Citation: IBMS BoneKEy **13,** Article number: 675 (2015) | doi:10.1038/bonekey.2015.42

www.nature.com/bonekey

ORAL POSTERS – PRE-CLINICAL

The 4th Joint Meeting of ECTS and IBMS
Rotterdam, The Netherlands
25–28 April 2015

OP21 (P224)

UBR5, an E3 Ubiquitin-Protein Ligase, Regulates Hedgehog-mediated Tendon Ossification

David Mellis, Natalie Dora, Michela Grillo, Elaine Kinsella, Antonia Sophocleous, Stuart Ralston, Donald Salter, Robert Hill, Mark Ditzel
IGMM, School of Molecular, Genetic and Population Health Sciences, Western General Hospital, University of Edinburgh, Edinburgh, UK

The objective was to determine the role of Hedgehog signalling in UBR5-associated heterotopic tendon ossification. Our studies into the N-end Rule Ubiquitin-protein ligase UBR5 revealed its role in controlling heterotopic tendon ossification in the mouse limb and hypothesise that UBR5 regulates stem/progenitor behaviour to control tendon homeostasis. Spatiotemporal skeletal development is tightly regulated to maintain a functioning skeleton. However, in certain diseases heterotopic ossification (HO) can occur in soft tissue. Using Prx-Cre combined with a floxed UBR5 mutant allele (UBR5mt) we deleted UBR5 function in the developing murine embryonic limb bud. Micro-CT analysis revealed HO in homozygous UBR5mt adult animals and histological analysis identified numerous chondrocytes within the tendon midbody. HO was progressive, identified in multiple tendons and first detected at 6 weeks of age. Importantly, no HO was observed in the Prx-Cre control animals (n=18). Our work in other tissues indicates UBR5 as an important regulator of stem/progenitor cell function, with UBR5 being highly upregulated in pericytes, the progenitors of mesenchymal stem cells. Furthermore, we revealed that UBR5 regulates both Hedgehog (HH) ligand production and signal transduction. Indian Hedgehog signalling plays a central role in controlling stem/progenitor cell function in various tissues including the adult skeleton. UBR5mt animals treated with the HH pathway antagonist cyclopamine (n=3) resulted in an enhanced level of HO in comparison with those treated with an inactive cyclopamine analog (n=4). Cyclopamine treatment in a wild-type background did not promote HO, suggesting that a UBR5mt background sensitises tendons to HH pathway inhibition and ossification. We therefore conclude that UBR5 and HH signalling normally act to suppress heterotopic tendon ossification. In conclusion, UBR5 regulates heterotopic tendon ossification through regulating HH signalling.
Disclosure: The authors declared no competing interests. This work was supported by The University of Edinburgh.

OP22 (P79)

Oxidative Stress Inhibits PTH Type 1 Receptor Signalling and Trafficking

Juan A Ardura[1,3], Veronica Alonso[2,3], Sergio Portal-Nuñez[1], Peter Friedman[3], Pedro Esbrit[1]

[1]Bone and Mineral Metabolism Laboratory, IIS- Fundación Jiménez Díaz, Madrid, Spain, [2]Institute of Applied Molecular Medicine, San Pablo-CEU University School of Medicine, Madrid, Spain, [3]Laboratory for G Protein-Coupled Receptor Biology, Department of Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

During ageing, an increase of reactive oxygen species (ROS) occurs, affecting several processes involved in bone homeostasis, namely osteoblast and osteoclast apoptosis, osteoblastogenesis and adipogenesis. Various signalling pathways are known to be enhanced or decreased by ROS and constitute potential therapeutic targets to limit oxidative damage effects in ageing-associated diseases. Transient administration of parathyroid hormone (PTH), a master regulator of bone remodelling, currently represents the only anabolic therapy in osteoporosis. However, the molecular mechanisms underlying the anabolic features of PTH are ill defined. In osteoblasts, PTH binds to the PTH type 1 receptor (PTH1R), a G protein-coupled receptor, and triggers classic G-protein signalling pathways. The aim of the present study was to analyse the effects of ROS on PTH1R signalling and trafficking. We used fluorescence resonance energy transfer (FRET)-based cAMP (Epac) and ERK biosensors, and the calcium fluorescent dye Fluo-4 to analyse by microscope live cell imaging cAMP, ERK and calcium signalling, respectively, triggered by PTH (1-34) in PTH1R-overexpressing human embryonic kidney (HEK)-293 cells in the presence or absence of H_2O_2. PTHR internalisation and recycling was measured in HEK-293 cells transiently transfected with HA-PTHR using an ELISA protocol based on an anti-HA antibody and an anti-IgG conjugated with alkaline phosphatase. An increase in cAMP production, ERK phosphorylation and accumulation of intracellular calcium was observed upon PTH (1-34) stimulation of HEK-293 cells. Preincubation of these cells with 1-500 μM H_2O_2 substantially inhibited all of these PTH (1-34)-dependent signalling pathways. These inhibitory effects were not a result of PTH (1-34) oxidation since PTH (1-34) incubated or not with H_2O_2 triggered similar cAMP responses. In addition, PTH (1-34) ligand induced about 25% internalisation and subsequent recycling of the PTH1R, and both events were significantly reduced by H_2O_2 preincubation in these cells. These findings highlight the role of H_2O_2 as an inhibitor of PTH signalling, and suggest the relevance of ROS as a putative target in bone diseases associated to oxidative stress such as age-related osteopenia.
Disclosure: The authors declared no competing interests.

This work was supported by RETICEF (RD12/0043/0008).

