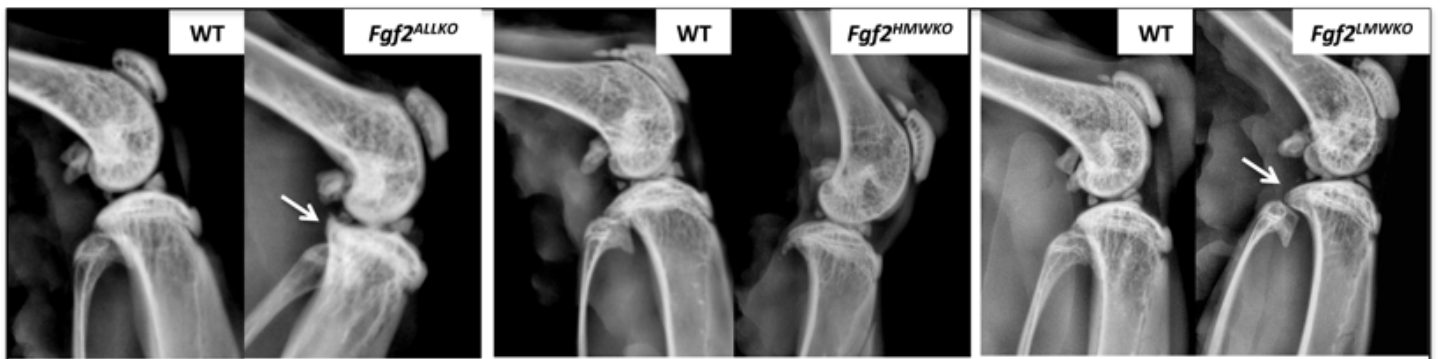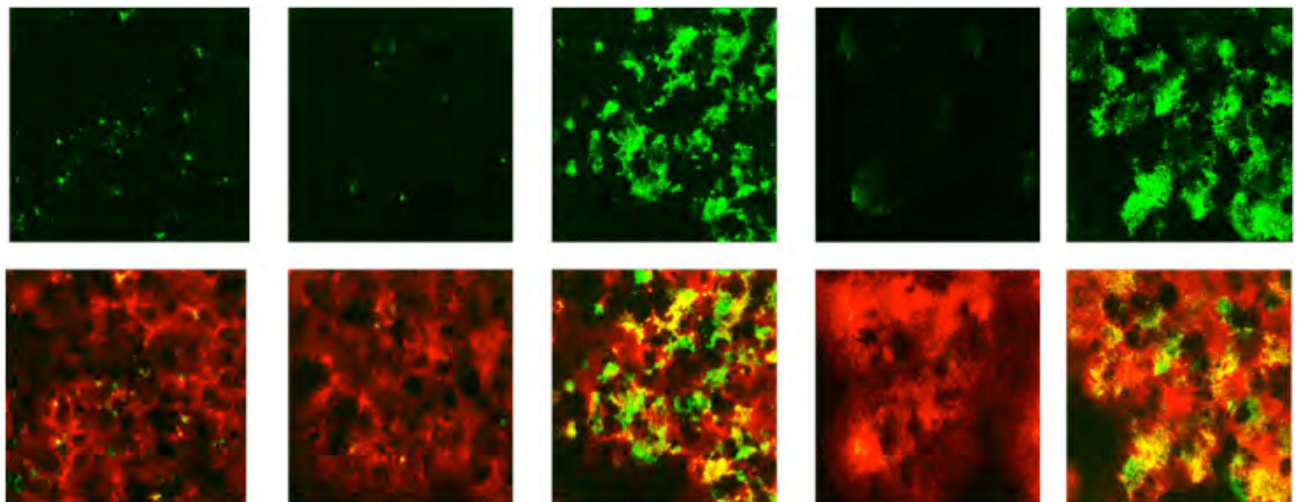# Skeletal, Craniofacial & Oral Biology Symposium 2015

Monday, June 8, 2015 • Low Learning Center
UConn Health
Farmington, CT

School of Dental Medicine

Welcome

Drs. Mina Mina, A. Jon Goldberg and William B. Upholt, Planning Committee for this Symposium, are pleased to welcome all participants and guests to the 2015 Skeletal, Craniofacial and Oral Biology Symposium.

