VVP) and 32 FR.
Anti-CCP test is equivalent to rheumatoid factor for sensitivity, but the specificity of anti-CCP2 is much better for the diagnosis of rheumatoid arthritis. This test allows effective discrimination between rheumatoid arthritis and other arthritic conditions generally anti-CCP2-negative but in which rheumatoid factor positive

P3004
Identification Of Differentially Expressed Genes In Mesenchymal Stem Cells Derived From Synovium, Meniscus And Ligament
***Yoichi Ezura**, Tadayoshi Hayata, Takuya Notomi, Masaki Noda*
Department of Molecular Pharmacology, Tokyo Medical and Dental University, Medical Resarch Institute, Tokyo, N/A, Japan

Stromal progenitor cells existing in various adult tissues possess multi-potency to differentiate into various cell types, and thus are called as mesenchymal stem cells (MSCs). In spite of

shared common features among these cells, detailed comparison indicated consistent differences between the Synovium derived human MSCs (SMSCs) and bone marrow (BM) derived MSCs (BM-MSCs). Gene expression and epigenetic status of these cells appeared to be different at some important loci. Here, to investigate further the basic differences among MSCs derived from different tissues, we compared global gene expression among hMSCs derived from synovium, meniscus and cruciate ligaments. Selectively expressed genes in synovium derived, meniscus derived, and cruciate ligament derived cells were extractable. Of note, 20 genes selectively expressed in meniscus derived cells included 3 genes that are implicated in joint development, cartilage formation, and tendon morphogenesis. These results indicate tissue specific functions of MSCs derived from various tissues.

P3005

Subchondral Bone Changes In Knee Osteoarthritis Can Be Quantitatively Detected From Plain Radiographs

Jukka Hirvasniemi[1], Ville Immonen[1], Tuomas Liikavainio[2], Jari Arokoski[3,4], ***Simo Saarakkala[1,5]***

[1]University of Oulu, Oulu, Finland; [2]Muonio Health Centre, Muonio, Finland; [3]University of Eastern Finland, Kuopio, Finland; [4]Kuopio University Hospital, Kuopio, Finland; [5]Oulu University Hospital, Oulu, Finland

Background: Radiography is cheap and widely-available imaging modality. Quantitative detection of osteoarthritic (OA) changes in subchondral bone from radiographs would be an advantage. We hypothesize that quantitative evaluation of bone changes would discriminate OA patients from controls.
Methods: Radiographs from 206 knees (110 healthy, 96 OA) were analysed using MATLAB software and graded using Kellgren-Lawrence (KL) grading. KL grades were not known during analyses. Joint space width (JSW) was measured from the centre of the condyles. Four region-of-interests (ROI) were placed below the condyles of the tibia (Figure 1). From the ROIs, normalized mean greyscale values (nGV) and texture parameters (Homogeneity [HI]) were calculated to evaluate bone density and structure. HI was derived from grey-level co-occurrence matrix which was separately calculated from unprocessed, local binary pattern (LBP), and Laplacian based images.
Results: JSW, nGV from medial ROIs, and HI from medial subchondral bone from LBP image differed significantly between different KL groups (Table 1). Systematic changes were not observed in trabecular bone, in lateral subchondral bone, or in texture parameters from unprocessed or Laplacian based images.
Conclusion: The results indicate that subchondral bone density and structure can be quantitatively evaluated from plain knee radiographs. For evaluation of bone structure, preprocessing of the radiograph is needed.

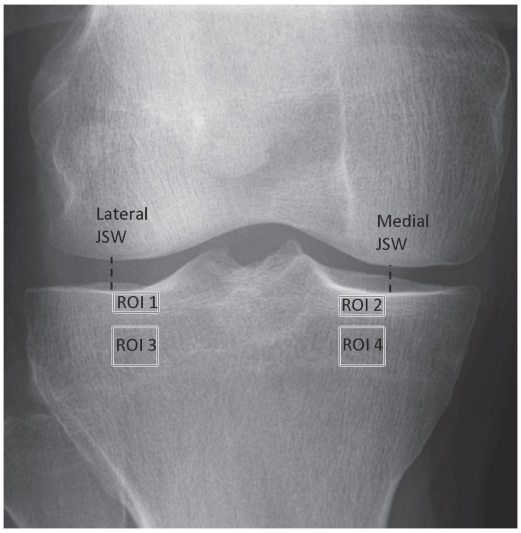


Figure 1 Location of the region-of-interests (ROIs).

P3006

Role Of Wnt/β-catenin Signaling In An *In Vivo* Model Mimicking Human Subchondral Bone And Osteocyte Pathophysiological Changes In Osteoarthritis

Anjali Jaiprakash[1], Nishant Chakravorty[1], Ross Crawford[1,2], Jian Feng[3], ***Yin Xiao[1]***

[1]Bone Biology, QUT-Institute of Health and Biomedical Innovation, Brisbane, Queensland, Australia; [2]Prince Charles Hospital, brisbane, Queensland, Australia; [3]Texas A&M Health Science Center, Baylor College of Dentistry, Texas, Dallas, TX, USA

Osteocyte Wnt/β-catenin signaling plays a central role in regulating joint physiology [1]. Subchondral bone sclerosis is an important clinical sign of osteoarthritis (OA), but its involvement in OA pathophysiology is poorly defined. Our recent studies suggest that dysregulated osteocytic proteins contribute to the

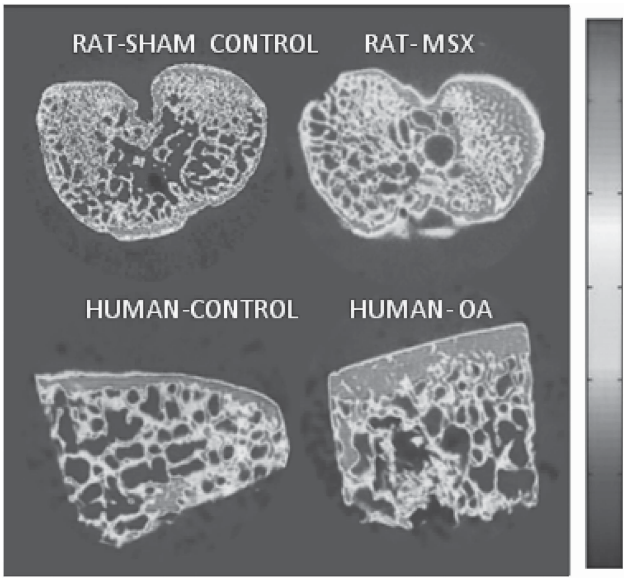
[P3005]

Table 1. Mean (± SD) values of medial and lateral joint space widths (JSW), normalized mean gray values (nGV), and homogeneity indices (HI) from subchondral bone ROIs.

KL	N	Medial JSW (mm)	Lateral JSW (mm)	Medial nGV	Lateral nGV	Medial HI	Lateral HI
0	109 - 110	5.1 ± 0.9	6.3 ± 1.3	0.64 ± 0.07	0.64 ± 0.06	0.49 ± 0.03	0.49 ± 0.03
1	28	4.7 ± 0.8	6.4 ± 1.0	0.67 ± 0.05	0.65 ± 0.07	0.48 ± 0.04	0.48 ± 0.04
2	27	3.9 ± 1.4	6.3 ± 1.0	0.69 ± 0.07	0.66 ± 0.08	0.47 ± 0.03	0.48 ± 0.03
3	32	2.9 ± 1.3	6.5 ± 1.3	0.69 ± 0.08	0.63 ± 0.06	0.47 ± 0.03	0.48 ± 0.03
4	9	3.1 ± 2.8	6.5 ± 2.7	0.70 ± 0.09	0.63 ± 0.10	0.46 ± 0.03	0.48 ± 0.03
ANOVA		<0.001	NS	<0.001	NS	<0.001	NS
Post-hoc*		0-2, 0-3, 0-4, 1-2, 1-3, 1-4, 2-3		0-2, 0-3		0-2, 0-3, 0-4	

*Differences between groups using Sidak's post-hoc test

pathological changes in subchondral bone of knee OA patients [2]. The aim of this study was to establish a suitable animal model that resembles the clinical situation and allows the investigation of progressive osteocyte changes and the involvement of Wnt/β-catenin signaling in OA pathophysiology. We induced OA in 12 week old Wistar Kyoto rats by removal of the medial meniscus (MSX, *n*=12). We investigated changes in subchondral bone and osteocytes 8 weeks post-surgery by SEM, microCT, histology, protein expression and compared it to sham controls and human knee samples.MSX and human OA demonstrated significantly higher subchondral bone volume compared to controls (Figure 1). Histological analysis of the MSX group demonstrated significantly higher numbers of osteocytes expressing DMP1, E11, AXIN2 and β-catenin whereas a decreased number of SOST and DKK1 expressing osteocytes were found compared to controls. These results indicate a potential regulatory role of osteocyte wnt/β-catenin signaling in subchondral bone remodeling. OA MSX experimental rat model resembles human OA pathophysiology and is suitable for studying the progression of OA subchondral bone pathogenesis.



P3007

Role Of Osteocyte Wnt/βcatenin Pathway In Long Term, Low Dose Inflammation And Its Potential Pathological Role In Osteoarthritis

Anjali Jaiprakash[1], *Nishant Chakravorty*[1], *Ross Crawford*[1,2], *Yin Xiao*[1]

[1]Bone Biology, QUT-Institute of Health and Biomedical Innovation, Brisbane, Queensland, Australia; [2]Prince Charles Hospital, Australia, Brisbane, Queensland, Australia

Cytokines play a pivotal role in the initiation and development of osteoarthritis (OA) and are important regulators of bone remodeling in disease conditions[1]. Osteocytes are postulated to play a key role in regulating bone remodeling and mineral metabolism. It is proposed that OA may be driven by a low-grade inflammatory processes associated with subchondral bone sclerosis[2]. TNFα is implicated in the pathophysiology of OA and is associated with bone loss due to lower expression of Wnt/βcatenin when treated on osteoblasts[3]. However, these changes are inconsistent with the clinical findings of increased bone mass in OA. Osteocytes are 90% of bone cells, thus we aim to explore the effects of TNFα on osteocytes and further evaluate their crosstalk with osteoblasts. Osteocyte cell line were cultured under the influence of low (0.5ngml^{-1}) and high (40ngml^{-1}) doses of TNFα for 7days. Low dose and long term stimulation increased osteogenic differentiation compared to controls in osteocytes. Further, soluble factors from day 7 low dose group were used to culture primary osteoblasts for 1,3,7days. The results demonstrated a dose dependent increase in osteogenic markers and up-regulation of Wnt/βcatenin pathway in osteocytes compared to controls. These results indicate that osteocytes preconditioned with low dose long term inflammatory mediators could potentially play a role in subchondral bone sclerosis. Hence osteocytes play an important role in inflammatory bone remodeling in OA.

P3009

Renal Calcification Induced By High Phosphorus Intake In Female Rats Is Related To High Levels Of Circulating FGF23

Satoshi Takasugi, *Miho Akutsu, Masashi Nagata*
Meiji Co., Ltd., Odawara, Kanagawa, Japan

This study aimed to investigate the effects of high phosphorus (HP) intake on ectopic calcification in sham-operated and ovariectomized (OVX) rats. Thirty-two 12-week-old female rats were divided into two groups: a sham-operated group and an OVX group. After the surgery, each group was divided into two subgroups: one that fed a control diet containing 0.3% P and one that fed a HP diet containing 1.2% P for 12 weeks (*n*=8 for each subgroup). At the end of the feeding period, we measured bone mineral density (BMD) of lumbar vertebrae, plasma FGF23 level, and calcium (Ca) concentration in the kidney and abdominal aorta. HP diet significantly decreased BMD and increased aortic Ca concentration in both Sham and OVX rats. Renal Ca concentration was significantly increased by HP diet in both Sham and OVX rats; however, the increase was much higher in Sham rats than in OVX rats. Furthermore, HP diet significantly increased plasma FGF23 level in Sham rats but not in OVX rats. The plasma FGF23 level was positively correlated with renal Ca concentration. These results suggest that estrogen deficiency alleviates the stimulating effects of HP intake on renal calcification and that the marked renal calcification induced by HP intake in intact female rats may be related to the increase in circulating FGF23 level.

P3010

Circulating FGF23 Is Regulated By Pth And Calcitriol During Fetal Development But Low FGF23 Does Not Significantly Alter Fetal Phosphorus Metabolism

Sandra Cooke-Hubley[1], *Beth Kirby*[1], *Subramanian Suppiah*[1], *Charlene Simmonds*[1], *Andrew Karaplis*[2], *Christopher Kovacs*[1]
[1]Memorial University of Newfoundland, St. John's, Newfoundland, Canada; [2]McGill University, Montreal, Quebec, Canada

FGF23 regulates adult phosphorus metabolism and is stimulated by PTH and calcitriol. Data are lacking about FGF23's role in fetal life. We therefore examined whether loss of PTH or calcitriol signaling alters circulating FGF23 and phosphorus homeostasis in fetal mice. We mated Pth+/- females and used an EIA (Kainos) to measure intact FGF23 at ED 18.5. Serum FGF23 ($pgml^{-1}$) was 65.6±11.2 in non-pregnant Pth+/– vs. 263.4±21.3 in pregnant Pth+/– mothers, 229.0±23.3 in WT fetuses, and was significantly reduced to 114.4±19.1 in Pth null fetuses ($p<0.01$ vs. maternal or WT values). Pth null fetuses are hyperphosphatemic which could be due to low FGF23, low calcitriol, or absent PTH. We then mated Vdr+/– females and found serum FGF23 ($pgml^{-1}$) was 142.8±13.3 in pregnant Vdr+/– mothers and 177.3±23.2 in WT fetuses, but was significantly reduced to 74.1±11.8 in Vdr null fetuses ($p<0.01$ vs. maternal or WT values). All parameters of fetal phosphorus metabolism were normal in Vdr null fetuses, including ionized calcium, serum phosphorus, PTH, amniotic fluid phosphorus, and skeletal ash weight and mineral content. In summary, FGF23 increases during pregnancy. Mothers and WT fetuses achieve equivalent levels, while loss of PTH or VDR each caused a >50% decline in fetal FGF23. Vdr nulls had normal phosphorus indices and normal PTH. We conclude that low FGF23 does not impact fetal phosphorus metabolism, and that it is likely loss of PTH that disturbs phosphorus metabolism in Pth nulls.

P3011

The Long Term Changes Of Bone Mass And Turnover Markers Suggest The Anabolic And Catabolic Actions Of Pth In The Patient With Parathyroid Carcinoma

Yasato Komatsu, *Yuji Hataya*
Endocrinology, Kyoto City Hospital, Kyoto, Japan

In order to investigate the dual actions of PTH on bone, a lot of studies have been done recently. We followed up the patient of parathyroid carcinoma (PC) for 15 years and observed the unique change of BMD and bone markers. A 45 y.o. female was admitted to our hospital because of hyperparathyroid crisis. She was diagnosed as PC and received total PTX at 23 y.o. Additional neck surgery was done twice because of local recurrence. The labo data at that time was Ca14.1$mgdl^{-1}$, P2.0$mgdl^{-1}$, iPTH1230$pgml^{-1}$ Her lumber spine (LS) and distal radius (DR) BMD were reduced; LS=0.708gcm^{-2} (T=-3.5DS) and DR=0.498gcm^{-2} (T=–2.1SD). A total bone scintigraphy showed enhanced uptake of cranium, spine, long bones and ribs. Intravenous zoledronate was intermittently given for hypercalcemia and osteoporosis. At 56 y.o., she had severe pain of hips and became difficult to walk because of atypical fracture of bilateral femurs and hip osteoarthrittis. The BMD was increased dramatically recently; LS=1.410gcm^{-2} (T=+2.4s.d.). femoral neck=1.117g/cm2 (T=1.8SD), ALP1299IUl^{-1}, Ca13.5$mgdl^{-1}$, P3.3$mgdl^{-1}$, iPTH1170$pgml^{-1}$, BAP 74.5μg/L, BGP 85 ng/mL, P1NP565.7 μgl^{-1}, urine NTX 574nMBCE/mMCr, TRACP-5b 1830 $mUdl^{-1}$ Her hipbone biopsy showed the unique histology; thinning of cortices and greater number of trabeculae. These findings suggest that increased bone formation by continuously elevated PTH and reduced bone resorption by bisphosphonates may lead to abnormal bone remodeling and cause osteosclerosis and bone fragility.

[P3010]

Table 1 Fetal Data showing mean ± s.e.

