ABSTRACTS

Symposium on Skeletal Biology, Development, and Disease

University of Kansas Medical Center
Kansas City, Kansas

September 21-22, 2017

SPONSORS

School of Medicine Bohan Visiting Professor Program
KUMC Musculoskeletal Research Program
Department of Anatomy and Cell Biology
Department of Orthopedic Surgery
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symposia Program</td>
<td>3</td>
</tr>
<tr>
<td>Abstracts</td>
<td>5</td>
</tr>
<tr>
<td>Index</td>
<td>15</td>
</tr>
</tbody>
</table>
Symposium on Skeletal Biology, Development, and Disease

Thursday, September 21, 2017:

3:30 p.m.  Registration, Outside SON Auditorium
4:00 p.m.  Welcome/Introductory Remarks – Bruce Toby, M.D./Dale Abrahamson Ph.D.

SCIENTIFIC SESSION 1:  SON Auditorium
Moderator: Jinxi Wang, M.D., Ph.D.
4:10 p.m.  Martin Lotz, M.D., Professor, Department of Molecular Medicine, The Scripps Research Institute, “FOXO Transcription Factors in Joint Maturation, Aging and Osteoarthritis”
4:50 p.m.  Xinmai Yang, Ph.D., Associate Professor, Department of Mechanical Engineering, Kansas University, “Pulsed High-Intensity Laser and Ultrasound to Relieve Post-traumatic Joint Contracture.”
5:10 p.m.  Mark Johnson, Ph.D., Professor and Chair, Department of Oral and Craniofacial Sciences, UMKC School of Dentistry, “Wnt Signaling in Bone and Muscle Crosstalk”
5:50 p.m.  Jinxi Wang, M.D., Ph.D., Harrington Distinguished Professor, Department of Orthopedic Surgery, K.U. Medical Center, "NFAT Signaling in Joint Health and Osteoarthritis"
6:10 p.m.  Poster Session/Reception, SON Atrium

Friday, September 22, 2017:

8:00 a.m.  Registration, Outside Beller Conference Center
8:00 a.m.  Breakfast, 1009 Beller

SCIENTIFIC SESSION 2:  Beller Conference Center
Moderator: Irfan Saadi, Ph.D.
8:30 a.m.  Maurizio Pacifici, Ph.D., Professor and Director of Orthopaedic Research, Children’s Hospital of Philadelphia, “Morphogenetic Mechanisms Regulating Synovial Joint and Articular Cartilage Development”
9:10 a.m.  Pamela Tran, Ph.D., Associate Professor, Department of Anatomy and Cell Biology, K.U. Medical Center, “Novel Ciliary Gene, Thm2, Interacts with Thm1 to Regulate Skeletal Development”
9:30 a.m.  Yang Chai, D.D.S., Ph.D., George and MaryLou Boone Professor of Craniofacial Molecular Biology, Ostrow School of Dentistry, University of Southern California, “Mesenchymal Stem Cells in Craniofacial Development and Tissue Homeostasis”
10:10 a.m.  Irfan Saadi, Ph.D., Associate Professor, Department of Anatomy and Cell Biology, K.U. Medical Center, “Genetic Dissection of SPECC1L Function in Craniofacial Morphogenesis”
10:30 a.m.  Break, 1009 Beller
SCIENTIFIC SESSION 3: Beller Conference Center
Moderator: Brian Andrews, M.D.

11:00 a.m.  James Iatridis, Ph.D., Professor and Vice Chair for Research, Mount Sinai Endowed Chair in Orthopaedic Research, Icahn School of Medicine at Mount Sinai, “Injury and Repair of Painful Intervertebral Discs”

11:40 a.m.  Brian Andrews, M.D., Associate Professor, Department of Plastic Surgery, K.U. Medical Center, “Cranial Bone Reconstruction vs. Regeneration: Why is it Important?”

12:00 p.m.  Christopher Evans, Ph.D., Professor and Director of the Rehabilitation Medicine Research Center, Mayo Clinic, “Gene Therapy for Disorder of Joints”

12:40 p.m.  Terence McIlff, Ph.D., Associate Professor, Department of Orthopedic Surgery, K.U. Medical Center, “TBA”

1:00 p.m.  Closing Remarks/Adjourn – Peter Smith, Ph.D.

1:10 p.m.  Lunch Box Distribution, 1009 Beller

www.kumc.edu/sbdd
SYMPOSIA ABSTRACTS
DNA-based injectable hydrogels for bone defect repair. Sayantani Basu¹, Settimio Pacelli¹, Arghya Paul¹*
¹BioIntel Research Laboratory, Department of Chemical and Petroleum Engineering, School of Engineering, University of Kansas, Lawrence, KS 66045, USA.