The purpose of the Symposium is to provide trainees in Skeletal, Craniofacial and Oral Biology and other research areas supported by the UConn NIH/NIDCR T90/R90 training grant, an opportunity to present their work in a formal symposium atmosphere. Mentors, faculty, colleagues and other students interact and provide feedback to our presenters. Individuals at different levels of research experience are represented at the Symposium, so some will present completed work, while others will present work in progress. For everyone this is an opportunity to network, discuss ongoing research and career goals, and have an enjoyable day.

This year we have a very special guest, *Dr. Martha Somerman, DDS, PhD, Director, National Institute of Dental & Craniofacial Research*. Dr. Somerman has kindly agreed to present a lecture on the future of NIH/NIDCR biomedical research to the entire UConn Health community, listen and interact to trainee presentations and have an informal luncheon/discussion with the trainees.

Welcome and thank you Dr. Somerman!

Mina Mina, DMD, MSD, PhD
Professor
Chair, Pediatric Dentistry
Director, Skeletal, Craniofacial and Oral Biology Training Grant

A. Jon Goldberg, PhD
Professor
Director, Center for Biomaterials
Interim Head, Biomedical Engineering—UConn Health
Co-Director, Skeletal, Craniofacial and Oral Biology Training Grant

William B. Upholt, PhD
Professor Emeritus
Advisor and Previous Co-Director, Skeletal, Craniofacial and Oral Biology Training Grant

Support

Acknowledgements

Schedule

Time	Event Presenter/Page Number
7:45 – 8:15 am	**Registration and Continental Breakfast** *Keller Lobby*
8:20 – 8:30 am	**Opening Remarks** *Low Learning Center* Dean MacNeil, Dr. Goldberg
8:30 – 9:45 am	**Oral Session I: Progenitors, Lineage** *Low Learning Center* Session Chair: Matthew Zambrello Sierra Root/6 Spenser Smith/8 Ivana Vidovic/10 Nathaniel Dyment/12
9:45 – 10:30 am	**Break and Poster Session** *Keller Lobby* Aja Aravamudhan/14 Anushree Banerjee/16 Eric James/18 Sandra Lopez/20 David Manz/22 Patience Meo Burt/24 Vanessa Scanlon/26 Xi Wang/28 Matthew Zambrello/30
10:30 – 11:45 am	**Oral Session II: Signaling** *Low Learning Center* Session Chair: David Manz Jason Gibson/32 Thomas Estus/36 David Paglia/38 Ryan Russell/40
12:00 – 1:00 pm	**Guest Speaker** *Keller Auditorium* Dr. Martha Somerman, DDS, PhD/42 Director, National Institute of Dental and Craniofacial Research *"The Future of Biomedical Research as seen through* *the Eyes of NIH/NIDCR"*
1:00 – 2:15 pm	**Trainee Luncheon with Dr. Somerman** (SCOB, T90, R90 Trainees only) *Onyiuke Dining Room*
1:00 – 2:15 pm	**AADR Connecticut Section, Dr. Tadinada** *Keller Auditorium*

Characterization of a newly identified bone marrow resident cell population with the potential to modulate skeletogenesis and hematopoiesis

Sierra Root, Elena Torreggiani, Brya Matthews, Ivo Kalajzic, and H. Leonardo Aguila

University of Connecticut

Abstract:

Objective: The project aims to characterize the developmental origin and physiological role of a newly identified cell population within the bone marrow space with the potential to be involved in the regulation of hematopoietic homeostasis, including the commitment of progenitors towards osteoclastogenesis. This population was initially identified as cells with dendritic cell morphology interspersed within the bone marrow space in a transgenic mouse system expressing cre recombinase under the control of DMP-1 promoter crossed with the visual reporter strain Ai9 (containing a targeted mutation of the *Gt(ROSA)26Sor* locus with a *loxP*-flanked STOP cassette preventing transcription of a CAG promoter-driven red fluorescent protein variant tdTomato).