PTH Colony	WT Fetuses [n]	Null Fetuses [n]	p value
Serum FGF23 [$pgml^{-1}$]	229.0 ± 23.3 [6]	114.4 ± 19.1 [8]	$p<0.01$
Serum Phosphorus [$mmol^{-1}$]	2.9 ± 0.2 [9]	3.5 ± 0.2 [12]	$p<0.02$
Serum PTH [$pgml^{-1}$]	19.5 ± 6.5 [6]	Undetectable [8]	$p<0.05$
VDR Colony	WT Fetuses	Null Fetuses	*p* value
Serum FGF23 [$pgml^{-1}$]	177 ± 3.2 [9]	74.1 ± 11.8 [8]	$p<0.001$
Serum Phosphorus [$mmol^{-1}$]	2.7 ± 0.2 [6]	2.8 ± 0.3 [6]	Not significant
Serum PTH [$pgml^{-1}$]	10.5 ± 1.6 [6]	9.3 ± 1.6 [9]	Not significant
Amniotic Fluid Phosphorus [$mmol^{-1}$]	1.99 ± 0.14 [9]	1.68 ± 0.14 [9]	Not significant

P3012

Effect Of Administration Frequency Of Teriparatide On Bone Metabolism In Ovariectomized Rats

***Aya Shimomura**, Hiroshi Yamane, Tomoya Tanaka, Katsuhiro Baba, Ryoko Takao-Kawabata, Yukihiro Isogai*
ASAHI KASEI PHARMA Co., Pharmaceuticals Research Center, Izunokuni-shi, Shizuoka-ken, Japan

Purpose: Teriparatide (TPTD) has shown the different effects on bone metabolism depending on administration frequency. To understand the pharmacological profile of TPTD on bone metabolism, we investigated the effect of dosing frequency on a variety of bone parameters in ovariectomized rats.
Methods: 3-month-old female Sprague-Dawley rats were divided into 15 groups, and then ovariectomized or sham operated. 3 weeks after surgery, rats were subcutaneously injected with 1.2, 6 or 30 μg/kg of TPTD once a day (D1 groups), twice a day (D2 groups),1.2 or 6 μg kg^{-1} of TPTD 3 times a day (D3 groups) or, 6 or 30 μg kg^{-1} of TPTD 3 times a week (W3 groups) for 4 weeks. Saline injection was given to sham (3 times a week) and each group as vehicle. Bone metabolic markers in serum and urine were measured during dosing with several time points. After dosing period, lumbar vertebra, tibia and femur were collected, and bone mineral density, structure and strength were analyzed, respectively.
Results: TPTD increased bone mineral density and strength in all groups. The increase was dependent on the total amount of TPTD per week rather than frequency. On the other hands, the increase of bone metabolic markers and bone histomorphometric parameters in vertebra and porosity in vertebral cortical area were frequency dependent.
Conclusion: These results suggest that TPTD affects bone volume and strength in a dose-dependent manner, but bone metabolism in a frequency-dependent manner in rats.

P3014

Possible Involvement Of Vitamin D Deficiency In Increasing Serum Levels Of Homocysteine In Patients With Primary Hyperparathyroidism

***Hisanori Suzuki**[1], Akira Takeshita[1], Megumi Miyakawa[1], Tsuguru Iwatani[2], Daishu Miura[2], Yasuhiro Takeuchi[1]*
[1]Toranomon Hospital - Department of Endocrinology and Metabolism, Tokyo, Japan; [2]Toranomon Hospital - Department of Breast and Endocrine Surgery, Tokyo, Japan

Backgrounds: An increased circulating level of homocysteine (Hcy) is a risk factor for osteoporotic fractures. Serum Hcy levels are positively correlated with an incidence of fractures, and their correlation is somewhat independent of bone mineral density (BMD). Hcy increases oxydative stress that could be involved in the accumulation of advanced glycation end-products in type I collagen in bone and its fragility. Thus, Hcy might deteriorate bone quality.
Purpose: To elucidate whether vitamin D metabolites are involved in serum Hcy levels in patients with primary hyperparathyroidism (pHPT).
Methods: We studied 31 patients who underwent parathyroidectomy at our hospital from Nov 2010 to March 2012 and had normal renal function (Cr $\leqq$1.0). We analyzed data of age, BMI, serum Hcy, corrected calcium, intact PTH, 25-hydroxyvitamin D (25OHD) , 1,25-dihydroxyvitamin D, bone ALP, osteocalcin, urine NTX, BMD in forearm and lumbar spine.
Results: 21 patients had vitamin D deficiency (25OHD < 20 ng/mL) and 10 patients had vitamin D insufficiency (20 $\leqq$ 25OHD < 30). With multiple regression analyses, serum Hcy levels were negatively and positively correlated with serum 25OHD and Cr, respectively. 25OHD levels were not correlated with Cr or BMD.
Limitations: We didn't measure serum vitamin B6, B12 and folic acid levels.
Conclusion: In pHPT, vitamin D deficiency is not only directly involved in the deterioration of bone metabolism but may impair bone quality via the increase in Hcy level.

P3015

Homozygous Deletion Of Dickkopf-1 Results In A High Bone Mass Phenotype Due To Increased Bone Formation

***Michelle McDonald**[1,2], Alyson Morse[2,5], Kathy Mikulec[2], Paul Baldock[1,4], Lauren Peacock[2], Alastair Aiken[2], Patrick Tam[3], Peter Croucher[1,4], David Little[2,5]*
[1]Bone Biology, The Garvan Institute, Darlinghurst, New South Wales, Australia; [2]The Kids Research Institute, The Children's Hospital Westmead, Sydney, New South Wales, Australia; [3]The Children's Medical Research Institute, Westmead, New South Wales, Australia; [4]University of New South WAles, Sydney, New South Wales, Australia; [5]Sydney University, Sydney, New South Wales, Australia

Dickkopf-1 (Dkk1) is an antagonist of osteoblast differentiation, acting through blockade of Wnt/β-catenin signaling via LRP5/6. Recently, viable homozygous Dkk1 KO mice were generated by regulating Wnt3 (1). We examined the bone phenotype in Dkk1-/-;Wnt3+/- (HOM/HET) compared to Dkk1+/+;Wnt3+/+ (WT/WT). Importantly Dkk1+/+;Wnt3+/- (WT/HET) mice showed no adult phenotype. Whole body BMD was increased 18% in male and 15% in female HOM/HET mice compared to WT/WT(p<0.01). Femoral BV/TV was increased 3-fold in female and 2-fold in male HOM/HET mice (p<0.01 vs WT/WT). Cortical BV was increased in both male (15%) and female (19%) HOM/HET mice (p<0.02 vs WT/WT). Vertebral BV/TV was increased in female (94%) and male (79%) HOM/HET mice (p<0.01 vs WT/WT). Bone formation rate was increased 67% in female and 54% in male HOM/HET mice compared to WT/WT (p<0.05) with no alterations in bone resorption parameters. Primary osteoblasts from neonate calvaria revealed increased proliferation and mineralization in HOM/HET compared to WT/WT. In conclusion, our findings reveal a robust high bone mass phenotype due to enhanced bone formation in the absence of DKK1. This confirms recent findings that neutralizing antibodies to Dkk1 show promising results in the treatment of skeletal disease and augmenting bone repair. (1)Lewis SL *et al.* Dkk1 and Wnt3 interact to control head morphogenesis in the mouse. Development. 2008 May;135(10):1791-801

P3016

Soluble Fibroblast Growth Factor Receptor 2 With Apert Mutation Inhibits Differentiation And Mineralization Of Osteoblast

Masako Yoshizaki[1,2], *Yukiho Kobayashi*[1,3], *Keiji Moriyama*[1,2]
[1]Dept. of Maxillofacial Orthognathics, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan; [2]Global Center of Excellence Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan; [3]Hard Tissue Genome Research Center, Tokyo Medical and Dental University, Tokyo, Japan

Apert syndrome (AS) is primarily caused by missense mutations in fibroblast growth factor receptor (FGFR) 2 through unknown mechanisms.
Objectives: To analyze the etiological mechanisms of craniosynostosis in AS and to investigate the effects of the purified soluble forms of FGFR2 with or without Apert mutation (S252W) (sFGFR2 or sFGFR2Ap) on MC3T3-E1 cells.
Methods: The expression of mRNA and protein in coronal sutures of AS model mice was analyzed by qPCR and Westen blotting. sFGFR2 and sFGFR2Ap proteins were purified by anti-FLAG affinity gel. The effect of these purified proteins on proliferation of MC3T3E1 cells stably expressing FGFR2Ap (MC-Ap) was analyzed by MTT assay. Phosphorylation of intracellular signaling molecules was examined by Western blotting, and mineralized nodule formation was observed by AR-S staining.
Results: Coronal sutures from AS model mice exhibited increased expression of Runx2, Osteopontin, Fgfr2IIIb mRNA and Fgf10 protein, and accelerated phosphorylation of Erk1/2 and MEK. Both sFGFR2 and sFGFR2Ap showed inhibition of FGF2 on MC-Ap proliferation. Administration of sFGFR2Ap more potently inhibited the phosphorylation of intracellular signaling molecules and mineralization of MC-Ap.
Conclusion: The ectopic expression of Fgf10 and Fgfr2IIIb might contribute to the aberrant Fgfr2 function in AS. The purified soluble FGFR2Ap could be a potential therapeutic agent for AS.

P3017

The Multiple Stages Of Basic Multicellular Units. Consequences For Haversian Canal Sizes And Tetracycline Double Labelling Measurements

Pascal Buenzli, *Peter Pivonka, David Smith*
Faculty of Engineering, Computing and Mathematics, The University of Western Australia, Perth, Western Australia, Australia

The life history of basic multicellular units (BMUs) is usually divided into three separate stages: initiation (resorption alone, early life), progression (resorption and formation, mid life), and termination (formation alone, late life). These stages depend on the presence and activity of osteoclasts and osteoblasts within the BMU. However, how these cell populations develop within a BMU is still unclear. Cell-cell communication mediated by a number of signalling molecules (such as RANKL and TGF-β) couple osteoclastogenesis and osteoblastogenesis in a non-trivial way. We develop a comprehensive mathematical model of the coupled development of osteoclasts and osteoblasts within a single BMU to investigate how osteoblast and osteoclast numbers vary during the initiation and progression stages of the BMU. The dynamics of cell populations predicted by the model suggests that the life history of a BMU can be decomposed into several levels of quasi-steady states until the progression stage is fully developed, and that a BMU may spend a significant portion of its lifetime in a developing stage. This may have two important consequences: (i) local enlargements of the Haversian canal can occur near the BMU's point of origin, where a fully-developed population of osteoclasts but a developing population of osteoblasts is experienced; (ii) tetracycline data collected from bone sites being refilled by BMUs at different stages of their lifetime can exhibit cross-sectional variability.

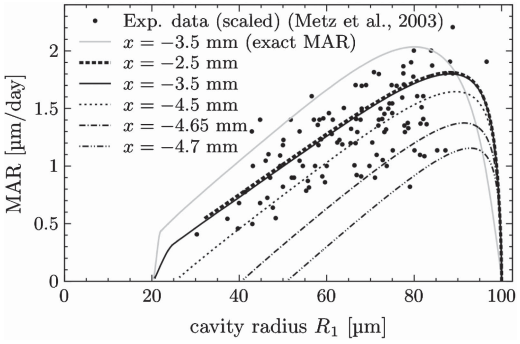


Figure 1 Matrix apposition rate versus BMU cavity radius obtained from the model (lines) and from tetracycline experiments (dots). Various positions x in bone experience the passage of the BMU at various stages of its lifetime. This may explain part of the cross-sectional variability in tetracycline data.

P3018

PP2A Cα Regulates Osteoblast Differentiation And Osteoclastogenesis Through The Expression Of Bone-related Genes

Hirohiko Okamura, *Di Yang, Tatsuji Haneji*
Histology and Oral Histology, Health Biosciences, Tokushima, Japan

The serine/threonine protein phosphatase 2A (PP2A) participates in the regulation of many important physiological processes. Here we examined the role of PP2A Cα subunit in osteoblast differentiation and its ability for osteoclastogenesis. Administration of okadaic acid, a specific inhibitor of PP2A, to the calvarial region in mice increased bone mineral density, mineral apposition, and bone thickness. The expression of PP2A Cα decreased in the initial step of osteoblast differentiation, which was in parallel with an increase in the expression of bone-related genes. Silencing of PP2A Cα dramatically accelerated osteoblast differentiation and mineralization, which were accompanied with increased expressions of Osterix, Bsp, and OCN. In contrast, the overexpression of PP2A Cα inhibited osteoblast differentiation and mineralization. Reduction of PP2A Cα in MC3T3-E1 cells (shPP2A) decreased RANKL expression and increased OPG expression. The conditioned medium from shPP2A cells failed to induce the expression of NFATc1 activation as well as the expression

of osteoclast marker genes cathepsin K and OSCAR in bone marrow macrophage cells. Treatment of bone marrow macrophage cells with the conditioned medium from shPP2A cells impaired osteoclastogenesis. Our data indicate that PP2A Cα plays an important role in osteoblast differentiation and osteoclastogenesis through regulating the bone-related genes.

P3019

MCP-1 Gene Expression Is Specifically Regulated Following Stress Fracture Initiation, But Blocked By The Dominant Negative Mutant, 7ND

***Mark Forwood**, Andy Wu, Wendy Kelly, Nigel Morrison*
School of Medical Science, Griffith University, Gold Coast, Queensland, Australia

MCP1 (or CCL2) is a CC chemokine and plays a critical role in recruiting immune cell precursors. We saw that within 4 hours of stress fracture (SFx) initiation, MCP1 gene expression was significantly elevated, followed by increased serum levels within 24h. Specific inhibition of MCP1 would test the significance of its expression for bone cell recruitment. We hypothesise that a plasmid DNA encoding a dominant negative mutant of MCP1 (7ND) will inhibit its gene expression associated with SFx. SFx was created in the right ulna of wistar rats using cyclic end-loading. Unloaded animals were used as a control. 24 h prior to loading, 7ND plasmid vector was injected in the thigh muscle to overexpress 7ND protein, which was then secreted into systemic circulation. Rats were euthanized 4h after loading (*n*=5/group) and RNA extracted for quantitative real time PCR analysis using TaqMan assays. In untreated rats, there was ~33 fold increase ($P<0.001$) in MCP-1 expression 4h after loading. Treatment with 7ND abolished the loading related increase in MCP-1, with gene expression levels lower than un-loaded control rats. We hypothesise that activation of the remodelling phase of SFx repair will be inhibited following this suppression of MCP-1. Because MCP-1 is markedly upregulated by SFx and by PTH, we propose that it provides important regulation of chemotaxis and osteoclast differentiation during initiation events of bone remodelling.

P3020

Effects Of TGF-β1 And LPS On BMP-2-induced Ectopic Bone Formation

***Akifumi Matsumoto**[1,2], Masamichi Takami[1], Keita Tachi[2], Kazuyoshi Baba[2], Ryutaro Kamijo[1]*
[1]Department of Biochemistry,Showa university, Shinagawa, Tokyo, Japan; [2]Department of Prosthodontics,Showa university, Shinagawa, Tokyo, Japan

We found that TGF-β1 strongly enhances ectopic and orthotopic bone formation induced by BMP-2 (5-fold greater than BMP-2 alone), which would be beneficial for performing bone regenerative treatments such as alveolar bone reconstruction in patients with periodontitis. However, patients often have bacterial infection in periodontal tissues that may affect bone regeneration. To examine the effects of bacterial infection on bone formation induced by BMP-2, we implanted collagen sponges containing BMP-2, TGF-β1, and various amounts of LPS under the fascia of the latissimus dorsi muscles of mice. LPS dose dependently reduced the volume of ectopic bone formed by BMP-2 + TGF-β1. The total volume of ectopic bone induced by BMP-2 + TGF-β1 with administration of LPS was less than 25% of that induced in the control group. Histological analysis of ectopic bone tissue formed in the presence of LPS revealed that bone volume/total volume and trabecular thickness were significantly decreased. Interestingly, osteoblast number and osteoid volume were significantly increased, while osteoclast number did not change. Since LPS induces production of TNF-α and IL-1, we implanted collagen sponges containing BMP-2, TGF-β1, and LPS into TNF-α or IL-1α/β deficient mice. LPS reduced the volume of ectopic bone in both TNF-α deficient but not in IL-1α/β deficient mice. These results suggest that LPS suppresses ectopic bone formation induced by BMP-2 and TGF-β1 in mice depending on IL-1 production.

P3021

Identification Of Proteins Binding To The Unique Domain In Rank Required To Establish Osteoclastogenic Signals

***Kazuaki Tsumura**[1], Yuu Taguchi[1], Masaaki Oyama[2], Hiroko Kozuka-Hata[2], Jun-ichiro Inoue[1,2]*
[1]Division of Cellular and Molecular Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [2]Medical Proteomics Laboratory, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

We previously identified a unique domain in RANK, which is essential to establish osteoclastogenic signals. The domain is highly conserved in various vertebrates, and was named HCR, which stands for highly conserved domain in RANK. This region is essential for long-term activation of NF-κB and ITAM signals, including PLCγ2 activation and calcium oscillations, which leads to NFATc1-induced osteoclastogenesis. We also demonstrated that protein complex containing Gab2 and PLCγ2 is formed on HCR in the late phase of RANK signaling. Because bone marrow cells derived from Gab2 deficient mice exhibit only partially impaired osteoclastogenic ability, it is highly possible that other protein(s) is involved in the HCR-mediated signal transduction. Based on the idea, we established tandem affinity purification system to purify HCR binding proteins in order to identify proteins involved in the HCR-mediated signal transduction.
HCR-peptide with multiple tag sequence was purified from *Escherichia coli* expression system and was immobilized on affinity resin. Proteins which bind to immobilized HCR peptide were collected and identified by mass spectrometry. The result of investigation of these proteins will be shown.