OP23 (P14)

A Stable Synthetic Sulforaphane Significantly Improves Bone Architecture and Gait in the Naturally Occurring Str/ort Model of Osteoarthritis

Behzad Javaheri[1], Blandine Poulet[2], Ahmad Aljazzar[1], Yu-Mei Chang[1], Roberto De Souza[1], Mark Hopkinson[1], Andrew Pitsillides[1]
[1]The Royal Veterinary College, London, UK, [2]University College London, London, UK

Osteoarthritis (OA), affecting joints and bone, causes physical gait disability with huge socio-economic burden; treatment remains palliative. Roles for antioxidants in preventing/reversing such chronic disorders have been examined previously. Sulforaphane is a naturally occurring antioxidant inflammation modulator. Herein, we explore whether Sulforadex®, a stable synthetic form of sulforaphane, modifies gait, bone architecture and slows/reverses articular cartilage destruction in a spontaneous OA model in Str/ort mice. Sixteen mice (n=8/group) were orally treated for 3 months with either 100 mg/kg Sulforadex® or vehicle (0.5% sodium carboxymethyl cellulose in H_2O). Gait was recorded and analysed using a DigiGait imaging system. Tibiae were microCT scanned using a Skyscan 1172 and architecture in a defined trabecular bone region and the entire cortical shaft analysed. Right knees were decalcified, wax-embedded and multiple 6µm coronal sections collected from across each entire joint stained with toluidine blue. OA lesion severity was graded using an Internationally recognised system. Analysis revealed development of asymmetric gait (hind limb paw area), normally linked to OA, in vehicle-treated Str/ort mice, which did not emerge in Sulforadex®-treated mice. Fore-limb asymmetry followed similar patterns. We found significantly increased trabecular bone volume/total volume, bone surface and trabecular number in Sulforadex®-treated mice. This was consistent with significantly increased indices of bone strength (cross-sectional thickness, Imin/Imax in many regions along the tibial shaft. Despite these marked improvements in gait and superior trabecular and cortical bone mass and architecture, we found that histologically-graded OA severity in articular cartilage was unmodified in the Sulforadex®-treated mice. This indicates that Sulforadex® improves bone microarchitecture and indices of mechanical strength and produces greater symmetry in gait without any marked effects on cartilage lesion development in these spontaneously osteoarthritic mice. Our findings support novel osteotrophic roles for Sulforadex® and beneficial gait effects that appear to be independent of articular cartilage lesion development in OA.

Disclosure: The authors declared no competing interests.

OP24 (P140)

Skin Inflammation Causes Bone Loss by IL17A-Mediated Inhibition of Bone Formation

Ozge Uluckan[1], Johannes Keller[2], Susanne Karbach[3], Osvaldo Graña[1], Andrew Croxford[3], Stephanie Finzel[4], Marije Koenders[5], Wim Van den Berg[5], Michael Amling[2], Ari Waisman[3], Georg Schett[4], Erwin Wagner[1]
[1]CNIO, Madrid, Spain, [2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [3]University Medical Center, Mainz, Germany, [4]University of Erlangen-Nuremberg, Erlangen, Germany, [5]Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Patients with chronic inflammatory diseases such as psoriasis are at high risk for developing osteoporosis. Psoriatic arthritis patients exhibit bone loss caused by increased bone resorption through activation of osteoclasts. However, it is not clear whether psoriasis can lead to bone loss in the absence of arthritis. Using mouse models with skin inflammation as well as psoriasis patient samples, we show that increased circulating IL-17A from the inflamed skin triggers bone loss through inhibition of bone formation. Osteocalcin (OCN), P1NP levels as well as bone formation rates were decreased in mice with an epithelial (Keratin5)-specific deletion of JunB (JunB$^{\Delta ep}$). Moreover, transgenic mice expressing IL-17A in keratinocytes exhibit decreased OCN and P1NP levels with no changes in TRAcP5 levels. Expression of Phex, Dmp1 and Sost, markers of osteocytes, were altered in both models of skin inflammation. The inhibition of bone formation by IL-17A was independent of its expression in T-cells, since JunB$^{\Delta ep}$ mice on a Rag-/- background displayed decreased levels of OCN. Importantly, pharmacologic IL-17A blockade rescued Ocn expression and bone formation rates in JunB$^{\Delta ep}$ mice. Mechanistically, IL-17A inhibits osteoblast maturation and mineralization *in vitro*. RNA-seq analyses from *in vitro* osteoblast cultures treated with IL-17A identified nitric oxide and lipocalin-2 (Lcn-2) as mediators of IL-17A-dependent osteoblast inhibition. *In vivo*, crossing JunB$^{\Delta ep}$ mice to Lcn-2-deficient mice reduced bone loss. Importantly, psoriasis patients without arthritis developed bone loss with decreased OCN levels and increased serum IL-17A levels. Therefore, this study suggests that IL-17A, upregulated in inflammatory and autoimmune diseases, provides a risk for bone loss and its blockade should be considered in such diseases to prevent the adverse consequences on the skeleton.

Disclosure: The authors declared no competing interests. ÖU: EMBO Long-term fellowship (LT-1244-2009), ECTS-AMGEN bone biology fellowship. AW by SFB: TRR 128 TPA7; EFW by the BBVA Foundation and a European Research Council Advanced Grant (ERC FCK/2008/37); GS and AW by DFG (Project SPP1468 Immunobone), the Marie Curie Osteoimmune and the IMI funded project BTCure.

OP25 (P141)

DLX3 is a Major Regulator of Bone Apposition and Homeostasis in the Appendicular Skeleton

J Isaac[1,2], J Erthal[1], J Gordon[3], O Duverger[1], H-W Sun[4], A.C. Lichtler[5], G.S. Stein[3], J.B. Lian[3], M.I. Morasso[1]
[1]Laboratory of Skin Biology, NIAMS, NIH, Bethesda, MD, USA, [2]Laboratory of Molecular Oral Pathophysiology, INSERM UMRS 1138, Team Berdal, CRC, Paris, France, [3]Department of Biochemistry, University of Vermont, Burlington, VT, USA, [4]iodata Mining and Discovery Section, NIAMS, NIH, Bethesda, MD, USA, [5]Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA

