



SESSION TIME: 1630 - 1800, Tuesday 24 Oct 2006

Oral Abstracts

4B (Basic) - Mechanisms of bone cell function and regulation I

- O35 IL-23 inhibits osteoclastogenesis indirectly through lymphocytes and is required for the maintenance of bone mass in mice**
Quinn, J.M.W., Sims, N.A., Miroso, D., Saleh, H., Walker, E.C., Martin, T.J. and Gillespie, M.T. (Australia)
- O36 Role of phosphate uptake through the sodium-dependent phosphate transporter 3 in chondrocyte differentiation**
Sugita, A., Kawai, S., Hayashibara, T., Tamura, D. Yoshikawa, H. and Yoneda, T. (Japan)
- O37 Manipulation of the anabolic and catabolic responses in an open fracture model**
Godfrey, C., McDonald, M., Amanat, N., Mikulec, K., Tagil, M. and Little, D.G. (Australia)
- O38 Gene expression in the pagetic osteoblast**
Matthews, B., Naot, D., Bava, U., Callon, K., Black, M., Song, S., Pitto, R., Cundy, T., Cornish, J. and Reid, I. (New Zealand and Australia)
- O39 The osteopetrotic incisor absent rat exhibits reduced osteoclast activity, resulting in normal fracture union with delayed hard callus remodeling**
McDonald, M.M., Seymour, A., Mikulec, A., Godfrey, C., Szytynda, T. and Little, D.G. (Australia)
- O40 Cyclic AMP/PKA signaling induces the activation of canonical Wnt pathway via phosphorylation of GSK3 β in osteoblastic cells**
Suzuki, A., Michigami, T., Kubota, T., Ozono, K. (Japan)
- O41 OS-9, an endoplasmic reticulum quality control protein, regulates maturation and expression of the calcium-sensing receptor**
Ward, B.K., Pedersen, B.T., Magno, A.L. and Ratajczak, T. (Australia)
- O42 Schnurri-2 promotes BMP-dependent osteoblastic differentiation**
Saita, Y., Takagi, T., Ezura, Y., Nakashima, K., Kurosawa, H., Ishii, S. and Noda, M. (Japan)

O35

IL-23 inhibits osteoclastogenesis indirectly through lymphocytes and is required for the maintenance of bone mass in mice

J.M.W. Quinn^{1,2}, N.A. Sims¹, D. Miroso¹, H. Saleh¹, E.C. Walker¹, T.J. Martin^{1,2} and M.T. Gillespie^{1,2}

¹St Vincent's Institute, Fitzroy, Vic, Australia, ²Department of Medicine, The University of Melbourne, Vic, Australia

IL-23 and IL-12 are pro-inflammatory cytokines containing a common p40 subunit. IL-23 (p19/p40 heterodimer) is produced by T cells; IL-12 (p35/p40 heterodimer) inhibits osteoclastogenesis via T cells. To determine the effects of IL-23 on osteoclastogenesis, we investigated the effects of IL-23 treatment, and IL-23 deletion in vitro, and in vivo.

IL-23 dose-dependently inhibited osteoclastogenesis in spleen cells stimulated by RANKL+M-CSF or co-cultured with 1,25 dihydroxyvitamin-D3 treated stromal cells. These effects were not seen in RAW264.7 cells, bone marrow (BM) cells or BM-derived macrophages, nor in spleen cells depleted of T cells or of the non-adherent cell fraction, indicating that IL-23 osteoclastogenesis inhibition requires T cells. Like IL-12, IL-23 was synergistic with IL-18 in inhibiting osteoclastogenesis. While IL-23 mRNA levels were robustly enhanced by IL-12 in spleen cells, IL-12 inhibited osteoclastogenesis in IL-23p19^{-/-} splenic cultures.

IL-23 inhibition of osteoclastogenesis appears to be important *in vivo*, since femora and tibiae of 12 week old male and female IL-23p19^{-/-} mice demonstrated 30% lower trabecular BMD (by pQCT) and 50% lower trabecular bone volume and a three-fold increase in osteoclast numbers compared to wild type controls. IL-23p19^{-/-} spleen and BM cells formed more osteoclasts than wild type controls in response to RANKL plus MCSF.