P3022

Low pH, Piceatannol And Heparin Activates Latent TGFβ In Lysed Platelets

***Ola Wahlström**[1,2], Ebba Frånlund[1,3], Per Magnusson[1,3]*
[1]Orthopaedics/IKE, Linköping University, Linköping, Sweden; [2]Orthopaedics/IKE, Linköping, Sweden; [3]Clinical Chemestry/IKE, Linköping, Sweden

Introduction: Platelets contain numerous growth factors essential for wound and fracture healing. Lysed platelet buffer

[P3022]

Table 1

	TGFβ in LPB3.5 (ngl^{-1})	TGFβ in LPB5.4 (ngl^{-1})	TGFβ in LPB7.4 (ngl^{-1})
Non-activated (=na)	870±83	<80	<80
Fully activated (=fa)	35000±3552	24000±718	22000±568
Piceatannol (na)	4700±1022	<80	<80
Piceatannol (fa)	73000±8131	24000±2071	28000±4227
Heparin (na)	5500±443	<80	<80
Heparin (fa)	74000±2512	26000±3925	33000±1842

mean±s.d.

(LPB) preparations at pH 5.4 (LPB5.4) increase proliferation of osteoblast-like cells (hFOB) in comparison with preparations in neutral pH (LPB7.4). Genome-wide microarray analysis has shown that stimulation of hFOB cells with LPB5.4 activates 8 pathways (e.g., TGFβ) common with bone formation and cancer growth. Piceatannol increase BMP synthesis in hFOB cells and interferes with TGFβ activation.
Hypothesis: The stimulatory effect of LPB5.4 is due to increased amounts of active TGFβ versus latent TGFβ. Piceatannol interferes with the release and activation of TGFβ.
Methods: The amount of active and latent TGFβ was investigated in LPB3.5, LPB5.4 and LPB7.4, in addition to the influence of piceatannol (1-100 mM) and heparin (0.1-1.0 IE/mL) on TGFβ activation.
Results: LPB3.5 released active TGFβ from latent TGFβ complexes. Piceatannol and heparin increased the active TGFβ concentration in LPB3.5 ($p<0.001$). Spontaneously activated TGFβ in LPB3.5 increased after treatment with both piceatannol and heparin. Piceatannol and heparin have anti-inflammatory properties and we suggest that they both act through activation of TGFβ. These results support the hypothesis that acidification increase the active TGFβ concentration by releasing active TGFβ from latent TGFβ complexes.

P3023

Gurucuronyl Carbohydrate Modification Commonly Attached On NAK ATPase Beta-subunit, FGFR1 And FGF-23 Is Recognized By alpha-Klotho

***Ryota Maeda**, Akihiro Imura, Yo-ichi Nabeshima*
Kobe, Kobe, Japan

Metabolic homeostasis is partially controlled via Klotho-dependent systems including endocrine fibroblast growth factor (FGF) signals and ion transporting machineries. There have been reported that alpha-Klotho interacts several mineral-related proteins including FGF-23, Na+,K+-adenosine triphosphatase (Na+,K+-ATPase). We questioned how alpha-Klotho interacts these distinct binding partners. We identified alpha-Klotho associates with destined glycosylated proteins including FGF receptor 1, Na+,K+-ATPase beta-subunit both on which glucuronyl (GlcA-) carbohydrates are attached. We show that physiologically active FGF-23 is glucuronydated as well and that GlcA-protein increases its affinity to alpha-Klotho, which selectively recognizes the GlcA moiety.
Alpha-Klotho transports the Na+,K+-ATPase complex to the cell surface in response to extracellular low calcium concentrations. The formed sodium gradient will be used for transporting calcium ion and releasing parathyroid hormone. With taking a glycan which alpha-Klotho specifically recognizes, we rationally explain the diverse functions of alpha-Klotho. Actually, we have identified that Na+,K+-ATPase beta-subunit, FGFR1 and FGF-23 is a glucuronyl modified protein and that the glucuronyl moiety is specifically recognized by alpha-Klotho.
In this way, alpha-Klotho functions as a novel GlcA-binding lectin, which accumulates circulating FGF-23 into kidney and recruits Na+,K+-ATPase complex on the cell surface.

P3024

Mutant ALK2 Receptors Identified In Patients With A Typical And A Variant Fibrodysplasia Ossificans Progressiva Show Different Sensitivities To Type II Receptors

***Mai Fujimoto**[1,2], Satoshi Ohte[1], Sho Tsukamoto[1], Arei Miyamoto[1], Naoto Suda[2], Takenobu Katagiri[1]*
[1]Division of Pathophysiology, Research Center for Genomic Medicine, Saitama Medical University, Hidaka, Saitama, Japan; [2]Division of Orthodontics, Meikai University School of Dentistry, Sakado, Saitama, Japan

Bone morphogenetic proteins (BMPs) induce heterotopic bone formation in skeletal muscle via type I and type II Ser/Thr kinase receptors. Type II receptor phosphorylates type I receptor, then the type I receptor phosphorylates Smad1/5/8. Fibrodysplasia ossificans progressiva (FOP) is a rare hereditary disorder characterized by progressive heterotopic ossification in skeletal muscle in childhood. An activated R206H mutation of a BMP type I receptor, ALK2, has been found in patients with FOP. Recently, a novel G325A mutation was identified in a patient with FOP who showed late-onset heterotopic ossification in adulthood. In the present study, we examined molecular mechanisms of the activation of G325A *in vitro*. G325A activated BMP-specific luciferase reporter and induced ALP activity without adding BMPs in C2C12 cells. G325A increased levels of phosphorylated (P) FLAG-Smad1. The stimulatory activities of G325A were weaker than those of R206H and were blocked by a specific inhibitor of BMP type I receptor kinases, suggesting a critical role of P-Smad1/5/8 in the activities. BMPR-II, a BMP type II receptor, cooperatively increased ALP activity with R206H, but did not with G325A. In contrast, ActR-IIB, another type of BMP type II receptor, synergistically increased the ALP activity with G325A. Our findings suggest that the sensitivities of ALK2 to type II receptors cause the variations of clinical features in patients with FOP.

P3025

Anti-Resorptive And Anabolic Actions Of Fasting-Induced Adipose Factor (FIAF) And Its Fragment On Bone Cells

***Jian-ming Lin**, Dorit Naot, Maureen Watson, Jessica Costa, Andrew Grey, Jillian Cornish*
University of Auckland, Auckland, New Zealand

Fat and bone mass are positively correlated and adipokines, such as leptin, likely mediate this relationship. This study aims to further explore the underlying mechanism by looking at the bone cell effects of the adipokine, fasting-induced adipose factor (FIAF) and its naturally truncated product coiled-coil domain (CCD). Our results show that CCD potently inhibits osteoclasts in mouse bone marrow and RAW264.7 cell cultures, and in isolated mature osteoclasts. The inhibitory rates by CCD (500 $ngml^{-1}$) were approximately 90%, 50% and 90% respectively in the above models. It also stimulated osteoblast proliferation by ~30% at this concentration. In comparison, intact FIAF (500 ng/mL) inhibited osteoclastogenesis by ~50% in bone marrow cultures, but was inactive in osteoclast resorption. In addition, it did not support osteoblast proliferation. CCD greatly reduced the expression of macrophage colony-stimulating factor (M-CSF), nuclear factor of activated T-cells c1 (NFATc1), dendritic cell-specific transmembrane protein (DC-STAMP), and mildly suppressed the expression of connective tissue growth factor (CTGF) in bone marrow cells. However, it did not change RANKL and OPG levels in the ways supporting its osteoclastic effect.
In conclusion, FIAF/CCD positively couples fat and bone mass by indirectly or directly acting on osteoclasts. CCD's action on osteoclasts is independent of RANKL/OPG system, but dependent of M-CSF, NFATc1, DC-STAMP and CTGF pathways.

P3026

Cartilage-Specific Overexpression Of CCN3 Negatively Regulates Endochondral Bone Formation

***Takako Hattori**[1], Mitsuaki Ono[2], Mitsuhiro Hoshijima[1], Koichi Kadoya[3], Miho Kuwahara[3], Haruka Oie[3], Yoshiko Miyake[1], Takuo Kuboki[2], Masaharu Takigawa[1]*
[1]Department of Biochemistry and Molecular Dentistry, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama City, Japan; [2]Department of Oral Rehabilitation and Regenerative Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama City, Japan; [3]Okayama University Dental School, Okayama City, Japan

CCN family protein 3/ Nephroblastoma OVerexpressed (CCN3/NOV) deletion mice (*Ccn3* ^{del3}) in which the 3rd domain is deleted showed abnormal endochondral ossification, indicating the important role of CCN3 in skeletal development. In our previous reports we showed that CCN2 enhances endochondral ossification, and CCN3 modifies production of cartilaginous extracellular matrices by binding to CCN2. Here we generated transgenic mice in which the *Ccn3* gene is expressed specifically under the type II collagen (*Col2a1*) promoter and analyzed their long bones. Embryonic long bone from *CCN3*^{Col2a1tg} mice showed thick and short bony part in skeletal preparation, and analysis of the skeletal phenotype by micro CT of femora from adults *CCN3*^{Col2a1tg} mice showed decreased bone volume, bone surface density, trabecular thickness, and trabecular number. *In situ* hybridization of E15.5 tibiae from *CCN3*^{Col2a1tg} mice showed delayed cartilage development and less osteoblastic markers. Furthermore, histological analysis of embryonic tibia from *CCN3*^{Col2a1tg} showed a reduced number of TRAP-positive osteoclasts and CD31-positive vascular endothelial cells in the spongiosa. Our data indicates that CCN3 overexpression in cartilage delayed endochondral ossification, possibly by inhibited vascular invasion and impaired osteogenesis by reducing osteoblastogenesis. CCN3 overexpression in cartilage may impair osteogenesis by modifying replacement of cartilage to bone during endochondral ossification.

P3027

1,25-dihydroxyvitamin D_3 Enhances Fibroblast Growth Factor-23 Expression By Both Transcriptional And Post-transcriptional Mechanisms

***Michiko Hori**, Yuichiro Shimizu, Yuka Kinoshita, Manabu Taguchi, Seiji Fukumoto*
University of Tokyo, Bunkyo-ku, Tokyo, Japan

FGF23 is a phosphaturic hormone that decreases circulating 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] and phosphate levels. It has been shown that serum FGF23 increases after treatment with $1,25(OH)_2D_3$ (1,25D) and 1,25D enhances promoter activity of murine *FGF23* gene. However, it is unclear whether 1,25D enhances *FGF23* expression only by transcriptional stimulation. The aim of this study was to elucidate the mechanisms by which 1,25D enhances *FGF23* expression. 1,25D stimulated *FGF23* mRNA expression in rat UMR-106 osteoblast-like cells in a dose-dependent manner as previously reported. About 25-fold increase of *FGF23* mRNA was observed in cells treated with 100 nM 1,25D compared to those with 1 nM 1,25D. In addition, 1,25D increased rat *FGF23* promoter activity of -500 and -1000 bps about twice. Longer promoter constructs up to -3500 bps did not show higher responsiveness to 1,25D. Furthermore, the decay of *FGF23* mRNA was assessed by measuring *FGF23* mRNA levels after actinomycin D treatment. 100 nM of 1,25D prolonged the half-life of *FGF23* mRNA also about twice compared to cells with 1 nM 1,25D. Therefore, 1,25D enhances *FGF23* expression by both transcriptional and post-transcriptional mechanisms. However, the stimulatory effect of 1,25D on *FGF23* mRNA expression was much potent than expected actions of 1,25D on *FGF23* promoter activity and mRNA stability. Therefore, further analysis of *FGF23* gene is necessary to fully understand the mechanisms of 1,25D on *FGF23* expression.

P3028

High-Dose BMP2 Reduces Cell Proliferation And Increases Apoptosis Via DKK1 And Sost In Human Primary Periosteal Cells

***Nobuhiro Kamiya**[1,2], Harry Kim[1,2]*
[1]Texas Scottish Rite Hospital for Children, Dallas, TX, USA; [2]University of Texas Southwestern Medical Center, Dallas, TX, USA

BMP2 is a FDA approved drug used in orthopaedics. Osteogenic function is well known; however, many complications of the

[P3028]

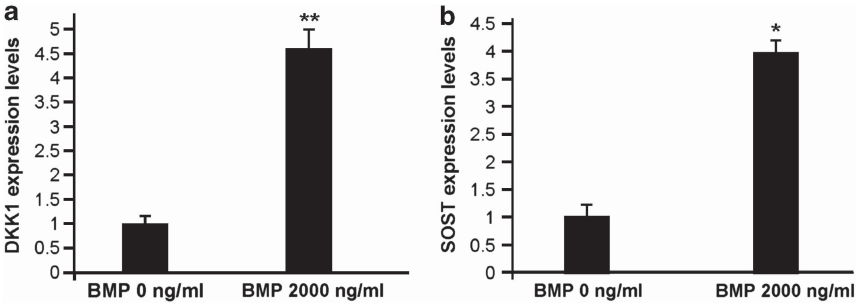


Figure 1 (**a**) Human periosteum-derived cells were isolated from three independent patients. After 24 hours of high-dose BMP2 treatment (2000 $ngml^{-1}$), RNA was isolated and cDNA was synthesized. Expression levels of DKK1 determined by qRT-PCR were significantly increased. (**b**) Expression levels of SOST by qRT–PCR were significantly increased. The values indicated the average from three independent cell lines. * $p < 0.05$, ** $p < 0.01$

BMP2 therapy have been reported recently. This is partly because the BMP2 concentration used for patients is much higher (>1000 μgml^{-1}) than that for basic studies (100~300 $ngml^{-1}$) and the effects of high-dose BMP2 on bone biology are largely unknown. This study investigated the effects of high-dose BMP2 on cell proliferation and apoptosis using human primary periosteal cells because BMP2 is generally applied around the periosteum in orthopaedic surgery. Interestingly, the cell proliferation by MTT activity was significantly reduced by high-dose BMP2 (~2000 ng/ml), while such a reduction was not observed by low-dose BMP2 (~200 ng/ml). The cell apoptosis by caspase activity was significantly increased by high-dose BMP2, while such an increase was not observed by low-dose BMP2. We found Wnt signaling activity was significantly reduced by high-dose BMP2 along with a dramatic increase in DKK1 and SOST, one of key Wnt inhibitors in bone. Silencing DKK1 or SOST normalized cell proliferation and apoptosis in the cells exposed to high-dose BMP2. These results suggest that high-dose BMP2 has a critical role in reducing cell number and inducing apoptosis via DKK1 and SOST in periosteal cells, which is not seen in low-dose BMP2. This study will advance the understanding of the high-dose BMP2 function and could improve the current high-dose BMP2 therapy in orthopaedics.

P3029
Low-Dose Vitamin K2 (MK-4) Supplementation For 12 Months Improves Bone Metabolism And Prevents Forearm Bone Loss In Postmenopausal Japanese Women

Noriko Koitaya[1], Mariko Sekiguchi[2], Yuko Tousen[1], Yoriko Nishide[1], Akemi Morita[2], Jun Yamauchi[1], Yuko Gando[3], Motohiko Miyachi[3], Mami Aoki[4], Miho Komatsu[4], Fumiko Watanabe[4], Koji Morishita[4], ***Yoshiko Ishimi[1]***

[1]Food Function and Labeling, National Institute of Health and Nutrition, Tokyo, Japan; [2]Nutritional Epidemiology, National Institute of Health and Nutrition, Tokyo, Japan; [3]Health Promotion and Exercise, National Institute of Health and Nutrition, Tokyo, Japan; [4]Kyowa Hakko Bio Co., Ltd., Tsukuba, Japan

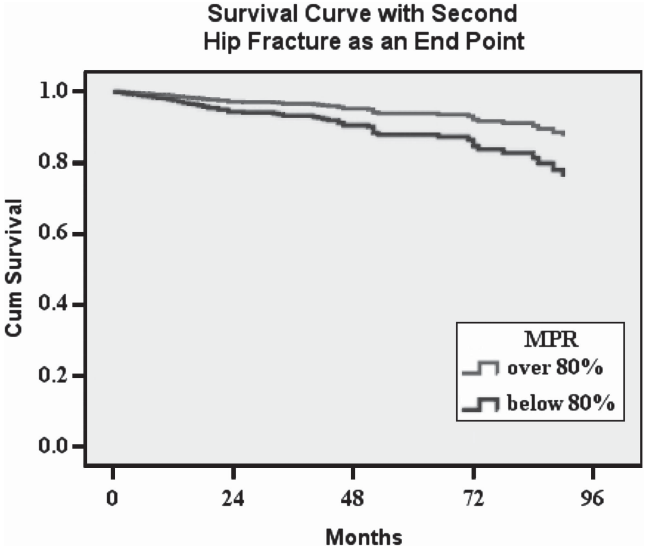
Menaquinone-4 (MK-4) administered at a pharmacological dosage of 45 mg per day has been used for the treatment of osteoporosis in Japan. However, it is not known whether a lower dose of MK-4 supplementation is beneficial for bone health in healthy postmenopausal women. The aim of this study was to examine the long-term effects of 1.5-mg daily supplementation of MK-4 on the various markers of bone turnover and bone mineral density (BMD). The study was performed as a randomized, double-blind, placebo-controlled trial. The participants (aged 50-65 years) were randomly assigned to 2 groups according to the MK-4 dose received: the placebo-control group (n = 24) and the 1.5-mg MK-4 group (n = 24). The baseline concentrations of undercarboxylated osteocalcin (ucOC) were high in both groups (>5.1 $ngml^{-1}$). After 6 and 12 months, the serum ucOC concentrations were significantly lower in the MK-4 group than in the control group. In the control group, there was no significant change in serum pentosidine concentrations. However, in the MK-4 group, the concentration of pentosidine at 6 and 12 months was significantly lower than that at baseline. The forearm BMD was significantly lower after 12 months than at 6 months in the control group. However, there was no significant decrease in BMD in the MK-4 group during the study period.These results suggest that the low-dose MK-4 supplementation for 6-12 months improved bone quality in the postmenopausal Japanese women.