Methods: We have used a combination of histology, gene expression analysis, flow cytometry, cell sorting and lineage progression assays in order to identify the phenotype and potential function of these cells *in vivo*. We have also begun defining the ontogeny of these cells by testing primary and secondary sites of hematopoiesis using flow cytometry and histology in adult, neonates and embryos.

Results: Initially, the hematopoietic fraction of tomato positive cells were isolated by Fluorescent Activated Cell Sorting (FACS) and found to express transcripts for important hematopoietic cytokines including: RANK ligand and IL-7. Flow cytometric analysis of various cell surface markers determined that these cells are heterogenous and express primarily markers of the myeloid/macrophage lineage. Markers included are CD11b, F4.80, CD169, Ly-6C, Ly-6G, CD11c, CD62L suggesting that these cells could correspond to a previously unidentified bone marrow resident macrophage-like cell population. Regarding studies to define the ontogenic origin of these cells: standard assays for lineage progression from adult hematopoietic stem cells have rendered negative results in generating this committed cell type. However, within the CD11b myeloid committed tomato negative population, generation of tomato positive cells does occur and they have the ability to expand *ex vivo* in coculture conditions. Hematopoietic dmp1-Cre-tomato+ cells exist in the bone marrow space of early neonates and late gestation embryos (day 15 to day 21) suggesting that they may have fetal origins.

Conclusions: We have identified a small resident cell population in the bone marrow using the transgenic DMP1-Cre-tdTomato mouse model. These cells are hematopoietic in origin and appear to be a tissue resident macrophage that is not maintained from adult hematopoietic stem cells but instead possibly established during early fetal life and then self-maintained throughout adulthood. These cells express genes that are important for the regulation of osteoclastogenesis and lymphopoiesis. Attempts to grow and expand this pure tdTomato+ cell population *ex vivo*

have only been successful from femur cocultures. Further functional studies are underway to understand their true function during homeostasis and altered bone marrow microenvironments *in vivo*.
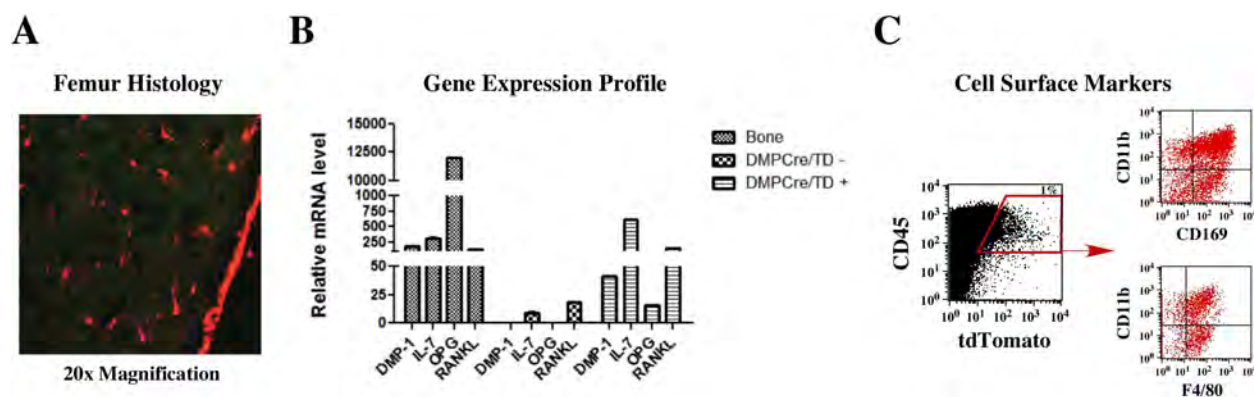

Figure 1. Characterization of hematopoietic DMP1-Cre-tdTomato expressing cells.
A) Histology of femur demonstrates tomato+ osteoblast cells as well as complex tomato+ cells with dendritic like processes throughout the bone marrow. B) Hematopoietic (CD45+) tomato+ and tomato- cells were sorted to purity, RNA extracted, amplified, reverse transcribed and then evaluated for genes involved in hematopoiesis and skelatogenesis. Hematopoietic cells expressing tdTomato express genes responsible for regulating osteoclastogenesis (RANKL) and lymphopoiesis (IL7). C) Flow cytometric evaluation of dmp1-cre-tdTomato+ bone marrow flush shows a 1% population co-expressing tomato and CD45. Within this tomato positive hematopoietic gate, expression of myeloid and tissue resident macrophage markers CD11b, F4/80 and CD169 are highly represented.