Although human mutations and *in vitro* studies suggest that DLX3 is involved in bone formation, its *in vivo* role has not been elucidated. To address the functions of DLX3 in the appendicular skeleton, we generated and analysed mice carrying conditional loss-of-function mutation of DLX3 in osteoblasts (*Dlx3*$^{OCN\text{-}cKO}$). Using dynamic bone formation, histological and micro-computed tomography analyses, we demonstrated that *in vivo* DLX3 deletion in osteoblasts results in significant increase in bone mass throughout the lifespan. In absence of DLX3, endochondral bone formation still takes place at the growth plate but we observed more trabeculae that extend deeper into the medullary cavity. Furthermore *Dlx3*$^{OCN\text{-}cKO}$ cortical bone is thicker with higher mineral apposition rate, decreased bone mineral density and increased cortical porosity. By combining *in vivo* site-specific gene profiling, TRAP staining and *ex vivo* culture of M-CSF-dependent mononuclear cells, we showed that the increase in trabecular bone mass in *Dlx3*$^{OCN\text{-}cKO}$ mice does not arise from impaired osteoclastic activity but from direct enhancement of bone-forming osteoblast activity with an imbalance in bone homeostasis in favour of bone apposition. *In vivo* RNA-seq analysis on *Dlx3*$^{OCN\text{-}cKO}$ metaphysis demonstrated that DLX3 deletion in osteoblasts results in up-regulation of genes encoding transcription factors essential for osteoblastogenesis as well as genes important to mineral deposition and bone turnover. Finally, using DLX3-deleted bone marrow stromal cells and ChIP-seq analysis, we demonstrated that DLX3 removal results in increased osteoblast differentiation associated with enhanced occupancy of key transcriptional activators of osteogenesis on the bone-specific osteocalcin (OCN) promoter. In conclusion, these results demonstrate for the first time that DLX3 plays a central role in the maintenance of bone homeostasis and skeletal integrity by attenuating bone mass accrual in the appendicular skeleton.

Disclosure: The authors declared no competing interests. This research was supported by the Intramural Research Program of the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the NIH. JB Lian is supported by NIH grant R37 DE012528.

OP26 (P142)

VEGF-Dependent Control of Osteoblast/Adipocyte Differentiation

Agnes Berendsen, Bjorn Olsen
Harvard School of Dental Medicine, Boston, MA, USA

Vascular endothelial growth factor A (VEGF) functions as a key factor in angiogenesis but also plays essential roles in cellular survival, cartilage and bone development, and bone maintenance. Apart from coupling angiogenesis and osteogenesis, VEGF regulates osteoblast progenitor cell fate by controlling the balance between osteoblast and adipocyte differentiation. Conditional deletion of VEGF in Osterix-expressing osteoblast progenitor cells in mice, carrying floxed *Vegfa* alleles and the Osx-Cre transgene, leads to age-related osteopenia characterised by loss of bone mass with increased marrow fat. Both *in vivo* and *in vitro* studies indicated that VEGF knockdown induces differentiation of mouse bone marrow stem cells (BMSCs) into adipocytes at the expense of osteoblasts. Experiments aimed at rescuing differentiation defects revealed that addition of exogenous recombinant VEGF had no effect on BMSC fate, suggesting that VEGF functions via intracrine rather than paracrine mechanisms. A role for intracellular VEGF was supported by detection of VEGF and VEGF receptors in nucleus and cytoplasm using immunostaining and western blotting of BMSC subcellular fractions. To assess the effect of modulating intracellular VEGF levels on BMSC differentiation, we generated a cell-permeable VEGF protein consisting of VEGF fused to TAT, a cell-penetrable peptide, followed by a nuclear localisation sequence. This cell-permeable VEGF proved to be effective in entering cells and translocating to their nucleus. Remarkably, cell-permeable VEGF, but not paracrine VEGF, stimulated expression of osteoblast marker genes in BMSCs. VEGF protein levels in nucleus and cytoplasm appeared to be regulated by mechanisms involving proteasome activity. In summary, we have identified a novel function for VEGF in controlling the fate of BMSCs involving intracrine mechanisms that are independent of its role as secreted growth factor. (Animal experiments were approved by Harvard Medical Area Standing Committee and in agreement with U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.)

Disclosure: The authors declared no competing interests. This work was supported by a NIH grant 5R01AR036819 (to B.R.O.) and an HSDM Dean's Scholarship (to A.D.B.).

OP27 (P186)

Gene Correction by Homologous Recombination in *TCIRG1*-Defective Induced Pluripotent Stem Cells

Francesca Ficara[1,2], Sharon Muggeo[1,2], Tui Neri[1,2], Maria Elena Caldana[1,2], Laura Crisafulli[1,2], Maria Luisa Focarelli[1,2], Francesca Faggioli[1,2], Camilla Recordati[3], Samantha Scaramuzza[4], Dario Strina[1,2], Eugenio Scanziani[3], Stefano Mantero[1,2], Chiara Buracchi[2], Cristina Sobacchi[1,2], Angelo Lombardo[4], Luigi Naldini[4], Paolo Vezzoni[1,2], Anna Villa[1,2]
[1]Milan Unit Istituto di Ricerca Genetica e Biomedica CNR, Milano, Italy, [2]Humanitas Clinical and Research Center, Rozzano (Mi), Italy, [3]Mouse&Animal Pathology Laboratory, Fondazione Filarete, Milano, Italy, [4]San Raffaele Telethon Institute for Gene Therapy and San Raffaele Scientific Institute and Vita Salute Univeristy, Milano, Italy

