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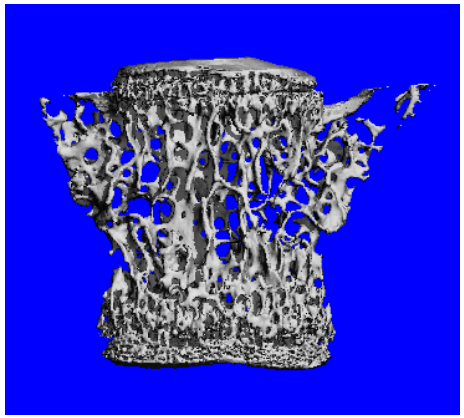
Archived Showcase Programs

Showcase of Medical Discoveries

A Focus on Bone Research



Wednesday
January 22, 2020
4:30—6:00 p.m.

A Wine & Cheese Reception Featuring UAMS Investigators Discussing their Research and Discoveries.

Location: Winthrop P. Rockefeller Cancer Institute
10th Floor Rotunda

UAMS
Research & Innovation

Poster #26

GPR109A Regulates Osteoclastogenesis and Bone Resorption in Mice

Jin-Ran Chen, Haijun Zhao, and Oxana P. Lazarenko

The G protein-coupled receptor 109A (GPR109A) is expressed robustly in osteoclastic precursor macrophages. We previously reported that GPR109A may mediate effects of diet-derived phenolic acids such as hippuric acid (HA) and 3-(3-hydroxyphenyl) propionic acid (3-3-PPA) on inhibiting osteoclastogenesis and bone resorption. However, the role of GPR109A in metabolic bone homeostasis and osteoclast differentiation has not been investigated. In this report, we found bone mass including bone mineral density (BMD) and content (BMC) were significantly higher (37% increased on trabecular) in tibia of 4 week old GPR109A gene deletion ($GPR109A^{-/-}$) male mice, compared to their wild type controls. Osteoclast numbers *in vivo* in bone and *in ex vivo* bone marrow cell cultures (in the presence of 50 ng/ml RANKL) after TRAPase staining were significantly decreased (by ~81%) in $GPR109A^{-/-}$ animals compared to wild type controls. In accordance with these data, we found significantly decreased levels of CTX-1 in bone marrow plasma and decreased gene expression of bone resorption markers (TNFα, TRAP, Cathepsin K) from $GPR109A^{-/-}$ mice compared to their wild type controls. cAMP levels in bone marrow plasma were increased in $GPR109A^{-/-}$ mice compared to their wild type controls. Non-adherent bone marrow hematopoietic osteoclast precursors isolated from 4 week old wild type mice were treated with HA or 3-3-PPA in the presence of RANKL. HA and 3-3-PPA substantially inhibited RANKL-induced GPR109A expression (mRNA and protein), and osteoclast differentiation. HA and 3-3-PPA had no additional effects on inhibiting osteoclastogenesis and osteoclast resorptive activity in *ex vivo* cultures of osteoclast precursors from $GPR109A^{-/-}$ mice, compared to cells without HA or 3-3-PPA treatments. These results suggest an important role for GPR109A during osteoclast differentiation and bone resorption, and GPR109A may mediate effects of HA and 3-3-PPA on inhibiting bone resorption.

Poster #25

Bone-derived Sclerostin has Endocrine Actions in Adipocyte Precursors and Pancreatic Beta Cells

Ashley L. Daniel, Adam Ferrari, Jessica H. Nelson, Kevin McAndrews, Meloney Cregor, Ziad Ghazzawi, William Thompson, Carmela Evans-Molina, Teresita Bellido, and Jesus Delgado-Calle

In this work, we investigated the crosstalk between bone and distant tissues, including the pancreas and peripheral fat depots. We found that mice with activated β-catenin in osteocytes (daβcatOt) exhibit high serum Sclerostin, an effect accompanied by increased in whole-body fat and peripheral white (WAT) and brown (BAT) adipose tissue mass and a marked impairment in glucose metabolism. Genetic deletion of the gene SOST/Sclerostin, an osteocyte-derived soluble factor that regulates bone formation locally in bone, restored to control levels the elevated body-fat mass and WAT mass, but not BAT, and improved glucose tolerance in daβcatOt mice. Treatment with recombinant Sclerostin enhanced adipogenic differentiation of primary adipocyte precursors in vitro. Further, treatment with Sclerostin fully prevented the increase in insulin mRNA expression and decreased insulin secretion induced by high-glucose media, demonstrating that Sclerostin acts directly on pancreatic β-cells to impair insulin production. In concert, these findings provide new evidence supporting that endocrine actions of Sclerostin mediate the crosstalk between bone and fat and the pancreas.

UAMS Research and Innovation is pleased to
co-sponsor the 27th Showcase of Medical Discoveries
on Bone research with the College of Medicine.

