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Poster Presentations

Basic abstracts

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P87

A Novel Microrna Regulates Osteoclast Differentiation via Targeting Protein Inhibitor of Activated STAT3 (PIAS3)

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Background: Study the function of a new identified miRNA (miR-9718) in osteoclast differentiation.
Methods: Small RNA isolation and cloning were performed to isolate small RNA. Bioinformatic analysis was carried out to identify new miRNA and predict its target gene. Luciferase reporter gene validated the prediction. QRT-PCR, Northern Blot and Western Blot experiments were used to detect the expression of aimed genes or miRNAs. We carried out overexpression or inhibition experiments by transfection to observe the effection of miR-9718. *In vivo* study showed the effection of miR-9718 in mice receiving ovariectomy (OVX) and in sham-operated controlmice.
Results: We identified a new microRNA named miR-9718. MiR-9718 promotes osteoclast differentiation by repressing protein inhibitor of activated STAT3 (PIAS3) at the post-transcriptional level. MiR-9718 was found to be transcribed in RAW 264.7 cells during macrophage colony stimulating factor (M-CSF) and nuclear factor-κB ligand (RANKL) induced osteoclastogenesis. Overexpression of miR-9718 promoted M-CSF and RANKL induced osteoclastogenesis, whereas inhibition of miR-9718 attenuated it. PIAS3 was predicted to be a target of miR-9718.
Conclusion: We identified a new miRNA named miR-9718 and our study showed that miR-9718 played an important role in osteoclast differentiation via targeting PIAS3 both *in vitro* and *in vivo*.

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Lactoferrin Promote Primary Rat Osteoblast Proliferation and Differentiation via Up-Regulation of Insulin-Like Growth Factor-1 Expression

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Background: The aim of this study was to explore the effect of lactoferrin (LF) in primary fetal rat osteoblasts proliferation and differentiation and investigate the underlying molecular mechanisms.
Methods: Primary rat osteoblasts were obtained from the calvarias of neonatal rats. Osteoblasts were treated with LF (0.1–1000 μg/mL), or OSI-906 (a selective inhibitor of IGF-1 (insulin-like growth factor 1) receptor and insulin receptor). The IGF-1 was then knocked down by small hairpin RNA (shRNA) technology and then was treated with adding recombinant human IGF-1 or LF. Cell proliferation and differentiation were measured by MTT assay and alkaline phosphatase (ALP) assay, respectively. The expression of IGF-1 and IGFBP2 (IGF binding protein 2) mRNA were analyzed using real-time PCR.
Results: LF promotes the proliferation and differentiation of osteoblasts in a certain range (1–100 μg/mL) in time- and dose-dependent manner. The mRNA level of IGF-1 was significantly increased, while the expression of IGFBP2 was suppressed by LF treatment. Knockdown of IGF-1 by shRNA in primary rat osteoblast dramatically decreased the abilities of proliferation and differentiation of osteoblasts and blocked the proliferation and differentiation effect of LF in osteoblasts. OSI906 (5 μM) blocked the mitogenic and differentiation of LF in osteoblasts.
Conclusion: Proliferation and differentiation of primary rat osteoblasts in response to LF are mediated in part by stimulating of IGF-1 gene expression and alterations in the gene expression of IGFBP2.

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The Regulatory Role of AMPK in Osteogenic Differentiation Of MC3T3-E1 Cells

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Background: Recent studies on bone have shown an endocrine role of the skeleton producing endocrine hormones that regulate energy metabolism and mineral homeostasis, which could be impaired in various human diseases including osteoporosis, obesity, and diabetes-associated bone diseases. As a sensor and regulator of energy metabolism, AMP-activated protein kinase (AMPK) may also play an important role in the regulation of bone metabolism. The current study aimed to study the role of AMPK in osteogenic differentiation.
Methods: (1) MC3T3-E1 cells were induced to diffenentiated into osteoblast and the gene expression profiles and phosphorylation pattern of AMPK subunits were determined in the process of osteogenesis. (2) AMPK activity in MC3T3-E1 cells was up or down regulated by metformin or Compound C as well as overexpression of AMPKα subunit or a constitutively negative AMPKα by lentivirus vectors. ALP and Alizarin Red S staining, as well as expression of various osteoblast-specific genes were determined to evaluate the osteogenic differentiation.
Results: (1) During Osteogenic differentiation of MC3T3-E1 cells, the expression of AMPKα1, β1, γ1 subunits and the activating pattern phospho-AMPKα (Thr172) were up-regulated. (2) Overexpression of AMPKα subunit promoted osteogenic differentiation of MC3T3-E1 cells, while forced inhibition of AMPK by overexpression of a constitutively negative AMPKα (CN-AMPK) or Compound C inhibited osteogenic differentiation of MC3T3-E1 cells.
Conclusion: (1) The activity of AMPK was significantly increased during osteogenic differentiation of MC3T3-E1 cells, which indicated the high demand of energy in osteogenesis. (2) Constitutively activation of AMPK promoted osteogenic differentiation of MC3T3-E1 cells, while constitutively inhibition of AMPK inhibited the osteogenic differentiation.

P90

Strontium Exerts *in Vivo* Anabolic Effect on Intact Rats Through Modulating Osteogenic and Osteoclastogenic Potential Of Bone Marrow Cells

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Background: Strontium (Sr) ralenate is a new agent for postmenopausal osteoporosis. The present study aims to investigate the effect of Sr on intact rats trabecular bone and the possible cellular mechanism by examining the osteogenic and osteoclastogenic potential of bone marrow cells after Sr treatment.
Methods: Twenty four 3-month old female Sprague-Dawley rats were randomly divided into Veh group (vehicle, 0.9% saline) and Sr group (strontium chloride, 4 mmol/kg/d) administrated for 12 weeks p.o.. Serum osteocalcin was measured 4, 8 and 12 weeks after Sr treatment. The effect of Sr on trabecular bone microstructure was analyzed by microCT on proximal tibiae. Static and dynamic histomorphometric analysis was performed to evaluate the bone formation and resorption markers in trabecular bone. Osteogenic and osteoclastogenic genes in the bone marrow were analyzed with real-time PCR assay. Bone marrow cells were cultured and colony formation assay (CFU-F and CFU-ALP) and TRACP staining was evaluated.
Results: Sr treatment resulted in a significant increase in osteocalcin compared with VEH at week 12 (P<0.05). Trabecular bone structure was improved after Sr treatment. Bone volume (BV/TV), trabecular number (Tb. N) and connectivity density (Conn. D) was greater in Sr-treated group compared with Veh-treated group (P<0.05 for all). At the tissue level, mineral apposition rate (MAR) and bone formation rate (BRF/BS) were greater while N. Oc/BS was less in Sr-treated group compared with Veh-treated group (P<0.05 for all). Bone sialoprotein and osteocalcin genes were significantly up-regulated while cathepasin K gene was significantly down-regulated in Sr group (P<0.05 for all). Colony formation assays demonstrated that bone marrow cells from Sr-treated group exhibited higher osteogenic colony (CFU-ALP, P<0.05) while fibroblast colony (CFU-F) was comparable between these two groups (P>0.05). Number of TRACP+ multinucleated cells was less in Sr-treated group than that of Veh-treated group (P<0.05).
Conclusion: Our study confirmed the dual effect of Sr on intact trabecular bone by providing the static and dynamic parameters of bone formation and resorption. Osteogenic potential was greater as assessed by osteoblast-related gene expression and CFU-ALP colonies and osteoclast potential was less.
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Icaritin Promotes Osteoblastogenesis of Bone Marrow Mesenchymal Stem Cells during Exerting Anabolic Effect on Osteoporotic Bone

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Background: Epimedium-derived Flavonoids (EFs) have a potential to treat established osteoporosis in postmenopausal

women. Whether Icaritin (ICT), an active molecular compound from EFs, can exert beneficial effect on osteoporotic bone and the underlying mechanism remains to be explored.
Methods: Eleven-month-old female Wistar rats were divided into five groups with ten animals per group: (1) sham operated (SHAM); (2) ovariectomized (OVX); (3) SHAM+ solvent vehicle (SHAM-SV); (4) OVX+ sv (OVX-SV); (5) OVX + ICT 40 mg/kg body weight/day (OVX-ICT). Groups 1 and 2 were sacrificed after three months to establish that bone loss in proximal tibia had occurred due to OVX. The remaining groups began four months of treatment, at the end of which the animals were also sacrificed. Trabecular BMD in proximal tibia was analyzed by microCT for static histomorphometric parameters and dynamic histomorphometry. The effect of the conditioned serum on proliferation / differentiation of rat-derived bone marrow MSCs were assessed for colony formation assay (CFU-F, CFU-Osteo and CFU-Adipo). Osteogenic and adipogenic related genes expression was analyzed by real-time PCR. MAPK and Wnt signaling pathways were examined by western blotting in MSCs cultures.
Results: A significantly low trabecular BMD in proximal tibia was found by MicroCT at three-month post ovariectomy in OVX Group compared to SHAM Group ($P<0.01$). ICT treatment significantly increased serum bone formation marker ICTP and decreased bone resorption marker TRACP 5b compared to OVX-SV rats ($P<0.05$ for both). Bone volume (BV/TV) and Trabecular Thickness (Tb.Th) were significantly increased in OVX-ICT Group compared to OVX-SV Group ($P<0.05$ for both) by MicroCT analysis. Un-decalcified histological examination demonstrated that ICT significantly increased trabecular bone mineralizing surface (MS/BS, $P<0.05$), bone formation rate (BFR/BS, $P<0.01$) and mineral apposition rate (MAR, $P<0.05$) compared to OVX-SV Group.
Conclusions: Our findings indicated that ICT could exert the anabolic effect on osteoporotic bone at the micro- and tissue level. The underlying mechanism was partially explained by increased osteogenic differentiation of bone marrow MSCs, which required Wnt signaling pathway.

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Effects of Lipoxin A4 on the Osteoclastic Differentiation and Maturation

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Background: Osteoclasts are responsible for the exacerbation of inflammatory bone diseases, since they not only resorb bone matrix, but also produce proinflammatory cytokines. Lipoxin A4 (LXA4) is an endogenous anti-inflammatory mediator that is biosynthesized during the course of inflammation. The present study aims to investigate whether LXA4 directly suppresses osteoclastogenesis.
Methods: Bone-marrow derived macrophages (BMMs) were obtained from 5-week-old mice. A varied concentration of LXA4 was treated during the osteoclast differentiation from BMMs. We evaluated changes in the proliferation, differentiation and maturation of the osteoclasts.
Results: MTT assay revealed that LXA4 did not have any cytotoxic effect on the BMMs. The cell proliferation was significantly reduced on day 3 in the LXA4 (1nM)-treated group. Tartrate-resistant acid phosphatase (TRAP) staining showed that LXA4 decreased both the number and size of differentiated osteoclasts in a dose-dependent manner. In the RT-PCR and western blot analyses, the markers involved in the maturation of preosteoclasts also decreased by the treatment with LXA4.
Conclusion: These results demonstrated that the anti-inflammatory mediator LXA4 directly affects osteoclastogenesis, suggesting that LXA4 can be exploited to develop new therapy for inflammatory bone diseases.