These data show IL-23, like IL-12, inhibits osteoclast formation in a T cell-dependent manner although IL-23 does not mediate IL-12 actions. Furthermore, markedly low bone mass due to elevated osteoclastogenesis in IL-23^{-/-} mice suggests a significant role for IL-23 in the maintenance of bone structure.

O36

Role of phosphate uptake through the sodium-dependent phosphate transporter 3 in chondrocyte differentiation

A. Sugita^{1,2}, S. Kawai¹, T. Hayashibara¹, D. Tamura², H. Yoshikawa² and T. Yoneda¹

1. Department of Biochemistry, Osaka University Graduate School of Dentistry, Japan

2. Department of Orthopaedic Surgery, Osaka University Graduate School of Medicine, Japan

Occurrence of rickets and retarded skeletal development in X-linked hypophosphatemia (XLH) suggests a potential role of phosphate in endochondral ossification. Here we studied *Hyp* mice, the murine homologue of human XLH, to examine the role of phosphate in chondrocyte differentiation. *Hyp* mice showed growth retardation with short long bones and disturbed endochondral ossification compared with wild-type (WT) mice. The extracellular phosphate is known to regulate the cellular metabolism by entry through the sodium-dependent phosphate transporter (NPT). *Hyp* chondrocytes showed decreased mRNA expression of NPT3, ³²P uptake and mineralization compared with WT chondrocytes. Overexpression of NPT3 cDNA in *Hyp* chondrocytes restored mineralization. Apoptosis, a prerequisite for the cartilage mineralization, and deposit of matrix vesicles in extracellular matrices were reduced in the growth plates of *Hyp* mice. A non-selective competitive inhibitor of NPT, phosphonoformic acid (PFA), caused hypophosphatemia and disturbed endochondral ossification with reduced apoptosis in WT mice. To understand the mechanism by which reduced intracellular phosphate levels cause decreased apoptosis, we determined the intracellular ATP levels in *Hyp* chondrocytes, since ATP is required for an activation of caspase 9-mediated apoptosis. Intracellular ATP levels in *Hyp* chondrocytes were significantly decreased. PFA reduced intracellular ATP levels in WT chondrocytes. 3-bromopyruvate, an inhibitor of ATP production, inhibited chondrocyte mineralization *in vitro* and administration of 3-bromopyruvate reduced apoptosis in the growth plates in WT mice. In conclusion, our results suggest phosphate uptake through the NPT3 modulates intracellular ATP levels, which in turn controls apoptosis and mineralization of chondrocytes, thereby regulates endochondral ossification.

O37

Manipulation of the anabolic and catabolic responses in an open fracture model

C. Godfrey, M. McDonald, N. Amanat, K. Mikulec, M. Tagil, and D.G. Little

The Children's Hospital Westmead, The University of Technology Sydney, Australia

High energy open fractures are responsible for the majority of non-unions. Anabolic and anti-catabolic approaches are being explored for the prevention of non-union in these challenging circumstances. We designed a study of open rat femoral fractures held with a single K wire. 35% of fractures in a Saline treated group did not unite.

Anabolic treatment was given as either PTH 10µg/kg or 50 µg/kg daily for 5 of 7 days, or a single dose of OP-I (BMP-7) 50 µg. Anti-catabolic treatment was given as a single dose of zoledronic acid (ZA) at 2 weeks.

PTH had no effect on the rate of fracture union in this open fracture model. Neither PTH 50 µg/kg for 6 weeks or lower doses nor combination therapies with ZA yielded increases in the rate or strength of union.

OP-I alone led to union in 95% of cases. OP-I followed by ZA led to union in all cases, with an increase of 49% in load to failure over OP-I alone. ZA alone without an anabolic did not increase union rate.

While PTH has been shown to increase the strength of fracture union in closed models, open fractures require an anabolic agent such as OP-I that can stimulate the recruitment, proliferation and differentiation of cells down the osteoblastic lineage. PTH likely stimulates only mature cells in an established microenvironment. Anti-catabolic agents such as ZA further increase the rate and strength of union in the presence of OP-I but are not useful in the absence of a robust anabolic response.

O38

Gene expression in the pagetic osteoblast

B. Matthews, D. Naot, U. Bava, K. Callon, M. Black, S. Song, R. Pitto, T. Cundy, J. Cornish and I. Reid
University of Auckland, New Zealand

Paget's disease is a focal disease of increased bone turnover. Although the aetiology of Paget's disease is unknown, it is regarded as an osteoclast disease due to the characteristic lytic lesions and abnormal appearance of pagetic osteoclasts. However, given the role osteoblasts play in the control of osteoclast development, it is possible that changes in osteoblasts contribute to the pagetic phenotype. To examine this possibility we studied differential gene expression in pagetic osteoblasts and bone marrow stromal cells.

