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Hot Musculoskeletal Research Topics Presentations

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HT1

Sclerostin and Sclerostin Antibody: From Human Genetics to Discovery of Therapeutics for Bone Disorders

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Wnt/beta-catenin signaling plays an important role in bone formation and resorption. Sclerostin (Scl) binds to co-receptors Lrp5/6 and inhibits wnt/beta-catenin signaling in osteoblast lineage, results in decreased bone formation. Inactivation of sclerostin leads to substantially increased bone mass in humans and in genetically manipulated animals. A series of preclinical studies demonstrated that monoclonal antibody targeting sclerostin (Scl-Ab) increases bone formation, decreases bone resorption, and increases BMD and bone strength in animal models of osteoporosis and fracture healing. It has been showed that increased bone formation induced by Scl-Ab was primary stemmed from activating bone formation on the quiescent bone surface (modeling-based bone formation) and prolong the formation of new bone on pre-existing bone remodeling surface in animal models. Scl-Ab treatment in OVX rats decreased osteoclastogenesis in bone marrow culture *in vitro*, and decreased osteoclast number and surface *in vivo*, suggesting that Scl-Ab treatment decreases bone resorption. Despite continuously increased BMD and bone strength, some trabecular and periosteal bone formation temporally increased and gradually returned to control level in a 26-week study while endocortical bone formation remained significantly elevated at end of the study in OVX rats. Furthermore, a humanized Scl-Ab (AMG 785, romosozumab) has been shown to simultaneously increase bone formation markers and decrease bone resorption markers, leading to a significant increase in BMD giving by monthly s.c. injection for 12 months. The increase in BMD was significantly greater in patients treated with romosozumab as compared with patients treated with alendronate or hPTH(1-34). In this clinical study, serum bone formation markers were temporally increased and returned to baseline within 6 to 12 months while serum bone resorption markers remained decreased throughout the 12 months duration. These data suggest that antagonizing sclerostin with a monoclonal antibody is an attractive approach in development of therapeutics for bone disorders. Late-stage clinical trials are ongoing to evaluate the efficacy of Scl-Ab in preventing skeletal fractures in patients with postmenopausal osteoporosis.

HT2

Analysis of miRNAs in Cartilage by TALEN/CRISPR Genome Editing Techniques

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microRNA (miRNA) is a member of ~22 nt non-cording RNA and play a critical role in the tissue or organ development and is also associated with human diseases. We recently found that miR-146 is highly expressed in rheumatoid arthritis synovium to regulate inflammation and that miR-140 is high in cartilage and important for tissue homeostasis against cartilage degradation. Our recent findings by next generation sequencing techniques revealed that series of miRNAs are involved in arthritis pathogenesis. To generate miRNA knockout mice in high-through put way, we applied TALEN and Cas9 system to delete miRNA gene in mice genome, which successfully leads us to analyze miRNA functions *in vivo*. To identify the target genes of each miRNAs, we developed cell based comprehensive gene screening and found molecular network regulated by miRNAs. Combination of above methods may provide the novel aspect of RNA regulatory system and should promote our understanding of arthritis pathogenesis.

HT3

Activin Receptor Type IIB (ACVR2B) Functions In Osteoblasts To Negatively Regulate Bone Mass

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Bone and skeletal muscle mass are highly correlated in mammals suggesting the existence of common anabolic signaling networks in these two anatomically adjacent tissues that serve to coordinate their development. Myostatin and related activin ligands in the TGF-β)/BMP superfamily are attractive candidates to fulfill such a role. Increased skeletal muscle mass in mice treated with myostatin/activin decoy receptors is accompanied by increases in bone mass which has been attributed to physical/mechanical stimuli emanating from the increased skeletal muscle mass. Here we show that major components of the myostatin/activin receptor are expressed in bone and

function independent of skeletal muscle to control bone mass postnataly. Immunohistochemistry localized ACVR2B to osteoblasts and osteocytes in trabecular and cortical bone. Primary mouse osteoblasts expressed both ACVR2B and ACVR IB (ALK4) and were expressed in primary osteoblasts and the expression of ACVR2B and demonstrated decreased pSmad2/3 levels upon exposure to activin ligands. To test the importance of ACVR2B signaling in bone *in vivo*, we analyzed the skeletal phenotype of mice deficient in this receptor in mature osteoblasts (OC-Cre x Acvr2b Fl/Fl). Femurs from 6 week old mutant mice had significantly increased trabecular and cortical bone volume but modest increases in bone formation rate measured histomorphometrically. *In vitro*, osteoblasts lacking ACVR2B exhibited enhanced differentiation by measures of alkaline phosphatase activity and mineral deposition. Taken together, these data indicate that activin receptor type IIB functions in osteoblasts as a negative regulator of bone mass.