P93

Proteoglycan Glypican-3 Inhibits BMP Activity and Osteogenesis

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Background: Signalling of bone morphogenic proteins (BMPs) is critical in bone; understanding its regulation is important. Previous studies in Drosophila suggested that glypican family (GPC1-6) of matrix proteoglycans may regulate BMP activity, and a GPC3 knockout mouse study reported some alterations in skeletal formation. However, it remains unclear whether and how GPCs regulate BMPs involved in bone formation. This study investigated roles of GPCs in regulating BMP activity and osteogenesis.
Methods: Expression of the major glypicans (GPC1 & GPC3) and osteogenic BMPs was examined by qRT-PCR during osteogenesis in rat bone marrow stromal cells. To examine effect of glypicans on BMP activity, BMP-2, 4, or 7-mediated Smad activation pID183-luc reporter assays were conducted in mouse osteogenic cells (C3H10T1/2 progenitor and MC3T3E1 osteoblastic cells) transfected with/without GPC-1 or GPC-3 transgene. GPC protein or neutralising antibody treatment effects on BMP-2-mediated BMP activity and osteogenesis were examined in MC3T3E1 osteoblastic cells. We are also currently examining bone phenotypes of GPC3 knockout mice.
Results: mRNAs of GPC1, GPC3 and BMPs were found to be differentially expressed during osteogenesis in rat. Bone marrow stromal cells. While BMP2 and BMP4 rose, BMP7 decreased; and while GPC1 increased, GPC3 declined during osteogenesis, suggesting an inverse relationship in levels of GPC3 with BMP2/BMP4. Ectopic expression of GPC3 (but not GPC1) was found to block activities of BMP-2, 4, and 7 in both C3H10T1/2 progenitor and MC3T3E1 osteoblastic cells. BMP2 activity was also found to be inhibited by treatment with GPC3 (but not GPC1) protein, but enhanced by anti-GPC3 antibody. GPC3 protein suppressed BMP2-mediated osteogenesis in MC3T3E1 cells. We are also currently examining bone phenotypes of GPC3 knockout mice.
Conclusion: The current study observed an inverse relationship in expression levels of BMP2/BMP4 and glypican-3 during osteogenesis, and that glypican-3 (but not glypican-1) inhibits BMP activity and suppresses osteogenesis.

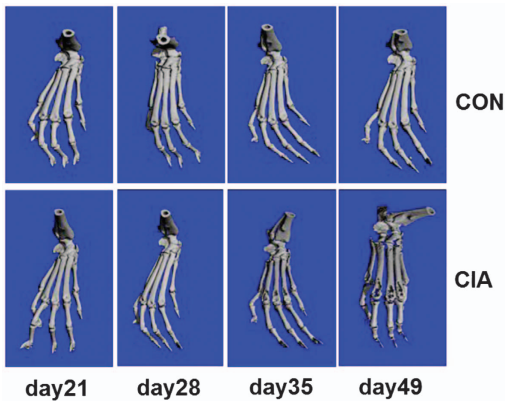

Figure 1 Time course change in bone architecture by microCT after immunization.

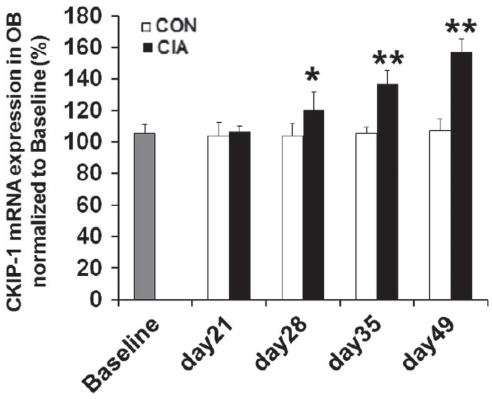

Figure 2 Time course change in CKIP-1 mRNA expression in osteoblasts after immunization.

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Aberrant Overexpression Of CKIP-1 in Osteoblast Correlated with Progressive Bone Erosion and Decreased Bone Formation in Collagen-Induced Arthritis of Mice

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Background: CKIP-1 is an intracellular negative regulator for bone formation. However, its effect in failure of osteoblast-mediated repair for articular bone erosion in rheumatoid arthritis is still unknown. This study is to investigate the changes of CKIP-1 expression in osteoblast, bone erosion and bone formation in mice with collagen-induced arthritis.

Methods: Male DBA/1J mice (8 weeks old) were randomly divided into the three groups: collagen-induced arthritis group (CIA), control group (CON) and baseline group (BL). CIA was induced by immunization twice with bovine type II collagen and complete Freund's adjuvant. On day 21, 28, 35 and 49 after immunization, the hind limbs of ten mice from CIA group and CON group were sacrificed. Mice in BL group were sacrificed on day 0. MicroCT, histology, bone histomorphometry and Q-PCR were used to detect bone architecture, bone erosion, bone formation and CKIP-1 mRNA expression in osteocalcin-positive cells, respectively.

Results: In CIA mice, the BMD, BV/TV, BFR/BS, MS/BS, MAR, and O.Pm/T.Pm in tarsal area were decreased over time from day 28 after primary immunization (see Figure 1). Furthermore, the levels of those examined parameters were all significantly lower in CIA group than those in CON group on day 35 and 49, respectively. In addition, the score of bone erosion in CIA group was continuously increased from day 28 after immunization. On the other hand, the CKIP-1 mRNA expression in osteoblasts from CIA group was increased over time from day 28 and also showed significantly higher than that in CON group on day 28, 35 and 49, respectively (see Figure 2).

Conclusion: The results showed that increased CKIP-1 expression in osteoblasts was accompanied by progressive bone erosion and decreased bone formation in CIA mice, implying the role of CKIP-1 in failure mechanism of osteoblast-mediated repair for articular bone erosion in rheumatoid arthritis.

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PTH Promotes Angiogenic Differentiation of Intravenous Delivery Mesenchymal Stem Cell During Bone Repair May via VEGF Induction

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Background: We showed that PTH enhances intravenous (IV) human mesenchymal stem cells (hMSC) homing to fracture callus. While PTH enhances osteogeneic and chondrogenic differentiation of IV delivered hMSC during fracture healing, our recently finding indicated that PTH could also significantly increase vascular formation within the new callus. To investigate the mechanism beneath, we performed quantitate Micro-CT scan and applied serials immunohistochemistry (IHC) staining on a murine femoral fracture that received IV MSCs followed a consecutive PTH treatment.

Method: Stabilized right femoral fractures were generated in 8-week-old C57BL/6 female mice for experimental groups of (1) vehicle (0.9% saline), (2) 10^5hMSCs IV, (3) PTH 40ug/kg/d subcutaneously for 14 days, (4) 10^5hMSCs IV + PTH 40ug/kg/d (14 days). At the day 14, mice were euthanized by ketamine &xylazine following perfused with lead based chromate dye Microfil® MV-120 (Flowtech Inc.) to show capillaries within fracture callus. Before and after decalcification, the Micro-CT (VivaCT40, Scanco USA Inc.) was employed to quantify the volume of new bone and vasculature, respectively. The distributions of vasculature within new bone callus were analyzed by Micro-CT 3-D reconstruction, which followed by histological confirmation of anti-CD31, *VEGF, VEGFR1* and *VEGFR2*

immunohistochemistry staining. The expression level of CD31, *VEGF, VEGFR1* and *VEGFR2* within trabecular bone, mesenchymal tissue and cartilage were quantitated by Visiopharm imaging software.
Results: PTH significantly increased total volume and the mass of new bone callus, as well as small vessels: Micro-CT analysis and 3-D reconstruction showed total bone volume of PTH with (36.8±5.0 mm^3) or without hMSC (34.9±6.1 mm^3) significantly (*P<0.05*) increased compared with vehicle or hMSCs alone. Coordinately findings were that trabecular bone number in hMSC, PTH or PTH+hMSCs groups were significantly increased. But there were no significant in bone mass density (BMD), trabecular bone thickness and separation in all. IHC was employed for angiogenic related factors CD-31, VEGF, VEGFR1, and VEGFR2 staining. CD-31 was highly expressed both trabecular bone and the mesenchymal tissue that treated with PTH and /or hMSCs. But none of group had shown in cartilage. PTH and hMSCs combination dramatically increased CD-31, VEGF and VEGFR1 expression in mesenchymal tissue, but not VEGFR2.
Conclusion: PTH induced IV hMSCs homing and significantly increased total bone volume and bone mass in fracture repair. It may through promotes IV hMSCs undergo angiogenic differentiation that increased capillary formation. The molecular mechanisms beneath are still under investigating.

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The Expressions of FGF2 And BMP2 in The Kidney and Bone of Osteoporosis Rats

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Background: To investigate the expressions of fibroblast growth factor 2 (FGF2) and bone morphogenetic protein 2 (BMP2) in the kidney and bone of osteoporosis rats.
Methods: Wistar female rats were randomly divided into sham control group (NS) and ovariectomy (OVX) group. After ovariectomy 4,8,12 weeks, each 5 rats chose randomly to separate the bone and renal tissues. Used immunohistochemistry to observe the renal tissue express sites of FGF2 and BMP2, using RT-PCR measured the mRNA levels of FGF2 and BMP2 in kidney and bone of each group, using western blot measured the protein levels of FGF2 and BMP2 in kidney of each group.
Results: Immunohistochemistry results showed FGF2 and BMP2 expressed mainly in the cytoplasm of renal tubular epithelial cells in renal tissues. The results of bone RT-PCR showed that at 4, 8 and 12 weeks, the BMP2 and FGF2 mRNA expression levels of OVX group were all lower than NS group ($P<0.05$), and the results of kidney RT-PCR and western blot showed that, the BMP2 and FGF2 mRNA and protein expression levels of OVX group were lower than NS group just at 12 weeks ($P<0.05$) but not 4 and 8 weeks ($P>0.05$).
Conclusion: The expressions of FGF2 and BMP2 decreased different rate in the bone and kidney of osteoporosis rats.

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The New Bone Formation And Microstructure Assessed by Combination of Confocal Laser Scanning Microscopy and Differential Interference Contrast Microscopy

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Background: Bone is a mineralized connective tissue that is continuously and micro-structurally remodelled. Altered bone formation and microstructure arise in pathological bone diseases such as osteoporosis, osteonecrosis, fracture repair and Paget's disease of bone. A proper and objective assessment of bone formation and microstructure will provide insights into the understanding of bone pathogenesis and remodeling.
Methods: Here, the new bone formation *ex vitro* and its microstructure was evaluated in *in vivo* multiple sequential polychrome-labelled samples using confocal laser scanning microscopy (CLSM), which generated clearer and more reliable images of thick bone sections than conventional fluorescence microscopy (CFM). Intriguingly, fine details of the bone microstructural features, including the mineralization fronts, quiescent versus active osteons, and Volkmann's channel, were elucidated using CLSM, which defines relationship between morphological changes and function, when combined with differential interference contrast (DIC) microscopy.
Results: CLSM provided objective evaluations of bone formation, such as the ratio of labelled areas of new bone formation in a rabbit model when compared with CFM. Altogether, new bone formation and its microstructure can be evaluated more adequately using combination of the CLSM and DIC microscopies.
Conclusion: The new bone formation and bone microstructure, including the mineralization fronts were investigated by CLSM, which advantaged in adjustments of operating parameters, thickness of optical sections and automatic image acquisition and analysis. Polychrome sequential fluorescence quantification is more accurate with higher repetitiveness than CFM and areas ratio of sequential fluorescent labels in new bone formation by CLSM in healing bone was calculated as new bone growth rate, a better estimation of bone growth in repairing. CLSM combined with DIC technique further generated sequentially fluorescent labeling images showing mineral apposition with details of microstructure, including osteonsand Volkmann's channel. Presence or absence of fluorescent labels in osteons reflect their activeness during the bone formation and remodeling, which renders a new imaging process for the study of bone mineral metabolism and fracture healing.