Check out the latest collaborative research projects by
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∞

Poster #1

Bone-Targeted Inhibition of Notch Signaling Blocks Tumor Growth and Prevents Bone Loss Without Inducing Gut Toxicity In Immunodeficient and Immunocompetent Murine Models of Established Multiple Myeloma

Adam Ferrari, Kevin McAndrews, Jessica Nelson, James Bell, Venkatesan Srinivasan, Frank H. Ebetino, Robert K. Boeckman Jr, G. David Roodman, Teresita Bellido, and Jesus Delgado-Calle

In this work, we generated a bone specific Notch inhibitor (BT-GSI) by using a hydrolyzable linker to conjugate GSI-XII to a targeting agent with high affinity for bone (BT). BT-GSI was first examined in immunodeficient mice injected with JJN3 human myeloma cells. BT-GSI decreased Notch in bone but not in the brain or gut, and decreased by 45% tumor burden and osteolytic area. Moreover, BT-GSI decreased resorption by 30%, but did not affect bone formation. BT-GSI was also tested in immunocompetent mice injected with 5TGM1 murine myeloma cells. BT-GSI decreased tumor burden and the number of osteolytic lesions by ~50%. These results show that bone-targeted Notch inhibition reduces MM growth and preserves bone mass in mice with established myeloma disease. Because BT-GSI inhibits Notch signaling selectively in the bone-myeloma niche and lacks gut toxicity, it is a promising therapeutic approach to inhibit tumor growth and prevent bone loss in multiple myeloma.

Poster #24

Flexion Instability after Knee Replacement

Jeffrey B. Stambough, Erin Mannen, Paul K. Edwards, C. Lowry Barnes, and Simon C. Mears

We have examined the reliability of physical examination in measuring flexion instability of the knee after replacement. Flexion instability is one of the most common complications of knee replacement that leads to pain and revision surgery. Revision surgery has a poorer chance of success when compared to revision for other diagnoses.

We determined the inter observer reliability of four surgeons examination using motion analysis capture. 10 patients with previous knee replacements were examined on the same day by each of the four surgeons, each using their standard physical exam technique.

We found that each surgeon used different amounts of force when examining the patients' knees. We found poor correlation between surgeons in measuring the amount of instability of the knee when compared to motion analysis measurement.

Our study indicates that the gold standard test for determining flexion instability of a knee replacement is unreliable when compared to biomechanical measurements.

Poster #23

Use of BT2-PEG2, a Novel Bone-targeting Agent for the Enhanced Delivery of Antibiotics and other Biological Agents for the Treatment of Bone Infections

Zaineb A.F. Albayati, Narsimha R. Penthala, Shobanbabu Bommagani, Ginell R. Post, Philip Breen, Mark S. Smeltzer and Peter A. Crooks

Osteomyelitis is defined as any inflammatory process in bone caused mostly by *Staphylococcus aureus*. Vancomycin is often the only effective treatment for bone infections. However, vancomycin has low bone bioavailability when administered systemically. Therefore, BT2-peg2, a bone-targeted agent, has been developed and conjugated to vancomycin as BT2-peg2-vancomycin. In a previous study, vancomycin has been administered i.v. and i.p. to Albino Wistar male rats as BT2-peg2-vancomycin. Data have demonstrated that BT2-peg2-vancomycin administration resulted in elevated levels of serum creatinine and blood urea nitrogen, decreased serum albumin, and produced an altered pharmacokinetic (PK) profile that afforded extremely high plasma concentrations of BT2-peg2-vancomycin. Additional studies afforded similar PK profiles after i.p. injection using a multiple dosing regimen (seven doses at 12 h intervals). We believe that the toxicity exhibited by BT2-peg2-vancomycin is due to vancomycin alone and not to BT2-peg2, since vancomycin is known to exhibit kidney toxicity at high doses and high plasma concentrations. The present study was intended to determine whether BT2-peg2 contributes to the observed nephrotoxicity of BT2-peg2-vancomycin. The results provides evidence that BT2-peg2 does not exhibit toxicological properties in and of itself and is a safe structural moiety that can be conjugated to other antibiotics and biological agents for targeted delivery to bone for treatment of bone infections, warranting further pre-clinical and clinical investigation.

Poster #2

An Antibody Against Oxidized Phospholipids Prevents Age Related Bone Loss and Improves Glucose Metabolism in Mice

Michela Palmieri, Teenamol E. Joseph, Horacio Gomez-acevedo, Joseph L. Witztum, Stavros C. Manolagas and Elena Ambrogini

Oxidized phospholipids containing phosphocholine (PC-OxPL) are pro-inflammatory lipid peroxidation products present on oxidized low density lipoproteins and apoptotic cells. IgM E06 is a natural antibody that blocks the deleterious effects of PC-OxPL. Overexpression of a single chain form of E06 IgM (E06-scFv) increases bone mass in mice by increasing bone formation. Age-related bone loss is characterized by reduced bone formation, increased oxidative stress and lipid peroxidation. In addition, anti-PC IgM decrease with age, suggesting that increased oxidized phospholipids and decreased antibodies against them contribute to age-related bone loss. To test this hypothesis, we aged E06-scFv transgenic mice and WT littermates. BMD analysis of up to 24 months showed that E06-scFv prevented age-related bone loss in both sexes. Moreover, E06-scFv mice accumulated less fat mass than WT littermates and had improved glucose tolerance. E06-scFv, therefore, may be a prototypic therapeutic intervention that simultaneously ameliorates more than one of the consequences of aging.

Poster #3

A Blueberry-enriched Diet Counteracts the Effects of Estrogen Deficiency in Mice on Bone, Skeletal Muscle, and Peripheral Fat, and Alters the Gut Microbiome.