Autosomal Recessive Osteopetrosis caused by mutations in the *TCIRG1* gene, is a severe bone disorder characterised by

bone marrow fibrosis and consequent pancytopenia, multiple spontaneous fractures, blindness and hearing loss. The *oc/oc* mouse well recapitulates the clinical signs of the disease. To date, haematopoietic stem cell (HSC) transplantation is the unique possible treatment, however the chance of cure is limited by the need for a matched donor. With the final aim to exploit novel therapeutic strategies allowing the use of corrected autologous HSC, we evaluated the feasibility and potentiality of induced pluripotent stem cells (iPSc), as alternative and unlimited source of autologous stem cells. To this end, we reprogrammed murine wild-type (wt) and *oc/oc* fibroblasts into iPSc, to genetically correct the *Tcirg1* mutation by homologous recombination, and to generate haematopoietic stem and progenitor cells able to give rise to functional osteoclasts. We employed a third generation polycistronic lentiviral vector carrying the reprogramming genes Oct4, Sox2 and Klf4, subsequently excisable by the Cre recombinase. After reprogramming, iPS clones with low vector copy number and normal numerical distribution of chromosomes were treated with Cre and sub-cloned. Obtained iPSc showed normal karyotype and pluripotency tested by teratoma formation assay, *in vitro* embryonic germ layers differentiation, and expression of stemness markers by immunocytochemistry and RT-PCR. Importantly, iPSc were successfully derived from *oc/oc* fibroblasts, and then corrected through homologous recombination upon transfection with a BAC containing the wt gene. iPSc were guided to differentiate towards haematopoietic belonging to different lineages. We obtained differentiation towards osteoclasts, the relevant cells in our model, which were functional as demonstrated by the dentine resorption assay. In conclusion, we provided the first evidence of targeted gene correction in osteopetrotic iPSc, supporting the rationale of using iPSc as future source of donor cells in the clinical setting.

Disclosure: The authors declared no competing interests. The research has received funding from the FP7 n.602300 (SYBIL), PRIN Project 20102M7T8X_003,and RF-2009-1499,542 to A.V.

OP28 (P187)

Abstract withdrawn

OP29 (P15)

In Vivo Bone Surface Monitoring Reveals Age-Related Changes in Adaptive Bone (Re)Modelling Sequences

Annette Birkhold[1], Hajar Razi[1], Georg Duda[1], Richard Weinkamer[2], Sara Checa[1], Bettina Willie[1]
[1]Julius Wolff Institut, Charité - Universitätsmedizin Berlin, Berlin, Germany, [2]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

Age-related bone loss is associated with a failure in bone (re)modelling (modelling and remodelling) processes. Bone (re)modelling occurs at spatially and temporally discrete sites to remove damaged or older bone, replacing it with new bone. However, the evaluation of bone (re)modelling has mainly been retrospective. The aim of this study was to identify the time kinetics of (re)modelling in response to loading at different ages. The left tibiae of female C57Bl/6J mice (10 wks: n=6, 26 wks: n=13, 78 wks: n=10) underwent two weeks of in vivo cyclic compressive loading [1]. The right tibia served as control. In vivo microCT at an isotropic voxel resolution of 10.5 µm was performed at the tibial mid-shaft (5% tibia's length; day 0, 5, 10, 15). Images were registered, binarised, and segmented. Two consecutive images in a common coordinate system were compared (day k–day k+1) to identify formation (F), resorption (R) and quiescence (Q) sites on the cortical endocortical and periosteal bone surfaces [2]. Comparing the three time intervals (d0–d5, d5–d10, d10–d15), 27 (re)modelling sequences (FFR, RRF, QQF, etc) were identified. Using this dynamic micro-tomography based technique we could show that during two weeks of skeletal loading, bone adaptation occurs predominantly by modelling-bases formation and resorption processes, which last less than 10 days. In young and adult bones adaptive formation processes can be extended to a longer time-interval, whereas the elderly mice lost this ability. Ageing reduced adaptive modelling (spatially unlinked formation and resoprtion) and increased remodelling (resorption followed by formation). This *in vivo* approach of tracking local movements of the endosteal and periosteal bone surface allows not only to detect how strong the local response to mechanical loading is, but when it sets in and how long it lasts. This should be of great help to find an adequate stimulation – not only mechanical, but also pharmaceutical – that results in a sustained response of bone formation on the cortical surfaces.

Disclosure: The authors declared no competing interests. This study was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft; WI 3761/1-1, WI 3761/4-1).

References

1. Willie *et al.* (2013). *Bone*.
2. Birkhold *et al*. (2014) *Biomaterials*.

OP30 (P216)

Evidence that the Human *SOST* Gene is 1α,25-Dihydroxyvitamin D Sensitive

Asiri Wijenayaka[1], Nobuaki Ito[1], Masakazu Kogawa[1], Matthew Prideaux[1], Paul Anderson[2], David Findlay[1], Gerald Atkins[1]
[1]The University of Adelaide, Adelaide, SA, Australia, [2]The University of South Australia, Adelaide, SA, Australia

Sclerostin, the *SOST* gene product, is a negative regulator of bone formation and a positive regulator of bone resorption. In a screen to identify novel regulators of *SOST* expression, we found that treatment of human primary osteoblasts with 1α,25-dihydroxyvitaminD_3 (1,25D) resulted in increased expression of *SOST* mRNA and sclerostin protein. This effect was also evident in the human osteosarcoma/osteocyte-like cell line SAOS2. Effects on *SOST* mRNA levels occurred as early as 3 hours post-stimulation, consistent with a direct effect of 1,25D on the *SOST* promoter. Sequence analysis of the published human *SOST* gene revealed a single putative vitamin D response element (VDRE) upstream of the transcription start site (TSS). Cloning of this sequence into a luciferase reporter construct upstream of the constitutive thymidine kinase (TK) promoter, and transfection into HEK-293T cells, identified the presence of a 1,25D responsive element with activity equivalent to that of the well characterised mouse osteopontin VDRE. Electrophoretic mobility shift analysis (EMSA) of HEK-293T nuclear extracts revealed a 1,25D dependent gel-shift, consistent with binding of the VDR/RXR heterodimeric com-

plex. Sequence substitution in the VDR/RXR half-sites abolished VDRE reporter activity and binding of nuclear proteins. In addition, transient expression of a 6.3 kb fragment of the proximal *SOST* promoter ahead of the TSS in a luciferase expression vector demonstrated a promoter responsive to 1,25D. However, addition of a known bone specific enhancer region ECR5 ahead of the cloned promoter fragment did not increase the level of responsiveness to 1,25D. Mutation or deletion of the predicted VDRE resulted in the 6.3 kb *SOST* promoter being unresponsive to 1,25D. We conclude that 1,25D is a direct regulator of *SOST* gene expression, extending the pathways of control of sclerostin expression.