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Gene Expression Profiling: Identification of Genes with Altered Expression in Tumor-Induced Osteomalacia (TIO)

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Background: Tumor-induced osteomalacia (TIO) is a rare paraneoplastic syndrome induced by excessive production of fibroblast growth factor 23 (FGF23), which causes acquired hypophosphatemic osteomalacia. TIO tumors are almost all benign mesenchymal tumors, occurred in soft tissue and bone sites. However, the mechanism for why excessive FGF23 is secreted by TIO tumors still remains unclear. Hence, we conducted this study to evaluate the possible roles and mechanisms involved in this process.

Methods: For gene expression analysis, 6 TIO tumors (3 from soft tissues and 3 from bone) were compared with 4 non-osteomalacia mesenchymal tumors (3 from soft tissues and 1 from bone). Total RNA from each sample was amplified and transcribed into fluorescent cRNA. Microarray analysis was performed by Agilent Array platform. The acquired data were analyzed by special softwares. Differentially expressed genes with statistical significance were identified. Pathway analysis and GO analysis were applied to determine the roles of these differentially expressed genes played in these biological pathways or GO terms.

Results: Microarray gene expression analysis showed 591 genes were downregulated and 574 genes were upregulated. *COL4A5, PLCL2, RBBP9, ZBTB32* and *ALDH5A1* were among the most downregulated genes, whereas DEFB103B, DFNB59, CEP85, LEUTX, PPP2R2A and ICAM5 were significantly upregulated in the TIO group compared with the control group. Functional analysis showed that the TNF signaling, carbohydrate digestion and absorption pathways were significantly downregulated, while Wnt signaling was upregulated in the TIO group. The GO terms related to macromolecule localization, anatomical structure morphogenesis, cellular developmental process and cell adhesion were significantly affected.

Conclusion: The results suggest that the comprehensive mechanisms were involved with complex gene expression-alterations encompassing many altered pathways and GO terms in TIO tumors, which provides some new insights into the study of TIO. However, future study is needed to elucidate how FGF23 is regulated by these tumors.

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Specific Effects of Adenovirus-Mediated Sirna Targeting Pparγ2 on Osteogenic Differentiation of C2C12 Cells Induced by BMP9

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Background: Bone morphogenetic protein 9 (BMP9) is one of the most potent osteoinductive factors in tissue engineering. Previous studies have demonstrated that peroxisome proliferator-activated receptor γ2 (PPARγ2), a critical adipogenic transcription factor, is the downstream target of BMP9. The aim of this study is to explore the specific effects of PPARγ2 on BMP9-induced osteogenic differentiation of myoblast C2C12.

Methods: Recombinant adeneviruses expressing BMP9 (AdBMP9) and small interfering RNA targeting PPARγ2 (AdsimPPARγ2) were used respectively to deliver BMP9 and silence PPARγ2. The expression level of PPARγ2 was examined using Reverse Transcription PCR (RT-PCR) and Western blotting in C2C12 cells infected AdsimPPARγ2. Then, histochemical staining was used to test the early osteogenic marker alkaline phosphatase (ALP) activities when C2C12 cells were infected with AdBMP9 and/or AdsimPPARγ2 for 3, 5 or 7 d. The protein level of osteopontin (OPN) and osteocalcin (OCN) in the C2C12 cells after treatment of BMP9 and/or PPARγ2 silencing for 1, 3 or 14d was detected by Western blotting. The mineralization in the cells was tested with Alizarin red S staining after the treatment for 14 d. The activation of BMP-Smad signaling was studied with BMPR-Smad binding site luciferase reporter assay and Western blotting to determine the phosphorylation level of Smad1/5/8. Finally, RT-PCR and Western blotting were performed to examine the expression of PTEN and insulin-like growth factor 1 (IGF1) in C2C12 cells infected with AdBMP9 and/or AdsimPPARγ2 for 12h.

Results: The endogenous expression of PPARγ2 was markedly decreased in C2C12 cells infected with AdsimPPARγ2 at both mRNA and protein levels. Knockdown of PPARγ2 effectively increased BMP9-induced ALP activities and expression of OPN and OCN at early stage in C2C12 cells. However, PPARγ2 silencing significantly inhibited BMP9-induced mineralization and OCN expression at 14 days post treatment. Mechanistically, PPARγ2 silencing enhanced BMP9-induced Smad signaling and attenuated PTEN activity. Moreover, PPARγ2 silencing reversed the inhibitory effect of BMP9 on IGF1, and IGF1 facilitated BMP9-induced ALP activities.

Conclusion: PPARγ2 silencing can enhance osteogenesis induced by BMP9 inearly phase through modulating BMP-Smad and IGF1 signaling pathways.

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Overexpression of Mir-378 Attenuates High Glucose-Suppressed Osteogenic Differentiation in MC3T3-E1 Cells and Activates the PI3K/Akt Signaling Pathway

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Background: Hyperglycemia is one of the possible causes for osteoporosis and bone fracture in diabetes mellitus. MicroRNAs are single-stranded RNA containing 18-24 nucleotides that play an important role in many physiological and pathological processes. In this study, the relationship between microRNA-378 and osteogenic differentiation has been investiageted to reveal the underlying mechanism of diabetes-induced osteoporosis.
Methods: (1) we modeled diabetes-induced osteoporosis *in vitro* using preosteoblastic cell line MC3T3-E1, (2) Lentivirus production and cell transfection, (3) Cell viability assay, (4) Cell apoptosis analysis, (5) Analysis of mineralization, (6) Quantitative real-time polymerase chain reaction (qRT-PCR), (7) Luciferase reporter assay.
Results: We found that in addition to reducing osteoblast viability and differentiation (mineralization), culture in elevated glucose down regulated microRNA-378 (miR-378) expression but ectopic miR-378 expression reverses the effects of high glucose. We identified caspase-3 (CASP3) as a target of miR-378 and show that miR-378 represses CASP3 mRNA and protein expression in MC3T3-E1 cells. We further show that both miR-378 expression and CASP3 silencing independently restored ALP activity and the expression of osteoblastic differentiation markers Runx2, Osteorix (Osx), Collagen I (Col I), Osteocalcin (OC), and Osteonectin (ON). We also found that under high glucose conditions miR-378 activates the PI3K/Akt signaling pathway and down regulates pro-apoptotic CytC, Apaf-1 and Bax proteins via the PI3K/Akt pathway.
Conclusion: Collectively, these results suggest that miR-378 overexpression attenuates high glucose-suppressed osteogenic differentiation through targeting CASP3 and activating the PI3K/Akt pathway to suppress additional pro-apoptotic proteins.

P101

Lactoferrin Inhibits Apoptosis through Insulin-Like Growth Factor I in Primary Rat Osteoblasts

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Background: The study is to excessive apoptosis of osteoblasts is the major cause of low bone mass, and bovine lactoferrin (bLF), an iron-binding glycoprotein, might protect osteoblastic cells from apoptosis induced by serum withdrawal. The aim of this study was to elucidate the mechanisms underlying the anti-apoptotic action of bovine LF in rat osteoblasts *in vitro*.
Methods: Primary rat osteoblasts were incubated in the presence of varying concentrations of bovine LF for 24 h. The expression of insulin-like growth factor I (IGF-I) and IGF-I receptor (IGF-IR) was measured uisng real time PCR and western blotting. Cell apoptosis was examined with flow cytometry. siRNAs targeting IGF-I was used in this study.
Results: Treatment of bLF (0.1–1000 μg/mL) dose-dependently increased the expression of IGF-I and IGF-IR in the osteoblasts. Treatment with bLF (10–100 μg/mL) markedly inhibited the osteoblast apoptosis (with the rate of total apoptosis of 70% at 10 μg/mL), but the high concentration of bLF (1000 μg/mL) significantly promoted the osteoblast apoptosis. Knockdown of the IGF-I gene in osteoblasts with siRNA markedly increased the osteoblast apoptosis.
Conclusion: Lactoferrin (10 and 100 μg/mL) effectively inhibits apoptosis of primary rat osteoblasts by upregulating IGF-I expression.

P102

Dose-Dependent Effect of Estrogen Suppresses the Osteo-Adipogenic Transdifferentiation of Osteoblasts via Canonical Wnt Signaling Pathway

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Background: Fat infiltration within marrow cavity is one of multitudinous features of estrogen deficiency, which leads to a decline in bone formation functionality. The origin of this fat is unclear, but one possibility is that it is derived from osteoblasts in bone, which transdifferentiate into adipocytes and further form bone marrow fat.
Methods: We used ALP staining and activity, Oil red staining, RT-PCR, Western-blot and immnocytochemistry examined the dose-dependent effect of 17 beta-estradiol on the capacity of MC3T3-E1 cells and murine BMMSCs derived osteoblasts undergoing osteo-adipogenic transdifferentiation.
Results: We found that 17beta-estradiol caused a significant ($P<0.05$) elevation of alkaline phosphatase (ALP) staining and activity, calcium deposition, and the transcriptional expression of *Alp, Col1a1*, *Runx2* and *Ocn* by dose-dependent manner. While 17beta-estradiol also decreased lipid sizes and numbers and the transcriptional expression of *Fabp4* and *PPARγ* in the presence of osteo-adipogenic transdifferentiation ($P<0.05$). Moreover, brown adipocyte markers such as *Myf5, Elovl3* and *Cidea* and undifferentiated adipocyte makers including *Dlk1, Gata2* and *Wnt10b* were also modulated by the effect of 17beta-estradiol during the osteo-adipogenic transdifferentiation process. Western bolt and immunostaining further showed that canonical Wnt signaling could be activated by estrogen to exert its inhibitory effect of osteo-adipogenesis.
Conclusion: This is the first study to demonstrate dose-dependent effect of 17beta-estradiol on the osteo-adipogenic transdifferentiation of MC3T3-E1 cells and BMMSCs via canonical Wnt signaling pathway partially or even dependently, suggesting that osteo-adipogenic transdifferentiation modulated by canonical Wnt signaling pathway in bone metabolism may be a new explanation for the gradually increased bone marrow fat in estrogen-inefficient condition.

P103

Osteoblast Proliferation Is Enhanced upon the Insulin Receptor Substrate 1 Overexpression via PI3K Signaling Leading to Down-Regulation of BAX

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Background: We previously showed the incidence of type 2 diabetes with osteoporosis maybe associated with inhibition of IGF-1, IRS-1 and IRS-2 expression, reduction of PI3K, AKT expression and increased expression of NFκB in bone tissue. In this study, we transfected of rat osteoblasts *in vitro*, observed NfκB, mRNA and protein expression in osteoblasts, to clarify a new mechanism under diabetes osteoporosis.
Methods: Primary rat osteoblasts were isolated from neonatal Wister rat calvariae. Osteoblasts were transfected with pEGFP-N1 or pEGFP-N1 encoding wild-type IRS1 (pEGFP-N1-IRS1). Cell cycle analysis was performed using flow cytometry. The IRS1 mRNA expression level was measured by RT-PCR. The expression levels of $pAkt^{Thr308}$ and BAX were measured by Western blotting.
Results: The level of IRS1 mRNA was significantly increased in pEGFP-N1-IRS1 cells compared to group (control) and group (N1). The level of $pAkt^{Thr308}$ protein, target receptor of IRS1 signaling, was increased in pEGFP-N1-IRS1 cells, and the effect induced by pEGFP-N1-IRS1 was abolished by incubation of LY294002 (P<0.05; n=3). Both the levels of IRS1 mRNA and pAktThr308 protein were increased in pEGFP-N1-IRS1 cells, which revealed that pEGFP-N1-IRS1 resulted in activation of PI3K/Akt signaling in osteoblasts. Recombinant pEGFP-N1-IRS1 stimulated osteoblast proliferation, as evidenced by an increase in the number of cells in the S phase compared to controls (P<0.05; n=3). Recombinant pEGFP-N1-IRS1 inhibited expression of BAX in osteoblast, and the effect was reversed in presence of LY294002 (a PI3K inhibitor) (P<0.05; n=3).
Conclusion: IRS1 down-regulates NFκB and BAX pathway through PI3K/Akt signaling, which may contribute to promoting osteoblast proliferation. The findings of the present study may contribute to better understanding the association between diabetes and abnormal bone metabolism.