DNA-based materials are enriched with inherent qualities such as biocompatibility, programmable sequences and precise recognition properties. The underlying chemical nature of the double stranded DNA facilitates for chemical and physical bonding to other bioactive substances. Moreover, the base-pairing interactions leading to the secondary double stranded structure is highly specific. Given these features, DNA can prove to be an attractive starting material for the fabrication of multifunctional hydrogels and scaffolds for tissue engineering and controllable drug delivery. In this study, we report a new approach to develop physically crosslinked DNA based hydrogels with sustained drug release properties. This approach utilizes the non-covalent interactions of the DNA backbone and silicate nanoparticles to form new injectable nanocomposite hydrogels. The system exploits the electrostatic interactions between the negatively charged phosphate backbone of DNA strands and positively charged surfaces of silicate nanoparticles. As a first step, a pre-gel is formed by heating and cooling of the double stranded DNA, which is further strengthened by the introduction of silicate nanoparticles (Laponite®). We have also investigated the effects of different concentration of Laponite (0.1 - 0.5% w/v) on the gel formation as well as on the physical and mechanical properties of the crosslinked structure. The inclusion of the discotic Laponite nanoparticles assisted in the formation of compact network structure, which in turn resulted in the reduction in pore size of the hydrogel network. Furthermore, we tested the ability of the nanocomposite to modulate the release of a model osteoinductive drug, dexamethasone (Dex), for promoting differentiation of human adipose stem cells (hASCs) to osteogenic lineage. The released drug maintained its activity and modulated gene expression in hASCs. Moreover, the encapsulation of Dex within DNA nanocomposite hydrogels allowed a higher retention of Dex, resulting in significantly increased alkaline phosphatase activity, calcium deposition, and alkaline phosphatase gene expression of hASCs. Overall, the nanoengineered injectable hydrogel obtained from physically crosslinked network is mechanically resilient and can be used for biomedical applications such as bone regeneration and therapeutic delivery.
ELUCIDATING ALTERATIONS IN BONE REMODELING IN WILDTYPE AND OSTEGENESIS IMPERFECTA MODEL MICE (oim/oim) FEMORA DUE TO PHARMACOLOGICAL INHIBITION OF ACTIVIN RECEPTOR IIB

Salah Daghlas¹, Youngjae Jeong¹, R. Scott Pearsall², Charlotte Phillips¹
¹Dept. of Biochemistry, University of Missouri, Columbia, MO
²Acceleron Pharma Inc, Cambridge, MA

Osteogenesis Imperfecta (OI) is a clinically heterogeneous disease characterized by compromised bone quality and strength. It is caused by mutations in genes involved in the synthesis and/or modification of type I collagen, a major constituent of bone. Current treatments come with significant adverse side effects and only partially improve clinical outcomes in OI patients; this necessitates exploring novel therapeutic alternatives. Our laboratory is investigating the effects of Activin Receptor Type IIB-mFc (ActRIIB-mFc), a fusion protein that inhibits the endogenous uptake of myostatin (GDF8), a negative regulator of muscle growth. We hypothesize that increased muscle load on bone will increase bone mass and strength due to bone’s mechanosensitive properties. We employed the oim mouse in our study, a commonly used OI mouse model. The homozygous (oim/oim) and heterozygous (+/oim) mice model moderate to severe and mild OI phenotypes, respectively. Mice were injected with either vehicle (Tris-buffered saline [TBS]) or 10 mg/kg ActRIIB-mFc twice a week from 2 months to 4 months of age, at which time the mice were euthanized and had their femora and tibiae harvested. uCT analyses demonstrated an increase in femoral trabecular bone volume and number with ActRIIB-mFc treatment. For analysis of bone formation, mice were injected with 5 mg/kg calcein green and 20 mg/kg alizarin red, 10 and 3 days prior to sacrifice, respectively. To evaluate the bone remodeling response to ActRIIB-mFc treatment, we employed histomorphometric analyses to quantify bone cell activity and bone growth in the four-month-old oim/oim and Wildtype (WT) femora. Preliminary results suggest ActRIIB-mFc treated mouse femora exhibited increased bone surface and bone formation rates in the presence of decreased osteoclast activity (osteoclast surface/bone surface [OC.S/BS] and osteoclast number/bone surface [OC.N/BS]) with no observed change in osteoblast activity (osteoblast surface/bone surface [OB.S/BS] and osteoblast number/bone surface [OB.N/BS]) regardless of genotype. However, total osteoblast number increased. Thus, our data suggest that ActRIIB-mFc’s mechanism for increasing bone mass includes both inducing bone formation and inhibiting bone resorption.
Cartilage-Specific NFAT1 Overexpression Delays the Onset and Attenuates the Progression of Posttraumatic Knee Osteoarthritis Induced by Meniscal Destabilization