Disclosure: The authors declared no competing interests. This work was supported by the National Health and Medical Research Council of Australia (grant numbers APP1004871, APP1047796).

OP31 (P217)

Estradiol Modulates and Recovers Osteocyte Metabolic/Lipid Profiles after Ovariectomy in Acute and Long-Term *In Vivo* Hormone Replacement Therapeutics

Ana M. Silva[1,2], Ana C. Moreira[3], Maria S. Santos[1,2], Juliana R. Dias[4,5], Nuno Alves[4,5], Romeu A. Videira[6], Rui A. Carvalho[1,2], Vilma A. Sardão[1]

[1]CNC - Center for Neuroscience and Cell Biology, Coimbra, Portugal, [2]Department of Life Sciences, University of Coimbra, Coimbra, Portugal, [3]IBMC.INEB, University of Porto, Porto, Portugal, [4]CDRSP - Centre for Rapid and Sustainable Product Development, Marinha Grande, Portugal, [5]IPL – Instituto Politécnico de Leiria, Leiria, Portugal, [6]CQ-VR – Chemistry Centre – Vila Real, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal

For the first time, we assessed the metabolic and lipid profiles of osteocytes *ex vivo*. During menopause, the appearance of an osteoporotic condition can be associated with an overall metabolic decline in bone cells, and we hypothesised that it is mainly attributed to osteocyte metabolic and lipid changes, which are attenuated after increasing blood oestradiol (E2) levels. To test this, we considered control and ovariectomised (OVX) female rats in order to compare metabolic/lipid profiles of bone-embedded osteocytes, in the presence or absence of E2. Animal groups (used accordingly with FELASA approved procedures) were: a) Controls SHAM, CTL; b) ovariectomised animals, OVX; and c) OVX+E2 (single bolus injection 30 µg/Kg, 24 hours prior sacrifice for the acute study. For the subchronic study, rats were implanted with 0.5mg E2 slow release pellets for 21 days. 24 hours prior sacrifice, animals were I.P. injected with deuterated water for metabolic fluxes analyses. Left and right femurs and tibia were surgically removed and freeze-clamped or preserved for DXA and µCT analysis. Extracted metabolites from those cells as well as total lipids were analysed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy and ^{2}H NMR for *de novo* lipogenesis analyses. Total lipids were extracted, quantified and analysed by HPLC-MS and fatty acids analysed by GC-MS. Ovariectomy clearly changed lipid profile inducing significant changes in both diacyl- and choline-plasmalogens content (comparatively with SHAM and OVX+E2 groups). Also, an increase of the relative proportion of long chain fatty acids was observed in the OVX group, being attenuated by 24h-treatment with E2. Total lipids analysis revealed that E2 was able to recover the CTL profile in OVX+E2, and decrease *de novo* synthesis of lipids after 21 days treatment. In terms of metabolites profile, the OVX group presented a slight decrease of lactate/alanine ratio, although osteocytes were forced to produce high levels of lactate after E2 treatment, increasing this ratio. Our results show a change in the process of lipid remodelling as a result of ovaries removal, with E2 partially compensating the alteration in long chain fatty acids. High levels of PC-Plasmalogens measured in OVX animals may be related to a signalling/protective action against the damaging effects of oxidative stress triggered by the E2 decline. Acute E2 administration in OVX animals induced osteocytes to increase aerobic glycolysis in an attempt to compensate for the metabolic deficit associated with ovaries removal. Long-term therapeutics supported the effects of the acute study, indicative of the high impact of this hormone in osteocytes metabolism.

Disclosure: The authors declared no competing interests. This Work was supported by PTDC/AGR-ALI/108326/2008 and PEst-C/SAU/LA0001/2013-2014 grants from Fundação para a Ciência e Tecnologia (FCT), co-funded by FEDER/Compete/National Funds. FCT PhD and Post-doc fellowships (SFRH/BD/33892/2009, SFRH/BD/76086/2011 and SFRH/BPD/31549/2006, respectively). The work was also supported by QREN project #4832 "Stemcell-based platforms for Regenerative and Therapeutic Medicine", reference CENTRO-07-ST24-FEDER-002008.

OP32 (P445)

Plasminogen Activator Inhibitor-1 is Involved in Glucocorticoid-Induced Osteopenia, Diabetes and Muscle Wasting in Mice

Yukinori Tamura[1], Naoyuki Kawao[1], Masato Yano[1], Kiyotaka Okada[1], Katsumi Okumoto[2], Yasutaka Chiba[3], Osamu Matsuo[4], Hiroshi Kaji[1]

[1]Department of Physiology and Regenerative Medicine, Kinki University Faculty of Medicine, Osakasayama, Japan, [2]Life Science Research Institute, Kinki University, Osaka, Japan, [3]Clinical Research Center, Kinki University Hospital, Osakasayama, Japan, [4]Kinki University Faculty of Medicine, Osakasayama, Japan