We examined gene expression in primary osteoblasts and bone marrow stromal cells cultured from pagetic lesions of 23 patients, and compared these to non-pagetic bone samples using microarrays and real time PCR. The Wnt signalling antagonist Dkk1 was upregulated 3-5 fold, similar to findings in multiple myeloma and other cancers that cause bone lesions. In addition, more than 2-fold upregulation of alkaline phosphatase was observed, while the late osteoblast markers osteocalcin and bone sialoprotein were downregulated. Interleukin-6, which has previously been implicated in Paget's disease was upregulated more than 2-fold, and Interleukin-1 β levels were also increased. There were also significant changes in expression of a number of other genes with previously unidentified roles in osteoblast biology.

The results suggest that Pagetic osteoblasts are abnormal, even after a number of weeks in culture, and may play a role in driving the overactivity of Pagetic osteoclasts. Alterations in the Wnt signalling pathway may be important in this process, as increased Dkk1 levels have also been identified in cancers that cause lytic lesions in bone.

O39

The osteopetrotic incisor absent rat exhibits reduced osteoclast activity, resulting in normal fracture union with delayed hard callus remodelling

M.M. McDonald, A. Seymour, A. Mikulec, C. Godfrey, T. Szynda and D.G. Little
The Children's Hospital Westmead, The University of Technology Sydney, Australia

A spontaneous mutation in the incisor absent (*ialia*) rat results in abnormal osteoclasts which display deficient resorption. We investigated the resulting osteopetrotic phenotype. Furthermore, we utilised this model to examine fracture repair in the absence of functional osteoclasts.

Samples were harvested from 3 weeks of age for extensive phenotype analysis. Closed fractures were performed on *ialia* and controls, with harvests at 1, 2 and 3 weeks post fracture.

Preliminary serum CTX analysis showed decreases at 7 and 9 weeks of age in *ialia* rats. Further, *ialia* primary osteoclast cultures revealed decreased differentiation and resorption pits at 9 weeks compared to control.

Femoral length showed up to a 14% decrease in *ialia* compared to control rats ($p < 0.01$). DEXA scans of tibial metaphyses revealed a 70-110% increase in BMD up to 24 weeks of age in *ialia* ($p < 0.01$). Further, histology at this site revealed a 176-441% increase in BV/TV in *ialia* rats ($p < 0.01$).

Initial fracture union was achieved by 3 weeks in *ialia* and controls. QCT revealed significant increases in BMC and volume in *ialia* calluses at all times ($p < 0.01$). Controls showed a reduction in callus volume between 2 and 3 weeks, whereas the *ialia* callus volume increased 41%.

In conclusion, the *ialia* rat exhibits a severe osteopetrotic phenotype resulting from reduced osteoclast activity. Although *ialia* rats achieved initial fracture union, hard callus remodelling was hindered resulting in a

larger, more mineralised callus. This study reveals that osteoclast function is not essential for initial union but is essential for hard tissue remodelling.

O40

Cyclic AMP/PKA signaling induces the activation of canonical Wnt pathway via phosphorylation of GSK3 β in osteoblastic cells

A. Suzuki¹, T. Michigami¹, T. Kubota¹ and K. Ozono²

¹Department of Environmental Medicine, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan,

²Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan.