P3030
Incidence Of Second Hip Fracture And Compliant Use Of Bisphosphonate

Young-Kyun Lee[1]*, Yong-Ki Min[4], Yong-Chan Ha[2], Jae Suk Chang[3], Dong-Won Byun[6], Ho Yeon Chung[5], Chan Soo Shin[7]*

[1]Seoul National University Bundang Hospital, Seongnam-si, Republic of Korea; [2]Chung-Ang University College of Medicine, Seoul, Republic of Korea; [3]Asan Medical Center, College of Medicine, University of Ulsan, Seoul, Republic of Korea; [4]Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea; [5]Kyung Hee University Medical Center, Seoul, Republic of Korea; [6]Soonchunhyang University College of Medicine, Seoul, Republic of Korea; [7]Seoul National University College of Medicine, Seoul, Republic of Korea

Purpose: Bisphosphonate has been used to prevent osteoporotic fracture, and is recommended for secondary prevention after hip fracture. However, little is known regarding secondary prevention after first hip fracture. Our purpose was to determine the incidence of second hip fracture and to evaluate



whether compliant use of bisphosphonate can reduce the risk of second hip fracture.

Methods: Eight hundred twenty six patients who sustained the first hip fracture from May 2003 to October 2011 were retrospectively evaluated. The incidence of second hip fracture was compared between compliant users of bisphosphonate and non-users.

Results: Seventy-one (8.6%) patients suffered a second hip fracture at mean 30.0 months (SD 24.6, range 1 to 90 months) after the initial hip fracture. The cumulative incidence of second hip fracture was 5.1% (42/826) at 2 year and 8.6% (71/826) at 8 years. The incidence of second hip fracture was 4.2% (12/283) in compliant users and 10.9% (59/543) in non-users (p = 0.001). (Figrure 1).

Conclusions: Compliant use of bisphosphonate is effective in prevention of second hip fractures. (This study was accepted at Osteoporosis Int.)

P3031

Bisphosphonate Use And Subsequent Hip Fracture In South Korea

*Yong-Taik Lim[3], **Young-Kyun Lee**[1], Yong-Chan Ha[2], Deog-Yoon Kim[4], Ho Yeon Chung[4], Chan Soo Shin[5], Dong-Won Byun[6]*

[1]Seoul National University Bundang Hospital, Seongnam-si, Republic of Korea; [2]Chung-Ang University College of Medicine, Seoul, Republic of Korea; [3]Yeouido St. Mary's Hospital, The Catholic University of Korea, Seoul, Republic of Korea; [4]Kyung Hee University Medical Center, Seoul, Republic of Korea; [5]Seoul National University, Seoul, Republic of Korea; [6]Soonchunhyang University College of Medicine, Seoul, Republic of Korea

Background: Bisphosphonate is prescribed worldwide to prevent osteoporotic fracture in terms of primary prevention. However, the association between adherent use of bisphosphonate and prevention of second hip fracture has not well known. The purpose of this study was to determine whether the adherent use of bisphosphonate was associated with a decreased risk of second hip fracture in South Korea, using nationwide database.

Methods: From 2007 to 2011, the first and second hip fractures were identified using the ICD-10 and procedure code form from nationwide database from Health Insurance Review and Assessment Service (HIRA). Compliant user of bisphosphonate use was defined as patients with medication possession ratio (MPR) of 80 or more. And, Persistent user was defined patients with refill gap of 30 days or less.

Results: The cumulative incidence of second hip fracture was 1.0 % (552/59,782) at 1 year, 1.9 % (1123/59782) at 2 year, and 2.2% (1336/59782) at 3 year. After multivariate analysis, compliant and persistent use of bisphosphonate showed significantly independent protectors for second hip fracture. (p<0.0103 and p<0.0001, respectively).

Conclusions: The results of this study show that adherent use of bisphosphonate decrease a risk of second hip fracture, in terms of secondary prevention.

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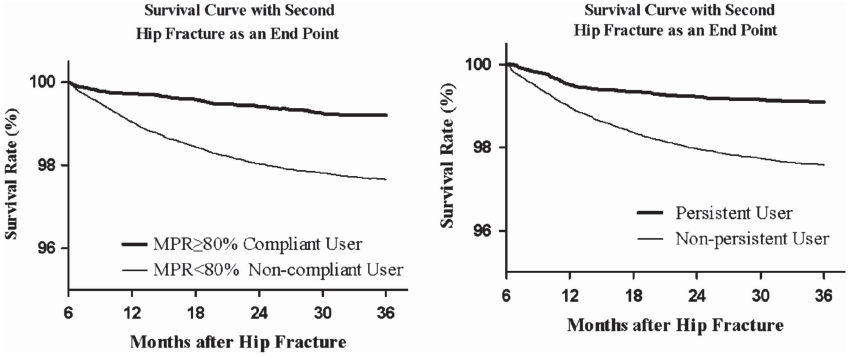

P3032

Early Changes In Biochemical Bone Formation Marker Predict The Long-term Response To Daily Teriparatide Therapy

Rui Niimi[1], *Toshibumi Kono*[1], *Atsushi Nishihara*[1], *Toshihiko Kono*[1], *Akihiro Sudo*[2]
[1]Tomidahama hospital, Yokkaichi, Mie, Japan; [2]Mie University Graduate School of Medicine, Tsu, Mie, Japan

Introduction: The correlations between early changes in biochemical bone formation markers and the subsequent bone mineral density (BMD) changes after daily teriparatide therapy in patients with osteoporosis were studied.
Materials and Methods: One hundred and eighteen patients diagnosed osteoporosis participated in this study. We measured BMD of the lumbar spine (LS) using dual-energy X-ray absorptiometry at baseline and 12 month, and biochemical bone formation marker (serum concentration of intact N-terminal propeptide of type I collagen [PINP]) at baseline and 1 month.
Results: The mean percent change in LS BMD from baseline to 12 months was 8.4%. The mean change in serum PINP from baseline to 1 month was 78.6 μgl^{-1}. There were positive correlations between changes in serum PINP and BMD. Using receiver operator curve analysis, we determined that 80.0μgl^{-1} increases in serum PINP was the most accurate predictors of the LS BMD response (Area under curve = 0.74). Using a serum PINP cut-off of more than 80.0μgl^{-1}, the sensitivity for 10% gain in LS from baseline to 12 months was 57%, the specificity was 81%. The positive predictive value was 64%.
Conclusion: Greater short-term changes in serum PINP with daily teriparatide therapy are associated with greater 12 months increases in LS BMD for osteoporotic patients.

P3034

Fracture Levels Of Atypical Femoral Fracture Are Associated With Alignment Of Lower Limb

Yoshitomo Saita, *Muneaki Ishijima, Masashi Nagao, Kazuo Kaneko*
Orthopaedics, Juntendo University, Tokyo, Japan

Atypical femoral fractures (AFFs) are suggested to be a stress-related fracture and associated with a long-term treatment with bisphosphonates (BPs) for osteoporosis. As they occur anywhere along the femoral shaft, it still remains unclear how the fracture levels of AFFs are determined. This retrospective study found fourteen AFFs in ten patients (four bilateral fractures) among 2238 hip and femoral shaft fractures treated in our associated hospitals during 2005-2010, the frequency of AFFs was 0.63%, similar to previous studies. A case-control study showed that the greater proportion of patients with AFFs used BPs and glucocorticoid (GCs) and were suffering from collagen diseases (CDs) (odds ratios; 36.0, 13.0 and 9.0, respectively). Interestingly, the fracture levels in the femora were almost the same in the patients with bilateral AFFs. The FTA of the patients with atypical subtrochanteric femoral fractures (ASFFs) was significantly smaller than that of those with typical proximal femoral fractures (TPFFs, 177°, $p<0.01$), and that of those with atypical diaphyseal femoral fractures (ADFFs) was significantly greater than that of those with typical diaphyseal femoral fractures (TDFFs, 175°, $p<0.01$). Furthermore, there was a correlation between the fracture levels in the femur and FTA in patients with AFFs ($r=0.81$). These results indicate that the fracture levels of AFFs are associated with the alignment of lower limb.

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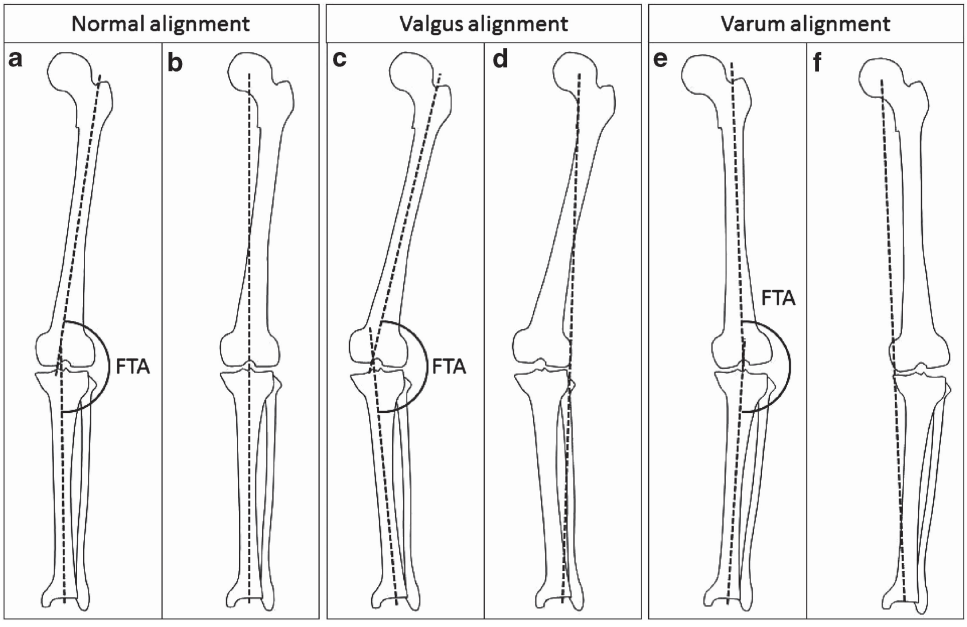

P3035

The Phase 2 Dose-Finding Study Of Odanacatib, A Potent Cathepsin-k Inhibitor, In Japanese Patients With Osteoporosis With A Model-based Pharmacokinetic (PK) Analysis

Shinji Uchida[1], *Masataka Shiraki*[2], *Masao Fukunaga*[3], *Tatsushi Tomomitsu*[3], *Go Fujimoto*[1], *Mariko Nakagomi*[1], *Albert Leung*[4], *Stefan Zajic*[4], *Arthur Santora*[4], *Julie Stone*[4], *Julie Passarell*[5], *Toshitaka Nakamura*[6]

[1]MSD K.K., Chiyoda-ku, Tokyo, Japan; [2]Research Institute and Practice for Involutional Diseases, Nagano, Japan; [3]Kawasaki Medical School, Okayama, Japan; [4]Merck Research Laboratories, Rahway, NJ, USA; [5]Cognigen Corp, Buffalo, NY, USA; [6]University of Occupational and Environmental Health, Kitakyusyu, Fukuoka, Japan

A double-blind, randomized, multi-center study in Japanese patients with osteoporosis was run to assess the efficacy and safety of oral odanacatib (ODN) 10, 25, or 50 mg or placebo (PBO) once-weekly (OW) for 52 weeks and to characterize the pharmacokinetic profile. The primary endpoint was the 52-week % change from baseline in lumbar spine (LS) BMD. A model-based population-PK analysis was conducted as an exploratory endpoint and was used to estimate predicted steady-state patient exposures. 287 patients were randomized to PBO (*n*=73) or ODN 10 mg (*n*=74), 25 mg (*n*=71), or 50 mg (*n*=69). The least-squares mean % changes from baseline in LS BMD were 0.5%, 4.1%, 5.7% and 5.9% and in total hip BMD were -0.4%, 1.3%, 1.8% and 2.7% with PBO or ODN 10 mg, 25 mg and 50 mg. Odanacatib reduced bone resorption markers in a dose-dependent manner. After 52 weeks, for patients receiving ODN 50 mg OW, % changes from baseline (SE) were -58.6 (3.3) for urine NTX/creatinine (*n*=51), but only -25.9 (3.2) for serum BSAP (*n*=54). Safety profiles were similar among all treatment groups. Covariate analysis of the pop-PK model suggested that ethnicity does not substantially influence odanacatib pharmacokinetics. In conclusion, treatment with odanacatib for 52 weeks OW increased LS and hip BMD in a dose-dependent manner and was well-tolerated in Japanese patients with osteoporosis. Pop-PK analysis suggests that there is little difference in pharmacokinetics between Japanese and non-Japanese patients.

P3036

The Evaluation Of Bone Mineral Density In Patients With Nonalcoholic Fatty Liver Disease

Tugrul Purnak[1], *Cumali Efe*[2], *Yavuz Beyazit*[3], *Merve Hayretci*[4], *Ersan Ozaslan*[1]

[1]Gastroenterology and Hepatology, Ankara Numune Education and Research Hospital, Ankara, Turkey; [2]Hacettepe University Medical School, Ankara, Turkey; [3]Yuksek Ihtisas Hospital, Ankara, Turkey; [4]Ankara Numune Research and Education , Department of Radiology, Ankara, Turkey

Background and aim: The primary goal of the present study was to evaluate the association between bone mineral density and liver function in patients with NASH.

Materials and Methods: Consenting patients with a diagnosis of NAFLD were included in the study. Extent of fatty change was graded based on ultrasonographic appearance (Grade 1, mild; Grade 2, moderate; Grade 3, severe). Bone mineral density was measured using the dual-energy x-ray absorptiometry method. ALT and hs-CRP were considered as non-invasive marker of NASH. According to ALT levels, patients were divided into two subgroups.

Results: A total of 102 patients with NAFLD and 54 healthy controls participated in the study. None of the patients with NAFLD had an abnormal bone mineral density. Furthermore, there was no difference between groups with regard to serum vitamin D levels. A subgroup analysis revealed that female patients with elevated serum ALT level had significantly lower bone mineral densities and higher hsCRP levels than female patients with normal ALT levels. The difference in vitamin D levels and body mass indices between the same subgroups was statistically insignificant.

Conclusions: Simple steatosis of the liver does not affect bone mineral density. However, in a subgroup of patients with NAFLD, the presence of elevated serum ALT and hs-CRP levels, which are suggestive of NASH, was associated with lower bone mineral densities.

P3037

Lean Mass But Not Fat Mass Is Associated With Hip Geometry In Japanese Women

Junichi Takada[1,2], *Kousuke Iba*[2], *Kouichi Sasaki*[2], *Kumiko Kanaya*[2], *Takayuki Douke*[2], *Takashi Yoshizaki*[1], *Toshihiko Yamashita*[2]

[1]Kitago Orthopaedic Clinic, Sapporo, Hokkaido, Japan; [2]Dept. of Orthopaedic Surgery, Sapporo Medical University, Sapporo, Hokkaido, Japan

Obesity is seemed to confer protection against osteoporosis and fracture. Although body weight consists of bone mass, lean mass and fat mass, most of these previous studies focused on body weight. This study is the first presentation for the relations between the body composition (bone mineral content (BMC), lean, fat) and hip geometry in Japanese women. 183 community-dwelling Japanese women (65.6 years of age) who had a checkup for osteoporosis were measured body composition (BMC, lean, fat) in lower leg and hip geometry parameters by whole-body scan DXA and hip structural analysis (HSA), respectively. The structural parameters were BMD, cross sectional area (CSA, index of resistance to long axis), section modulus (Z, index of bending forces), buckling ratio (BR, index of cortical stability). The correlations between body compositions and HSA parameters were analyzed by Pearson's R and adjusted for age.

BMC and lean mass at neck and intertrochanter were positively correlated with BMD, CSA, Z (r = 0.441 – 0.852), and BMC were inversely correlated with BR (r = –0.562 to –0.624). On the other hand, fat mass did not show significant correlation with CSA, Z, BR. The highest quartile (Q4) in Lean/Fat ratio was significantly higher in Z compared to other quartiles (Q1-3).

The protective effects of body weight in preventing fracture and osteoporosis are mediated by BMC and lean mass, not fat mass.

P3038

Mandibular Trabecular And Cortical Bone Used As Fracture Predictors

Grethe Jonasson[1], *Valter Sundh*[2], *Magnus Hakeberg*[3], *Azar Hassani-Nejad*[4], *Lauren Lissner*[3], *Margareta Ahlqwist*[4]
[1]Research and Developement Unit, Borås, Sweden; [2]Dept. of Public Health and Community, Institute of Medicine, Gothenburg, Sweden; [3]Dept. of Behavioral and Community Dentistry, Institute of Odontology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [4]Dept. of Oral and Maxillofacial Radiology, Institute of Odontology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Introduction: Bone mineral density (BMD) only partly explains bone quality and strength.
Materials and Methods: 1003 women were included in the present prospective study (born in 1914, 1922, or 1930). Panoramic radiographs from 1968/69, 1980/81 and 1992/93 were evaluated visually: the trabecualtion as sparse, alternating sparse and dense, and dense, and the cortex as normal, moderately eroded, and severely eroded. All women with fracture before 1968 were excluded.
Results: The group with severely eroded cortex increased from 0.5 % in the youngest group (38 year olds) to 75.4 % in oldest group (78 year olds) whereas the sparse trabeculation group increased from 21% to 46% during the same 24 years. At all examinations, the sparse trabeculation group had more fractures (71-78 %) than the non-sparse group (27-31 %), whereas the severely eroded compact group showed more fractures than the less eroded groups 24 years later. Sparse trabecular pattern was associated with future fractures both in peri-menopausal and older women (relative risk (RR): 1.47-4.37) and cortical erosion in older women (RR: 1.35-1.55).
Conclusions: The mandibular trabecular pattern seems to be a highly significant predictor of future fracture risk both in peri-menopausal and older women whereas cortical erosion performed well for older women. Sparse trabeculation indicated that 71-78 % of these women would suffer a bone fracture.