Glucocorticoids (GC) have been widely used for the treatment of inflammatory disease. Despite high efficacy of GC treatment, its clinical use is limited by the numerous adverse effects, including osteoporosis, diabetes and muscle wasting. However, its pathogenesis remains unclear, and the evidence for systemic mediators in GC effects are lacking. Plasminogen activator inhibitor-1 (PAI-1) is adipocytokine, which is induced by GC treatment. Previous studies suggest that elevated circulating PAI-1 level is associated with several metabolic disorders, such as diabetes and osteoporosis. Therefore, in the present study, we examined the role of PAI-1 in GC-induced osteoporosis, glucose/lipid abnormalities and muscle wasting by using PAI-1-deficient mice. GC treatment for 4 weeks markedly increased the levels of circulating PAI-1 and PAI-1 mRNA in white adipose tissues in wild-type mice. Quantitative CT and histological analysis revealed that PAI-1 deficiency blunted GC-induced bone loss and the number of osteoblasts decreased by GC treatment in tibia of mice. Moreover, exogenous PAI-1 treatment induced apoptosis in primary os-

teoblasts obtained from mouse calvaria in vitro, suggesting that PAI-1 deficiency protects from GC-induced bone loss presumably through a decrease in apoptosis of osteoblasts. PAI-1 deficiency significantly improved insulin resistance but not hyperlipidaemia induced by GC treatment in mice. In vitro study revealed that exogenous PAI-1 treatment inhibits insulin-induced phosphorylation of Akt and glucose uptake in hepatocytes, but not in adipocytes and myotubes, suggesting that PAI-1 is involved in GC-induced insulin resistance by affecting hepatocytes. Moreover, PAI-1 deficiency blunted GC-induced muscle loss in mice. In conclusion, we first demonstrated that PAI-1 is involved in the metabolic adverse effects of GC treatment, such as bone loss, insulin resistance, and muscle wasting in mice. PAI-1 may be a novel therapeutic target for decreasing GC-induced adverse outcomes and also a diagnostic marker of GC-induced osteoporosis, diabetes and muscle wasting.
Disclosure: The authors declared no competing interests.

OP33 (P225)

HIF1α Down-Regulates MMP-13 Expression through Blockade of Wnt Canonical Signalling

Wafa Bouaziz[1,2], Johanna Sigaux[1,2], Dominique Modrowski[1,2], Caroline Marty[1,2], Hang-Korng Ea[1,2], Sylvain Provot[1,2], Martine Cohen-Solal[1,2], Eric Hay[1,2]
[1]NSERM UMR-1132, Paris, France, [2]University Paris-Diderot, Paris, France

Background: Chondrocyte catabolism and MMP-13 expression are triggered by activation of Wnt pathway in osteoarthritis (OA) along with a loss of hypoxic environment. The mechanism by which Wnt/β-catenin pathway is lost down-regulated physiologically is unknown. We speculated that Hypoxia Inducible Factor 1α (HIF1α) regulates Wnt activation and catabolism. Here we investigated the effect of HIF1α/β-catenin interaction in the regulation of MMP-13 expression in OA.
Methods: Murine chondrocytes from WT and ΔHIF1α were cultured with Wnt3a in 21% O_2 and 1% O_2 (hypoxic) and we analysed the expression of the catabolic markers. The binding of TCF4 to *MMP13* regulatory region was assessed by Chip assay. To determine the role of this interaction *in vivo*, $\Delta HIF1\alpha^{chon}$ and $HIF1\alpha^{fl/fl}$ mice underwent DMM and received articular injection of PKF 118-310, an inhibitor of β-catenin/TCF4 interaction.
Results: Hypoxia abolished the Wnt induced decrease of *COL2* and the increase of *MMP13* expression. HIF1α knockout enhanced the expression of *Mmp13* while HIF1α over-expression inhibited it. In hypoxic chondrocytes, Chip assay reveals that HIF1α lowered β-catenin/TCF4 binding to Mmp13 regulatory region by interacting directly with β-catenin. Induced OA resulted in a decreased HIF1α expression in articular cartilage of WT mice. Furthermore, DMM in $\Delta HIF1\alpha^{chon}$ mice induce more sever cartilage lesions with higher expression of β-catenin and Mmp13. Local administration of PKF 118-310 prevented cartilage lesions and reduced Mmp13 expression.
Conclusion: Here, we show that HIF1α prevents cartilage degradation through blockade of β-catenin/TCF4 binding and decrease of Mmp-13 expression. HIF1α is an inhibitor of Wnt signalling and should be targeted in OA to reduce chondrocyte catabolism.
Disclosure: The authors declared no competing interests.

OP34 (P226)

UBR5, an E3 Ubiquitin-Protein Ligase, Regulates Hedgehog-mediated Articular Cartilage Homeostasis

David Mellis, Michela Grillo, Natalie Dora, Elaine Kinsella, Antonia Sophocleous, Stuart Ralston, Donald Salter, Robert Hill, Mark Ditzel
IGMM, School of Molecular, Genetic and Population Health Sciences, Western General Hospital, University of Edinburgh, Edinburgh, UK

Our objective was to investigate UBR5's role in regulating articular cartilage homeostasis. Our work has revealed the N-end Rule Ubiquitin-protein ligase UBR5 as a potent suppressor of osteoarthritis-associated changes in murine articular cartilage (AC). Using Prx-Cre combined with a floxed UBR5 mutant allele we deleted UBR5 function in the developing murine limb buds. Homozygous UBR5 mutant (UBR5mt) limbs appeared morphologically normal, but exhibited reduced Hedgehog signalling (*IHH, PTCH1, GLI1*) and perturbed expression two master regulators of chondrocyte biology (*MSX2* and *RUNX2*). Six-week-old UBR5mt animals exhibited chondrocyte clustering, massively increased numbers of hypertrophic-like chondrocytes, osteophytes, vascular invasion and cartilage fibrillation (n=6). By 12 weeks of age, UBR5mt animals exhibited dramatic AC loss down to the subchondral bone (n=6). We hypothesise that UBR5 influences stem/progenitor-mediated control of AC homeostasis. Our work in other murine tissues indicates UBR5 as an important regulator of stem/progenitor cell function, with UBR5 being highly upregulated in pericytes, the progenitors of mesenchymal stem cells. Furthermore, we revealed that UBR5 regulates both Indian Hedgehog (IHH) ligand production and signal transduction. IHH-mediated signalling plays a central role in governing stem/progenitor cell function in various tissues, including juvenile and adult bone. Based on IHH's role in the growth plate, we hypothesise that UBR5 normally functions to promote IHH-mediated suppression of chondrocyte hypertrophy and AC homeostasis. Our current work addresses this hypothesis by (i) utilising Hedgehog pathway agonists and antagonist and (ii) Hedgehog signalling-associated gain-and loss-of-function alleles to modify the UBR5mt AC phenotype. We conclude that UBR5 regulates AC homeostasis and suppresses chondrocyte hypertrophy.
Disclosure: The authors declared no competing interests. This work was supported by The University of Edinburgh.