Yi Feng1, Qinghua Lu1, William C. Kramer1, Nicholas C. Barnthouse1, Jinxi Wang1

1Department of Orthopedic Surgery, University of Kansas Medical Center, Kansas City, KS, USA

Introduction: The risk of posttraumatic osteoarthritis (PTOA) after joint injuries ranges from 20% to more than 50% even with the best current care of joint injuries. Our recent studies revealed that mice lacking NFAT1 exhibit normal skeletal development but display dysfunction of articular chondrocytes and OA-like changes in adults, with early osteoarthritic changes in the knee joints at 6-8 months of age. To develop therapeutic strategies for NFAT1 deficiency-mediated PTOA, this study aimed to test our hypothesis that cartilage-specific Nfat1 overexpression will prevent the onset or attenuate the progression of knee PTOA after surgical destabilization of the medial meniscus (DMM) in mice.

Methods: Cartilage-specific Nfat1-expressing (Col2-CA-Nfat1+/−) mice were generated in our laboratory using constitutively active Nfat1 (CA-Nfat1) cDNA under the control of the collagen-2 promoter. We generated bilateral DMM by surgical transection of the medial meniscotibial ligament (MMTL) as described by Glasson et al. [3] in 2 to 3-month-old (both sexes), Col2-CA-Nfat1+/−/Nfat1−/−, Col2-CA-Nfat1−/−/Nfat1−/−, and wild-type (WT) mice. Animals were euthanized at 2, 4, 8, and 16 weeks after surgery. The knee joints were harvested and processed for histochemical and histomorphometric analyses to evaluate the severity of knee osteoarthritis (OA). Statistical analyses were performed with Student t-test and ANOVA. Results: At 2 weeks after DMM, WT and Col2-CA-Nfat1−/− mice (rescue group with cartilage-specific NFAT1 expression) displayed no histopathologic OA changes. At 4 weeks, early OA changes and chondrogenic differentiation of synovial cells were observed in the medial compartment of the knee joints in all Col2-CA-Nfat1−/− mice, but not in Col2-CA-Nfat1+/− mice. At 8 weeks, OA changes further progressed to loss of articular cartilage and formation of osteophytes in the medial side of the knee joints in all Col2-CA-Nfat1−/− mice, while Col2-CA-Nfat1+/− mice only displayed focal loss of safranin-O staining and synovium reaction with no apparent structural changes. At 16 weeks, Col2-CA-Nfat1−/− mice displayed late-stage OA changes in the medial compartment of the knee joints, while Col2-CA-Nfat1+/− mice showed mild OA changes.

Discussion: The onset of PTOA is delayed and the severity of OA is attenuated in Col2-CA-Nfat1+/− mice (rescue group) in which CA-Nfat1 is only expressed in cartilage, but not in any other tissues. Significance: This study identifies the rescue effect of cartilage-specific NFAT1 overexpression on PTOA. This will direct us to develop new anti-OA therapies using NFAT1 as a target.

Acknowledgments: This work was supported in part by the U.S. Department of Defense medical research grant W81XWH-12-1-0304.
Potential mitochondrial dysfunction in mouse models of Osteogenesis imperfecta.
Victoria Gremminger¹, Youngjae Jeong¹, Rory Cunningham², Grace Meers², R. Scott Rector², Charlotte Phillips¹,³
¹Department of Biochemistry, University of Missouri-Columbia
²Departments of Nutrition and Exercise Physiology and Medicine-GI, University of Missouri;  Harry S Truman Memorial VA Hospital
³Department of Child Health, University of Missouri-Columbia

Osteogenesis imperfecta (OI), also referred to as brittle bone disease, is a heritable connective tissue disorder characterized by short stature, fragile bones, craniofacial deformity and muscle weakness. OI is most often the result of autosomal dominant gene defects in the type I collagen genes: COL1A1 and COL1A2. The remaining rare autosomal recessive forms result from defects in gene products responsible for modification and packaging of type I collagen. Muscle weakness not only impacts a patient’s quality of life, but likely negatively affects bone health via altered bone-muscle crosstalk and/or mechanotransduction. The oim/oim mice model severe human type III OI with reduced body mass, skeletal fragility, and reduced muscle mass and function compared to their wildtype (WT) counterparts. Whereas, the G610C OI mice model the less severe human type I/IV OI with skeletal weakness, but normal muscle function. The differential presence of inherent muscle pathology between oim/oim and G610C mice suggest mutation specific mechanisms. To begin to investigate the mechanisms we examined myostatin (mstn, a known negative regulator of muscle growth) expression and citrate synthase activity, a known marker of mitochondrial activity. mstn expression was significantly increased in the soleus muscle of oim/oim, but not G610C mice, compared with WT. Elevation of mstn expression may contribute to reduced muscle size in oim/oim mice. Similarly, citrate synthase activity in the mixed fiber type gastrocnemius muscle was significantly increased only in the oim/oim mice compared to WT, despite evidence of reduced muscle function. G610C mice did not have altered activity. The unexpected increases in citrate synthase activity leads to the question of whether or not mitochondrial biogenesis and/or degradation may be altered in OI.
Moderate SPECC1L deficiency results in palate defects through changes in both oral epithelium and cranial neural crest derived palatal mesenchyme