We have investigated the effects of cyclic AMP (cAMP)/PKA signaling on the canonical Wnt pathway in osteoblasts using human osteoblastic cell line SaOS-2. In reporter assays, treatment with 5-50 μ M forskolin, an activator for adenylyl cyclase, induced TCF-dependent transactivation in a dose-dependent manner, which was abolished by pretreatment with PKA inhibitor H89. Western blot analyses demonstrated the nuclear accumulation of β -catenin by treatment with forskolin. Treatment with both Wnt3a and forskolin synergistically increased the TCF-dependent transactivation, suggesting the cross talk between cAMP/PKA and canonical Wnt signaling pathways. To identify the convergence point of these signaling pathways, we investigated the involvement of glycogen synthase kinase 3 β (GSK3 β), which phosphorylates β -catenin to promote its degradation. GSK3 β itself is inactivated by phosphorylation at Serine-9. Western blot analyses demonstrated that GSK3 β was rapidly phosphorylated at Serine-9 by the treatment with forskolin. Then, to investigate the physiological roles of this crosstalk, the effects of forskolin on the expression of endogenous target genes of Wnts were examined. Treatment with forskolin resulted in the increased expression of wnt-induced secreted protein 2 (Wisp2), which is a target gene of Wnt signaling and involved in osteoblast function. We also investigated the effects of the Wnts on the expression of RANKL, a downstream target gene of cAMP/PKA signaling. Interestingly, co-treatment with Wnt3a markedly reduced the forskolin-induced RANKL expression. These results suggest that cAMP/PKA signaling activates canonical Wnt pathway through the inactivation of GSK3 β and that Wnt signaling might inhibit bone resorption through negative impact on cAMP/PKA signaling in osteoblasts.

O41

OS-9, an endoplasmic reticulum quality control protein, regulates maturation and expression of the calcium-sensing receptor

B.K. Ward, B.T. Pedersen, A.L. Magno and T. Ratajczak

Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital and Centre for Medical Research, University of Western Australia, Nedlands, Western Australia 6009

The calcium-sensing receptor (CaR), is a classically structured G protein-coupled receptor with a large intracellular tail containing determinants important for cell surface expression. In a recent yeast two-hybrid analysis we confirmed several overlapping clones of the endoplasmic reticulum (ER)-associated protein, OS-9, as binding interactors of the CaR tail. We cloned full length human OS-9 by RT-PCR into a mammalian expression vector (pcDNA3) containing an EGFP tag and confirmed *in vivo* interaction by co-immunoprecipitation of CaR and OS-9 in HEK293 cell lysates co-expressing EGFP-OS-9 and FLAG-tagged CaR. In addition, yeast two-hybrid deletion mapping studies demonstrated that the OS-9 clones, which share an 89 amino acid carboxyl terminal domain, bound to a region of the CaR important for its cell surface expression and stability. OS-9 has a quality-control function in the ER lumen, sorting aberrant glycoproteins for targeted proteosomal destruction via the ER-associated protein degradation (ERAD) pathway. The CaR glycoprotein is under ERAD control through its association with the E3 ubiquitin ligase, dorfins, a downstream ERAD component that ubiquitinates CaR, allowing its degradation. Since overexpression of dorfins causes reduced CaR expression (presumably by upregulating ERAD), we hypothesized that overexpression of OS-9 might also reduce CaR expression. We demonstrate that graded overexpression of OS-9 in HEK293 cells transfected with CaR-FLAG, results in a progressive decrease in expression of mature and immature forms of

CaR. We conclude that OS-9 may have profound effects on CaR maturation and expression through its role in sorting immature CaR forms for plasma membrane expression or targeted proteosomal degradation.

O42

Schnurri-2 promotes BMP-dependent osteoblastic differentiation

Y. Saita, T. Takagi, Y. Ezura, K. Nakashima, H. Kurosawa, S. Ishii and M. Noda

Molecular Pharmacology, Tokyo Medical and Dental University, Medical research institute, Japan

The zinc finger protein Schnurri (Shn) has been known to be required for BMP signaling in drosophila. In mammals, three Shn homologs are known (Shn-1, Shn-2, Shn-3) and Shn-2 was recently demonstrated to control BMP-dependent adipogenesis via interaction with Smad proteins. In addition, Shn-3 was recently reported as a negative regulator of osteoblasts and Shn-3 deficient mice increase adult bone mass. We previously reported that Shn-2 deficient mice decreases in total bone mass and Shn-2 deficient primary osteoblasts were suppressed in the level of the BMP-induced ALP activity. Here, we further investigated the function of Shn-2 in osteoblast differentiation. Overexpression of Shn-2 in MC3T3E1 cells enhanced BMP-induced enhancement in ALP, the expression levels of Runx2 and Osterix. We also examined whether Shn-2 targets transcriptional events. Overexpression of Shn-2 enhanced BMP-dependent transcriptional activity and additional transfection of Smad I further enhanced it. These observations revealed that Shn-2 promotes BMP-dependent osteoblastic differentiation in coordination with Smad I. In conclusion, Shn-2 is a novel modulator of BMP-dependent osteoblast differentiation.