OP35 (P227)

PiT1/Slc20a1 Mediates Survival of Chondrocytes from Endoplasmic Reticulum-Induced Stress *In Vivo* and *In Vitro*

Greig Couasnay[1,2], Sarah Beck-Cormier[1,2], Sophie Sourice[1,2], Jérôme Guicheux[1,3], Laurent Beck[1,2]
[1]Inserm U791, Nantes, France, [2]University of Nantes, Nantes, France, [3]CHU PHU4 OTONN, Nantes, France

The synthesis of an abundant extracellular matrix (ECM), together with a harmful microenvironment triggers an evolutionary conserved mechanism known as the unfolded protein response (UPR). Activation of UPR allows an endoplasmic reticulum (ER) homeostasis leading to cell survival and appropriate ECM synthesis. If sustained, activation of the UPR leads to apoptosis. Recently, we have shown that PiT1, mostly described as a phosphate transporter, is expressed in the ER of chondrocytes. To

elucidate its cellular functions and physiological role, we have generated inducible chondrocyte-specific PiT1 knockout mice ($PiT1^{cKO}$) by crossing $PiT1^{lox/lox}$ with $Agc1^{tm(IRES\text{-}creERT2}$ mice and treated by tamoxifen at postnatal day (P) 3 (french ethical approval #02286.01). Histological analysis of humerus sections at P5 revealed the presence of a hypocellular zone in the center of the growth plate due to massive cell death. Analysis of the phenotype at earlier stages showed an upregulation of the UPR-associated pro-apoptotic factor CHOP in PiT1-depleted cells. The ultrastructure of $PiT1^{cKO}$ chondrocytes analysed by electron microscopy showed a massively distended ER, a hallmark feature of ER-stressed cells. *In vitro*, triggering of an ER stress in primary chondrocytes and ATDC5 chondrogenic cells led to a strong induction of *PiT1* expression which was dependent on the UPR transducers $ATF6_N$, ATF4 and XPB1s. Furthermore, PiT1-depleted ATDC5 cells showed an increased sensitivity to ER stress-induced apoptosis as demonstrated by CHOP upregulation. Importantly, overexpression of the wild-type PiT1 or phosphate transport-deficient PiT1 mutant rescued this phenotype, illustrating that the implication of PiT1 in UPR is independent from its phosphate transport function. Our results suggest that under ER stress conditions, PiT1 regulates balance between chondrocyte survival and death. Of importance, the phenotype of $PiT1^{cKO}$ mice is reminiscent of HiF1α and PTEN deletion in cartilage. The mechanistic links between HiF1α/ PTEN pathways and PiT1 are currently being investigated in our lab.

Disclosure: The authors declared no competing interests. This work was supported by Région Pays de la Loire ("Nouvelles Equipes nouvelles Thématiques" grant). Greig Couasnay was the recipient of a Région Pays de la Loire doctoral fellowship.

OP36 (P237)

Circulating Microvesicles from Elderly Donors Modulate Osteogenic Differentiation of Mesenchymal Stem Cells Through the Delivery of microRNAs

Sylvia Weilner[1,2], Elisabeth Schraml[1], Matthias Wieser[1], Paul Messner[3], Andrea Maier[4], Heinz Redl[5,6], Peter Pietschmann[7], Matthias Hackl[8], Regina Grillari-Voglauer[1,2], Johannes Grillari[1,2]

[1]Department of Biotechnology, CD laboratory on Biotechnology of Skin Aging, BOKU - University of Natural Resources and Life Sciences Vienna, Vienna, Austria, [2]Evercyte GmbH, Vienna, Austria, [3]Department of NanoBiotechnology, Vienna Institute of BioTechnology, University of Natural Resources and Life Sciences Vienna, Vienna, Austria, [4]Department of Gerontology and Geriatrics, Leiden University Medical Center, Vienna, Austria, [5]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, AUVA Research Center, Vienna, Austria, [6]Austrian Cluster for Tissue Regeneration, Vienna, Austria, [7]Department of Pathophysiology and Allergy Research, Center of Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria, [8]TAmiRNA GmbH, Vienna, Austria

Ageing is a complex process that results in the decline of physiological functions due to accumulation of damage in cells and tissues as well as due to reduced repair capacities. The regenerative power of stem and progenitor cells has been found to decline with age and to be influenced by the systemic environment. In particular, the osteogenic differentiation capacity of mesenchymal stem cells (MSCs) has been shown to decrease with age thereby contributing to decelerated bone formation and the development of osteopenia or osteoporosis. The objective of this study was to identify circulating factors of the aged systemic environment that influence the functionality of adult stem cells. In order to identify such factors, the microRNA content in serum of young versus elderly healthy individuals was analysed using quantitative PCR. Levels of hsa-miR-31-5p were found to be strongly induced in serum of elderly donors. As a possible source senescent endothelial cells that secrete exosomal miR-31-5p were identified in vitro, since it could be shown that exosomal miR-31-5p can be transferred to target cells such as MSCs. Subsequently, exosomes with elevated levels in miR-31-5p were harvested either from senescent endothelial cells or elderly donors, and used for treatment of MSCs prior and during osteogenic differentiation, resulting in slowed differentiation. The effect of the vesicles could be rescued by antagonistic miR-31-5p, and mimicked by delivery of miR-31-5p alone. One of the novel targets of miR-31-5p in this context is the WNT ligand FZD3 whose knock-down leads to a similar inhibition of osteogenic differentiation as miR-31-5p. In conclusion, we could identify a novel mechanism by which circulating microvesicles and their content might impact tissue physiology during ageing. Furthermore these data show that microvesicles might represent a source for biomarkers as well as therapeutic targets in age-related diseases like osteoporosis.