P104

The Influence of Pparγ Gene Silencing on The Osteogenic and Adipogenic Differentiation of Rat Bone Marrow Stromal Cells

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Background: Peroxisome proliferator-activated receptor γ (PPARγ), considered to be the master regulator, is both necessary and sufficient for adipogenesis. However, there are few reports regarding the effect of PPARγ regulation on osteogenic and adipogenic differentiation in rat bone marrow stromal cells (BMSCs). The purpose of this study is to investigate the effect of PPARγ gene silencing on osteogenic and adipogenic differentiation in rat BMSCs.
Methods: We use short hairpin RNA (shRNA) sequences targeted to PPARγ in the pSilencerTM2.1-U6 expression vector. Cultured BMSCs were transfected with the PPARγ-shRNA or a non-target control shRNA (shNTC), cells were cultured in osteogenic or adipogenic medium for 14 days, we determine the osteogenic and adipogenic gene and protein levels by RT-PCR and Western blot assays, the differentiation of BMSCs were analyzed by alkaline phosphatase (ALP), Oil red O staining, Alizarin red S staining, calcium deposition.
Results: The expression of the adipogenic factors adipocyte determination and differentiation-dependent factor 1 (ADD1) and recombinant CCAAT/enhancer binding protein alpha (C/EBPα) were decreased as measured by RT-PCR and Western blot assay following PPARγ silencing. In contrast, expression of the osteogenic genes encoding collagen I and Cbfa1/Runx2 were increased. In adipogenic medium, PPARγ-shRNA transfection reduced the lipid droplet count as measured by Oil red O staining when compared to the control groups. In osteogenic medium, PPARγ-shRNA increased the activity of alkaline phosphatase and the amount of calcium deposition as measured by Alizarin red S staining.
Conclusion: These results indicate that the downregulation of PPARγ by shRNA decreases adipogenic differentiation and promotes osteogenic differentiation in a rat BMSC model system.

P105

Osteogenic Differentiation of Human Mesenchymal Stromal Cells Promoted by Acoustic Vibration

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Background: Bone ingrowth and absorption are closely related to the mechanical environment. Mechanical vibration has been shown to be effective in treatment and prevention of osteoporosis. However, current vibration has largely been limited to lower frequency (1-100 Hz). In this study, we aimed to delineate whether acoustic vibration signals could influence human mesenchymal stromal cells (hMSCs) toward bone formation *in vitro*.
Methods: hMSCs were exposed to repeated bouts of the stimulus at an acceleration of 0.3 g and different frequencies of 30, 400 and 800 Hz for 30 min/day for 7 or 14 days *in vitro*. hMSCs markers of osteoblastic differentiation, and their functional capacity to form mineralized bone nodules were examined.
Results: Expression of ALP, Col I, OPN, Runx2, and Osx were up-regulated in all vibration groups (30, 400 and 800 Hz) at 7 days. The highest response of these related genes was due to the 800 Hz regimes. It enhanced ALP, Col I and Osx mRNA expression most significantly, which exhibited 5.3-, 2.4 and 4.6-fold increases, and significantly larger than the response to 30 and 400 Hz. After 14 days, hMSCs vibrated at 800 Hz also displayed significantly higher osteogenic-related gene expression, with increases of 2.3-fold at ALP, 1.2-fold, Col I, 1.8-fold at OPN and 1.8-fold at Osx, and calcium was deposited

nearly 1.5-fold. But 30 and 400 Hz groups significantly inhibited osteogenesis.
Conclusion: The findings suggested that the acoustic vibration was responsible for hMSCs osteogenic differentiation, and acoustic vibration at 800 Hz was the most favorable for hMSCs osteogenic differentiation. Further studies are necessary to verify the long-term viability and commitment to osteogenic differentiation of hMSCs, with the purpose of determining whether acoustic vibration could potentially become a novel means to treat osteoporosis.

P106

Decreased Bone Mass of Femora in Both High Fat Diet-Induced and Genetic Hyperlipidemia Mice Possibly through Different Mechanisms

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Background: This study was designed to determine and compare the effects of hyperlipidemia induced by high-fat diet and low-density lipoprotein receptor gene knockout on bone metabolism in mice.
Methods: Wild-type mice and low-density lipoprotein receptor gene knockout (*LDLR–/–*) mice at age of 8 weeks were placed on either control diet or high-fat diet for 12 weeks. Bone structural parameters were determined with μCT. Bone-related gene expression including runt-related transcription factor 2 (Runx2), collagen type I alpha 1 (Col1a1), osteoprotegerin (OPG) and the receptor activator of nuclear factor-kappaB ligand (RANKL) from distal metaphyses of bone was studied by qRT-PCR. Bone marrow stromal cells (BMSC) were cultured to observe the expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and peroxisome proliferator-activated receptor gamma (PPARγ).
Results: Our research showed that the trabecular bone mass was significantly lower in both diet-induced and genetic hyperlipidemia mice. In hyperlipidemia mice, the expression of Runx2 and Col1a1 was reduced, indicating impaired bone formation in these mice. The expression of PPARγ and HMGCR in BMSC was significantly up-regulated in *LDLR–/–* mice on either diet.
Conclusion: We concluded that both diet-induced and genetic hyperlipidemia decreased bone mass as a result of impaired bone formation in mice. The up-regulation of PPARγ in BMSC may be responsible at least partly for impaired bone formation in *LDLR–/–* mice.

P107

Ginsenoside-Rb2 Displays Anti-Osteoporosis Effects through Reducing Oxidative Damage and Bone-Resorbing Cytokines During Osteogenesis

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Background: Reactive oxygen species (ROS) is a significant pathogenic factor of osteoporosis. Ginsenoside-Rb2 (Rb2), a 20(S)-protopanaxadiol glycoside extracted from Ginseng, is a potent antioxidant, which raises popular concern among the bone metabolism area. We try to testify anti-osteoporosis effects that Rb2 might possess and its underlying mechanisms by this study.
Methods: We built an oxidative damage model induced by hydrogen peroxide (H_2O_2) in osteoblastic MC3T3-E1 cells to testify the essential anti-osteoporosiseffects of Rb2. Murine MC3T3-E1 cells were purchased from the Center Laboratory for Tissue Engineering, College of Stomatology, Fourth Military Medical University, Xi'an, China. H_2O_2 acted as exogenous ROS treatment, while N-acetyl-L-cysteine (NAC) acted as a ROS cleaner.
Results: The results showed that Rb2 promoted proliferation of MC3T3-E1 cells significantly ($P<0.05$), improved alkaline phosphatase (ALP) expression, elevated calcium mineralization and mRNA expression of Alp, Col1a1, osteocalcin (Ocn) and osteopontin (Opn) against oxidative damage induced by H_2O_2. More importantly, Rb2 reduced expression of receptor activator of nuclear factor kappa-B ligand (RANKL), IL-6 and inhibited H_2O_2 -induced production of ROS to some degree.
Conclusion: Taken together, these results demonstrated that the anti-osteoporosis effects of Rb2are linked to a reduction of oxidative damage and bone-resorbing cytokines, suggesting that Rb2 may be effective in anti-osteoporosis area.

P108

Leonurine Hydrochloride Inhibits Osteoclastogenesis and Prevents Estrogen Deficiency-Induced Osteoporosis through Inhibition of NF-κb And PI3K/Akt Signaling Signaling Pathways

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Background: Osteoclasts, the primary bone-resorbing cells, are responsible for bone destructive diseases such as postmenopausal osteoporosis, rheumatoid arthritis, and periodontitis. Many plant-derived medicines used in traditional medicinal systems that may suppress the formation and/or function of osteoclasts is promising for the treatment of osteoclast-related diseases.
Methods: In this study, we investigated changes in RANKL-induced osteoclastogenesis and ovariectomy-induced bone loss in response to Leonurine Hydrochloride (LH), is a chemical compound synthesized based on the structure of leonurine in motherwort.
Results: In RAW264.7 cells and mouse bone marrow monocytes (BMMs), LH suppressed RANKL-induced osteoclastogenesis and actin ring formation in a dose-dependent manner. Specifically, LH targeted RANKL-induced osteoclastogenesis and bone resorption at an early stage. Further molecular analysis revealed that LH attenuated RANKL-induced nuclear factor-kappaB (NF-κB) signalling by inhibiting the phosphorylation and degradation of IκBα and NF-κB p65 nuclear translocation. Moreover, LH also inhibited the RANKL triggered RANK-TRAF6 association and the phosphatidylinositol 3-kinase (PI3K)/Akt axis, without significantly affecting the extracellular signal-regulated kinase (ERK)/mitogen-activated

protein kinase (MAPK) and AP-1 signalling pathway. The RANKL stimulated the expression of osteoclast-related genes including NFATc1, TRAP, cathepsin K, and osteoclast-associated receptor (OSCAR) also diminished by LH. Consistent with the *in vitro* results, administration of LH prevented estrogen deficiency-induced bone loss in mice by attenuating osteoclast activity.

Conclusion: Our results demonstrated that LH suppresses RANKL-induced osteoclastogenesis *via* RANK-TRAF6, NF-κB, and PI3K/Akt signaling signaling pathways. Therefore, these data indicated that LH is a promising therapeutic compound for the treatment of osteoclast-related diseases, such as osteoporosis.

P109

Effect of Zoledronate on Particle-Induced Osteolysis *in Vivo* and *in Vitro*

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Background: Aseptic loosening is a key factor that causes implant failure of joint replacement. This study was aimed to investigate the effect of zoledronate on particle-induced osteolysis *in vivo* and *in vitro*.

Methods: First, osteoclast precursors were isolated from the long bones of six-week-old C57BL/6J mice and assigned into 6 groups with different component: in Group A, the culture solution was added; in Group B, the macrophage colony stimulating factor (M-CSF), the receptor activator of nuclear factor kappa ligand (RANKL) and culture solution was added; in Group C, the titanium particles and culture solution was added; in Group D, the solution (the supernatant of cultured mouse macrophages induced by titanium particles after 24h) and culture solution was added; in Group E, the M-CSF, RANKL, ZOL and culture solution was added; in Group F, the solution, ZOL and culture solution was added. After 10 days coverslips were stained by the tartrate-resistant cultured acid phosphatase (TRAP). And the activity of the osteoclast cells was detected with the area of osteo-assay surface resorption. The TRAP positive multinucleated cells and the resorpion lacunae were observed in group B, group D, group E and group F.