Biography:

BS Chemistry-Biology (University of Hartford, 2005)
PhD Biomedical Sciences-Genetics and Developmental Biology (UCONN Health, 2014)
Post-Doctoral Fellow (Year 1)

It is my career objective to advance the field of monocyte and osteoclast development, their supporting cell types and their role in pathological conditions, especially inflammation. During my PhD, I focused primarily on human embryonic stem cell derived monocytes and osteoclasts as well as monocyte/osteoclast progenitors from human peripheral blood. I chose a postdoctoral fellowship at Uconn Health to further develop my doctoral projects and study the role of monocytes, osteoclast and bone supporting cells within the hematopoietic compartment of the murine bone marrow within the collaborative initiative at Uconn Health specifically in the Muscoskeletal Biology Research labs. I look forward to studying and advancing these projects within this collaborative and translational research community.

microRNA Regulation of Circadian Rhythm in the Osteoblastic Lineage

Spenser S Smith, Neha S Dole, Tiziana Franceschetti, Anne M Delany

Center for Molecular Medicine, UConn Health, Farmington, CT

Disruption of circadian rhythm is associated with many diseases, including cancer, metabolic syndrome and bone loss. In bone, genes critical for normal homoeostasis display circadian rhythm. Rhythm is maintained by clock genes, including Bmal1 and Per1-3, which interact in a series of transcriptional and post-transcriptional feedback loops. In non-skeletal tissues, microRNAs (miRNAs) were shown to be important for controlling circadian rhythm; however, miRNAs maintaining rhythm in bone have not been determined. Here, we identify miR-433 as a regulator of rhythmic gene expression in the osteoblast lineage.

Hif1α and Igf1 are critical osteoblast genes displaying circadian rhythm. Using Luciferase reporter-3'UTR assays, we demonstrated that these genes are novel miR-433 targets. In vivo, we saw that miR-433 displays robust rhythmicity in mouse calvaria. Its expression pattern was anti-phasic in relation to Bmal1, peaking after light removal. However, in primary mouse mesenchymal cells synchronized with a short pulse of dexamethasone, miR-433 did not display rhythmicity. This suggests that a systemic factor establishes miR-433 rhythm in vivo.

miR-433 was recently shown to target the glucocorticoid receptor (Riester et al.), and rhythmic secretion of glucocorticoids is critical for synchronizing local clocks in peripheral tissues by activating transcription of Per genes. Moreover, as circulating glucocorticoid levels fluctuate diurnally, so does the sensitivity of tissues to glucocorticoids. We hypothesized that miR-433 helps maintain circadian rhythm in osteoblasts by regulating glucocorticoid signaling.

To determine if miR-433 regulates circadian rhythm in vitro, its activity was inhibited using a doxycycline-inducible competitor (miR-433 tough decoy) in stably transduced C3H10T1/2 cells. Although miR-433 inhibition did not affect Bmal1 rhythm, it dramatically altered Per2 amplitude, period length, and phase (Figure 1). miR-433 inhibition also dampened rhythmicity of Runx2 mRNA. Inhibiting miR-433 activity amplified the dexamethasone-stimulated expression of glucocorticoid responsive genes Dusp1 and Per2, demonstrating increased glucocorticoid sensitivity. Overall, we found that miR-433 displays a circadian rhythm in calvaria, targets mRNAs with rhythmic expression and regulates sensitivity to glucocorticoid signaling. We speculate that miR-433 can modify responsiveness of peripheral tissues to variations in circulating glucocorticoids and alter bone metabolism.