Sato AY, Nakatsu CH, Pellegrini GG, Cregor M, McAndrews K, Bosco S, McCabe LD, McCabe GP, Ferruzzi M, Lila MA, Peacock M, Burr D, Weaver CM, and Bellido T.

Diets containing natural plant products exhibit protective effects on the adult skeleton by unclear mechanisms. We identified a blueberry cultivar (Montgomery= Mont) that protects from ovariectomy (OVX)-induced bone loss. Bones from Mont-fed mice exhibited increased endogenous antioxidant response (EAR), represented by phase-II detoxifying and antioxidant enzymes. Mont did not prevent OVX-induced reduction in the expression of C3, an estrogen receptor ERE-containing responsive gene demonstrating that protection was achieved without activating canonical estrogenic actions. Mont-fed mice were also protected from OVX-induced bone microarchitectural deterioration and increased resorption. Mont-fed mice were also protected from OVX induced skeletal muscle loss, and the increase in body weight and peripheral fat mass. The diet effects on the musculoskeletal system were associated with gut microbiome changes known to affect homeostasis of several tissues, and studies by analyzing fecal bacterial DNA using 16S rRNA gene sequences from high throughput paired end MiSeq technology. OVX did not induce significant microbiome changes. In contrast, Mont-fed mice exhibited a statistically significant alteration of the microbiome compared with control-fed mice (axis1=54.4% per MANOVA, p=0.001). Analysis of the phylogenetic diversity detected higher prevalence of the bacterial communities *Alloprevotella*, *Ruminococcus* (starch fermenters), and an unclassified taxon *Coriobacteriales Incertae* in Mont-fed mice. By contrast, control-fed mice exhibited higher prevalence of *Bifidobacterium* and *Coriobacteriaceae UCG-002* communities. These findings highlight the impact of nutrition on the musculoskeletal system and the gut microbiome, and suggest nutritional interventions to prevent the harmful effects of estrogen deficiency.

Poster #22

Rapid Recovery After Total Joint Arthroplasty Using General Anesthesia

Jeffrey B. Stambough, Barnes Bloom, Paul K. Edwards, Gregory R. Mehaffey, C. Lowry Barnes, and Simon C. Mears

Multiple papers have purported the superiority of spinal anesthesia used in total joint arthroplasty (TJA). There is a paucity of data available for modern general anesthesia (GA) regimens.

We retrospectively reviewed 1527 consecutive primary TJAs (644 total hip arthroplasties and 883 total knee arthroplasties) performed over a 3-year span at UAMS.

From the elective TJAs performed using a modern GA protocol, 96.3% (n = 1471) of patients discharged on postoperative day 1, and 97.2% (n = 1482) of subjects were able to participate with physical therapy on the day of surgery. Only 6 patients (0.4%) required an intensive care unit stay postoperatively. The 90-day readmission rate over this time was 2.4% (n = 36), while the reoperation rate was 1.3% (n = 20).

Our data support the notion that modern GA techniques that limit narcotics and certain inhalants can be successfully used in short-stay primary total joint arthroplasty.

Poster #21

Mesenchymal Stem Cell-derived Extracellular Vesicles as Therapeutic Agents for Skeletal Tissue Repair

Elisabeth Ferreira, Landon Gatrell, and Ryan M. Porter

Mesenchymal stem/stromal cells (MSCs) are being evaluated for treating tissue injuries with insufficient endogenous repair response. Preclinical evidence suggests that the contributions of exogenous MSCs to tissue repair are mainly indirect, through paracrine effects on local progenitors. Extracellular vesicles (EVs) have been implicated in such paracrine actions, suggesting their use as an alternative to direct MSC therapy. The goal of this project is to determine whether MSCs can be preconditioned to produce EVs enriched in signals that stimulate endochondral bone repair. EVs were harvested from the conditioned media of human bone marrow MSCs cultured as spheroids in 21% or 1% O2 with or without chondrogenic stimulus. Under all conditions tested, MSCs secreted abundant numbers of 50-200 nm vesicles displaying established EV markers. EV yields were higher from chondrogenic spheroids, while hypoxic challenge did not alter EV production substantially. Among these preconditions, differences in EV miRNAs and mRNAs that regulate vasculogenesis and osteochondrogenesis are being determined.

Poster #4

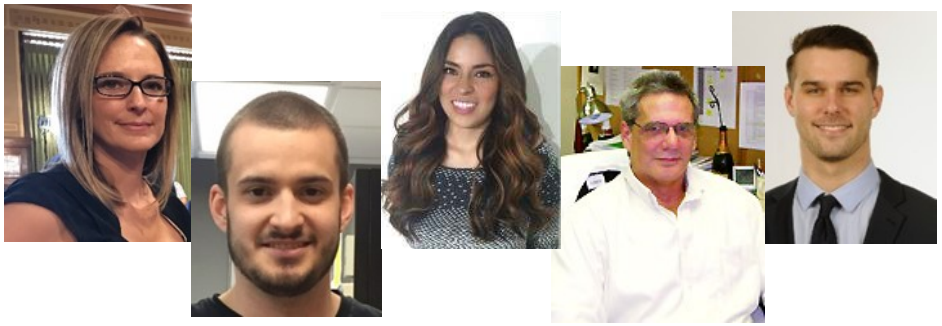
Osteocyte Transcriptome Dysregulation in Two Mouse Models of Osteogenesis Imperfecta