Everett G. Hall¹, Nathan R. Wilson¹, S. Undurty², J. Standley² E.A. Augustine-Akpan³, Youssef Kousa⁴, Diana S. Acevedo¹, Lenore Pitstick⁵, N. Natsume⁶, Shahnaawaz Yousa⁴, T. Busch², E. Kosa¹, C. Nguyen⁷, M. Nguyen⁷, Wasiu L. Adeyemo⁴, M.A. Eshete⁹, A. Czirok¹, Bryan C. Bjork⁵, Brian C. Schutte⁴, Satoshi Suzuki²,⁶, Jeffrey C. Murray², Azeez Butali³, Irfan Saadi¹.

¹Dept. of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS. ²Dept. of Pediatrics, Craniofacial Anomalies Research Center, University of Iowa, Iowa City, IA. ³Dept. of Oral Pathology, Radiology and Medicine/Dow Institute for Dental Research, College of Dentistry, University of Iowa, Iowa City, IA. ⁴Dept. of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI. ⁵Dept. of Biochemistry, Midwestern University, Downers Grove, IL. ⁶Division of Research and Treatment for Oral and Maxillofacial Congenital Anomalies, Aichi Gakuin University Hospital 2-11 Suemori-Dori, Chikusa-ku, Nagoya, Japan. ⁷Odonto Maxillo Facial Hospital, 263-265 Tran Hung Dao Street, District 1, Ho Chi Minh City, Vietnam. ⁸Dept. of Oral and Maxillofacial Surgery, College of Medicine, University of Lagos, Lagos, P.M.B. 12003, Nigeria. ⁹Dept. of Plastic and Reconstructive Surgery, Addis Ababa University, Addis Ababa, P.O. Box: 26493, Ethiopia.

Clefts of the lip and palate (CL/P) are among the most common craniofacial defects. SPECC1L mutations in patients with rare atypical clefts or syndromic CL/P cluster in the second coiled coil (CCD2) and calponin homology domains, disrupting SPECC1L association with acetylated microtubules. To elucidate the role of SPECC1L in palatogenesis we generated null and hypomorphic Specc1l deficient mouse alleles. Null mutants are lethal at embryonic day 9.5 with defective neural tube closure and cranial neural crest cell (CNCC) delamination. Moderate deficiency of SPECC1L in Specclnull/hypo compound mutants results in palate elevation defects at E14.5, transient oral adhesions, and subepidermal blebbing. In addition, pups with a new allele specifically disrupting CCD2 exhibit cleft palate at birth. We hypothesize that the palate phenotypes are caused by a combination of defects in early CNCC function, CNCC-derived palatal mesenchyme, and oral epithelium. Indeed, live-imaging of E8.5 null mutant embryos indicates reduced CNCC specification and migration. In moderate mutants, live-imaging of primary mouse embryonic palatal mesenchyme cells show impaired collective cell migration, a novel mechanism for palate closure defects. The oral adhesions in our mutants resemble those found in heterozygotes for two known clefting genes, If6 and Grhl3, and we show that SPECC1L expression in the oral epithelium requires IRF6. Together, our data show that deficiency of SPECC1L, specifically disruption of CCD2, is sufficient to affect palatogenesis, and that SPECC1L functions in the emerging IRF6/GRHL3 pathway in lip and palate development. Funding: NIH NIDCR (R01DE026172), NIH COBRE (P20 GM104936).
Characterization of Tibial gene expression patterns of in Osteogenesis Imperfecta Murine (OIM) and G610C models of OI
Catherine Omosule1, Youngjae Jeong 1, Ferris Pfeiffer2 and Charlotte Phillips1, 3
Department of Biochemistry1, Orthopedic Surgery2 and Child Health3
University of Missouri- Columbia