Disclosure: Johannes Grillari acts as scientific advisor to TAmiRNA GmbH and CSO of Evercyte GmbH. This work was supported by: EU-FP7 Health Project FRAILOMIC 305483; and EU-FP7 Health Project SYBIL 602300.

OP37 (P238)

Circulating microRNAs that are Induced by Osteoporotic Fractures Modulate Osteogenic Differentiation of Mesenchymal Stem Cells

Sylvia Weilner[1,2], Susanna Skalicky[1,3], Peter Dovjak[4], Peter Pietschmann[5], Johannes Grillari[1,2], Matthias Hackl[1,2]

[1]TAmiRNA GmbH, Vienna, Austria, [2]Department of Biotechnology, CD laboratory on Biotechnology of Skin Aging, BOKU - University of Natural Resources and Life Sciences Vienna, Vienna, Austria, [3]Evercyte GmbH, Vienna, Austria, [4]Salzkammergut-Klinikum Gmunden, Gmunden, Austria, [5]Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University Vienna, Vienna, Austria

MicroRNAs (miRNAs) regulate gene expression on a post-transcriptional level and are known to take part in the control of bone formation and bone resorption. In addition, it is known that miRNAs are secreted by many cell types and can transfer "messages" to recipient cells. Thus, circulating miRNAs might not only be useful as surrogate biomarkers for the diagnosis or prognosis of pathological conditions, but could be actively modulating tissue physiology. The objective of this study was to test whether circulating miRNAs that exhibit changes in recent osteoporotic fracture patients could be causally related to bone metabolism. For this purpose an explorative qPCR analysis of 175 miRNAs in serum samples obtained from 7 female patients with recent osteoporotic fractures at the femoral neck, and 7 age-matched controls was performed. Unsupervised cluster analysis revealed a high discriminatory power of the top 10 circulating miRNAs for patients with recent oste-

oporotic fractures. In total 6 miRNAs, miR-10a-5p, miR-10b-5p, miR-133b, miR-22-3p, miR-328-3p, and let-7g-5p exhibited significantly different serum levels in response to fracture (multiple testing adjusted p-value < 0.05). These miRNAs were subsequently analysed in a validation cohort comprising 23 patients (11 control, 12 fracture), which confirmed significant regulation for miR-22-3p, miR-328-3p, and let-7g-5p. A set of these and of other circulating miRNAs previously reported in the context of osteoporosis were subsequently tested for their effects on osteogenic differentiation of human mesenchymal stem cells (MSCs) *in vitro*. The results show that 5 out of 7 tested miRNAs could modulate osteogenic differentiation *in vitro*. Overall, these data suggest that levels of specific circulating miRNAs change in the context of recent osteoporotic fractures and that such perturbations of "normal" levels might affect bone metabolism or bone healing processes.

Disclosure: Matthias Hackl is employed by TAmiRNA GmbH. The work was supported by: EU-FP7 Health Project FRAILOMIC 305483; and EU-FP7 Health Project SYBIL 602300.

OP38 (P390)

Development of a Lentiviral Vector to Express RANKL in Mesenchymal Stem Cells for the Therapy of RANKL-Dependent Osteopetrosis

Ciro Menale[1,2], Lorenzo Diomede[1,2], Francesca Schena[3], Lucia Sergi Sergi[4], Eleonora Palagano[5,1], Lucia Susani[1,2], Cristina Sobacchi[1,2], Anna Villa[1,2]

[1]Genetics and Biomedical Research Institute - CNR UOS Milan, Milan, Italy, [2]Humanitas Clinical and Research Hospital, Rozzano, Milan, Italy, [3]Laboratory of Immunology and Rheumatic Disease - Istituto G. Gaslini IRCCS, Genova, Italy, [4]San Raffaele Telethon Institute for Gene Therapy, Milan, Italy, [5]Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy

Autosomal Recessive Osteopetrosis (ARO) is a rare bone disease characterised by an increase in bone density due to the failure of bone resorption by impaired osteoclast development or function. The only therapy is haematopoietic stem cell transplantation, which, however, is not effective in osteoclast-poor RANKL-dependent ARO, since in bone RANKL is produced mainly by stromal cells. On the other hand, mesenchymal stem cells (MSCs) transplantation (MSCT) could represent a possible effective therapy. To verify this hypothesis, we established bone marrow derived MSCs (BM-MSCs) lines from the Rankl$^{-/-}$ mouse model, which recapitulates the human disease, and we developed a third generation lentiviral vector expressing human soluble RANKL (hsRANKL) for their correction. This vector carries hsRANKL under the CMV promoter and GFP under the hPGK promoter. The lentivirus production was performed by calcium phosphate transfection in HEK293T cells with the pMDL-g/pRRE, pMD2-VSVg, pRSV-Rev plasmids. In order to evaluate transduction efficiency, the produced vector was tested by transducing HEK293T cells at different multiplicity of infection (MOI). Fluorescence microscopy and FACS analysis showed about 100% GFP^{+} cells, while hsRANKL production, assessed by western blot and ELISA on the culture supernatant, increased proportionally to the MOI (ranging from 1 to 100) and was stable over time. However, the higher the MOI (50 and 100 MOI), the higher the cytotoxicity observed. Based on these data, we performed a lentiviral hsRANKL transduction in Rankl$^{-/-}$ BM-MSCs at 20 and 50 MOI, to define the optimal transduction conditions. After transduction 99.5% of MSC were GFP^{+}. While in Rankl$^{-/-}$ control cells the cytokine was not detected, in corrected cells RANKL production and secretion was measurable and comparable with sRANKL levels in wild type (WT) mouse and human BM-MSCs. We are currently testing the transduced MSCs by *in vitro* functional assays; then we will use them in Rankl$^{-/-}$ mice.

Disclosure: This work was supported by FP7 [HEALTH.2013.2.1.1-1] -SYBIL-"System biology for the functional validation of genetic determinants of skeletal diseases".