Sarah M. Zimmerman, Milena Dimori, Melissa E. Heard-Lipsmeyer, and Roy Morello

Osteogenesis imperfecta (OI), the most commonly inherited bone fragility disorder, is a congenital disease characterized by osteopenia/frequent fractures with a severity spectrum (mild to lethal). Studies point to osteoblasts as the primary dysfunctional cell type in OI, but osteocytes' role in OI pathogenesis is still poorly understood. We hypothesized that OI osteocytes are dysregulated and may contribute to the disease process. In this study, we took an unbiased approach and set out to compare the transcriptome of osteocytes from control mice with that of osteocytes from two OI mouse models (i.e., *CrtapKO* and *oim/oim* mouse). RNA was extracted from osteocyte-enriched cortical femurs and tibias harvested from WT, *CrtapKO* and *oim/oim* mice, sequenced and subsequently analyzed to identify differentially expressed transcripts. A large number of transcripts were dysregulated in either model of OI (544 and 855 transcripts in *oim/oim* and *CrtapKO* mice vs WT, respectively) but 281 of them were similarly up- or down-regulated in both compared to WT controls. Bioinformatics analyses identified several critical dysregulation hubs that were enriched in annotation terms such as development/differentiation, ECM and collagen fibril organization, cell adhesion, signaling, regulatory processes, pattern binding, chemotaxis, and cell projections. The osteocyte transcriptome appears greatly dysregulated in OI. The data further showed alterations in important signaling pathways (i.e., WNT and TGF-β), but also highlighted new candidate genes to pursue in future studies. Conversely, very few transcripts were differentially expressed between the *Crtap*KO and *oim/oim* mice, indicating that distinct alterations in type I collagen can lead to shared pathogenic processes and phenotypic outcomes.

Poster #5

The Staphylococcal Accessory Regulator (sarA) plays a Critical Role in Staphylococcus aureus Bone Infection by Repressing the Production of Extracellular Proteases

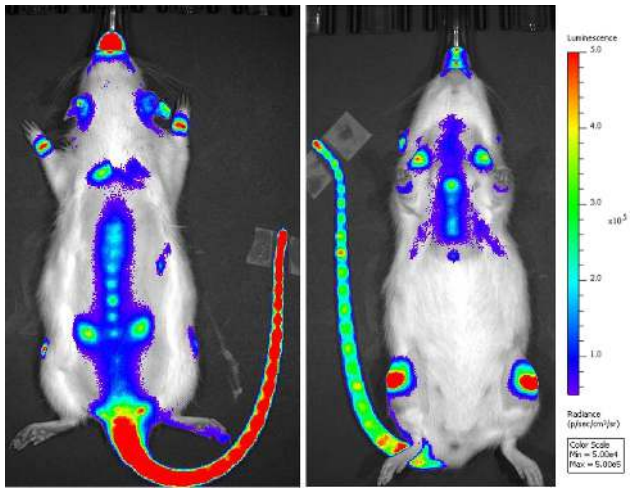


Karen E. Beenken, Joseph S. Rom, Aura M. Ramirez,
Daniel G. Meeker, and Mark S. Smeltzer

Osteomyelitis is a complicated infection that requires an inter-disciplinary clinical approach that includes long-term systemic antibiotic therapy and, in most cases, surgical debridement to remove damaged and contaminated bone. Even after such extensive medical and surgical intervention, recurrence of the infection is an all too common occurrence that can lead to amputation. *Staphylococcus aureus* is the most prominent cause of osteomyelitis and the bacterial pathogen that causes the most bone destruction in the shortest period of time. We have demonstrated that the staphylococcal accessory regulator (*sarA*) plays a key role in this regard owing to its ability to repress the production of extracellular proteases that would otherwise limit the accumulation of the *S. aureus* virulence factors required for biofilm formation and cortical bone destruction. These results demonstrate that *sarA* could be an important therapeutic target for the prevention and treatment of osteomyelitis.

Poster #20

A Transgenic Rat for Noninvasive Assessment of Chondrogenic Activity In Vivo



Elisabeth Ferreira and Ryan M. Porter

Chondrogenic differentiation, or chondrogenesis, represents a bottleneck to skeletal tissue repair. Rat models are frequently employed in the preclinical development of treatment strategies for stimulating new chondrogenesis. To support these efforts, we generated a transgenic rat strain that can report chondrogenic differentiation nondestructively. The specificity of type II collagen expression in chondrogenic lineage cells was employed to produce a conditional expression construct incorporating bioluminescent (firefly luciferase) and fluorescent (mCherry) reporters, and the resulting DNA was injected into Lewis rat embryos. For two candidate founders and their offspring, substantial bioluminescence signal was detected noninvasively from cartilaginous tissues, including the limb synovial joints, tail, sternum, nose, and ears. Signal strength was intense in weanlings, but declined rapidly with continued development. Even in adults, however, luminescent signal remained readily detectable. mCherry expression was also observed in these tissues upon fluorescence imaging of whole explants or frozen sections. Ongoing studies are evaluating this strain in models of skeletal repair and heterotopic bone formation.