Results: In group F (5.54%±1.25%), the area of resorption lacunae was smaller than that in group D (10.34%±1.69%, $P<0.01$, t=5.61). We demonstrated that the zoledronate could inhibit the activation of osteoclasts induced by the titanium particles *in vitro*. On the other hand, the model of particle-induced osteolysis in mice skull was established, and then a single dose of zoledronate was locally administered to investigate its effect on particle-induced osteolysis. Ninety-six 12-week-old C57BL/6J mice were equally allocated into eight groups, and different dosages of zoledronate were added. Fourteen days after operation, the animals were sacrificed in CO_2. According to the CT scan and Micro-computed Tomography, it showed an inverse correlation between dosage of zoledronate and the extent of bone resorption.

Conclusion: According to these results, zoledronate may have the potential to be a therapeutic target for particle-induced osteolysis.

P110

GPR40 Protects from Bone Loss via Promotion of Osteogenic Differentiation of Bmmscs

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Background: In osteoporosis, bone marrow fat increased dramatically and nearly filled up in marrow cavity. However, the relationship between fat and bone remained controversial. GPR40, as the G protein-coupled receptors of long chain unsaturated fatty acids, has demonstrated to have anti-inflammatory effect of macrophages and activating Ca^{2+} signaling response to fatty acids.

Methods: By demonstrating that the expression level of GPR40 can be affected by osteogenesis of BMMSCs, we hypothesized that this receptor may take a part in promoting osteogenic differentiation of BMMSCs and play a positive role in bone remodeling *in vivo*.

Results: In primary cultured BMMSCs, we showed that GW9508, a selective GPR40 agonist, promote osteogenic differentiation of BMMSCs. What's more, *in vivo* administration of GW9508 rescued estrogen-deficient bone loss, indicating the essential role of the GPR40 receptor.

Conclusion: Therefore, in the prevalence of age and metabolic related disorders, especially osteoporosis, our findings suggest for the first time that GPR40 is not only a key to understand the link between bone and fat but also an underlying target for the treatment of bone complications in the foreseeable future.

P111

The Effects of Atorvastatin in the Prevention of Osteoporosis and Hyperlipaemia in the Ovariectomized Rats Fed with High Fat Diet

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Background: Previous studies have reported statins showed positive effects on bone in human and animal models. This study was to investigate the effects of atorvastatin in the prevention of osteoporosis and hyperlipaemia in the ovariectomized rats fed with high fat diet.

Methods: 3-month-old SD female rats were subjected to either sham operation (n=8) or ovariectomized operation (OVX, n=24). The OVX rats were orally administered deionised water (n=8) or 5ml/kg/d standardized high fat emulsion (n=16). Then the high fat diet-treated rats were orally administered without addition (n=8) or 3.6mg/kg atorvastatin (n=8). After 12 weeks, all rats were killed under anesthesia. Blood, femur, tibia, 5th and 6th lumber vertebra were collected for biochemistry, four-point-bending mechanical test, histomophometry and micro CT analysis, respectively.

Results: Cholesterol and low density lipoprotein cholesterol were significantly decreased while high density lipoprotein cholesterol was significantly increased in the atorvastatin-treated rats compared to that of the rats without treatment. Micro CT data showed significant increase in the bone mass (BV/TV) and better organized microarchitecture were found in the atorvastatin treated rats than that of the rats without treatment. Histomophometric data showed significantly increased bone formation (MS/BS, MAR, BFR/BS, and BFR/BV) happened in the atorvastatin treated rats compared to that of the rats without treatment. Biomechanical data showed increased maximum loading and fracture loading happened in the atorvastatin treated rats compared with that of the high fat diet-treated OVX rats.
Conclusion: Low body weight, hyperlipidaemia, low bone mass and poor bone mechanical properties were observed in the rats treated with high fat diet-treated OVX rats. Atorvastatin showed preventive effects on the hyperlipidaemia and bone loss, which suggested that atorvastatin may not only play a role in the treatment of hyperlipidaemia but also play a role in the prevention of osteoporosis.

P112

The Effects of Galangin on Osteoblast Proliferation, Differentiation and Apoptosis *in Vitro*

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Background: Galangin is a strong antioxidant natural active molecules with flavonoid structure. This study was undertaken to investigate the effects and mechanism of Galangin on osteoblast proliferation, differentiation and apoptosis within the condition of oxidative stress *in vitro* culturing osteoblasts from SD rats.
Methods: Osteoblasts were obtained from craniums of newly born SD rats and were cultured by multiple enzyme digestion method *in vitro*. The effects of different doses of galangin (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3}g/ml) on osteoblast proliferation, differentiation and mineralization were determined by MTT, p-nitrophenyl phosphate (PNPP), alizarin red staining respectively. The effects of different doses of galangin on osteoblast proliferation, apoptosis, and reactive oxygen species were also determined by MTT, flow cytometry with Hoechst 33258 fluorescent probe and DCFH - DA fluorescence probe respectively within the condition of oxidative stress induced by H_2O_2 (100uM/L).
Results: Compared with the control group, 10^{-5} g/ml galangin promoted osteoblast proliferation (P<0.05) and increased the activity of alkaline phosphatase significantly (P<0.01); 10^{-5} g/ml and 10^{-4} g/ml galangin both increased the mineralized nodule number and mineralization area significantly (P<0.01) without H_2O_2 intervention. Compared with H_2O_2 treatment group, 10^{-5} g/ml galangin promoted osteoblast proliferation within the condition of oxidative stress induced by H_2O_2. Flow cytometry results demonstrated that 10^{-5} g/ml galangin decreased reactive oxygen species by 56.77% significantly (P<0.05) and also reduced apoptotic bodies and the nucleus fragmentation in osteoblasts.
Conclusion: Galangin could promote osteoblast proliferation, differentiation and mineralization *in vitro*. The machanism is related to the inhibition of osteoblast apoptosis and descreasing the generation of reactive oxygen species within the condition of oxidative stress, alleviating the damage of oxidative stress on osteoblast.

P113

Effect of Chinese Herb Eucommia Ulmoides Oliver on Proteins Expression of MMP2-EGFR Signal Pathway in Lumbar Vertebra of Ovariectomized Rat

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Background: To observe the level of proteins expression of MMP2-EGFR signal pathway in lumbar vertebra of ovariectomized rat by Chinese Herb Eucommia ulmoides Oliver.
Methods: 105 female SD rats in six months, were randomly divided into model group with 75 rats, 30 rats in operation group. 12 weeks after the operation, each group were randomly selected 15 rats were sacrificed. The remaining 60 rats were divided into model group, saline group (4 ml/d) 20, traditional Chinese medicine low concentration group (1 g/kg/d) 20, high concentration of traditional Chinese medicine group (4 g/kg/d) 20, were killed after 24 weeks. Rats were anesthetized and then measured by dual energy X-ray absorptiometry in bone mineral density. After the sacrifice of animal, the third lumbar was quickly achieved, excluding the surrounding soft tissue, rapid homogenate; total RNA was extracted by Trizol method. The level of protein expression by Western-blot was detected in signal pathway.
Results: Levels were significantly higher than those in the saline group and sham operation group, MMP2, EGFR, AREG, ERK, MEK, RAF expression of high concentration of traditional Chinese medicine group (P<0.01); Chinese medicine low concentration group of MMP2, EGFR, AREG, ERK, MEK, RAF expression levels were higher than those in the saline group and sham operation group (P<0.05).
Conclusion: Chinese Herb Eucommia ulmoides Oliver can significantly improve the levels of protein expression of the ovariectomized rats in matrix metalloproteinase 2-epidermal growth factor receptor protein transduction pathways. And its mechanism should be discussed in future.

P114

The Inhibitory Activity of Benzophenone Compound Against Osteoclastogenesis

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Background: Osteoclasts play an important role in bone resorption. The imbalance between bone resorption and bone formation triggers osteoporosis. Therefore, substances, which can suppress osteoclast differentiation are potential candidates for osteoporosis drug development.
Methods: Cell study for osteoclast differentiation was performed. The transcriptional and translational expressions for

bone-related genes were measured by real-time PCR and Western blotting.
Results: Benzophenone compound efficiently inhibited RANKL-induced osteoclast differentiation without cellular toxicity and down-regulates protein and mRNA expression levels of main transcription factor, NFATc1, as well as fusion-related molecules including TRAP, Cathepsin K, c-Src and DC-STAMP. Also, it inhibits translational expression of NFATc1, which is well known as master key factor in RANKL-mediated osteoclastogenesis.
Conclusion: these results suggest that benzophenone compound could obstruct RANKL-induced osteoclatogenesis via blocking NFATc1 activity. This compound may be new drug candidate for the prevention of osteoporosis.

P115
Combined Aspirin and Diethylstilbestrol Prevented Osteoporosis Induced by Ovariectomy and D-Galactose in Rats
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Background: The study was to establish a new animal model of osteoporosis induced by ovariectomy combined with D-galactose in rats and to determine whether the combination of aspirin and diethylstilbestrol would have a preventive effect on bone mass in these animal models.
Methods: Three-month-old female Sprague–Dawley rats were randomized assigned into five groups of 9 animals each, including a sham operation group (CON), an ovariectomy group (OVX), an OVX + D-galactose (100 μg/kg s.c., daily) group (MOD), a MOD+ diethylstilbestrol (30 μg/kg i.g., daily) group (MOD+D30), and a MOD + aspirin (9 μg/kg i.g., daily) + diethylstilbestrol (10 μg/kg i.g., daily) group (MOD+A-D10). After the 8-week experimental period, histomorphometric analysis on the tibial proximal metaphysis was measured. The trabecular bone microarchitecture were evaluated by Micro CT. Bone biomechanical test was performed by three-point bending test and compression test.
Results: Compared with the sham-operated rats, ovariectomy and D-galactose significantly increased body weight, trabecular separation (Tb.Sp), percent labeled perimeter (%L.Pm), bone formation rate (BFR/BV), while significantly decreased percent trabecular area (%Tb.Ar), trabecular thickness (Tb.Th), and trabecular number (Tb.N) of tibial trabecular bone. Micro CT data showed OVX and D-galactose markedly decreased bone volume (BV/TV), Tb.Th, Tb.N, trabecular bone mineral density (BMD), and connectivity density (Conn.D.) and markedly increased Tb.Sp and structure model index (SMI) of tibial trabecular bone. Furthermore, OVX and D-galactose significantly decreased peak load and stiffness of femur, and decreased maximum compressive load of lumbar vertebra.
Conclusions: Significant bone loss and deteriorated mechanical properties were observed in the rats treated with OVX and D-galactose. 9 mg/kg/d aspirin combined with 10 μg/kg/d diethylstilbestrol showed effective prevention effects on cancellous bone loss and promoted bone strength as the same effects of high dose diethylstilbestrol (30 μg/kg/d).