P3039

Trabecular Bone Score Predicts Vertebral Fracture Over 10 Years Independently Of Bone Density In Japanese Women: The Japanese Population-based Osteoporosis (Jpos) Cohort Study

Masayuki Iki[1], *Junko Tamaki*[1], *Yuho Sato*[2], *Eiko Kadowaki*[1], *Namiraa Dongmei*[1], *Renaud Winzenrieth*[3], *Sadanobu Kagamimori*[4], *Yoshiko Kagawa*[5], *Hideo Yoneshima*[6]
[1]Department of Public Health, Kinki University Faculty of Medicine, Osaka-Sayama, Osaka, Japan; [2]Jin-ai University, Echizen, Fukui, Japan; [3]Med-Imaps, Bordeaux, France; [4]University of Toyama, Toyama, Toyama, Japan; [5]Kagawa Nutrition University, Sakado, Saitama, Japan; [6]Shuuwa General Hospital, Kasukabe, Saitama, Japan

Aims: To examine whether trabecular bone score (TBS) predicts the risk of vertebral fractures (VFx) over 10 years in Japanese women.

Methods: Among 900 women aged 50 to 79 years selected randomly from 3 areas, 728 completed the baseline and at least one follow-up surveys during 10 years. Each survey included spine imaging by absorptiometry and spine BMD measurement (QDR4500A, Hologic, USA). Spine TBS was calculated at baseline using TBS iNsight software (Med-Imaps, France). Incident VFx was determined when a vertebra reduced its height by 20% or more during follow-up, and satisfied McCloskey-Kanis criteria or Genant's grade 2 fracture.
Results: Age, BMD and TBS of 685 women without any condition affecting bone metabolism were 64.2±8.2 years, 0.815±0.144 gcm^{-2} and 1.193±0.097, respectively. 102 women suffered incident VFx (18.0/1000 person-years) and were older and had lower BMD and TBS than those without VFx. Crude odds ratio of VFx for 1 SD decrease in TBS was 2.04 (1.62-2.57) with the area under ROC curve (AUC) of 0.686. TBS and BMD in combination (AUC=0.702) afforded a better fit to the data than TBS or BMD alone according to Akaike information criterion. Incidence rate of VFx was higher in lower TBS groups in each stratum classified by BMD (Fig). Osteopenic women in the lowest TBS group had a higher risk of VFx than osteoporotic women in the highest group.
Conclusions: Lower TBS was associated with higher risk of VFx over 10 years independently of BMD in Japanese women.

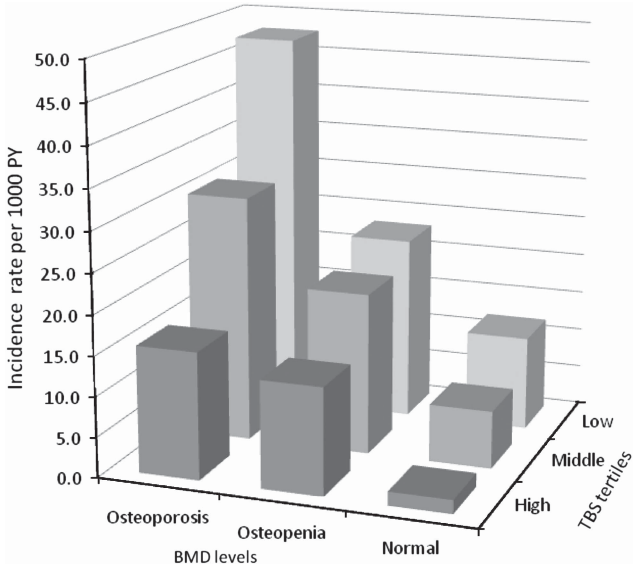

Figure 1 Incidence rates of vertebral fractures in TBS tertile groups in normal, osteopenic and osteoporotic BMD strata. The JPOS Cohort Study. PY: person-years, BMD: bone mineral density at the spine, TBS: trabecular bone score

P3040

Trabecular Homogeneity Index Derived From Plain Radiographs To Evaluate Bone Quality

*Jerome Thevenot[1], Jukka Hirvasniemi[1], Mikko Finnila[1], Volker Kuhn[2,3], Felix Eckstein[2,4], Timo Jämsä[1,5], **Simo Saarakkala**[1,5]*
[1]University of Oulu, Oulu, Finland; [2]Ludwig-Maximilians-University, Munich, Germany; [3]Medical University, Innsbruck, Austria; [4]Paracelsus Medical University, Salzburg, Austria; [5]Oulu University Hospital, Oulu, Finland

Aims: We tested an improved method to assess trabecular bone homogeneity index (HI) from hip radiographs by correlating it with bone mineral density (BMD), volumetric computed tomography (CT) parameters and femoral strength.
Material and Methods: Volumetric CT parameters, femoral strength and BMD were assessed from 178 cadaver femora (mean age 79.3±10.4 years). A gradient-based semi-automatic custom algorithm was applied to the radiographs to calculate the HI along the trabecular fibers by site. Statistical analysis was performed to compare HI with the other parameters.
Results: Regression analysis between the HI versus site-specific BMD showed relationships of R^2=0.67, R^2=0.59 and R^2=0.66 in the neck, trochanteric and total hip areas, respectively. Neck HI and BMD were well correlated with the volumetric parameters from CT scans. Regression analysis between total HI and failure load resulted in R^2=0.50, which improved to R^2=0.57 for cervical fractures alone. Area under the ROC curve was ~0.87 in the discrimination of bones with higher risk of fractures (load <3000N) for models using HI, similarly to BMD-based discrimination.
Conclusion: Homogeneity index can be considered as a promising method to evaluate trabecular bone properties at the hip showing good correlation with BMD and moderate correlation with volumetric CT parameters. HI can explain 50% of the experimental failure load and determine bones with high fracture risk with similar accuracy than BMD.

Table 1 Mean and SD of volumetric CT parameters and their correlation with neck homogeneity index (HI) and neck BMD.

	Mean (SD)	Neck HI	Neck BMD
HU	348 (100)	0.62*	0.84*
SMI	0.36 (0.24)	-0.59*	-0.64*
TbPF	-0.23 (0.10)	-0.55*	-0.59*
BV/TV	48.3 (2.7)	0.53*	0.65*
TbN	0.13 (0.01)	0.47*	0.51*
TbSp	3.82 (0.22)	-0.41*	-0.50*

HU average Hounsfield unit, *SMI* structure model index, *TbPF* trabecular pattern factor, *BV/TV* bone volume fraction, *TbN* trabecular number, *TbSp* trabecular separation. * $p < 0.001$

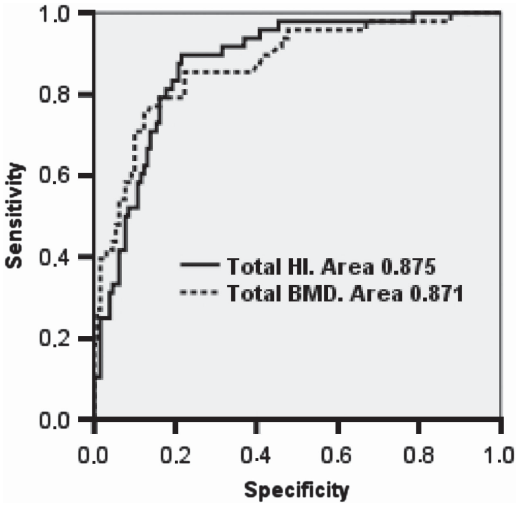

Figure 1 Receiver operating characteristic curves for the prediction of femurs at high fracture risk (hip strength less than 3000N) (48 bones versus 130 controls).

P3041

Cortical Thickness Of Trabecular Bone Assessed By A Novel Ultrasonic Bone Densitometry Is A Possible Predictor Of Vertebral Fracture In Type 2 Diabetes

***Koka Motoyama**[1], Yasuo Imanishi[1], Takashi Mishima[1], Yuki Nagata[1], Shinsuke Yamada[1], Nagato Kuriyama[2], Yoshiyuki Watanabe[2], Masanori Emoto[1], Masaaki Inaba[1]*
[1]Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Osaka, Japan; [2]Department of Neurology, Kyoto Prefectural University of Medicine, Kyoto, Kyoto, Japan

In patients with type 2 diabetes, a bone fracture is one of well-recognized complications. As recent epidemiological studies have found that bone mineral density (BMD) cannot predict bone fractures in type 2 diabetes, further assessments of bone quality are required. Recently, an ultrasonic wave propagation phenomenon described as Biot's theory that focuses on two longitudinal waves (fast and slow waves) has been actively studied and adopted for assessment of bone strength. The LD-100 system (Oyo Electric, Kyoto, Japan) is a newly developed ultrasonic bone densitometry based on the waves, which can non-invasively evaluate trabecular bone density, cortical thickness, and elastic modulus of trabecular bone. We studied BMD, cortical thickness, and elastic modulus of trabecular bone by LD-100 system and vertebral fractures in 31 type 2 diabetic patients. There was no significant difference between fracture group and non-fracture group in BMD (145±30 vs. 163±53 mgm^{-3}; P=0.381). On the other hand, cortical thickness of trabecular bone was significantly decreased in the fracture group. (2.74±0.86 vs. 3.64±0.98 mm; P=0.029). The elastic modulus of trabecular bone exhibited border significance. (2.68±0.29 vs. 2.96±0.44 GPa; P=0.102). In conclusion, cortical thickness of trabecular bone evaluated by a novel ultrasonic bone densitometry can be a predictor of bone fragility in type2 diabetes.

P3042

The Rate Of Bone Loss During The Trans- Or Post-Menopausal Period Assessed By QCT

***Kyoung Min Kim**[1,2], Sujin Lee[1,2], Won Jin Kim[1,2], Jo Eun Kim[1,2], Kwang Joon Kim[2,3], Yumie Rhee[1,2], Sung-Kil Lim[1,2]*
[1]Yonsei University College of Medicine, Seoul, Republic of Korea; [2]Endocrine Research Institute, Yonsei University College of Medicine, Seoul, Republic of Korea; [3]Severance Hospital Executive Healthcare Clinic, Seoul, Republic of Korea

Estrogen deficiency is one of major cause of osteoporosis majorly happened in elderly women. The aim of this study

was to describe the rate of bone loss in the transition from pre-menopause to post-menopause. Healthy 150 pretrans-, trans- or postmenopausal women aged 45 to 65 years were recruited and their volumetric bone mineral densities (vBMDs) were measured by quantitative computed tomography (QCT). For comparing the rate of bone loss, the subjects were divided into two groups by 10-year ages interval (45 to 55 years as pretrans-/trans-menopausal group and 56 to 65 years as post-menopausal group). The slopes of bone loss were calculated as pearson correlations between age and vBMD after adjusting for body mass index. Cumulative bone loss was 36.6 % for lumbar, and the rate of bone loss in pretrans-/trans-menopause group was faster than that of postmenopausal group (r=–0.305 vs. –0.192). Total amount of bone loss was 21.8% for femur neck, and the rate of bone loss was also greater in pretrans-/trans-menopause group (r=–0.356 vs. –0.144). Next, we also examined the changes of geometry, and femur neck cortical thinning was happened by 31.8% in this period with greater loss during pretrans-/trans-menopause and slower rate during postmenopause. Significant amount of bone loss was happened during the premenopause to postmenopause transition, and those rates were greater in pretrans- to transmenopausal period than those of postmenopausal period both at lumbar and femur.

P3043

Effect Of Visceral Adiposity To One Year Change Of Bone Mineral Density

Kwang Joon Kim[1]*, Sung-Kil Lim*[1]*, Kyoung Min Kim*[1]*, Han Seok Choi*[2]*, Yumie Rhee*[1]
[1]Severance Hospital, Seoul, Republic of Korea; [2]Dongguk University Medical Center, Koyang, Republic of Korea

Several studies have documented relationships between adipose tissue and bone mineral density (BMD). However, there has been no longitudinal study that evaluated the effect of visceral adipose tissue on skeleton. Therefore, we investigated how the change of visceral adipose tissue effected on bone mass. A total of 87 healthy subjects have been enrolled in this study (45 male, 42 female) from Feb, 2011 to May, 2011. Visceral and subcutaneous adiposity was analyzed by computed tomography (CT), and bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry for 2 years in a row. (2011 and 2012) Blood samples were collected to measure lipid profile, insulin resistance (HOMA-IR), hepatic enzymes, and hsCRP. In the cross sectional study, we found that baseline hsCRP, HOMA-IR, and visceral fat area but not subcutaneous fat area were inversely correlated with BMD and BMC. In the simple regression analysis, an increase in total visceral fat area which was measured by CT scan was negatively correlated with the change of BMD and BMC. (r=-0.34. p <0.01) Furthermore, Δ insulin resistance (HOMA-IR) also negatively related with ΔBMC. (r=-0.37, p <0.01) In the multiple regression analysis, the change of visceral fat area was an independent predictor of changes in BMD and BMC. Visceral fat, but not subcutaneous fat, might be another risk factor of low BMD in Korean men and women.

P3044

Comparison Of Bone Mineral Density In Hip Fracture Patients With Or Without Prior Osteoporotic Spinal Compression Fractures

Soo Uk Chae*, Yeung Jin Kim, Jae In Park*
Orthopedic Surgery, Wonkwang University, Gunsan, Republic of Korea

Objectives: To compare the characteristics in a group who had hip fracture patients with or without prior osteoporotic spinal fractures.
Methods: From Jun. 2006 to Jun. 2012, 240 patients who had operated hip fractures were evaluated, 127 patients who had with prior osteoporotic spinal compression fractures were in group I, and 113 patients who had without prior osteoporotic spinal compression fractures were in group II. In each group, we measured age and gender, body mass index (BMI), BMD, type of hip fractures, concomitant diseases, and whether of secondary hip fracture and vertebroplasty.
Results:The mean age of group I was 79.0 years(M/F:28/99), and that of group II was 77.5 years(M/F:37/76). The mean BMI of group I was 21.3 and that in group II was 22.0. The mean BMD and t-score of group I was 41.1, -4.45 and those in group II was 50.9, -4.17. The type of neck and intertrochanter fracture of group I was 31 and 91 patients, and those in group II were 37 and 76 patients. Have a concomitant diseases of group I and II were 86 and 65 patients. Eighteen patients had undergone vertebroplasty and 18 patients had second hip fractures. We found that there were significant difference in BMD and T-score between two groups.
Conclusion: We concluded that BMD and T-score in hip fracture patients with prior osteoporotic spinal compression fractures were significantly lower and these multiple osteoporotic fracture patients should be clinically evaluated and treatment for osteoporosis.

P3045

Bone Marrow Adipocyte Progenitors Subpopulation Isolated By Silica Microspheres Incubation Displayed High Potential For Phenotypic Conversion To Osteogenic Cells

Qiqi Lu[1]*, Hua Liu*[2]*, Tong Cao*[1]
[1]NUS Graduate School for Integrative Scienve and Engineering, National Univeristy of Singapore, Singapore, Singapore; [2]Zhejiang University, Hangzhou, Zhejiang, China

Excessive bone marrow adipocytes formation may promote the progression of osteoporosis. Since bone marrow mesenchymal stem cells (BMSCs) are highly heterogeneous, characterization of the bone marrow adipocyte progenitors (BMAPs) subpopulation may offer better cellular targets for osteoporosis research and therapy. In this study, we discovered the BMAPs subpopulation could be effectively isolated from heterogeneous BMSCs by a novel silica microspheres selection method. The selected BMAPs possessed high basal adipogenic genes expression and could homogeneously and rapidly differentiate into matured adipocytes. But after long-term *in vitro* culture,

BMAPs would gradually convert to highly osteogenic cells, accompanied with change of surface marker pattern from Sca-1+CD73-CD90-CD105+ to Sca-1-CD73+CD90-CD105- and reduction of basal adipogenic genes expression. Further studies revealed that expression of CD105 was critical for maintaining the strong adipogenic potential of BMAPs. During transition, most of the CD105+CD73- BMAPs would first transform to highly osteogenic CD105-CD73- intermediate cells and then further converted to the terminal CD105-CD73+ cells. The existence and plasticity of BMAPs offer the possibilities to specifically target the highly adipogenic BMSCs and induce their conversion to osteoblasts for osteoporosis therapy.