Poster #19

CXCL12 Deletion in Osteoprogenitors Causes a Dramatic, Albeit Balanced, Increase in the Rate of Bone Remodeling and Attenuates the Loss of Cortical Bone Mass Caused by Estrogen Deficiency in Mice

F. Ponte, H.N. Kim, S. Iyer, L. Han, M. Almeida,
and S.C. Manolagas

CXCL12 is abundantly expressed in reticular cells associated with the perivascular niches of the bone marrow, promotes osteoclastogenesis and has been implicated in pathologic bone resorption. We have now generated female mice with conditional deletion of a *Cxcl12* allele in Prrx1 targeted cells ($Cxcl12^{\Delta Prrx1}$). Ovariectomy increased the expression of *Cxcl12* and B cell number in the $Cxcl12^{f/f}$ control mice, but these effects were abrogated in the $Cxcl12^{\Delta Prrx1}$ mice. Cortical or trabecular bone mass was not affected in $Cxcl12^{\Delta Prrx1}$ mice. Albeit, the cortical bone loss caused by ovariectomy was greatly attenuated. Most unexpectedly, the rate of bone turnover in sex steroid sufficient $Cxcl12^{\Delta Prrx1}$ mice was dramatically increased, as evidenced by a more than two-fold increase in several osteoblast- and osteoclast- specific mRNAs, as well as increased mineral apposition and bone formation rate and increased osteoclast number in the endosteal surface. The magnitude of the $Cxcl12^{\Delta Prrx1}$ induced changes were much greater than those caused by ovariectomy in the $Cxcl12^{f/f}$ mice. These results strengthen the evidence that CXCL12 contributes to the loss of cortical bone mass caused by estrogen deficiency. Moreover, they reveal for the first time that in addition to its effects on hematopoiesis, CXCL12 restrains bone turnover – without changing the balance between resorption and formation – by suppressing osteoblastogenesis and the osteoclastogenesis support provided by cells of the osteoblast lineage.

Poster #6

Osteoblast Production of Osteoprotegerin Suppresses Osteoclast Formation

Keisha M. Cawley, Nancy Cecile Bustamante-Gomez, Anveshi Guha, Ryan S. MacLeod, Jinhu Xiong, Igor Gubrij, Linda Liu, Robin Mulkey, Michela Palmieri, Jeff D. Thostenson, Joseph J. Goellner, and Charles A. O'Brien

Osteoprotegerin (OPG) inhibits osteoclast formation by binding RANKL. To identify the cellular sources of OPG, we crossed OPG-flox mice with different Cre-driver stains and compared the skeletal phenotype with OPG-null mice. Deletion of OPG using Dmp1-Cre mice, which delete in osteoblasts and osteocytes, reduced cancellous and cortical bone volume to levels similar to OPG-null mice. To distinguish between osteocytes and osteoblasts as sources of OPG, we deleted OPG using Sost-Cre mice, which delete target genes in osteocytes and hematopoietic cells, but not osteoblasts. Mice lacking OPG in Sost-Cre-targeted cells exhibited only slightly lower cancellous bone in the spine compared to controls. Similarly, femoral cortical bone was reduced compared to controls but the changes were less than those observed in OPG-null mice. Deletion of OPG from B lymphocytes had no impact on bone mass. This evidence suggests that osteoblasts are a major source of the OPG involved in suppressing osteoclast formation.

Poster #7

Abaloparatide is More Effective than PTH in Promoting Bone Gain under Physiological Conditions and in Established Type 1 Diabetes in Male Mice.

Ucer Ozgurel S, McAndrews K, Halladay D, Sato AY, Nelson J, Cregor M, and Bellido T

Therapeutic approaches that prevent bone fragility induced by diabetes are needed. Using a preclinical murine model of established type 1 diabetes (T1D) induced by Streptozocin (STZ), we compared the effect of PTH (1-34) and abaloparatide (ABL), ligands of the PTH 1 receptor (PTH1R) and FDA approved bone anabolic agents. After 4 wks of established T1D, bone mineral density (BMD) was decreased compared to C mice. Mice were then injected daily with PTH (50 ng/g/day), ABL (47.5 ng/g/day), or vehicle (veh). No changes in BMD were found after 2 wks of treatment in C mice, but both PTH1R ligands increased BMD to a similar extent in diabetic mice. After 4 wks, PTH and ABL increased BMD in both C and T1D mice; but ABL was more effective than PTH. T1D mice exhibited bone microarchitectural deterioration, which was improved in ABL-treated mice compared to veh or PTH treated mice in both C and T1D conditions. Both PTH and ABL increased circulating bone resorption and bone formation markers in diabetic mice after 2 wks; but only ABL sustained the increase after 4 wks. Both PTH and ABL prevented the upregulation of Sost mRNA expression induced by T1D in bone. In contrast, only ABL increased the expression of osteoclast and osteoblast markers in bone of T1D mice. Thus, at equal molar doses ABL is more effective than PTH in increasing bone mass and restoring the cortical and trabecular bone lost with diabetes, due to higher and longer lasting increases in bone remodeling.