HT4

Trabecular Plates and Rods Fully Determine Elastic Modulus and Yield Strength of Human Trabecular Bone

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Microstructurally, trabecular bone is mainly composed of individual trabecular plates and rods, which can be determined from the emerging high-resolution skeletal imaging modalities such as micro computed tomography (μCT) or clinical high-resolution peripheral quantitative CT (HR-pQCT) using the individual trabecula segmentation (ITS) technique. It has been shown that the ITS-based plate and rod parameters are highly correlated with elastic modulus and yield strength of human trabecular bone. In the current study, plate-rod (PR) finite element (FE) models were constructed completely based on ITS-based individual trabecular plates and rods. Their ability and efficiency in predicting elastic modulus and yield strength of human trabecular bone were determined. Human Trabecular bone cores from proximal tibia (PT), femoral neck (FN) and greater trochanter (GT) were scanned by micro computed tomography (μCT). Specimen-specific ITS-based PR FE models were generated for each μCT image and corresponding voxel-based FE models were also generated in comparison. Both types of specimen-specific models were subjected to nonlinear FE analysis to predict the apparent elastic modulus and yield strength using the same trabecular bone tissue properties. Then, mechanical tests were performed to experimentally measure the apparent modulus and yield strength. The ITS-based PR models predicted accurately both elastic modulus and yield strength determined experimentally across three distinct anatomic sites ($P > 0.15$). Strong linear correlations for both elastic modulus (r^2=0.97) and yield strength (r^2=0.96) were found between the PR FE model predictions and experimental measures, suggesting that trabecular plates and rods morphology adequately captures three-dimensional (3D) microstructure of human trabecular bone. In addition, the PR FE model predictions in both elastic modulus and yield strength correlated highly to the voxel-based FE models (r^2=0.99, r^2=0.98, respectively), resulted from the original 3D images without the PR segmentation. Taking these together, trabecular plates and rods fully and accurately determine elastic modulus and yield strength of human trabecular bone.

HT5

EGFR Signaling in Cartilage Remodeling

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The epidermal growth factor receptor (EGFR) is an essential player in the development of multiple organs during embryonic and postnatal stages, but its function in cartilage development and homeostasis is largely unknown. Recent studies from our group and others have demonstrated that EGFR and its cognate ligand, TGFα, in chondrocytes consist of a critical signaling pathway governing endochondral ossification and articular cartilage maintenance. Using pharmacological and genetic approaches, we have shown that rodents with deficient EGFR activity in chondrocytes exhibit profound defects in postnatal endochondral ossification, characterized by massive accumulation of hypertrophic chondrocytes in the growth plate and delayed formation of secondary ossification center in the long bones. Gene expression analysis and immunohistochemistry revealed that amounts of matrix metalloproteinases (MMP9, -13, and -14) and RANKL in the hypertrophic chondrocytes close to the marrow space are reduced, resulting in decreased cartilage matrix degradation by chondrocytes and osteoclasts. Analyses of EGFR downstream signaling pathways in primary epiphyseal chondrocytes demonstrated that up-regulation of MMP9 and RANKL by EGFR signaling is partially mediated by the canonical Wnt/β-catenin pathway. In line with this result, deficiency in chondrocyte-specific EGFR activity reduces β-catenin amount in hypertrophic chondrocytes *in vivo*. In adult mice, reduction in chondrogenic EGFR activity leads to thinner articular cartilage with more disorganized hypertrophic chondrocytes. Interestingly, after surgical induction of osteoarthritis through destabilization of the medial meniscus, these mice developed much more severe osteoarthritis phenotypes, such as deprivation of the cartilage layer and subchondral bone sclerosis, than wild-type mice, implicating a primarily protective role of EGFR during osteoarthritis progression by regulating chondrocyte survival and cartilage degradation. In conclusion, chondrocyte-specific TGFα/EGFR signaling is an emerging key pathway in cartilage remodeling at both developmental and adult stages.

HT6

Nanomaterial Mediated Nucleic Acid Delivery to Cartilage Joint for Early Diagnosis of Osteoarthritis

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Osteoarthritis (OA) is one of the most common causes of disability. However, the lack of tools for early diagnosis of OA hampers the prevention and treatment of the disease to decelerate articular cartilage loss and alleviate suffering of patients. The OA Biomarker Initiative has identified a series of biomarkers, including Matrix metalloproteinases (MMP), which are elevated in articular cartilage during OA pathogenesis. However, detection of MMP protein levels or activities in serum may not be sensitive enough, while the more sensitive detection of MMP transcripts requires invasive procedure to obtain biopsy of articular joint tissue. Therefore, there is an urgent need to develop sensitive *in vivo* imaging technology to detect molecular changes at early stages of arthritis without harming articular cartilage. Molecular beacon (MB) technology provided an intriguing possibility to detect the changes of mRNA levels in live animals *in vivo*. However, there is no report of detection of OA using MB due to the significant challenge of *in vivo* delivery of MB into joint tissues. In this study, we showed the feasibility of early detection of OA in the Destabilizing Medial Meniscus (DMM) mouse OA model using MB to detect induction of MMP-13 transcript, a major matrix proteinase that degrades interstitial collagen matrix during arthritis. *In vivo* delivery of MMP13 MB was made possible by a novel nanomaterial named Nanopieces that derives from rosette nanotubes. It is especially impressive that this technology detects pathogenesis of OA at an early stage (within a week) in a mild OA model (DMM). The high sensitivity may be due to the detection at the mRNA level and the high efficiency of MB intracellular delivery by Nanopieces.