Biography: I graduated from The College of Idaho in 2008 with a B.A. in Biology and History. I am currently a third year Ph.D Biomedical Science student in Dr. Anne Delany's lab in the Skeletal, Craniofacial, and Oral Biology Area of Concentration. I was attracted to UCHC because of the bone research program. My career aspirations are to obtain a position in academia as a professor teaching as well as researching bone biology.
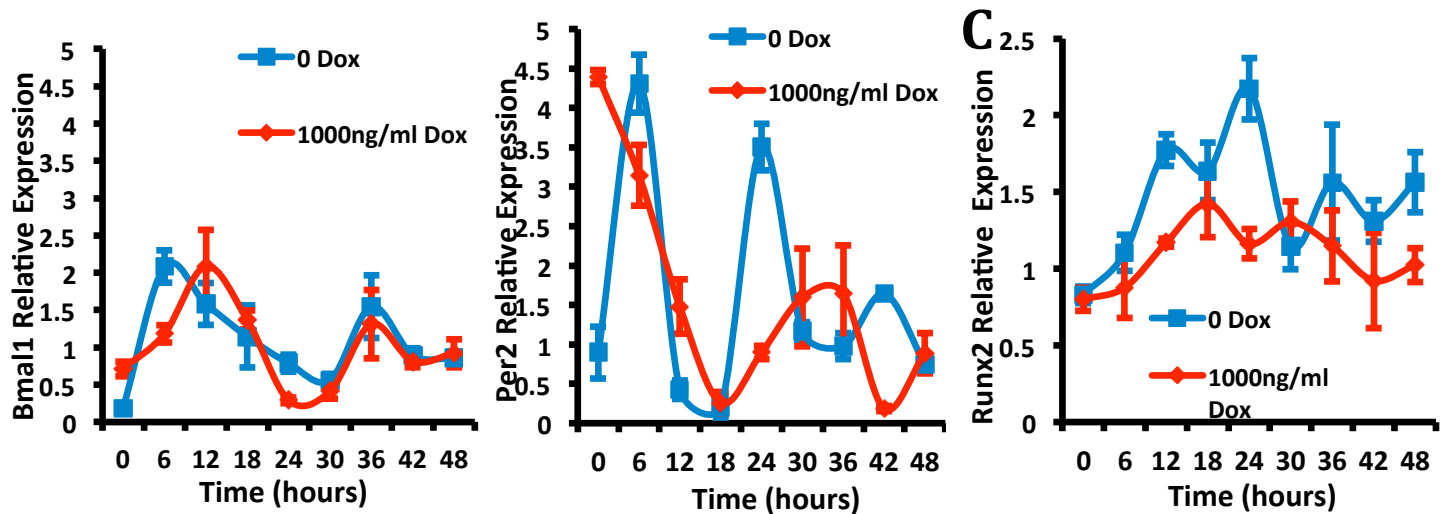

Figure 1. Disruption of miR-433 activity affects Per2 and RUNX2 mRNA rhythmic expression.
C3H10T1/2 cells were stably transduced with a Doxycycline (Dox)-inducible miR-433 decoy construct, to inhibit miR-433 activity.

A. miR-433 decoy did not dramatically impact the cycling of Bmal1 mRNA.
B. miR-433 decoy profoundly altered the amplitude and period length of Per2 mRNA cycle.
C. miR-433 decoy decreased the amplitude the Runx2 mRNA cycle.

Lineage tracing of αSMA perivascular cells during reparative dentinogenesis

Vidovic Ivana, Banerjee Anushree, Matthews Brya, Dyment Nathaniel, Kalajzic Ivo, Mina Mina

University of Connecticut Health Center

Abstract:

Introduction: Reparative dentinogenesis is a regenerative process that leads to dentin bridge formation and the maintenance of the dental pulp vitality. Reparative dentinogenesis occurs after intense injuries that lead to odontoblast death and involves the recruitment and proliferation of progenitor cells to the site of injury and their differentiation of odontoblasts or "odontoblast-like cells". At the present time the origin/identity of the progenitor cells and signaling pathways involved in reparative dentinogenesis remain elusive.

Purpose: The goal of our studies was to examine the roles of perivascular cells in the pulp expressing αSMA-tdTomato+ during reparative dentinogenesis using lineage tracing experiments in transgenic mice.