Introduction. Bone is a highly dynamic tissue that is constantly being remodeled by the coupled actions of osteoblasts and osteoclasts, which regulation from osteocytes. In Osteogenesis Imperfecta (OI), a clinically heterogeneous disorder characterized by bone fragility, osteoblast and osteoclast functions are thought to be dysregulated. µCT analyses and biomechanical testing demonstrate compromised bone microarchitecture and biomechanical integrity in OI mouse models, respectively. However, the underlying molecular mechanisms in two molecularly distinct OI mouse models (osteogenesis imperfecta murine [OIM] vs. G610C) that lead to the generation of brittle bones is not yet well understood. The mutations that give rise to the G610C and OIM mouse models differ. In the G610C model, missense mutations in Col1a2 cause a glycine to cysteine substitution at the 610 position of the triple helical domain. The defective collagen is then incorporated into heterotrimeric type I collagen of heterozygote +/-G610C mice 50 percent of the time. However, the OIM model has a single nucleotide deletion in the COL1A2 gene which leads to the generation of functional null prod2 (I) chains that cannot be incorporated into normal heterotrimeric type I collagen, thus leading to the generation of homotrimeric type I collagen in homozygous mice (oim/oim). In this study, we investigated gene expression patterns of specific osteoblast, osteoclast and osteocyte markers in the OIM and G610C mouse models. Method. We examined snap-frozen right tibias from 4month-old male WT, +/-G610C and oim/oim mice. RNA was extracted according to a modified TRizol protocol. Quantitative RT-PCR was performed with 200ng of cDNA per well. Data was analyzed using the delta delta Ct method with ATP6 as the reference gene. Results. Oim/oim mice exhibited significant upregulation in the expression of osteoblast (RUNX2, ALPL, DLX5, BGLAP, SP7, and SERPINH1) and osteoclast (RANKL and CSF1) differentiation markers as compared to WT littermates. However, +/-G610C mice did not exhibit any significant changes in expression of these listed markers. Conclusion: Our data demonstrated that the two molecularly distinct mutations in COL1A2 of OI mouse models (OIM vs. +/-G610C) differentially impacts osteoblast, osteoclast and osteocyte gene expression.
Nanodiamond-Gelatin Hydrogel for Drug Delivery and Bone Tissue Engineering
Ryan Maloney1, Settimio Pacelli2, Aparna Chakravarti1, Flavia Castanho1, Vijayan Manoharan1, Arghya Paul1,2 1Bioengineering Graduate Program, University of Kansas, Lawrence KS, USA. 2Department of Chemical and Petroleum Engineering, Bioengineering University of Kansas, Lawrence, KS, USA.

Background. Nanodiamonds (NDs) have attracted considerable attention as drug delivery nanocarriers due to their low cytotoxicity and facile surface functionalization as compared to other carbon nanomaterials. Given these features, NDs have been recently investigated for the fabrication of nanocomposite hydrogels and scaffolds for tissue engineering. Aim of the study. Assessing the influence of ND-Dexamethasone complexes within the gelatin methacrylamide (GelMA) scaffold on the osteogenic differentiation of encapsulated human adipose mesenchymal stem cells (hASCs) with respect to GelMA scaffolds loaded with only Dex. Results and discussion. In this study, a novel nanocomposite hydrogel was synthesized using photocrosslinkable gelatin methacrylamide (GelMA) hydrogel and NDs as a three-dimensional scaffold for drug delivery and stem cell-based bone regeneration. We investigated the effect of different concentration of NDs (0.05-0.2% w/v) on the physical and mechanical properties of the GelMA hydrogel network. The inclusion of NDs increased the network stiffness, which in turn augmented the traction forces of human adipose stem cells (hASCs) seeded on the surface of the scaffolds. As a next step, we tested the ability of NDs to adsorb and modulate the release of a model drug dexamethasone (Dex) to promote the osteogenic differentiation of hASCs. The ND-Dex complex modulated gene expression, cell area, and focal adhesion number in hASCs. Moreover, the integration of the ND-Dex complex within GelMA hydrogels allowed a higher retention of Dex over time, resulting in significantly increased alkaline phosphatase activity, calcium deposition, and osteocalcin expression of encapsulated hASCs. These results suggest that conventional GelMA hydrogels can be coupled with conjugated NDs to develop a novel platform for bone tissue engineering that enables precise control over the osteogenic differentiation of hASCs. Conclusions. NDs enhanced the mechanical properties of GelMA hydrogels as demonstrated by compressive and rheological studies. Furthermore, the adsorption of Dex onto the surface of NDs enabled a sustained release of Dex in comparison with conventional GelMA scaffolds that were not able to retain Dex over time. Finally, the inclusion of the ND-Dex complex enhanced osteogenic differentiation of encapsulated hASCs in 3D.
A novel compound that shows synthetic lethality for p53 loss in osteosarcoma cells
Alejandro Parrales, Peter McDonald, Mitch Brown, Anuradha Roy, Melinda Broward, Douglas Thamm, Tyce Bruns, Scott J. Weir, Joy Fulbright, and Tomoo Iwakuma

Department of Cancer Biology, University of Kansas Medical Center, Kansas City, KS, USA