P3046

Plasminogen Activator Inhibitor-1 Contributes To The Pathogenesis Of Diabetic Osteoporosis In Female Mice

Yukinori Tamura[1], *Naoyuki Kawao*[1], *Kiyotaka Okada*[1], *Masato Yano*[1], *Katsumi Okumoto*[2], *Osamu Matsuo*[1], *Hiroshi Kaji*[1]
[1]Department of Physiology and Regenerative Medicine, Kinki University Faculty of Medicine, Osakasayama, Japan; [2]Life Science Research Institute, Kinki University, Osakasayama, Japan

An impaired osteoblastic bone formation is responsible for an increased fracture risk in diabetes. However, the pathogenesis of diabetic osteoporosis remains to be clarified in detail. Plasminogen activator inhibitor-1 (PAI-1) is principal inhibitor of fibrinolysis, and several reports suggest that PAI-1 contributes to the development of diabetes. In this study, we investigated the role of PAI-1 in the pathogenesis of diabetic osteoporosis using PAI-1-deficient mice. In quantitative computed tomography, PAI-1 deficiency reversed streptozotocin-induced (STZ-induced) bone loss in female mice, but not in male mice. PAI-1 deficiency reversed the levels of osteogenic genes in the tibia suppressed by diabetic state only in female mice. STZ treatment markedly elevated the levels of PAI-1 mRNA in liver tissues only from female mice, but not in other tissues from both gender of mice. Moreover, recombinant active PAI-1 treatment suppressed osteogenic differentiation and mineralization in primary osteoblasts from female mouse calvaria. In conclusion, this study indicates that PAI-1 plays an important role in the pathogenesis of diabetic osteoporosis in female, and this pathological importance may be a gender-dependent. The production of PAI-1 from liver tissues and the sensitivity of bone cells to PAI-1 might be responsible for mechanisms of gender difference. This study also proposed novel mechanism that liver might be involved in diabetic osteoporosis.

P3047

Prevalence And Risk Factors For Self-Medication Of Corticosteroids

Kawtar Nassar, *Saadia Janani, Wafaa Rachidi, Noufissa Etaouil, Ouafaa Mkinsi*
Rheumatology, Casablanca, Morocco

By their efficiencies, corticosteroids are the treatment for most inflammatory diseases. However, the severity of their side effects are major issues of concern during long-term use.

Purpose: Evaluation of prevalence and factors favoring self-medication with corticosteroids.
Material and methods: Descriptive survey, conducted among 125 patients in the department of Rheumatology of Casablanca receiving prolonged systemic corticosteroid therapy. between December 2011 and may 2012.
Results: The mean age was 46.5 years±10years old. Majority of patients received prednisone dose ≥20 mg a day, for at least one year in 71, 2% of patients. 100 patients recognize they had found out before treatment.Predominant complaint was neuropsychiatric and weight gain. 46.4% were taking corticosteroids for self-medication. The reasons identified were rapid efficacy of corticosteroid therapy during attacks (36.2%), for lack of information (25.8%), appointment of remote consultation (19%), easy access to the pharmacist (10.4%), treatment not expensive to 8.6%. Adherence was noted in 36%. Therapeutic education was performed in all patients in the study. Our study raises the frequency of self-medication with steroids and side effects attributed to them. The need for specific support and regular patients is needed, as well as the creation of laws prohibiting marketing without a prescription at pharmacies.

P3048

Clinical Characteristics Of Ankle Fracture In Osteoporotic Patients Over Sixty-five Years Of Age

Soo Uk Chae, *Yeung Jin Kim, Byung Soo Kim*
Orthopedic Surgery, Wonkwang University, Gunsan, Republic of Korea

Purpose: To investigate clinical characteristics of ankle fracture in osteoporotic patients over sixty-five years of age.
Materials and Methods: From Jan. 2009 to Dec. 2011, 40 patients who were diagnosed the osteoporosis and after ankle surgery. We measured age, gender, body mass index(BMI), bone mineral density (BMD) and T-score. Type of fracture was classified into Danis-Weber and AO classification and whether or not of syndesmotic injury and concomitant disease. Surgery performed under AO principle. We investigated by wound healing, fixation failure and bone union.
Results: The mean age was 70.3 years(M/F:21/19) occurred by low energy rotational injury. Mean BMI was 24.1, mean BMD was 63.6, and T-score was -3.77. Lateral malleolar fracture was 19 cases, bimalleolar was 10 cases, medial malleolar was 7 cases, and trimalleolar was 4 cases. Weber type B was 28 cases, AO classification type B1 was 18 cases, type B2 was 5 cases, type B3 was 5 cases. There was no case with syndesmotic injury. In case of concomitant disease were 24 cases. There were no wound infection and fixation failure. We obtained bone union in all case after 6month.
Conclusion: Ankle fracture in osteoporotic patients over sixty-five years of age occurred by minor trauma, in all cases, there were mostly Weber AO B1 type and there were no syndesmotic injury. Surgery was performed under only AO principles without augmentation method for osteoporosis, and we obtained bone union in all cases without complication.

P3049

Endoxifen Inhibits Bone Loss In Ovariectomized Animals

Anne Gingery[1], *Malayannan Subramaniam*[1], *Kevin Pitel*[1], *Laurence Lindemaier*[2], *James Ingle*[3], *Matthew Goetz*[3], *Russell Turner*[2], *Urszula Iwaniec*[2], *Thomas Spelsberg*[1], *John Hawse*[1]

[1]Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN, USA; [2]Oregon State University, Corvallis, OR, USA; [3]Mayo Clinic, Rochester, MN, USA

Endoxifen (END) is a selective estrogen receptor modulator (SERM) which offers a new modality for treatment of osteoporosis. Gene expression profiles of MC3T3 osteoblasts treated with END relative to treatment with estradiol, tamoxifen, raloxifene or lasofoxifene showed little overlap. Proliferation and mineralization studies confirmed significantly different mechanism of action for END compared to other SERMs. To evaluate the skeletal effects of END, we examined 3 month old ovariectomized (ovx) C57BL/6 mice treated with END (50mg/kg/day) for 45 days. Bone analyses by DXA, pQCT and uCT revealed significant increases in BMD and BMC, trabecular density, cortical content, area, and thickness in the tibial metaphysis and diaphysis, and increased BV/TV, trabecular number, thickness, and decreased spacing in the femoral metaphysis. Serum markers of bone formation and resorption were increased in END treated animals indicating active bone turnover. We have now employed a pre-clinical rat model using 4 month old ovx and intact Sprague Dawley rats treated with END (10mg/kg/day) for 30 days which revealed significant protection against cancellous bone loss following estrogen depletion. In contrast to other SERMs, END treatment enhanced cancellous bone formation in ovary intact animals. These are the first studies to evaluate END's effect on the skeleton and suggest a strong potential for therapeutic treatment of osteoporosis.

P3051

Phenotypic Change In A Patient With Hypophosphatasia With The Onset Of Renal Failure

Toshimi Michigami[1], *Tim Cundy*[2], *Kanako Tachikawa*[1], *Michael Dray*[3], *John Collins*[4]

[1]Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Japan; [2]Department of Medicine, FMHS, University of Auckland, Auckland, New Zealand; [3]Department of Pathology, Middlemore Hospital, Auckland, New Zealand; [4]Renal Medicine, Auckland City Hospital, Auckland, New Zealand

Hypophosphatasia is an inherited disorder with a wide phenotypic manifestation, largely determined by the nature of *ALPL* mutations. We describe a previously asymptomatic adult whose phenotype dramatically changed after he developed renal failure. A 50 year old man was diagnosed with IgA nephropathy. At age 52 (eGFR 50ml min^{-1}) he suffered his first metatarsal fracture. A DXA scan showed osteopenia, and he was prescribed alendronate. His renal failure progressed and he began dialysis (CAPD) at age 55. He suffered multiple non-traumatic fractures. Alendronate treatment was stopped. Serum PTH levels were low and there was discordance between the formation markers ALP 56 U/L (N 40-120) and P1NP180ugl^{-1} (N 20-85). ALP levels had been low before starting alendronate. A bone biopsy showed osteomalacia. Genetic analysis showed compound heterozygosity for missense mutations in *ALPL* (T117H and G438S). In transfection experiments, T117H mutant had almost no enzymatic activity, but G438S mutant retained similar activity to wild-type ALP. Six-month treatment with teriparatide produced a significant increase in ALP activity and histological improvement in bone but significant side effects. After the restoration of renal function by transplantation there was complete symptomatic and histological resolution. It is probable that as the patient developed renal failure, phosphate retention inhibited his residual ALP enzyme activity, resulting in a marked clinical deterioration.

P3052

Enhanced FcγRIII Signaling Drives Osteoclastogenesis And Pathological Bone Resorption During Critical Illness

Ineke Vanhees, *Jan Gunst, Andy Wauters, Erik Van Herck, Sophie Van Cromphaut, Greet Van den Berghe, Helen Owen*

KU Leuven, Leuven, Belgium

During critical illness, circulating bone formation markers are reduced and bone resorption markers are increased, indicating an uncoupling between osteoblast and osteoclast activity, resulting in excessive bone loss. In addition, we have previously shown that increased circulating osteoclast precursors in critically ill patients result in increased osteoclastogenesis *in vitro*, through Fc gamma receptor type III (FcγRIII) signaling, a specific marker for osteoclast precursors in diseases of extreme bone resorption. In the current study, we analyzed the effect of critical illness on bone metabolism at the tissue level in a rabbit model of prolonged critical illness. This *in vivo* model showed a reduction in serum ionized calcium and osteocalcin levels, as is seen in humans. Trabecular area, bone mineral content and density were decreased in sick rabbits, as was the trabecular expression of osteoblast and angiogenesis markers, indicating decreased bone formation and impaired vascularization. There was no change in the expression of the osteoclast differentiation markers from the canonical RANKL/RANK/OPG pathway; however, there was an increase in expression of markers from the non-canonical FcγRIII pathway. The reduction in trabecular bone in the current study may be partly due to a combination of reduced osteoblast differentiation, increased osteoclast formation and reduced vascularization. These findings may help to unravel mechanisms behind bone loss during critical illness.

P3053

A Case Of Postmenopausal Osteoporosis Complicated By Hypophosphatasia

Takeshi Ibata[1], *Takehisa Yamamoto*[2], *Jun NIshikawa*[1], *Hiromi Kitai*[1], *Chihiro NIshii*[1], *Ryutaro Komuro*[1], *Kazushige Adachi*[3], *Takayuki Tsuda*[4], *Toshimi Michigami*[5], *Keiichi Ozono*[6]

[1]Department of Internal Medicine, Minoh City Hospital, Minoh, Osaka, Japan; [2]Department of Pediatrics, Minoh City Hospital, Minoh, Osaka, Japan; [3]Department of Obsterics and Gynecology, Minoh City Hospital, Minoh, Osaka, Japan; [4]Department of Orthopedics, Minoh City Hospital, Minoh, Osaka, Japan; [5]Department of Bone and Mineral Research, Osaka Medical Center and Research Institute for Maternal and Child Health, Izumi, Osaka, Japan; [6]Department of Pediatrics, Osaka University Graduate School of Medicine, Suita, Osaka, Japan

Background: Adult type of hypophosphatasia was reported to have a risk of bilateral atypical bone fractures by the bisphosphonate therapy. Thus, it is preferable to find hypophosphatasia patients prior to the therapy.

Patient: The patient was a 61 years old lady. She was referred to the osteoporosis clinics of Minoh City Hospital for detailed examination. She had neither past history of bone fractures and teeth abnormalities. Bone X-P in lumbar and thoracic spine revealed no fractures. Lumbar BMD was 61% YAM. Laboratory data revealed extremely elevated u-NTX (140mmolBCE/mmolCr) in the presence of relatively decreased serum BAP values (7.0IUl^{-1}), suggesting a state of uncoupling for bone metabolism. Urinary NTX/serum BAP ratio was 20, which was significantly elevated compared with controls matched with age, gender and BMD of the patient (99.9%C.I: 0.89-4.4 *n*=10). These normal values were derived form 494 patients in 8 years. After 3 years of bisphosphonate therapy, serum ALP values turned out to be 67 IUl^{-1}. A gene analysis for TNSALP revealed a heterozygous mutation of c.1559delT in exon 12, confirming this patient to be a carrier of hypophosphatasia. Fortunately, she had no bone fracture during the bisphosphonate therapy. BMD increased from 61% to 70% YAM. At present, we treat her by eldecalcitol instead of bisphosphonate.

Conclusion: The higher rate of u-NTX/serum BAP may be a useful marker for the early diagnosis of apparently healthy adult type of hypophosphatasia patient.

P3056

Keratan Sulfate Levels In Plasma And Urine As A Biomarker In Morquio A

Shunji Tomatsu

Biomedical Research and Orthopedics, Nemours/Alfred I. duPont Hospital for Children, Wilmington, Delaware, USA

Mucopolysaccharidosis IVA (MPS IVA) leads to skeletal dysplasia through excessive storage of chondroitin-6-sulfate and keratan sulfate (KS). KS is synthesized mainly in cartilage and released into circulation, making it a critical biomarker for MPS IVA to evaluate clinical course and effectiveness of therapies. Therefore, an accurate and sensitive method is required to measure KS levels. Using liquid chromatography tandem mass spectrometry (LC/MS/MS) assays, we measured KS levels in blood and urine from MPS IVA patients and healthy controls to evaluate comparability of results. Blood (patients, *n* = 110; controls, *n* = 364) and urine (patients, *n* = 103; controls, *n* = 326) specimens were obtained. Plasma and urine KS measurements in patients were age-dependent and higher than age-matched controls. A moderate correlation between blood and urine KS measurements in the same individual was observed. We have suggested that previous ELISA and current LC/MS/MS methods of measuring urine KS in MPS IVA patients yield comparable results, although plasma KS results are less comparable between methods. The ELISA method is less effective at measuring total KS. However, both methods have reproducibility, accuracy, and high throughput for MPS IVA patients. Overall, both methods should provide a useful tool to measure blood and urine KS to assess the clinical status of MPS IVA patients and to measure the response to treatments such as ERT, HSCT, SRT, and gene therapy.

P3057

Assessment Of Vertebral Fractures In Glucocorticoid-induced Osteoporosis (GIO) Using Semiquantitative Analysis (SQ)

Ikuko Tanaka[1], *Shigenori Tamaki*[1], *Mari Ushikubo*[2], *Harumi Kuda*[2], *Keisuke Izumi*[2], *Kumiko Akiya*[2], *Hisaji Oshima*[2]

[1]Nagoya Rheumatology Clinic, Nagoya, Aichi, Japan; [2]Tokyo Medical Center, Tokyo, Tokyo, Japan

Purpose: Severity and pathophysiology of vertebral fracture in glucocorticoid induced osteoporosis (GIO) were evaluated using semiquantitative analysis (SQ).

Patients and Methods: 136 patients with connective tissue diseases other than rheumatoid arthritis were recruited and observed for 2 years (114 females, age: 61+/–15yo (mean+/–SD), disease duration:12+/–11ys, total prednisolone (PSL) dosage: 34+/–34g, daily PSL dosage: 8+/–6mg/day). SQ method (Gerant et al. JBMR, 1993) was used for evaluation of diagnosis and severities of vertebral fractures.

Results: 1) Incident vertebral fractures were seen in 64 patients (46%) at the baseline of the observation. 42 (31%), 15 (11%), and 6 (4%) patients revealed the grade G1, G2, and G3, respectively. 2) In two years of follow up one, two, and three grade deterioration were observed in 83 %, 8 %, and 9% of the patients, respectively. 3) The patients with more than the two-grade deterioration showed higher age, higher SQ, and lower bone mineral density (BMD) than one-grade deteriorated patients at the baseline. 4) A logistic regression analysis showed age (odds ratio: 1.5/5yo), total PSL dosage (1.1/5g), daily PSL dosage (2.6/5mg), and BMD (1.3/5% decrease) as risk factors, and treatments with bisphosphonates (0.03) and vitamin K2 (0.08) as preventing factors ($p<0.05$).

Conclusions: Evaluation of the vertebral fractures with SQ method was suggested to be an important aspect in pathogenesis and prevention of GIO.

P3058

Trabecular Bone Microarchitecture Is Deteriorated In Patients With Type 2 Diabetes Mellitus

Kenichiro Matsuzaki[1,2], *Toshihide Kawai*[2], *Takeshi Miyamoto*[2], *Shu Takeda*[2], *Hironori Kaneko*[3], *Akio Yokoi*[1], *Keisuke Horiuchi*[2], *Morio Matsumoto*[2], *Hiroshi Ito*[2], *Masako Ito*[4], *Yoshiaki Toyama*[2]

[1]Dept. of Orthopedic Surgery, National Hospital Organization Tokyo Medical Center, Tokyo, Japan; [2]Keio University, Tokyo, Japan; [3]Kitasato Institute Hospital, Tokyo, Japan; [4]Nagasaki University, Nagasaki, Japan

Introduction: Fracture risk is increased in type 2 diabetes mellitus, although areal bone mineral density is elevated. There is a possibility that some aspect of bone quality plays a role here. These include bone turnover, material property such as cross - links of collagen, cortical and trabecular microarchitecture. We investigated the trabecular microarchitecture of lumber spine of patients with type 2 diabetes mellitus (T2DM).
Methods: 28 postmenopausal women with BMD < 80%-YAM with T2DM and 28 control postmenopausal women with BMD < 80%-YAM without T2DM were evaluated in a cross-sectional study. Clinical multi-detector row CT (MDCT) was applied to capture differences in three-dimensional (3D) trabecular bone architecture and volumetric density in lumber supine. We measured/calculated 3D trabecular bone architecture parameters. Statistical significance was set at $P<0.05$.
Results: Trabecular and cortical vBMD significantly increased in T2DM patients. On the other hand, BV/TV was lower in T2DM patients significantly compared with control patients. V*tr, which indicates the connectivity of trabecular, was significantly decreased inT2DM compared with control. All other parameters did not reach significance but showed the tendency related decreased bone strength in T2DM patients.
Discussion: It is suggested that deterioration of trabecular bone microarchitecture may be one of the reasons why fracture risk is elevated despite high BMD in T2DM patients.