Poster #18

Straight from the Shoulder: A Bare Bones Approach to Shoulder Joint Instability

Surbhi Raichandani, MD, Gitanjali Bajaj, MD, Roopa Ram, MD, Kedar Jambekhar, MD, and Tarun Pandey, MD

This educational exhibit serves to function as a ready reference guide to approaching shoulder joint instability.

TEACHING POINTS

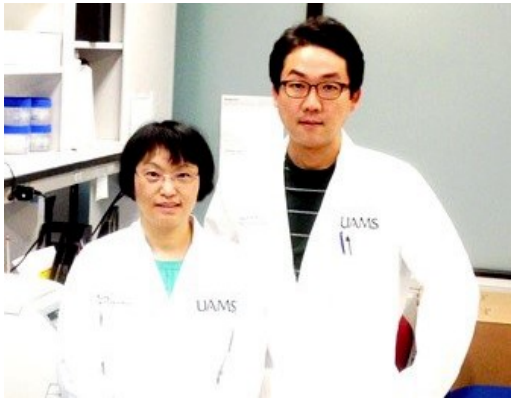
1. Understand functional anatomy and relevant biomechanics of the shoulder joint
2. Recognize the contribution of core soft tissue support to maintain stability
3. Discern various patterns of instability and their subtypes
4. Visualize imaging features of the most common lesions resulting in instability

OUTLINE

1. Graphical review of essential clinical anatomy & shoulder biomechanics
2. Classification of shoulder instability 1. Unidirectional - Anterior - Posterior - Inferior 2. Multidirectional 3. Microinstability
3. Illustrative review of shoulder instability patterns with discussion of relevant pathological lesions
4. Case based review of shoulder instability on MRI, CT and conventional imaging
5. Review of potential pitfalls and normal anatomical variants

Poster #17

Deletion of the Mitochondrial Deacetylase Sirt3 Prevents Age-related Bone Loss in Mice



Kimberly Krager, Wen Ling, Aaron Warren, Nukhet Aykin-Burns, Maria Almeida, and Ha-Neui Kim

Excessive bone resorption can cause bone loss and lead to osteoporosis. Old age plays a prominent role in the pathogenesis of osteoporosis in both sexes. However, the cellular and molecular mechanisms responsible are only partially understood. Sirt3 is the primary mitochondrial protein deacetylase involved in stimulating mitochondrial function and energy homeostasis, most likely by promoting Complex I activity and cellular ATP levels. However, its physiological role in bone cells and skeletal homeostasis during aging remains largely unknown. To determine if Sirt3 plays a role in age-associated bone loss, we generated mice with global deletion (KO) of Sirt3 gene and characterized their bone phenotype. We found that global deletion of Sirt3 prevented the bone loss caused by old age (16 months of age) in both male and female mice by decreasing osteoclast number and bone resorption. In contrast, Sirt3 KO mice exhibited no overt skeletal changes at 6 months of age. Consistent with this, the Sirt3 KO mice did not develop age-associated cortical bone porosity, an important component of bone quality that deteriorates with aging. Mechanistic *in vitro* studies demonstrated that suppression of Sirt3 prevents the effects of RANKL on the differentiation and activation of cultured osteoclast precursors from aged mice, accompanied by a decrease in mitochondrial function. However, we did not observe obvious changes in RANKL-induced osteoclast formation in young mice between the genotypes. Taken together, these results indicate that Sirt3 activation in aged mice stimulates bone resorption, most likely by promoting mitochondrial function.

Poster #8

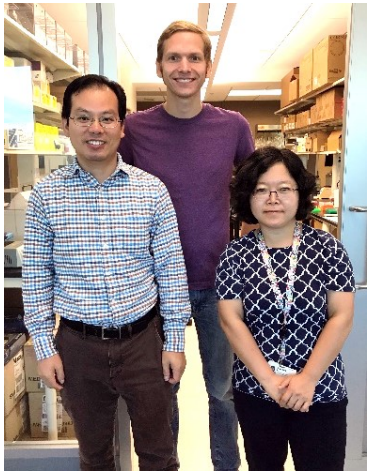
Chaperone-Mediated Autophagy Contributes to Skeletal Remodeling in Growing Female Mice

Nisreen Akel, Ryan MacLeod and Melda Onal

Chaperone-mediated autophagy (CMA) is a protein degradation mechanism that can eliminate soluble cytoplasmic proteins that are damaged, incorrectly folded, or targeted for selective proteome remodeling. CMA has been proposed to contribute to DNA repair, cellular reprograming and cellular stress response. However, the role of CMA in skeletal remodeling under physiological and pathophysiological conditions are unknown. To start addressing the role of CMA for skeletal remodeling, we produced mice that lack CMA in all tissues. Five-week-old CMA-deficient female mice had lower cortical and cancellous bone mass compared to their wild type controls. Bones of female CMA-deficient mice exhibited higher mRNA levels of the pro-resorptive cytokine RANKL and osteoclast marker genes compared to controls. Unlike the female mice, five-week-old male CMA-deficient mice did not have a skeletal phenotype. Taken together these results suggest that lack of CMA may cause increased bone resorption that leads to low bone mass in growing female mice.