Osteosarcoma is the second-highest cause of cancer-related death in children and adolescents. At the time of diagnosis, micro-metastasis is common in patients with osteosarcoma, making chemotherapy the first choice of treatment. Despite advances in chemotherapy and surgery, the survival rate for metastatic osteosarcoma remains below 30% for the last three decades. Thus, discovery of new chemotherapy agents is crucial for improving osteosarcoma therapy. Here, using high-throughput screening, we have found a new compound, called 2-{2-[(3,5-Dimethoxybenzyl)sulfanyl]-1,3-thiazol-4-yl}-N-(2-thienylmethyl)acetamide (referred to as KU0171032), as an inducer of apoptosis in various canine and human osteosarcoma cells. This compound shows minimal effects on non-transformed osteoblast and fibroblast cells. Intriguingly, the apoptotic effects of KU0171032 are observed only in osteosarcoma cells having mutant p53 or null for p53, but not in cells with wild-type p53. Notably, knockdown of wild-type p53 in U2OS and SJSA-1 cells enhances sensitivity to KU0171032 with increase in caspase-3 cleavage and DNA damage. Our results strongly suggest that KU0171032 shows synthetic lethality for loss of wild-type p53 activity in osteosarcoma cells.
Ribosomopathies are tissue-specific congenital anomalies that result from perturbations in ribosome biogenesis, a multistep global process that is critical for cell growth and survival. Ribosomopathies present with an array of defects with the most common being craniofacial and skeletal anomalies. One example is Treacher Collins Syndrome (TCS), which is caused by mutations in TCOF1, POLR1C and POLR1D. TCOF1 plays an important role in ribosomal RNA (rRNA) transcription by RNA polymerase I (pol I), a rate-limiting step of ribosome biogenesis, while POLR1C and POLR1D are subunits of pol I and III. TCS is also classified as a neurocristopathy as it results from deficient neural crest cell (NCC) development that leads to hypoplasia of the facial bones and cartilage, together with cleft or high-arched palate. NCC comprise a multipotent, migratory population that generates many tissues including most of the craniofacial bone and cartilage. Tcof1, polr1c and polr1d loss-of-function mouse and zebrafish mutants exhibit craniofacial anomalies consistent with TCS. However, how factors involved in rRNA transcription function in a spatiotemporal manner remains poorly understood. Specifically, whether pol I and III have intrinsic roles in NCC or other tissues, remains unknown. We hypothesize that the cell type-specific phenotypes observed in ribosomopathies arise through differential regulation of pol I and III activity and tissue-specific threshold sensitivities to disruptions in ribosome biogenesis. Therefore, we are investigating the roles of pol I and III transcription, Tcof1, Polr1c and Polr1d during NCC, bone and cartilage embryonic development. Our results will provide information on the spatiotemporal dynamics of rRNA transcription and give a deeper appreciation of the etiology and pathogenesis of congenital ribosomopathies and neurocristopathies, including TCS.
The regulation of Reserve hematopoietic stem cells by N-cadherin expressing mesenchymal stem cells in bone marrow niche

Fang Tao2,3, *, Meng Zhao1,2, #, Aparna Venkatraman2, Zhenrui Li2, Shiyuan (Cynthia) Chen2, Sarah Smith2, Christina Ward2, Pengxu Qian2, Allison Peak2, John Perry2, Xi He2, Linheng Li2,3*

1The Third Affiliated Hospital, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong, China. 2Stowers Institute for Medical Research, Kansas City, Missouri, USA. 3Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, Kansas, USA.

# these authors contribute equally to this work.

Regulation of hematopoietic stem cells (HSCs) by the bone marrow (BM) niche has been substantially studied, however, whether and how HSC subpopulations are distinctively regulated in the BM niche remain largely unclear. Here, we functionally distinguished reserve HSCs (rHSCs) vs. primed HSCs (pHSCs) and further examined their respective BM niches. We found that both pHSCs and rHSCs can support long-term hematopoiesis under homeostasis, however, pHSCs are sensitive to chemotherapy whereas rHSCs can survive from chemotherapy and support subsequent regeneration after myeloablation. The whole-mount HSC distribution study revealed that rHSCs were preferentially maintained in the endosteal region that enrich N-cadherin+ bone-lining cells during homeostasis and post-chemotherapy. pHSCs were associated with blood vessels which were vulnerable to chemotherapy compared to bone. Our transcriptome profiling and in vivo lineage tracing results showed that N-cadherin+ stromal cells are functional mesenchymal stem cells, which can give rise to osteoblast, adipocyte and chondrocyte during development and regeneration. Finally, we further demonstrated that deletion of either N-cadherin+ niche cells or Scf and Cxcl12 respectively from N-cadherin+ niche cells affects HSC maintenance.
**Understanding the pathogenesis and prevention of Acrofacial Dysostosis, Cincinnati type**

Kristin Watt¹, Cynthia Bezier¹, Cynthia Neben³,⁴, Shawn Hall¹, Amy Merrill³,⁴, Paul Trainor¹,²