P3059

A Prediction Model Using Serum Chemokine Ligands 4, 5, 6 And 8 Can Identify Osteoporotic Women

Gazelle Crasto[1], *Marcin Mizianty*[2], *Lukasz Kurgan*[2], *Sean Peel*[1]

[1]Faculty of Dentistry, University of Toronto, Toronto, Ontario, Canada; [2]University of Alberta, Edmonton, Alberta, Canada

Bone mineral density is useful at predicting fracture risk once significant bone loss has occurred. Current bone serum biomarkers provide information on the bone turnover rate and are useful for monitoring treatment efficacy.
Serum samples from three groups of women, young (aged 30-45), postmenopausal (aged 53 to 68) and diagnosed osteoporotic (aged 56-82) were obtained and levels of Type-I collagen (CTX), osteocalcin (OC), chemokine ligands (CXCL 1, 4, 5, 6, 7 and 8), insulin-like growth factor (IGF-1), insulin-like growth factor binding protein (IGFBP-3) and bone morphogenetic proteins (BMP) -2, 4 and 7 were measured by ELISA. These results were then used to develop a decision tree-based prediction method to generate a human readable diagnostic model. The predictive quality was quantified using accuracy, sensitivity, specificity and Mathews Correlation Coefficient (MCC). Machine learning experiments revealed the best multivariate diagnostic model used four chemokine markers (CXCL-4, 5, 6 and 8) to accurately predict osteoporosis in women, achieving cross-validation accuracy of 91.3±0.2%, MCC of 0.81±0.0 sensitivity of 90.9±0.2% and specificity of 92.2±0.4%. The resubstitution test gave an accuracy of 96.6%, an MCC of 0.93, sensitivity of 95% and specificity of 100%. These results indicate the CXCL-4,5,6,8 multivariate model correlates strongly with osteoporotic BMD scores and these chemokines should be investigated predictors of low BMD and fracture risk.

P3060

Randomize Controlled Clinical Study Of Osteoporotic Vertebral Fractures By Using Magnetic Resonance Imaging

Yoshihatu Kato

Orthopaedics, Tokyo Women's Medical University, Tokyo, Japan

Introduction: Magnetic resonance imaging (MRI) allows us to diagnose OVFs correctly because bone marrow bleeding and edema are demonstrated.
Materials and Methods: A total of 86 patients were divided into two groups. Group A (n=44) received Ca and VitD3, while group B (n=42) received these medicines + bisphosphonates. A diagnosis of OVF was confirmed by a low intensity on T1WI and a high intensity on STIR images. Follow up was performed for at least one year (group A: n=21; group B: n=20), and patients were evaluated for new fractures, bony union, and collapse.
Results: In 17 patients, it was difficult to diagnose OVFs on plain radiographs, but MRI enabled correct diagnosis; and a single fracture was found in 10 patients at the initial hospital visit. There were 18 patients with multiple fresh fractures (≥2), 12 with continuous multiple fractures, and 11 with other fractures. At follow up, MRI showed almost completed bony union within 6 months in both groups, while new OVFs occurred in four patients from group A and two patients from group B. The collapse rate ranged from 28.5% to 48.0% in group A and from 36.5% to 52.6% in group B. If the intensity of the fracture is low on T1WI images and mix of low, isointense and high on T2WI and STIR images, this indicates acute severe collapse due to a burst fracture.
Conclusion: We can avoid severe vertebral body collapse resulting in spinal deformity by employing MRI, even if it is more expensive.

P3061

Assessment Of Endocortical Bone Loss In The Femur Midshaft During Aging: An Experimental-Computational Approach

Peter Pivonka[1], *Pascal Buenzli*[2], *David Thomas*[3], *John Clement*[3]

[1]Australian Institute for Musculoskeletal Science, North West Academic Centre, University of Melbourne, Melbourne, Victoria, Australia; [2]Computational Bone Biology Group, University of Western Australia, Perth, Western Australia, Australia; [3]Melbourne Dental School, University of Melbourne, Melbourne, Victoria, Australia

It is well known that cortical porosity of bone increases in osteoporosis leading to reduction in bone strength and

ultimately to increased fracture risk. Several mechanisms have been hypothesized to drive porosity changes including increased activation frequency, reduction of bone formation at the cellular level, reduction of bone formation at the tissue level, and increased volume of bone resorbed by an individual basic multicellular unit. Assessment of cortical bone cross sections indicates that changes in cortical porosity are not uniformly distributed across the cortical thickness, but are biased towards the endocortical surface which is the interface between trabecular and cortical bone. Current bone biology literature suggests that changes in cortical porosity occur faster near the endocortical surface compared to the periosteal surface due to increased metabolic activity in trabecular bone. Here we utilize experimental and computational techniques to better understand how porosity evolves in osteoporosis across a cortical bone section.

P3062

Alveolar Bone Mineral Density Predicts Fracture Of Spine And Extremities

Yoshitomo Takaishi[1,4], *Seizaburo Arita*[2], *Mitsugi Honda*[3], *Takeshi Sugishita*[1], *Aiko Kamada*[4], *Takashi Ikeo*[4], *Takami Miki*[5], *Takuo Fujita*[6]

[1]Takaishi Dental Clinic, Himeji, Japan; [2]Department of Life and Medical Sciences, Doshisha University, Kyoutanabe, Japan; [3]Department of Radiology, Okayama University Hospital, Okayama, Japan; [4]Department of Biochemistry, Osaka Dental University, Hirakata, Japan; [5]Department of Geriatric Medicine, Osaka City University, Osaka, Japan; [6]Katsuragi Hospital, Kishiwada, Japan

Introduction: Tooth loss and systemic osteoporosis are linked with advancing age but no clear causal each other. We aimed to characterize the correlation between alveolar and spinal bone density with reference to fractures.

Methods: We used computerized radiogrammetry (Bone Right) to measure alveolar bone mineral density (al-BMD) and analyzed lumbar bone mineral density (L-BMD) using dual energy X-ray absorptiometry. L-BMD and al-BMD in 30 female patients (average age: 59±5yrs) were correlated with various patient attributes. Statistical analysis included area under the curve (AUC) and probability of asymptotic significance (PAS) in a receiver operating characteristic curve (ROC) followed by a multivariate analysis to compare the predictive strength of L-BMD(T)-scores and al-BMD for fracture occurrence, using category weight scoring.

Results: L-BMD and al-BMD were significantly correlated with age, years since menopause and alveolar bone thickness. Both were also negatively correlated with fracture incidence. The category weight score was –0.275 for a LBMD(T) <80%, and +0.183 for a LBMD(T) >80%. Category weight score was –0.860 for al-BMD <84.9 (Brightness), and +0.860 for al-BMD >84.9. AUC and PAS analyses suggested that al-BMD was a better predictor of fracture occurrence than LBMD.

Conclusions: Our results suggest that dental screening investigations could be used to predict susceptibility to future osteoporotic fractures.

Table 1. ROC(Receiver Operationg Caracteristics) Analysis

Fracture predictors	ROC curve (AUC)	95% confidence interval	SE	P-value (PAS)
al-BMD	0.932	0.831-1.033	0.052	0.00006
L-BMD(T)	0.792	0.626-0.957	0.084	0.007
100-Age	0.747	0.571-0.922	0.090	0.0226
al-T	0.710	0.710-0.992	0.095	0.0516

P3063

Analysis Of Correlation Between Bone Mineral Density And Polymorphic Variants Of Immune Cytokines In Young And Elderly Japanese Women

Yoshie Oishi[1], *Yoko Watanabe*[1], *Shoko Shinoda*[1], *Mamiko Naka*[2], *Yoshiteru Ozawa*[3], *Takeshi Matsuyama*[3], *Kyoei Morozumi*[3], *Yoko Fuke*[1]

[1]Tokyo Metropolitan University, Hachioji, Tokyo, Japan; [2]ihc Marunouchi Integrated Health Clinic for Women, Chioda-ku, Tokyo, Japan; [3]Fussa Hospital, Fussa, Tokyo, Japan

An important clinical risk factor in the pathogenesis of osteoporosis is the presence of genetic polymorphisms in susceptibility genes. Recent studies have clarified the interaction between bone and immune cells. In this study, we examined the associations between bone mineral density (BMD) and polymorphisms in genes encoding interleukin (IL)6 (-634C>G; rs1800796), tumor necrosis factor (TNF)α (-308G>A; rs1800629), IL17F (7488T>C; rs763780), transforming growth factor (TGF)β (869T>C; rs1800470), osteoprotegerin (OPG) (163A>G; rs3102735) and methylenetetrahydrofolate reductase (MTHFR) (677C>T; rs1801133) in young and elderly Japanese women. Whole-body, lumbar spine, and femoral neck BMD were 1.13±0.06, 1.14±0.12, and 1.00±0.11 gcm^{-2} in the young subjects, and 0.92±0.09,

[P3062]

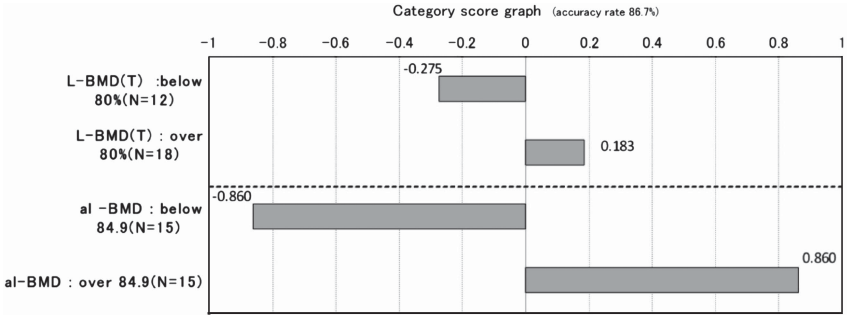

Figure 1

0.86±0.15, and 0.63±0.10 gcm^{-2}, in elderly subjects. The frequencies of the IL6 CC, CG and GG genotypes were 48%, 49%, and 3%, in young women. The frequencies of the IL17F TT, TC and CC genotypes were 79%, 15%, and 6%, in young women and 78%, 21%, and 1%, in elderly women. Polymorphisms of the IL6 and IL17F genes were significantly associated with BMD in young women. The significance of IL17F gene polymorphism was also indicated in elderly women, therefore polymorphism in the IL17F 7488T>C (rs 763780) may be useful as a new candidate gene for preventing primary osteoporosis.

P3064

Relation Of Bone Mineral Density To Vitamin D Receptor Gene Polymorphism And Lifestyle Factors In Japanese Female Workers Aged 22-44 Years

Yayoi Funakoshi[1], *Hisamitsu Omori*[2], *Hironori Yada*[3], *Takahiko Katoh*[2]

[1]Gifu City Women's College, Gifu, Japan; [2]Kumamoto University, Kumamoto, Japan; [3]Kumamoto Health Science University, Kumamoto, Japan

Bone mineral density (BMD) reflects both genetic and lifestyle factors. The aim of this cross-sectional study was to investigate the influence of vitamin D receptor (VDR) gene polymorphism and lifestyle factors on BMD in premenopausal female workers. The subjects were 162 premenopausal female employees aged 22-44 years who worked at a large-scale integrated manufacturing facility in Japan. BMD was measured at the nondominant radius by dual energy X-ray absorptiometry. Genomic DNA was isolated from peripheral leukocytes. BMD was positively correlated with age (r=0.22, p<0.001), weight (r=0.45, p<0.001), and body mass index (BMI) (r=0.48, p<0.001). The genotype frequencies of VDR gene polymorphism detected by TaqI analysis were 77.2%, 22.8%, and 0.0% for TT, Tt, and tt, respectively. According to multiple linear regression analysis, the independent determinants of BMD were age (coefficient=0.001, p<0.05), BMI (coefficient=0.006, p<0.001), and VDR gene polymorphism (TT vs. Tt; coefficient=-0.016, p<0.05). Our data show that BMD is negatively correlated with the Tt genotype of the VDR gene, but positively correlated with age and BMI. These findings suggest that analysis of VDR gene polymorphism may be useful for identifying individuals who are susceptible to osteoporosis so that early preventive measures can be provided.

P3066

Acute BMP2 Upregulation Following Induction Of Ischemic Osteonecrosis In Immature Femoral Head

Nobuhiro Kamiya[1,2], *Harry Kim*[1,2]

[1]Texas Scottish Rite Hospital for Children, Dallas, TX, USA; [2]University of Texas Southwestern Medical Center, Dallas, TX, USA

Juvenile ischemic osteonecrosis of the femoral head (IOFH) is one of the most serious hip conditions. Little is known about BMP signaling following ischemic osteonecrosis. In this study, we found acute BMP2 upregulation in the femoral head cartilage surrounding the necrotic bone 24 hours after ischemic induction in our immature pig IOFH model as well as in our mouse ischemic osteonecrosis model. BMP2 was increased in cartilage explants and primary chondrocytes under hypoxia (1% O2) compared with normoxia (21% O2). Addition of the hypoxia inducible factor 1 (HIF1) activator DFO significantly increased BMP2 while HIF1 silencing (siHIF1) only partially reduced BMP2, suggesting other mechanisms of BMP2 upregulation being present. Hypoxia is known to induce the production of free oxygen radicals, which are converted to hydrogen peroxide (H2O2) by superoxide dismutase 2 (SOD2). As an alternative mechanism, we investigated the effect of H2O2/ SOD2 production on BMP2 upregulation. Chondrocytes produced more H2O2 under hypoxia than normoxia. H2O2 addition to the chondrocyte culture also significantly increased BMP2 expression. SOD2 was also dramatically increased in the ischemic pig cartilage at 24 hours following surgery. Moreover, DFO significantly increased SOD2 while HIF1 silencing only partially reduced SOD2. We conclude that the acute BMP2 response of chondrocytes to ischemic osteonecrosis is more dominantly through the H2O2 production and only partly through the HIF1 pathway.

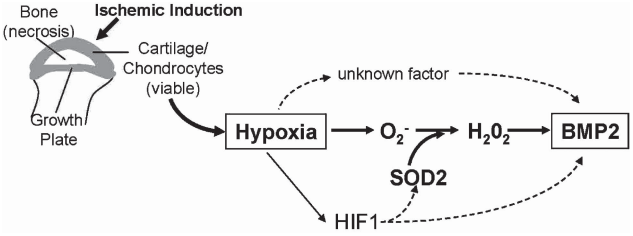

Figure 1 A potential model of acute BMP2 upregulation under hypoxia in chondrocytes. Hypoxia increases superoxide anion (O2-), which is converted to hydrogen peroxide (H2O2) via superoxide dismutase 2 (SOD2). By responding to the H2O2 upregulation, the BMP2 expression levels could be increased physiologically in chondrocytes. HIF1 is partially involved in BMP2 upregulation under hypoxia, along with unknown factor (s). Dotted lines indicate partial effects.

P3067

Survey About Patients Adherence On Long Term Corticosteroids

Kawtar Nassar, *Saadia Janani, Wafaa Rachidi, Noufissa Etaouil, Ouafaa Mkinsi*

Rheumatology, Casablanca, Morocco

Non-adherence factors are numeros, espacially with long term corticosteroids their use remains controversial. we performed cross-sectional survey, conducted among 125 patients monitored service Rheumatology Casablanca,between december 2011,and may 2012.

Result: Patients interviewed received corticosteroids for an average 73.6 months. The average maximum dose prescribed was 44.87 mg per day. Most often for inflammatory arthritis (50.4%).80% patients have recognized knowledgeable about the disease and treatment with corticosteroids before the start. 22 cases had non therapeutic compliance, following side effects Corticoid (nine cases), fear and reluctance of the medication (seven), or lack of information.

Conclusion: Improving the management and optimization of patient compliance in long-term systemic corticosteroids are required and necessary. This imposes the need for regular monitoring of adverse effects, specific support for patients who must be assigned so systemically.

P3068

Role For Circulating Versus Locally-activated 1,25-dihydroxyvitamin D In Bone And Lipid Metabolism: A Sub-Analysis Of Chiba (Coronary Heart Disease Of Ischemia And Bone Association) Study

***Daisuke Inoue**, Reiko Watanabe, Nobuyuki Tai, Junko Hirano, Toshihiro Amaki, Fumitaka Nakamura, Ryo Okazaki*
Third Department of Medicine, Teikyo University Chiba Medical Center, Ichihara-shi, Chiba, Japan

Serum 25 D, but not 1,25 D, is associated with various metabolic indices and a predictor of osteoporosis, metabolic syndrome and cardiovascular events. This can be explained by local 25D activation by extra-renal CYP27B1 and PTH-mediated effect, but their relative contribution, and particularly, the role of circulating 1,25D is not fully understood. We have reported that in 300 males who visited our department for coronary angiography, an MMP-dependent bone marker ICTP, is a good predictor of both cardiac damage (proBNP) and dysfunction (LVEF). In the current sub-analysis of 168 males with eGFR>60 ml/min, we investigated distinct roles of serum 25D and 1,25D. 25D was correlated positively with 1,25D and negatively with PTH. Osteocalcin, ICTP and CTX were significantly correlated with 25D but not with 1,25D or PTH. 25D correlation remained significant even after adjusted for 1,25D and PTH, confirming little contribution of circulating 1,25D to bone turnover. There were only three factors correlating only with 1,25D but not with PTH or 25D: FGF23, leptin and HDL-C. Correlation between 1,25D and HDL-C was independent of age, eGFR, 25D, PTH, Ca, P and leptin. We detected CYP27B1 expression in bone but not in liver by human tissue RT-PCR. Given the presence of functional VDRE in Apo A-1 gene, our results suggest that HDL-C is subject to regulation by circulating 1,25D and may contribute to anti-atherosclerotic effect of vitamin D.