Poster #9

Piezo1 is Essential for Bone Formation Induced by Mechanical Signals



Xuehua Li, Han Li, Intawat Nookaew, Erin Mannen, Matthew J. Silva, Maria Almeida, and Jinhu Xiong

In humans and mice, bone size and strength depend greatly on mechanical signals caused by physical activity. Reduced physical activity due to disuse causes profound bone loss and increases the risk of fractures. Evidence suggests that osteocytes, cells buried in bone matrix, sense changes in mechanical load and orchestrate bone adaptation. However, the mechanisms involved in skeletal mechanosensation are poorly understood. We have identified Piezo1 as an ion channel that is essential for attainment of normal bone size and strength during growth. It is also essential for the response of bone to exercise or disuse in adult mice. Importantly, activation of Piezo1 with a small molecule is sufficient to increase bone formation and bone mass in mice, mimicking the effects of exercise. These results demonstrated that Piezo1 plays an important role in the skeletal response to changes in mechanical load and provide a novel target for anabolic bone therapy.

Poster #16

A Decrease in NAD+ Contributes to the Loss of Osteoprogenitors and Bone Mass with Aging

Ha-Neui Kim, Filipa Ponte, Rebecca Ring, Srividhya Iyer, Li Han, and Maria Almeida

Age related osteoporosis results from a decrease in bone formation due to a deficit in osteoblasts, the cells that secrete bone matrix. The number of osteoblast progenitors also declines with age associated with increased markers of cell senescence. The forkhead box O (FoxO) transcription factors attenuate Wnt/β-catenin signaling and the proliferation of osteoprogenitors, and thereby decrease bone formation. The NAD+ dependent Sirt1 deacetylates FoxOs and β-catenin in osteoblast progenitors and, thereby, increases bone mass. However, it is unknown whether dysregulation of the Sirt1/FoxO/β-catenin pathway contributes to age-related bone loss. We found that the levels of NAD+ were decreased in osteoblast progenitor cultures from old mice associated with increased acetylation of FoxO1 and markers of cell senescence. Addition of the NAD+ precursor nicotinamide riboside (NR) to the cultures decreased the acetylation of both FoxO1 and β-catenin and several markers of cellular senescence. Consistent with these effects, NR increased the osteoblastogenic capacity of cells from old mice. NR administration to aging mice attenuated the decline in osteoblastogenic markers and the loss of bone mass. Attenuation of NAD+ levels via genetic or pharmacological means in osteoprogenitor cultures from young mice mimicked the effects of aging. Mice with decreased NAD+ in mesenchymal lineage cells exhibited accelerated skeletal aging. These findings suggest that the decrease in bone formation with old age is due to a decrease in NAD+ and dysregulated Sirt1/FoxO/β-catenin pathway in osteoblast progenitors. NAD+ repletion, therefore, represents a rational therapeutic approach to skeletal involution.

Poster #15

Genome-wide Approach to Defeating Staphylococcus aureus as an Orthopaedic Pathogen

Duah Alkam, Piroon Jenjaroenpun, Karen Beenken, Anthony R. Richardson, Thidathip Wongsurawat, David Ussery, and Mark Smeltzer

Staphylococcus aureus is the most problematic of all orthopaedic pathogens, and this implies that *S. aureus* has specific attributes that allow it to survive and persist in the microenvironment of bone. *S. aureus* has many attributes at its disposal, which makes identifying those that are most important extremely complicated. The development of next generation sequencing technology and powerful bioinformatics tools has provided a means of addressing this on a genome-level scale. One example of this is the ability to use a transposon mutant library and bioinformatics tools to identify mutants that have a reduced capacity to survive in specific microenvironments within the human host (Tn-Seq). Given the extreme clinical significance of *S. aureus* as a cause of osteomyelitis we used this technology to identify *S. aureus* mutants that exhibit a reduced capacity to avoid host defenses and survive within the specific microenvironment of bone.

Poster #10

Evaluation of a Polyurethane-based Bone Regeneration Scaffold for Local Antibiotic Delivery to Prevent Staphylococcus aureus Infection in a Contaminated Segmental Bone Defect

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Open fractures and penetrating wounds that damage the underlying bone are extremely complex injuries that involve the bone itself and surrounding soft tissues. Penetration through the skin resulting in exposure to the environment also makes such injuries highly susceptible to infection. Thus, two critical clinical concerns in the treatment of open fractures and penetrating injuries involving bone are avoiding infection and restoring the structural integrity of damaged bone. Our group developed a novel scaffold technology that promotes bone regeneration and can be used to locally deliver bioactive agents including antibiotics, but to date it's efficacy with respect to preventing infection has not been experimentally tested. Here we demonstrate that vancomycin can be incorporated into this scaffold in sufficient amounts to prevent infection from developing in a bone defect contaminated with *Staphylococcus aureus* without compromising its bone regenerative properties.

Poster #11

Biomechanical Changes following Pavlik Harness Treatment for Developmental Dysplasia of the Hip

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Developmental dysplasia of the hip (DDH) occurs in 5.5/1000 newborns, with many receiving treatment via Pavlik harness which holds infants' legs in a flexed/abducted position to encourage proper hip development. However, failure rates are 20%, and the mechanisms are not understood. The purpose of this study was to measure lower-extremity muscle activity and hip positions of DDH infants (treated and mild) at onset and after successful treatment and compare them with age-matched controls. Infants (n=3 DDH; n=2 mild; n=20 controls) underwent biomechanical testing: lower-extremity muscle activity and hip positions were measured during a 60s supine-lying task, and a range-of-motion activity was performed. At the initial and follow-up visits, DDH infants demonstrated similar or higher muscle activity to healthy controls, while infants with mild instability demonstrated lower muscle activity and abduction/adduction range-of-motion, which may put them at risk for residual dysplasia. This study is currently enrolling infants to increase sample size.