¹Stowers Institute for Medical Research, Kansas City, MO, ²Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS, ³Center for Craniofacial Molecular Biology, Ostrow School of Dentistry, University of Southern California, Los Angeles, CA, ⁴Department of Biochemistry and Molecular Biology, Keck School of Medicine, University of Southern California, Los Angeles, CA

Acrofacial dysostosis-Cincinnati type is a recently described syndrome characterized by craniofacial and limb anomalies. Mutations in the RNA Polymerase (Pol) I subunit POLR1A were identified in affected individuals, but how mutations in POLR1A specifically affect craniofacial development is not well understood. POLR1A is the largest subunit of Pol I and is critical for transcription of ribosomal RNA (rRNA), which is necessary for ribosome production in all cells. In order to understand the etiology and pathogenesis of Acrofacial dysostosis, we established zebrafish with a mutation in the polr1a gene. Remarkably, we found that polr1a is dynamically expressed during embryonic development. polr1a homozygous mutants exhibit deficient 47S rRNA transcription, alterations in ribosome biogenesis, and reduced protein synthesis. Consequently, this induces Tp53-dependent neuroepithelial apoptosis and diminished survival and proliferation of cranial neural crest cells (NCC), which results in hypoplasia of the craniofacial skeleton. Genetic inhibition of tp53 prevents NCC death partially ameliorating the polr1a mutant phenotype. However, hypoplasia of the craniofacial skeleton still occurs due to the failure to improve rRNA transcription and NCC proliferation in the tp53 null background. Altogether, this data reveals specific functions for polr1a in ribosome biogenesis and NCC during craniofacial development in the pathogenesis of Acrofacial dysostosis – Cincinnati type. Furthermore, our work sets the stage for investigating cell specific roles for Polr1a and ribosome biogenesis during embryogenesis and for identifying Tp53-independent therapies for the potential prevention of Acrofacial dysostosis – Cincinnati type.
Dynamic epigenetic mechanisms regulate age-dependent SOX9 expression in mouse articular cartilage
Mingcai Zhang\textsuperscript{a}, Qinghua Lu\textsuperscript{a}, Andrew H. Miller\textsuperscript{a}, Nicholas C. Barnthouse\textsuperscript{a}, Jinxi Wang\textsuperscript{a,b,}\textsuperscript{*}
From \textsuperscript{a}Harrington Laboratory for Molecular Orthopedics, Department of Orthopedic Surgery, \textsuperscript{b}Department of Biochemistry & Molecular Biology, University of Kansas Medical Center, Kansas City, KS 66160
While the developmental role of the SOX9 transcription factor in chondrocyte differentiation and cartilage formation is well documented, age-dependent SOX9 expression in articular chondrocytes (ACs) and its regulatory mechanisms remain unclear. This study aimed to explore epigenetic regulatory mechanisms for age-related changes in SOX9 expression in ACs of mice, spanning from the developmental stage to 18 months of age. Sox9 mRNA and protein were highly expressed in ACs during joint development but significantly decreased after 2 months of age. Histopathological features of osteoarthritis were not observed in examined hip and shoulder joints by 18 months of age. Epigenetic studies revealed that DNA methylation levels were increased at specific CpG islands of the Sox9 gene at 6 and 12 months; treatment of cultured ACs from 6-month-old mice with 5-Azacytidine (an inhibitor of DNA methylation) elevated the level of Sox9 expression in ACs by lowering DNA methylation levels in the Sox9 promoter region. Histone 3 lysine 4 dimethylation (H3K4me2, a histone modification for transcriptional activation) in the Sox9 promoter region was decreased with age, which was associated with the age-dependent decrease in SOX9 expression in ACs. Knockdown of lysine-specific demethylase-1 up-regulated SOX9 expression in ACs of adult mice through increased recruitment of H3K4me2 in the Sox9 promoter region. Our results suggest that SOX9 expression in mouse ACs is significantly decreased after the completion of joint development. These age-dependent changes in SOX9 expression are dynamically regulated by DNA methylation and histone methylation.
\textbf{Keywords:} SOX9; epigenetics; chondrocyte; articular cartilage; osteoarthritis
[ABSTRACT 1] DNA-based injectable hydrogels for bone defect repair. Sayantani Basu¹, Settimio Pacelli¹, Arghya Paul¹². BioIntel Research Laboratory, Department of Chemical and Petroleum Engineering, School of Engineering, University of Kansas, Lawrence, KS 66045, USA. (p 6).

[ABSTRACT 2] ELUCIDATING ALTERATIONS IN BONE REMODELING IN WILDTYPTE AND OSTEOGENESIS IMPERFECTA MODEL MICE (oim/oim) FEMORA DUE TO PHARMACOLOGICAL INHIBITION OF ACTIVIN RECEPTOR IIB. Salah Daghlas¹, Youngjae Jeong¹, R. Scott Pearsall², Charlotte Phillips¹. Dept. of Biochemistry, University of Missouri, Columbia, MO. ²Accelereron Pharma Inc, Cambridge, MA (p 7).