P116
Strontium Promote the Osteogenic Differentiation Of MSC through Attenuating the Cell Quiescent and Enhancing Asymmetric Differentiation
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Background: Strontium ranelate has been shown to reduce the risk of vertebral fracture in postmenopausal women. The beneficial effects of strontium on promoting bone formation are closely related to its capability to increase the osteogenic differentiation of mesenchymal stem cells. However, the molecular mechanisms of strontium underlying such beneficial effects were still not fully understood. The aim of this study is to investigate the mechanism underlying the transition from stemness to osteogenic lineage in the early stage of mesenchymal stem cells induced by strontium.
Methods: Human fetal BMSC provided by Saiye Biotechnology Company Limited and characterized by multi-differentiation. Cells were cultured in control group, osteogenic group and strontium promoted osteogenic group. The stemness, osteogenic and asymmetric differentiation related genes were analyzed by real-time quantitative PCR. The cell division was analyzed through EdU staining and cell cycle analysis. The CFU-OB was performed by ARS staining.
Results: In the first week of hfBMSC culture, the genes involved in stemness including *Oct4*, *Sox2*, *Nanog* and *Nestin* were kept in control group of untreated hfBMS, but they were lost in osteogenic induced groups. Alternatively, the related osteoblastic genes such as *Osterix*, *OPN* and *BSP* were significantly enhanced in osteogenic induced group from day 3. With the addition of Strontium, these genes were expressed in even higher level. The cell growth rate and the quiescent percentage of these cells were between the control group and osteogenic group. The asymmetric differentiation related genes in strontium groups were enhanced in day 3. In CFU-OB test, strontium induced more clones than the osteogenic induced group.
Conclusion: During the osteogenic differentiation of hfBMSC, the promoting effect of strontium is related to its attenuate effect in quiescent of stem cell and the enhanced effect in asymmetric differentiation of stem cell in the early stage.

P117
Preventive Effect Study of Complex Coenzyme Q10 on Bone Loss in Cyclophosphamide Induced Rats by Micro CT
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Background: To investigate the preventive effects of complex coenzyme Q10 (CCoQ10) on osteoporosis of rats induced by

cyclophosphamide and comparing with the positive drug of alendronate sodium (ALD).
Methods: 32 three-month-old Sprague-Dawley (SPF Grade) rats were randomly divided into 4 groups with 8 rats per group. Group 1 was treated with vehicle as control (NS group). Other groups were treated with cyclophosphamide (4.5 mg/kg/d) first, and then with vehicle (CP group), ALD (alendronate sodium 1 mg/kg/d) and CCoQ10 (3 ml/kg/d, combination of CCoQ10, panaxsaponin and semen oil) once a day for 15 days. At the end of the experiment, the femoral epiphysis end (FED) was analyzed by micro-CT.
Results: Compared with NS group, the micro-CT results of FED: bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular bone mineral density (BMD) and connectivity density (Conn.D.) was decreased by 34.0%, 21.2%, 22.1%, 18.8%, 25.7% and trabecular separation (Tb.Sp) and structure model index (SMI) was increased by 51.4% and 34.8%, respectively, suggesting that the bone mass lost and microstructure degenerated in CP group. Compared with CP group, the micro-CT results of both ALD and CCoQ10 indicated that both of them could prevent the bone loss of rats induced by cyclophosphamide. In ALD group, the BV/TV, Tb.N, Tb.Th and BMD was raised by 180.2%, 21.6%, 159.8% and 76.2% respectively, while Tb.Sp and SMI was reduced by 56.7% and 61.7%. However, Conn.D. was unimproved comparing with NS group. On the other hand, in CCoQ10 group, the BV/TV, Tb.N, Tb.Th, BMD and Conn.D. was raised by 53.1%, 24.0%, 35.3%, 23.1% and 13.1%, respectively and Tb.Sp and SMI was reduced by 31.5% and 26.2%, respectively. All above index of the prevented group were improved significantly.
Conclusion: Cyclophosphamide induces bone mass loss and microstructure degeneration in FED of rats. CCoQ10 and ALD have protective effects of FED in cyclophosphamide-treated rats.

P118

Effects of Guilu Erxian Glue on the Proliferation and Osteogenic Differentiation of Rat Bone Mesenchymal Stem Cells

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Background: Osteoporosis (OP) is considered the inevitable consequence of aging particularly in postmenopausal women. Guilu Erxian Glue (GLEXJ), a traditional Chinese herbal medicine formula, has been used for the treatment of OP for a thousand years following the expected therapeutic effects. In our previous study, we have observed that GLEXJ can enhance the bone strength through optimize bone microstructure and improve the bone mineral density, but the mechanisms of action is still not clear.
Methods: In this study, we used the serum pharmacological method to investigate the effects of GLEXJ on the proliferation and osteogenic differentiation of the bone mesenchymal stem cells (BMSCs) *in vitro*. BMSCs of rats were isolated by adherent cell cytopheresis and identified through FCM by the expression of CD45 and CD90. After the identification, BMSCs were subsequently divided into two groups: the blank serum group and GLEXJ-containing serum group. After treated with the corresponding serum, the proliferation of cell was detected by MTT assay and FACS to exam the best intervention condition of GLEXJ serum. Then, the ALP activity examination, alizarin red staining (ARS) and RT-PCR on markers of osteogenic differentiation were used to detect whether the GLEXJ-containing serum has the ability of inducing the BMSCs differentiate to osteoblasts.
Results: We found that the best promoting BMSCs proliferation condition was 10% concentration of GLEXJ serum. The ALP activity of 10% concentration DHJSD serum group were significantly increased compared to that in the blank serum group and the FBS group, and the calcium deposits of red color were observed in the 10% concentration of GLEXJ serum, the relative mRNA expression level of markers of osteogenic differentiation showed that the mRNA level of ALP and OC were higher than the other two groups.
Conclusion: These results of our study indicate that GLEXJ-containing serum of rats has the ability of enhancing the proliferation and osteogenic differentiations of rBMSCs which contribute to explain the clinical efficacy of OP partly, but our experiment is just a preliminary study on GLEXJ and its effects of BMSCs, there are still further researches need to be done in the future for the targets of the herbs.

P119

Strontium Ranelate Promotes Osteogenic Differentiation of Rat Bone Mesenchymal Stem Cells through Hedgehog/Gli1 Signaling Pathway

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Background: To explore whether strontium ranelate (Sr) promotes osteoblast lineage differentiation of rat bone mesenchymal stem cells (BMSCs) through the hedgehog/Gli1 signaling pathway.
Methods: BMSCs were isolated from 4-week-old rats by adherent culture. The cells in 3-5 generations were induced to differentiate into osteoblasts. Accrodding to the experimental purposes, the cells were exposed to different concentrations of Sr, cyclopamine (Cy, an inhibitor of the ligand of hedgehog) or Gli1 siRNA. The activity of alkaline phosphatase (ALP) was detected by colorimetry, and the mineralized nodules were observed by alizarin red staining. The expressions of Gli1 and Runx2 in the cells were detected by Western blotting.
Results: Exposure to Sr at 0.1 to 10 mmol/L for 7 d markedly increased the expression of Gli1 in the BMSCs, and the increment was the most obvious following 3 mmol/L Sr exposure. Otherwise, Sr at a concentration of 3 mmol/L showed a time-dependent increase in the expression of Gli1 from 1 d to 7 d. Cy at a concentration of 10 μmol/L inhibited Sr-induced up-regulation of Gli1 expression. Transfection of the BMSCs with Gli1 siRNA obviously inhibited Sr-induced up-regulation

of Gli1 and Runx2 expressions as well as Sr-induced enhancement of ALP activity and formation of mineralized nodules.
Conclusion: The hedgehog/Gli1 pathway is involved in Sr-induced osteogenic differentiation of rat BMSCs.

P120

Potential Strategy Regulating Mirnas to Improve Impaired Fracture Healing in Aged Females: A Systematic View for Timing and Targets

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Background: Fractures in aged women are associated with a high rate of morbidity and mortality impaired fracture healing in aged female skeleton is still a clinical challenge. Angiogenesis and osteogenesis are the two key stages during fracture healing, which are impaired in aged female. MicroRNAs (miRNAs) are key post-transcriptional non-coding regulators of gene expression, which has demonstrated important roles in angiogenesis and osteogenesis. Understanding how non-coding regulatory RNA in fracture healing changes with age will help identifying novel therapeutic targets that can be exploited to improve fracture healing in the aged females.
Methods: We compared the difference in expression profiles of miRNAs during fracture healing between adult and aged female mice. Meanwhile, in order to analyze the angiogenesis and osteogenesis in the early stage of female fracture healing, microCT-based angiography and micro-CT examination was performed on the femur callus samples in each group for evaluating callus neovascularization and the mineral content of the mineralized callus during fracture healing.
Results: Angiography showed smaller blood vessel volume in aged mice at early stage when compared to that in the adult mice. Reconstructed calluses showed lower bridging mineralization tissues within the gap in aged mice than that in the adult mice at the later stage. We found hub miRNAs were activated in adult female mice but not in aged ones during fracture healing. Moreover, the differential expression of the top hub miRNAs was only observed at early stage during fracture healing in adult female mice. The person correlation coefficient analysis revealed that there was five co-expression miRNA modules ($r>0.8$) participated in female fracture healing. The top hub miRNAs in fracture-healing-related molecular network were all included in the two largest modules.
Conclusion: This study reveals the possibility to improve impaired fracture healing in aged females by regulating key miRNAs at early stage.

P121

Mi-Gu Capsule Improves Bone Fracture Healing in a Mouse Model Of Osteoporosis: A Micro-Computed Tomography and Biomechanical Study

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Background: Mi-Gu Capsule (MG) is the water extract of a Chinese herbal formula Bushenfang (BSF), which composed of *Herba Epimedii*, *Radix Polygoni*, *Radix Astragali*, *Herba Dendrobii*, *Herba Cistanches*, *Rhizoma Drynariae*, and *Flos Chrysanthemi*. MG has been used in treatment of osteoporosis (OP) as an approved hospital prescription for many years in Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, China. Previous study showed MG could shorten the healing duration of osteoporotic fracture. The aim of this study was to investigate the effects of MG on bone fracture healing in the mouse osteoporosis model.
Methods: 45 female C57/BL mice were randomly divided into three groups. The first group was sham operated (SO), while the others were ovariectomized. Osteoporosis was confirmed by a reduction of bone mineral density after 3 weeks. Subsequently, the right femora of all animals were fractured and fixed with intramedullary pins. The SO and ovariectomized-control rats (OVXC) were given 0.9% normal saline, while MG group were given MG, at the dose of 630 mg/kg/day via oral gavages 6 days per week. Six weeks after surgical induction of fracture, all animals were euthanized and the femora dissected out for micro-computed tomography (micro-CT) scan and biomechanical testing.
Results: Micro-CT analyses showed that the SO and MG groups had significantly smaller volume and higher vBMD of callus than the OVXC group ($P<0.05$). Biomechanical analysis revealed the ultimate load parameter of the SO and MG groups was significantly higher than the OVXC group ($P<0.05$).
Conclusion: These results suggested that MG could improve the quality of callus mineralization and the biomechanical properties of callus. In summary, MG is associated with better fracture healing in the osteoporotic mouse model.

P122

Ovariectomy-Induced Osteoporosis Influences the Middle and Late Periods of Bone Healing in a Mouse Femoral Osteotomy Model

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Background: It is known that bone healing was delayed in the presence of osteoporosis in humans. However, due to the complexities of the healing of osteoporotic fractures, animal models may be more appropriate to study the effects of

osteoporosis in more details and to test drugs on the fracture repair process. The purpose of this study was to investigate the influence of ovariectomy-induced osteoporosis in bone healing in an open femoral osteotomy model, and to test the feasibility of this model for evaluating the healing process under osteoporotic conditions.

Methods: In assessing the effects of osteoporosis on fracture healing, ovariectomized mouse models were employed. A mid-shaft femur osteotomy model was also established 3 weeks after ovariectomy as an osteoporotic fracture group (OVX group). Femurs were then harvested at 2 weeks and 6 weeks after fracture for X-ray radiography, micro-computed tomography (micro-CT), histology and biomechanical analysis. A sham-operated group (Sham group) was used for comparison.