P3069

Carborane BA321, One Of The Carbon-Containing Polyhedral Boron-Cluster Compounds, Binds To Both Androgen Receptor And Estrogen Receptor, And Recovers Bone Loss Due To Sex Steroid Deficiency

***Chiho Matsumoto**, Masaki Inada, Michiko Hirata, Chisato Miyaura*
Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Koganei, Japan

Carboranes are a class of carbon-containing polyhedral boron-cluster compounds having exceptional hydrophobicity, and their features may allow a new medical application as a biologically active molecule that interact with steroid hormone receptors. We have synthesized carborane compounds having affinity with androgen receptor (AR) and estrogen receptor (ER) to search effective compounds for osteoporosis. BA321 is a putative AR antagonist, in which a benzene ring with electron-withdrawing group (-NO2) and a hydroxyl group are placed at the opposite vertices of the hydrophobic carborane cage. In the competitive binding assay, BA321 exhibited binding affinity to AR, and the affinity is 10-fold higher than that of hydroxyflutamine. In NIH3T3 cells transfected with AR, BA321 exhibited anti-androgenic activity in reporter gene assay. Orchidectomized (ORX) mice showed severe bone loss due to androgen deficiency measured by femoral BMD and micro-CT, and the bone loss was completely recovered by the treatment with BA321. The weight of seminal vesicle was reduced in ORX mice, and not influenced by the treatment of BA321, indicating that BA321 does not exhibit androgenic action in sex organ in the male. On the other hand, BA321 bound to ER, and prevented the bone loss due to estrogen deficiency in ovariectomized mice. BA321 may selectively bind to AR and ER, and act on bone tissues as selective androgen receptor modulator (SARM) or selective estrogen receptor modulator (SERM).

P3070

Role Of CCN3 / NOV In Bone Regeneration

***Yuki Matsushita**[1,2], Kei Sakamoto[1], Kenichi Katsube[1], Kiyoshi Harada[2], Akira Yamaguchi[1]*
[1]Section of Oral Pathology, Tokyo Medical and Dental University, Tokyo, Japan; [2]Section of Maxillofacial Surgery, Tokyo Medical and Dental University, Tokyo, Japan

CCN family proteins are secretary proteins composed of 6 members. The name of CCN family is originated from the initial letters C of CYR61, C of CTGF and N of NOV. In our study, we focus CCN3/NOV. We explored the gene expression profile during bone regeneration by microarray analysis using bone regeneration model in mouse. We identified that mRNA for CCN3 is highly upregulated at early stage of bone regeneration. This experiment prompted us to investigate the role of CCN3 in bone regeneration *in vivo*. We investigate the skeletal phenotype in Tg and KO mice of CCN3. We also generated skeletal injuries to these mice and examined the role of CCN3 in bone regeneration. Histomorphometric analysis suggested osteopenia in Tg mice, but KO mice exhibit no apparent skeletal changes. In our skeletal injury model, bone regeneration in KO mice was accelerated compared with that in WT mice. mRNA expression of osteoblast-related genes was upregulated earlier in KO mice than in WT mice. Histomorphometric analysis demonstrated that bone regeneration in KO mice was accelerated. In conclusion, CCN3 is upregulated at early phase of bone regeneration, and it inhibits bone regeneration.

Comparative Endocrinology of Calcium Regulation Workshop

CE01

Development And Application Of A Fish Scale *In Vitro* Assay System

Nobuo Suzuki
Kanazawa Univrrsity, Noto-cho, Japan

We recently developed an *in vitro* assay using fish scales that contain osteoclasts, osteoblasts, and bone matrix, all of which are similar to those found in mammalian membrane bone. Using the assay, we demonstrated that melatonin suppressed osteoclastic and osteoblastic activities. These findings are in agreement with the reports from *in vivo* studies in mice and

rats. In an attempt to develop molecules that increase bone mass, novel bromomelatonin derivatives were synthesized, and the effects of these chemicals on osteoclasts and osteoblasts using the scale assay were examined. As a result, novel bromomelatonin derivatives with the ability to possibly increase bone formation were identified. In scale osteoclasts, particularly, 1-benzyl-2,4,6-tribromomelatonin had a more potent activity than melatonin. In reference to osteoblasts, this agent (10-9 - 10-6 M) significantly activated osteoblasts. The effect of 1-benzyl-2,4,6-tribromomelatonin on bone formation was confirmed in ovariectomized rats. The oral administration of 1-benzyl-2,4,6-tribromomelatonin augmented the total bone mineral density of the femoral metaphysis of ovariectomized rats. In rats fed a low-calcium diet, the total bone mineral density of the femoral metaphysis significantly increased following the oral administration of 1-benzyl-2,4,6-tribromomelatonin. These studies identified a melatonin derivative that may have potential use in the treatment of bone diseases, such as osteoporosis.

CE02

Chicken Calcium Regulation And Bone Metabolism

Toshie Sugiyama
Deaprtment of Agrobiology, Niigata University, Niigata, Japan

The chicken (Gallus gallus domesticus) is a domesticated fowl which is mainly divided into two types, egg-laying hen and meat-type chicken (broiler). They are a source of eggs and meat. As a result of the genetic improvement of their productive efficiency, modern chickens have a number of defects, especially related to the calcium and bone metabolism, eggshell thinning and leg weakness. Laying-hens can produce up to 300 eggs per year. The eggshell consists of 5.7 g calcium carbonate of which 2.3 g is purely calcium. Therefore, the egg-laying hens require a large amount of calcium to support eggshell formation and their calcium metabolism is quite different to all other classes of vertebrates. Interestingly, the most of calcium in eggshell is derived not only from dietary sources but also from skeletal stores, called “medullary bone”. Tibial dyschondroplasia (TD), a major cause of leg weakness, is one of most severe diseases in meat-type chickens. TD appears in 2-6 percent of meat-type growing chickens, and it is characterized by an abnormal, unmineralized and unvascularized mass of cartilage occurring in the epiphyseal growth plate of the tibia. TD is closely related to the disturbance of normal endochondral ossification. In order to prevent eggshell thinning and TD, it is important to clarify the calcium regulation and bone metabolism in the chicken.

CE03

Novel Metabolism Of Vitamin D: mRNA Expression Of 1α-hydroxylase In Extra-Renal Tissues Of The Chicken And Pig

Chiharu Miyakawa[1], *Thau Chung*[2], *Toshie Sugiyama*[1]
[1]Deaprtment of Agrobiology, Niigata University, Niigata, Japan; [2]DSM Nutritional Products Asia Pacific, Singapore, Singapore

Egg-laying hens require a large amount of calcium to support the formation of eggshell, with each eggshell consisting of 2.3 g calcium. Vitamin D plays an important role of calcium homeostasis in the egg-laying hen. In general, the biologically active form (that is, 1α,25 (OH)2D3) is converted from vitamin D3 (cholecalciferol) via a two-step hydroxylation process catalyzed by 25-hydroxylase in the liver and 1α-hydroxylase in the kidney. Recently, the mRNA expression of 1α-hydroxylase has also been demonstrated in the extra-renal tissues of mammals such as pigs. These results indicated that locally-synthesized 1α,25 (OH)2D3 directly regulates cellular differentiation and functions in these tissues. The present study employed real-time PCR techniques to detect the comparative mRNA expression of 1α-hydroxylase in the kidney, intestines and skeletal muscle of egg-laying hens and pigs. The results showed that the relative mRNA expression levels of 1α-hydroxylase in egg-laying hens were: kidney, 1.000 > colon, 0.453 > ileum, 0.443 > caecum, 0.397 > jejunum, 0.333 > duodenum, 0.246 > skeletal muscle, 0.029. Likewise in the pig, the highest expression was detected in the kidney. However, the level of expression in other porcine tissues relative to the kidney was lower when compared to the results obtained in the egg-laying hen. These results suggested that the role of locally-synthesized 1α,25 (OH)2D3 regulating calcium metabolism may differ between avian and mammalian species.

CE04

Histochemical Analysis Of Thiram-Induced Tibial Dyschondroplasia In Chickens

Hidemi Okajima, *Toshie Sugiyama*
Deaprtment of Agrobiology, Niigata University, Niigata, Japan

Tibial dyschondroplasia (TD) is one of most severe diseases in meat-type chickens. TD appears in 2-6 percent of meat-type growing chickens, and it is characterized by an abnormal, unmineralized and unvascularized mass of cartilage occurring in the epiphyseal growth plate of the tibia. Although there is extensive data describing the morphological characteristics of the TD lesion, the mechanism of lesion formation has not been

fully clarified. In the present study, we fed meat-type chicks (aged 8 days) with commercial diet containing 100ppm tetramethylthiuram disulfide (Thiram) for two days and TD was artificially induced. As a result, almost all chicks fed Thiram represented typical TD lesions in the epiphyseal growth plate of the tibia. The growth plate, especially the hypertrophic chondrocyte zone, was significantly enlarged in TD. TUNEL assay detected a large number of apoptotic chondrocyte in TD lesion of hypertrophic zone and their extracellular matrix was not calcified. Moreover, immunohistochemical studies also demonstrated that both type II collagen and type X collagen were codistributed in hypertrophic chondrocyte zone. These characteristics of TD lesions were clearly distinct from those of normal growth plate. Consequently, these results suggested that TD is caused by the disturbance of normal chondrocyte differentiation with extracellular matrix modifications and cell death.

CE05

The Regulation Of Osteoclastogenesis By The Alteration Of Bone Marrow Cells In Avian Medullary Bone

Shinji Hiyama[1]*, Yumiko Kadoyama*[2]*, Takashi Uchida*[1]
[1]Hiroshima University Institute of Biomedical & Health Sciences, Hiroshima, Japan; [2]Faculty of Dentistry, Hiroshima University, Hiroshima, Japan

Avian medullary bone (MB) is quickly remodeled in the bone marrow cavity during the reproductive period under the control of circulating estrogen. Interestingly, a single administration of estrogen (E2) to male birds is induced MB formation and subsequent bone resorption during a couple of days. In the process of MB remodeling, although it is known that osteoclasts are differentiated from hematopoietic cells, the process of osteoclastogenesis is not clear. To address this question, we examined the osteoclast differentiation from bone marrow cells (BMCs) and the alteration of BMCs during MB formation using E2-treated male Japanese quails. At 0, 1, 2 and 3 days after E2 administration, BMCs that were derived from each days differentiated into osteoclasts in the presence of RANKL/M-CSF. In particular, BMCs of 3 days after E2 administration were formed large multinucleated cells and numerous bone resorption pits as compared with BMCs of other days. These bone resorption activity were completely inhibited by OPG. The number of CFU-GM/CFU-M was gradually increased and was observed most in BMCs of 3 days after E2 administration. Additionally, monocytes/macrophages were increased until 2 days and were remarkably reduced at 3 days after E2 administration. These results suggest that the osteoclastogenesis in avian MB may be dependent on RANKL/RANK/OPG system and the population of BMCs may shift from undifferentiated cells to osteoclast precursor cells during MB formation.

CE06

Bone-Invasive Spontaneous Cancers In Dogs And Cats: Mouse Models Of Prostate Cancer, Oral Squamous Cell Carcinoma And Osteosarcoma

Thomas Rosol*, Wachi Supsavhad, Wessel Dirksen, Blake Eason Hildreth, Chelsea Martin, Tobie Wolfe, Lisa Lanigan, Ramiro Toribio*
Ohio State University, Columbus, OH, USA

Cancer is a cause of death in older dogs and cats and clinical trials in pets are useful to predict efficacy of novel drugs for humans. Three prostate carcinoma cell lines have been developed from dogs. The Ace-1 cells formed mixed osteoblastic/osteolytic bone metastases in nude mice. Zoledronic acid (ZA) did not reduce bone metastases, but did inhibit bone resorption. Transfection with hDKK-1 (Wnt antagonist) increased bone metastases and eliminated new bone formation. The Leo cells metastasized to the brain, spinal cord, and bones, and death was due to brain metastases. The Probasco cells are an

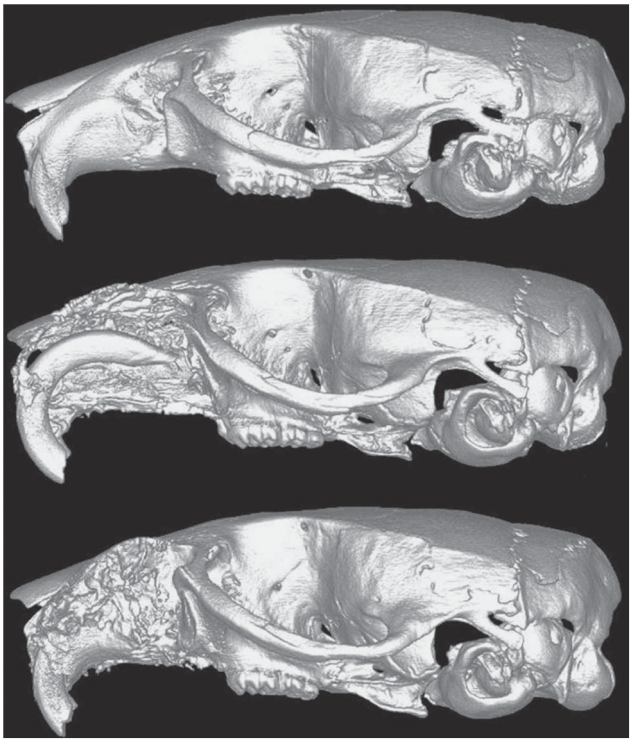

Figure 1 MicroCT image of mouse skulls. TOP: Control mouse. MIDDLE: Mouse with feline oral squamous cell carcinoma SCCF2 xenograft with bone resorption of the maxilla. BOTTOM: Mouse with SCCF2 and treatment with zoledronic acid. Note preservation of maxillary bone, but tumor invasion was still present.

osteoblastic cell line and do not produce PTHrP. Transfection with hPTHrP-141 resulted in osteolytic bone metastases. Oral squamous cell carcinoma (SCC) with invasion into the mandible or maxilla is common in older cats and mimics head and neck SCC in humans. Three feline oral SCC cell lines have been developed (SCCF1, 2, 3). The SCCF-2 cells produce PTHrP and invade the maxillary bone of nude mice. TGF-beta increased PTHrP and cancer cells close to bone had increased nuclear PTHrP. ZA inhibited bone loss, but did not inhibit local bone invasion. Canine osteosarcoma lines were screened *in vivo*. The OSCA-40 cells formed osteolytic intratibial tumors that metastasized to the lungs. ZA reduced bone resorption, but there was no reduction in lung metastases. The data demonstrated that ZA reduced bone resorption in bone-invasive cancers, but did not reduce metastasis or bone invasion.

CE07

What I Have Learnt From Dogs

Janine Danks[1,2]*, Kristi Milley*[1]
[1]Medical Sciences, RMIT University, Bundoora, Victoria, Australia; [2]The University of Melbourne, Dept of Medicine, Heidelberg, Victoria, Australia

Breast cancer is the second most common cancer among Australian women with one in nine women to be diagnosed with breast cancer before the age of 85. Breast cancer metastasis is associated with morbidity and mortality, with up to one third of women with early stage breast cancer eventually dying of the disease. Advanced breast cancer is less common in Australia since the introduction of screening mammography in 1994. Breast tumors are the most common tumors in female dogs and the second most common after bone tumors in all dogs. It is suggested that advanced disease is common in dogs. We are using canine breast tumors (CBT) as a model for human disease and we are subtyping the CBTs using the new human molecular subtyping system. We have developed an immunohistochemical panel that allows this subtyping of the CBTs. We have also demonstrated if they are parathyroid hormone-related protein positive. We have recruited over 150 veterinarians around Victoria and three veterinary pathology services. This has resulted in the collection of 240 fixed samples and >50 fresh frozen samples in less than two years. This has lead us to set up the first veterinary cancer biobank in Australia. We have found the veterinary community to be very cooperative and willing research partners. We plan to use this network to recruit canine patients for drug trials that will translate into better treatments for both dogs and humans.

CE08

Regulation Of Bone Metabolism During Pregnancy, Lactation, And Post-Weaning

Christopher Kovacs
Memorial University of Newfoundland, St. John's, Newfoundland, Canada

Pregnancy and lactation place significant demands upon mammals to supply calcium to the fetus and neonate, respectively. Despite a similar magnitude of calcium demand incurred by pregnancy and lactation, the adjustments made in each of these reproductive periods differ significantly. Upregulation of intestinal calcium absorption dominates during pregnancy, whereas the skeleton rapidly resorbs during lactation in order to provide calcium to milk. The magnitude of loss is 5-10% of skeletal mineral content during 6 months of lactation in humans, and a 25-55% loss during 3 weeks of lactation in rodents. In all mammals, the skeletal mineral content is fully restored after weaning. Novel mechanisms appear to be invoked to upregulate intestinal calcium absorption independent of calcitriol during pregnancy. An elegant interaction between breast, brain and bone programs the skeletal resorption that occurs during lactation. The post-weaning recovery of the skeleton is also regulated by novel mechanisms that do not require the known calciotropic hormones, but do involve upregulation of Wnt signaling.