HT7

Tendon Stem Cells Are Responsible for Loading-Induced Degenerative Tendinopathy

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Tendinopathy at advanced stages often manifests degenerative changes in tendons. The tendon disease affects millions of Americans each year in both occupational and athletic settings. In general, it is thought that excessive mechanical loading is a major etiological factor in tendinopathy, but the precise pathogenic mechanisms leading to tendinopathy remain unclear. Consequently, current clinical treatments for tendinopathy are largely palliative. For a number of years we have been studying the cellular mechanisms that lead to tendinopathy onset and progression. Recently, we and others showed that in addition to tenocytes, tendons contain adult stem cells, termed tendon stem/progenitor cells (TSCs). Since mechanical loading is an integral part of the tendon, we were interested in investigating the mechanobiology of TSCs. Using an *in vitro* model system, we found that small mechanical loading induces differentiation of TSCs into tenocytes, but large mechanical loading also causes TSCs to differentiate into non-tenocytes including adipocytes, chondrocytes, and osteocytes. Furthermore, using a mouse treadmill running model, we were able to show that moderate treadmill running (MTR) causes upregulation of tenocyte-related gene expression; however, intensive treadmill running (ITR) induces TSCs to undergo non-tenocyte differentiation in addition to tenocyte differentiation. Finally, using an irradiation and injection approach, where mouse tendons were irradiated and then injected with GFP-TSCs, we confirmed that TSCs are responsible for upregulation of non-tenocyte gene expression under ITR loading conditions. Therefore, our data indicate that the primary contributor to the development of *degenerative* tendinopathy is the aberrant differentiation of TSCs into non-tenocyte cell lineages, which are caused by excessive mechanical loading placed on the tendons.

HT8

Pamidronate Attenuates Muscle Loss After Pediatric Burn Injury

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Children burned over 40% of total body surface area lose bone and muscle due to inflammation-induced bone resorption and inflammation and glucocorticoid-mediated sarcopenia. Administration of the biosphosphonate pamidronate within 10d of burn injury prevents bone loss in a double-blind randomized control trial (RCT). Because several of the patients participating in the RCT also underwent stable isotope infusion studies of muscle protein kinetics, we reviewed the findings of muscle protein synthesis, breakdown, and net balance in those who participated in the pamidronate RCT. In those who received a single dose of bisphosphonate $\leq$ 10d post-burn fractional protein synthesis rate and muscle protein breakdown were lower than in controls. Net muscle protein balance was positive in the pamidronate group and negative in the placebo group. Moreover, muscle fiber diameter was significantly greater in the pamidronate group and leg strength at 9 m post-burn was not different between pamidronate subjects and normal physically fit age and sex-matched children. Leg strength in burned subjects who received placebo tended to be lower ($P=0.053$).[1] Furthermore, the slope of serum sclerostin concentration from 6-60d post-burn was positive in the pamidronate group and negative in the placebo group ($P=0.016$ by ANCOVA). These data suggest that bisphosphonates preserve osteocyte viability in burn patients and that osteocytes may play a role in the maintenance of muscle mass, possibly by production of a paracrine factor.

Reference

1. Børsheim *et al*., *J Bone Miner Res* 2014; **29**: 1369–1372.

HT9

Mesenchymal Stem Cells Promote the Growth and Pulmonary Metastasis of Osteosarcoma

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Stem cells, especially for the bone marrow derived mesenchymal stem cells (MSCs), can promote the tissue repair or regeneration with great application potential in orthopaedic clinic. However, recent studies indicated that stem cells could contribute to the tumor growth. We have investigated the interactions of MSCs with osteosarcoma (OS) cell line (Saos-2 cells) in tumor microenvironment. We found that the co-injection of MSCs and Saos-2 cells into nude mice could promote the tumor growth and progression. *In vitro*, the proliferation of Saos-2 and MSCs was promoted by each other's conditioned medium, in which IL-6 played an important role. The STAT3 signal pathway in Saos-2 was activated by IL-6 from MSCs. The inhibition of STAT3 in Saos-2 cells by siRNA or AG490 (a JAK2 inhibitor) decreased the proliferation, migration and invasion, down-regulated the mRNA expression of Cyclin D, Bcl-xL and Survivin and enhanced the apoptotic response of Saos-2 cells. Furthermore, AG490 could inhibit the growth and pulmonary metastasis of OS in an nude mice model and prolong the survival time of these mice. MSCs also promoted the anoikis resistance of Saos-2 cells *in vitro* and *in vivo*, which contributed to the pulmonary metastasis of OS. IL8-CXCR1-Akt pathway played an important role in the regulation of the anoikis resistance, and blocking the IL8-CXCR1-Akt pathway with shRNA targeting CXCR1 could inhibit the pulmonary metastasis of OS and prolong the survival time of tumor-bearing mice. Altogether, our data demonstrated that MSCs promote the growth and metastasis of OS and the involved signal pathway would be a new target for the OS treatment.