Results: The OVX mice had significantly lower BVF, vBMD and TMD in the fracture calluses at 6 weeks ($P<0.05$), and similar trend was observed in 2 weeks. Additionally, larger calluses in OVX animals were observed via micro-CT and X-ray, but these did not result in better healing outcomes as determined by biomechanical test at 6 weeks. Histological images of the healing fractures in the OVX mice found forward of broken end resorption and delay of hard callus remodeling. The impaired biomechanical measurements in the OVX group ($P<0.05$) were consistent with micro-CT measurements and radiographic scoring, which also indicated delay in fracture healing of the OVX group.

Conclusion: This study provided evidences that ovariectomy-induced osteoporosis impairs the middle and late bone healing process once more. These data also supported the validity of the mouse femoral osteotomy model in evaluating the process of bone healing under osteoporotic conditions.

P123

Bone Morphogenetic Protein 9 Stimulates Fracture Healing in Osteoporotic Rats

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Background: Osteoporosis is a common disease characterised by a systemic impairment of bone mass and microarchitecture that significantly increases the incidence of fragility fractures. The aim of this present study is to evaluate healing of osteoporotic fracture in response to the direct injection of adenoviral vectors containing coding regions for human bone morphogenetic protein 9 (BMP9).

Methods: The gelatin sponges soaked in recombinant adenovirus expressing BMP9 (AdBMP9) were evaluated both *in vitro* and in the femoral fracture of a rat model of postmenopausal osteoporosis for the osteogenic potential. *In vitro* studies, matrix mineralization assay, RT-PCR and western blot were conducted to examine whether mouse embryonic fibroblasts (MEFs) were responsive to these gelatin sponges. *In vivo*, 3 months after bilateral ovariectomy, the open fracture of the left femur was created in all osteoporotic Sprague-Dawley rats, and the fracture gap was filled with gelatin sponges soaked with AdBMP9 or recombinant adenovirus expressing green fluorescent protein (AdGFP). After 4 weeks, all the rats were euthanized and the femora were harvested for radiography, histology, immunohistochemistry, micro-computerized tomography (Micro-CT), and biomechanical analysis. Statistical analysis was performed about the correlation between results.

Results: *In vitro*, BMP9 effectively increased expression of osteogenic markers and induced mineralized nodules formation in iMEFs. *In vivo*, BMP9 treatment showed the strongest ability to improve microstructural parameters and biomechanical strength in the callus. Additionally, we also found that BMP9 increased the expression of receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) in both vitro and vivo.

Conclusion: The results of this study suggest that BMP9 can enhance the bone tissue formation, promote fracture healing and maintain an early mechanical stability in osteoporotic rat. This effect may be associated with the regulatory effect of BMP9 on the balance between RANKL and OPG.

P124

Repair of Dog Radial Bone Defects using True Bone Ceramics Combined with BMP-2-Related Peptide

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Background: Bone defects caused by osteoporotic fracture often require bone graft treatment to shorten healing and reduce complications. Although common, there are problems with BMP2. Our group has invented BMP-2-releated peptides, provings higher activity, safety, low cost, and specific binding properties. The true bone ceramic (TBC) developed by our group has favourable histocompatibility, osteoconductivity and completely retains the fine porous structure of natural bone. To investigate true bone ceramics (TBC)/BMP-2-related peptide composite scaffold material for enhancing healing of critical-size defects in canine radius, in order to explore the feasibility of their further applications.

Methods: A total of 8 beagle dogs were processed to prepare the critical-size defects (20 mm) in middle section of bilateral radials, and these dogs were divided into 4 groups according to their preparation sides randomly: pure TBC material was implanted into TBC group, the rhBMP2/TBC material was implanted into BMP2 group, the bone-seeking P28/TBC material was implanted into P28 group and no implant placing into the control bone defect group. After 12 weeks and 24 weeks of implantation, evaluations were performed to evaluate the bone repair capability of each kind of material.

Results: In TBC group, X-Ray showed no callus and no obvious fusion; A little osteoid and woven bone, but no lamellar bone structure were observed in defects. In rhBMP-2 group, the bone-material interface was unclear and there was a little callus formation at 12 week; there was much callus formation

with good osteointegrated at bone-material interface at 24 week. Histomorphometric examination showed much newer bone formation, a large number of inteconnected lamellar bones, numerous active osteoblasts can be found, and the bone-material interface had been integrated. The results of P28 group were similar to those of rhBMP-2 group. And no new bone was found in the control group.
Conclusion: The bone-seeking TBC/P28 composite scaffold material has good osteoinductive activity. It is a beneficial bone repair material for tissue engineering and deserves further pre-clinical research.

P125
Viability, Proliferation, Differentiation of Preosteoblast MC3T3-E1 on Three Dimensional Plottednha/SA Porous Scaffolds Loaded with BMP-2-Related Peptide
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Background: The BMP-2-related peptide, P28, is designed according to the amino acid composition of BMP-2 core regions by our group. It aims to stimulate bone formation in situ with improved bone-seeking and prolonged half-time. To evaluate the bioactivity of nHA/SA porous scaffold loaded with P28 compared with that of scaffold loaded with BMP2 or scaffold without p28 and BMP2 *in vitro*.
Methods: Three groups were set up: the group of scaffolds loaded with P28 (p28 group), the group of scaffolds loaded with BMP2 (BMP2 group) and the group of scaffolds without BMP2 or P28 (control group). After cells seeded on three types of materials, cell morphology on composite surfaces was observed by scanning electron microscope (SEM) at the assay time. The viability of cell on the scaffold was determined by Calcein-AM/PI viability kit. The proliferative ability was assessed by MTT assay. And alkaline phosphatase (ALP) activity assay was performed to evaluate the differentiation of the preosteoblast toward the osteoblast.
Results: SEM examination showed cells on three types of scaffolds were highly flattened and covered on the surface. The result of viability assay which showed no significantly difference between three groups indicated P28 had no cell-toxicity. The datum of MTT assay and ALP assay suggested that P28 group promoted MC3T3-E1 cell to proliferate and differentiate, with no significant difference compared to BMP2 group ($P>0.05$), but distinctly higher than control group ($P<0.01$).
Conclusion: P28 can stimulate the proliferation and osteogenic differentiation of MC3T3-E1 cell. In this point, it is similar to BMP-2. Furthermore, nHA/SA porous scaffolds have good biocompatibility. Owing to osteoinductivity, biocompatibility and individualization, nHA/SA porous scaffold loaded with P28 fabricated by 3DP technique may be potential substitute for repair material of osteoporotic fracture.

P126
Synchronization of Calcium Sulphate Cement Degradation and New Bone Formation Is Improved by External Mechanical Regulation
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Background: A major challenge faced in the bone materials of weight bearing without internal fixture support is the mismatch of material degradation and new bone formation, leading to weakening or even failure of the overall bony structure. The purpose of this study was to evaluate if calcium sulphate cement degradation and new bone formation could be better synchronized by external mechanical force.
Methods: 12-week-old female *Sprague Dawley rats* were anesthetized by 10% Chloral hydrate intraperitoneally. A hole of 3 mm in diameter and length was drilled into the left leg on the lateral side of the distal femur. Afterwards, calcium sulphate cement (CSC) scaffolds were implanted inside the holes. The rats were equally divided into two testing groups and a control. In the testing groups, the mechanical force was applied through a treadmill exercise. The exercise started at day 7 postoperatively for 30 consecutive days. One group was at a constant speed 8 m/min for 45 min/day. The other had a regulating mechanical force: an ascending treadmill speed from 8 m/min to 24 m/min with the increment of 8 m/min in every 10 days. In the control, the rats were restricted in the cages. The investigation was performed systematically ranging from micro-CT structural analysis *in vivo*, histological examination, to biomechanical evaluation.
Results: An ascending force in line with calcium sulphate cement degradation could achieve bone healing in 37 days with ultimate load to failure of 87.00±7.30 N, similar to that of intact femur (80.46±2.79 N, $P=0.369$). In contrast, the healing process under either a constant force or no force was not synchronized well with significant residual defect areas of 0.64±0.19 mm^2 and 1.78±0.39 mm^2 ($P<0.001$), and weaker ultimate loads to failure of 69.56±4.74 N and 59.17±7.48 N, respectively ($P<0.001$).
Discussion: Our results suggest that the mechanical regulation approach deserves further investigation and may potentially offer a clinical strategy to improve synchronization.

P127
The Drug Release Speed and *in Vitro* Bioactivity of a Novel Three-Dimensional Graphene Oxide Scaffold Loaded with BMP2-Related Peptide
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Background: Osteoporotic fractures are difficult to heal, owing to the lack of bone formation, thus in clinic, osteogenic growth

factors are urgently needed to facilitate the healing process. To investigate the peptide release speeds between TBC and GO-TBC, and to evaluate the different biological effects of the pure GO-TBC and GO-TBC loaded with P28 on the MC3T3-E1 proliferation and osteogenic differentiation.

Methods: The GO was prepared by a modified Hummers method; afterwards, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were used to activate hydroxyl in the surface of GO to carboxy. GO was covered on the internal and external surfaces of the TBC materials by physical adsorption. In the release kinetics experiment, the P28 peptide released from different scaffolds were determined by high-performance liquid chromatography (HPLC). To evaluate the biologic activity between the pure GO-TBC and GO-TBC loaded with P28, the MC3T3-E1 cells were seeded on the GO-TBC and GO-TBC/P28 scaffolds. The proliferation rates of MC3T3-E1 were assessed by MTT assay. Meanwhile, the osteogenic differentiation levels of cells on different scaffolds were determined by an alkaline phosphatase (ALP) activity assay.

Results: The release experiment showed that the release of P28 in GO-TBC was significantly slower than that in TBC ($P<0.05$). The proliferation rates and the ALP activities of both groups increased in a time-dependent manner, and the results in GO-TBC/P28 group were significantly higher than those in pure GO-TBC group ($P<0.05$).

Conclusion: The P28 peptide could markedly enhance the proliferation and osteogenic differentiation of MC3T3-E1 cells, indicating that the P28 peptide could be used as a potential alternative of rhBMP2 to facilitate the healing of osteoporotic fracture safely and efficiently. The GO-TBC loaded with P28 could be used as a potential complex scaffold to accelerate the bone formation and bone repairing process, especially in those osteoporotic fractures with lower bone formation capability.

P128

Reinforcement of Calcium Phosphate Cement using Silk Fibroin (SF) and Self-Assembled SF-Hydroxyapatite Complex

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Background: Vertebral augmentation has been widely used to treat vertebral compression fractures caused by osteoporosis, multiple myeloma, and osteolytic metastases. Biodegradable calcium phosphate cement (CPC) may achieve orchestrated cement resorption and new bone formation to restore vertebral body. However, the mechanical strength of CPCs is generally low. Therefore, we aimed to develop a strong CPC using silk fibroin (SF) and self-assembled SF-hydroxyapatite (HAP) complex as reinforcing components.

Methods: SF-HAP complex was prepared by reacting calcium hydroxide, phosphoric acid, and SF solution. The CPC contained 75% α-tricalcium phosphate, 20% tetra calcium phosphate, and 5% dicalcium phosphate anhydrous. SF solution or a combination of SF-HAP complex and SF solution were used to prepare CPC/SF or CPC/SF/SF-HAP composites.

Results: Self-assembled SF-HAP has been obtained. When SF and SF-HAP were supplemented into CPC, the microstructure of CPC was markedly altered, including reduction of flake-like crystals and increase of needle-like crystals. Consequently, the compressive strength of CPC was improved. Addition of 1%-3% SF-HAP to CPC/SF dramatically reinforced CPC, with a strength increase of up to 50%. The reinforcing effect deteriorated when SF-HAP exceeded 3%.