[ABSTRACT 3] Cartilage-Specific NFAT1 Overexpression Delays the Onset and Attenuates the Progression of Posttraumatic Knee Osteoarthritis Induced by Meniscal Destabilization. Yi Feng¹, Qinghua Lu¹, William C. Kramer¹, Nicholas C. Barnthouse¹, Jinx Wang¹. Department of Orthopedic Surgery, University of Kansas Medical Center, Kansas City, KS, USA (p 8).

[ABSTRACT 4] Potential mitochondrial dysfunction in mouse models of Osteogenesis imperfecta. Victoria Gremminger¹, Youngjae Jeong¹, Rory Cunningham², Grace Meers², R. Scott Rector², Charlotte Phillips¹−³. Department of Biochemistry, University of Missouri-Columbia. ²Departments of Nutrition and Exercise Physiology and Medicine-GI, University of Missouri; ³Harry S Truman Memorial VA Hospital. Department of Child Health, University of Missouri-Columbia (p 9).

[ABSTRACT 5] Moderate SPECC1L deficiency results in palate defects through changes in both oral epithelium and cranial neural crest derived palatal mesenchyme. Everett G. Hall¹, Nathan R. Wilson¹, S. Undurry¹, J. Standley² E.A. Augustine-Akpan³, Youssef Kousa⁴, Diana S. Acevedo¹, Lenore Pitstick⁵, N. Natsume⁶, Shahnawaz Yousaf¹, T. Busch², E. Kosa¹, C. Nguyen⁷, M. Nguyen⁷, Wasiu L. Adeyemo⁸, M.A. Eshete⁹, A. Czirok¹, Bryan C. Bjork⁸, Brian C. Schutte⁹, Satoshi Suzuki⁷⁸, Jeffrey C. Murray⁷, Azeez Butali³, Irfan Saadi³ (p 10).

[ABSTRACT 6] Characterization of Tibial gene expression patterns of in Osteogenesis Imperfecta Murine (OIM) and G610C models of OI. Catherine Omosule¹, Youngjae Jeong¹, Ferris Pfeiffer² and Charlotte Phillips¹,². Department of Biochemistry¹, Orthopedic Surgery² and Child Health³ University of Missouri-Columbia (p 11).

[ABSTRACT 7] Nanodiamond-Gelatin Hydrogel for Drug Delivery and Bone Tissue Engineering Ryan Maloney¹, Settimio Pacelli² Aparna Chakravarti¹, Flavia Castanho¹, Vijayan Manoharan¹, Arghya Paul¹². Bioengineering Graduate Program, University of Kansas, Lawrence KS, USA. ²Department of Chemical and Petroleum Engineering, Bioengineering University of Kansas, Lawrence, KS, USA. (p 12).


[ABSTRACT 9] RNA polymerase I and III transcription is spatiotemporally regulated during neural crest cell, bone and cartilage development. Karla Terrazas¹, Annita Achilles⁰¹, Paul Trainor¹ (p 14).

[ABSTRACT 10] The regulation of Reserve hematopoietic stem cells by N-cadherin expressing mesenchymal stem cells in bone marrow niche. Fang Tao²³, Meng Zhao¹², Aparna Venkatraman², Zhenrui Li², Shiyuan (Cynthia) Chen², Sarah Smith², Christina Ward², Pengxu Qian², Allison Peak², John Perry², Xi He², Liheng Li²³. The Third Affiliated Hospital, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong, China. ²Stowers Institute for Medical Research, Kansas City, Missouri, USA. ³Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, Kansas, USA. (p 15).

1Stowers Institute for Medical Research, Kansas City, MO, 2Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS, 3Center for Craniofacial Molecular Biology, Ostrow School of Dentistry, University of Southern California, Los Angeles, CA, 4Department of Biochemistry and Molecular Biology, Keck School of Medicine, University of Southern California, Los Angeles, CA (p 16).


aHarrington Laboratory for Molecular Orthopedics, Department of Orthopedic Surgery, bDepartment of Biochemistry & Molecular Biology, University of Kansas Medical Center, Kansas City, KS 66160 (p 17).
“Skeletal Biology, Development and Disease” is a major strategic focus of the NIH, and is the area of study of many clinical and basic scientists at KU School of Medicine and surrounding institutions. We have organized an exciting symposium, including 3 scientific sessions and a reception/poster session. We have invited 5 nationally renowned external speakers with expertise spanning the fields of Bone and Craniofacial Development, Malformation and Regeneration. Further, 7 local speakers will highlight clinical and basic research here at KUMC and greater Kansas City area.