Poster #14

Metallosis – A Post-Operative Bone Problem of the 21st Century

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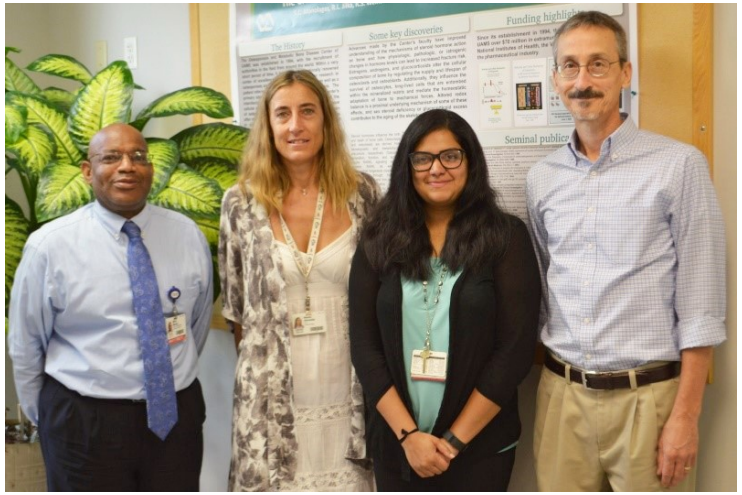
Metal poisoning is a condition in which debris shed from metallic implants within artificial hips, build up in soft tissue and leak into the bloodstream, manifesting as depression, suicidal tendencies and psychosis. Great controversy exists regarding the evaluation, radiological interpretation and therapeutic approach of patients with hip pain with mental health changes. These factors emphasize the importance of imaging of hip arthroplasty to identify clues for the origin of metal debris which could seriously impact morbidity and mortality. Imaging is used as a screening tool in asymptomatic and symptomatic hip prosthesis for infection exclusion and aseptic loosening as a cause of symptoms, assessment of component positioning and identification of solid or fluid-filled pseudotumor. Various types of metal debris associated reactions like Metallosis, Trunnionosis, Pseudotumor and ALVAL (Aseptic Lymphocyte - dominated Vasculitis- Associated Lesion) can be identified on imaging and help surgeons in making educated decisions to improve success of the surgery and overall prognosis.

Here we define metal poisoning and discuss/differentiate types of Metal Debris associated reactions, interpretation of early imaging findings and its impact on patient morbidity and mortality.

Poster #13

Center for Musculoskeletal Disease Research (CMDR)



Jinhu Xiong, Erin Mannen, Ryan Porter, Ha-neui Kim, Melda Onal, Simon Mears, Roy Morello, Intawat Nookaew, Dhivya Suresh, Mark Mosby, Maria Almeida, and Charles O'Brien

The CMDR advances understanding and treatment of musculoskeletal diseases by promoting development of project leaders and creating research teams. The CMDR is composed of the Administrative Core, three research cores, and four research projects, led by junior investigators. The Administrative Core oversees operation of the center, the three research cores, and a faculty development plan. The faculty development plan includes a mentoring program that guides Project Leaders to independence, with the key milestone being independent funding. The Administrative Core also operates a pilot project program to attract new investigators at UAMS. The Administrative Core also works with UAMS leadership to coordinate recruitment of new investigators whose expertise complements that of the Center. The CMDR research cores include a Genetic Models Core, which genetically manipulates cells and mice, a Bone Histology and Imaging Core to analyze hard tissues, and a Bioinformatics Core to analyze large datasets.

Poster #12

Vitamin D Receptor Signaling Prevents the Adverse Actions of Glucocorticoid Excess in Bone, Skeletal Muscle, and the Heart, by Interfering with the Atrogene Pathway

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Glucocorticoid (GC) excess has adverse effects in bone and skeletal muscle that lead to increased risk of bone fractures. GCs upregulate in both tissues the expression of the proteasomal degradation inducers MuRF1, atrogin1, and MUSA1 (atrogenes), thus providing a targetable pathway to prevent GC musculoskeletal actions. We report here that Vitamin D receptor (VDR) activation blocks GC-induced activation of the atrogene pathway and prevents bone and muscle loss. $1,25D_3$ (calcitriol) or the less hypercalcemic VDR ligand eldecalcitol-71 (ED) prevented atrogene upregulation *in vitro*, *ex vivo* and *in vivo*, and protect from GC-induced decrease in bone mineral density (BMD) and loss of lean body mass and the decrease in muscle strength. Further, GC increased atrogene expression also in the heart *in vivo* and *ex vivo* in murine left ventricular (LV) organ cultures, which was prevented by the VDR ligands. These findings demonstrate that atrogene upregulation is a common mechanism underlying the damaging effects of GC excess in bone, skeletal muscle, and heart; and suggest that activation of VDR signaling preserves tissue mass and function by interfering with GC actions on the atrogene pathway in each of these organs.