Conclusion: We have successfully developed a new type of CPC derived from a composite of CPC, SF and self-assembled SF-HAP complex. The compressive strength of this cement was far higher than the trabecular bone of human vertebral body and close to cortical bone, which is ideal for vertebral augmentation.

P129

Targeted Delivery of Simvastatin for Enhanced Fracture Healing: Mechanisms Involved in Osteotropism and Therapeutic Efficacy

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Background: Though known for their pleiotropic effects, bone anabolic statins have not been used for metabolic skeletal diseases due to the lack of tissue specificity. Recently, we developed a novel micelle-forming macromolecular prodrug of simvastatin (SIM). When administered systemically, this nano-sized drug delivery system passively targets to the fracture site with potent and locally sustained anabolic effects. This study was undertaken in an attempt to validate the osteotropism and the therapeutic efficacy of this prodrug in an osteotomized femur mouse model.

Methods: The simvastatin-containing prodrug, SIM-mPEG was synthesized and further formulated into micelles loaded with free SIM (SIM/SIM-mPEG). Osteotomized mice were treated with SIM/SIM-PEG (equiv. SIM 6 mg/kg/d, weekly i.v.), SIM (equiv. SIM 6 mg/kg/d, daily i.p.) and saline for two weeks. Then the fractured femurs were collected and processed for u-CT analysis. To define the biodistribution of SIM/SIM-mPEG, the prodrug, conjugated with different imaging labels, was given to the mice and analyzed. Isolated fracture calluses were evaluated by fluorescence-activated cell sorting (FACS). Cellular uptake of SIM/SIM-mPEG and its effects on SMAD signaling and production of BMP2 etc. were examined using a murine pre-osteoblast cell line MC3T3.

Results: For therapeutic efficacy, the u-CT morphometric parameters of the longitudinal fracture callus indicated that SIM/SIM-mPEG significantly enhanced fracture healing by improving the callus formation and organization. For the targeting at tissue/organ level, *in vivo* near infrared imaging demonstrated a selective accumulation of SIM/SIM-mPEG at fracture sites. For the targeting at cellular level, FACS analysis revealed that SIM/SIM-mPEG was mainly internalized by inflammatory cells and other activated resident cells involved in fracture healing in the callus.

Conclusion: Passive-targeting of SIM to fracture sites using the SIM/SIM-mPEG nano-formulation provides potent local bone anabolic effects and accelerates fracture healing in a mouse femur fracture model. The therapeutic efficacy and the observed fracture-tropism of the formulation may be explained by the accelerated cellular uptake and activation at the fracture site during the early stage of the fracture healing process.

P130

A Novel Highly Sensitive ELISA Allows The Measurement of Free, Bioactive, Human Soluble RANKL

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Background: RANKL, the receptor activator of nuclear factor kappa B ligand, is an essential factor for the formation of mature osteoclasts. Together with its receptor RANK and its antagonist Osteoprotegerin (OPG) RANKL is a key regulator in bone metabolism. RANKL is a membrane-bound protein that can be segregated to a soluble form (sRANKL), whereas only the latter has been reported to be bioactive. Due to its low circulating levels and the nature of the analyte binding to OPG, free sRANKL has been proven difficult to measure: the accuracy of sRANKL measurement is compromised by the very low or undetectable levels, as observed in some patient cohorts.
Methods: Our aim was to develop a highly sensitive and specific assay that enables the direct measurement of free, bioactive, soluble RANKL in serum and plasma samples. We have taken advantage of the high affinity and specificity protein-protein interaction between sRANKL and OPG and used immobilized, recombinant OPG to capture free sRANKL, which subsequently is detected with a biotin labeled anti-sRANKL antibody.
Results: The data presented here, demonstrate that 98% of all samples from an unselected healthy population (n=210) had detectable free sRANKL values within the calibration range of the assay (0–2 pmol/L). The median of serum samples, prepared immediately after blood collection and stored at –25°C until measurement, was 0.14 pmol/L, with a lower limit of quantification (LLOQ) of 0.01 pmol/L. Assay characteristics, such as intra/inter-assay precision, dilution linearity and spike/recovery as well as sample stability have been analysed.
Conclusion: Our novel ELISA provides a reliable and accurate tool for the quantitative determination of free, soluble, bioactive RANKL in human samples.

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Induction of Osteoporosis in Collagen-Induced Arthritis Rats Is Associated with Upregulation of Dickkopf-1

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Background: It is evident for rheumatoid arthritis (RA) to induce osteoporosis (OP) during its process and treatment. However the mechanism of how RA induced OP is not yet clear. The aim of this study was to investigate the changes of different sites of skeleton in Lewis rats with type II collagen-induced arthritis (CIA), and the effect of Dickkopf-1 (DKK-1) in bone loss induced by CIA.
Methods: Thirty-two 8-week-old Lewis rats, randomly selected 12 rats as control group, and used other 20 rats for model of CIA. After four weeks immunization, selected 12 rats successfully infected with arthritis (arthritis index ≥ 4) as CIA group, and randomly killed 6 rats on the 30 days and 90 days after CIA, respectively. Bone histomorphometry, bone biomechanics, bone mineral density (BMD) and micro-computer tomography (micro-CT) were used to examine joint or bone destruction. Western blot and immunohistochemical stain were performed to examine protein expression of DKK-1, RANKL and OPG.
Results: Lewis rats with CIA developed erosive cartilage and bone in ankle joints. CIA rats had reduced BMD of femur and lumbar vertebrae, biomechanical properties of femur, cancellous bone mass and cortical bone mass. Furthermore, the expression of DKK-1 and RANKL in ankle of CIA rats were significantly increased compared with control group, but the protein expression of OPG was significantly decreased. However, the expression of DKK-1 in right femur of CIA rats was significantly increased by 30 days after immunization, while no significance occurred between control groups and CIA groups by 90 days after immunization.
Conclusion: CIA in rats could lead to generalize bone loss. The mechanism may be related to the increase of bone resorption in cancellous bone by upregulating the expression of DKK-1 and increasing RANKL/OPG rate, and the decrease of formation in cortical bone by increasing the expression of DKK-1.

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Effects of Aging on Destabilized Medial Meniscus Induced Osteoarthritis in Mice

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Background: Aging is the most important risk factor for primary osteoarthritis (OA). This study was undertaken to validate the effects of aging on destabilized medial meniscus induced osteoarthritis from three different ages of mice and on the cultured chondrocytes from four weeks old mice.
Methods: Destabilized medial meniscus (DMM) in the in-life part were achieved by performing transection of the medial anterior meniscotibial ligament on the right knee joint. Bilateral knee joint were collected four weeks after surgery for histopathology to assess the degree of damage to the knee joint articular cartilage.
Results: The semi-quantitative evaluations on the histology slides showed the expected aging effects on articular cartilage degeneration of both right (operated) and left (intact) knees. The surgery induced changes were significant ($P<0.05$) in all three groups, as compared to the contralateral intact ones. Young animals, less than 12-week old when surgery induced injury occurred, seemed to possess limited auto-repair capabilities to maintain the pathology at a similar level in the early stage of articular degeneration. The aging effects on loss of these capabilities to recover from the injury were displayed in articular cartilage of the right knee joints. The data of the right knee joints from the ones operated at age 18-week old and terminated at 22-week old were significantly different ($P<0.01$) to the other two groups. Furthermore, the similar trend of

significant differences was also observed in the left joints (p<0.05), showing the aging effects on the articular cartilage degeneration.
Conclusion: Our study indicates that age is an important factor affecting the development and severity of OA changes after destabilized medial meniscus. Three selected ages of mice, especially for C57Bl/6 mice after 18 weeks old, can differentiate aging effect. It can be considered using this experimental model in aging-related OA studies.

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Longitudinal Alterations of Micro-Architecture and the Spatiotemporal Distribution of TGF-ß in the Bone-Cartilage Unit during Spontaneous Osteoarthritis in Dunkin-Hartley Guinea Pigs
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Background: Tis study aims to investigate the time-related adaptations of the tibial bone-cartilage unit andthe spatiotemporal distribution of transforming growth factor-beta (TGF-β) using Dunkin-Hartley (DH) guinea pig spontaneous osteoarthritis model.
Methods: Knee joints of DH guinea pigs at 3, 6, 9 and 12-month old were collected for micro-computed tomography (CT) analysis and histology. The distribution of TGF-β, osteoprogenitors and osteoclasts were studied via immunohistochemistry and histochemistry.
Results: The subchondral plate was increased in the bone mineral density (BMD), thickness and porosity. Meanwhile, the trabecular bone became denser, thicker and more separate whereas less connective and anisotropic. However, the severity of cartilage degeneration was only weakly correlated with the bone volume fraction (γ=0.290, P=0.021) and thickness (γ= 0.301, P=0.017) of subchondral trabeculae. The activity of TGF-β was decreased with age in the cartilage while it reached the highest level at 6-month old in the subchondral bone. The distribution and alteration of the osteoprogenitors followed the same pattern as TGF-β. Furthermore, Tartrate-resistant acid phosphatase (TRAP) staining showed the area of osteoclasts was continuously increased until 9-month old.
Conclusion: The changes of subchondral bone in DH guinea pig were different from normal aging and only weakly correlated to the progressive destruction of cartilage. The alterations of TGF-β in the cartilage and subchondral bone were not synchronous. The similarity of the spatiotemporal distribution between TGF-β and osteoprogenitors suggests TGF-β may play a role to induce the elevated bone formation during spontaneous osteoarthritis.

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A Recurrent Mutation and a Novel Mutation in the HPGD Gene in Nine Patients with Primary Hypertrophic Osteoarthropathy
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Background: Hypertrophic Osteoarthropathy (HOA) is characterized with digital clubbing, periostosis and hyperdermia. Primary hypertrophic osteoarthropathy (PHO) is a hereditary bone disease, which shares the same symptoms with Secondary Hypertrophic Osteoarthropathy. *HPGD* encoding 15-prostaglandin dehydrogenase (15-PGDH) and *SLCO2A1* encoding a kind of PGT were found responsible for PHO. The mutations of either of the two genes would lead to increased level of PGE2, which might be the cause of the constellation of the symptoms. The aim of this study was to analyze the *HPGD* gene and the clinical and radiological findings with 9 patients with the diagnosis of PHO.
Methods: Nine patients, including 2 siblings and the other 6 unrelated patients, were enrolled in the study. Sanger method was used to sequence the candidate *HPGD* gene to detect any mutations. We also analyzed the serum and urinary prostaglandin E2 (PGE2) and prostaglandin metabolite (PGE-M) levels for each of the 9 patients. Only one of these patients are female and her symptoms are less severe than the others, though she shared the same homozygous mutation with her brother and her PGE2 level were at the same level with the others.
Results: We identified a recurrent c.310_311delCT mutation presenting in all of the nine patients, of which 6 patients are homozygous, 2 patients heterozygous as well as 1 patient is compound heterozygous with this mutation and a novel heterozygous missense mutation c.488G>A (p.R163H) in one of these nine patients with PHO. The onset age is between 1 year old and 16 years old. And the PGE2 is significantly higher than normal with lower PGE-M level.
Conclusion: We identified a recurrent mutation and a novel mutation in *HPGD* gene responsible for PHO. It is likely to be a hot-spot mutation site for PHO patients.