Methods: We used Cre-mediated genetic lineage tracing, using a Tamoxifen (TM)-inducible αSMA-CreERT2 crossed with Cre-dependent Ai9 reporter mice (Rosa26-tdTomato).αSMA-Cre activity was induced in 4–6 week old αSMACreERT2; Ai9 double transgenic mice by 2 serial intraperitoneal (IP) injection of TM. The untreated (vehicle-injected) αSMACreERT2 mice and αSMACreERT2 negative; Ai9 TM-treated mice served as controls. At various time points animals were sacrificed, and image analyses were used to compare the location, number and the contribution of αSMA-Tomato^{+} descendants to reparative dentinogenesis in molars.

Results: In control vehicle – injected αSMACreERT2 αSMA-tdTomato+ cells were not detected in the dental pulp or in alveolar bones. In animals injected with TM, without injury two, seven and fourteen days following TM administration αSMA-tdTomato+ cells were detected around the blood vessels in the dental pulp of teeth and in the alveolar bones. In TM injected animals after pulp injury, αSMA-tdTomato+ descendants and number of αSMA-tdTomato+ cells increased with time as compared to control without injury. Most of αSMA-tdTomato+ cells were not located around blood vessels in the pulp with injury. By seven and fourteen days following pulp exposure, αSMA-tdTomato+ descendants migrated towards exposure site and were increased in number. Fourteen days following pulp exposure αSMA-tdTomato+ cells were detected in close proximity to the reparative dentin.

Conclusion: In dental pulp, the αSMA+ perivascular cells are one of potential progenitors to odontoblast-like cells and reparative dentin.

Biography:
DMD, Dental School, Medical Faculty, University of Rijeka, Croatia
Post-doctoral Fellow in Pediatric Dentistry – 2nd year

I decided to come to UConn because I was interested in the research being conducted by Dr. Mina Mina and I wanted to contribute to scientific area. I also appreciate the scientific culture at the School. Although I have limited research experience, I hope to improve my research skills and to be able to implement this knowledge conducting my own research program when I return to Croatia. I am sure that experience gained at the UConn Health Center will allow me to become a well-rounded dental practitioner and researcher.
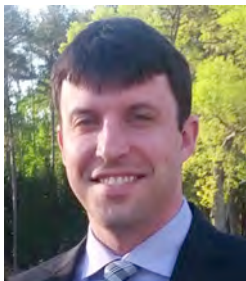
Gdf5 progenitors give rise to fibrochondrocytes that mineralize via hedgehog signaling to form the zonal enthesis

Nathaniel A Dyment,[1] Andrew P Breidenbach,[2] Andrea G Schwartz,[3] Ryan P Russell,[1] Lindsey Aschbacher-Smith,[4] Han Liu,[4] Yusuke Hagiwara,[1] Rulang Jiang,[4] Stavros Thomopoulos,[3] David L Butler,[2] David W Rowe,[1]

Affiliations
[1] University of Connecticut Health Center
[2] University of Cincinnati
[3] Washington University in St. Louis
[4] Cincinnati Children's Hospital Research Foundation

OBJECTIVE: The objective of this study was to demonstrate the progression of enthesis growth, as measured by clonal expansion of Gdf5 progenitors, spatiotemporal collagen gene expression and mineralized fibrocartilage apposition that leads to a mature zonal enthesis

METHODS: 1) Cellular expansion and collagen expression. 1a) Gdf5Cre x R26R-Confetti mice [1-3] were used to measure the clonal expansion of enthesis progenitors. 1b) Triple transgenic Col1a1-YFP:Col2a1-CFP:Col10a1-RFP mice were assessed at postnatal days P1, P14, and P28 to monitor temporal expression of collagen synthesis during growth. 2) Hh signaling regulation of mineralized fibrocartilage. 2a) Gli1-CreERT2 crossed with Ai14-tdTomato Cre reporter mice were injected with tamoxifen and calcein at 4.5 weeks of age and demeclocycline at 8.5 weeks of age to trace the maturation of Hh responsive cells during growth. 2b) Constitutive scleraxis (ScxCre) crossed with floxed smoothened (Smo$^{f/f}$) mice were injected with mineralization labels (2, 4, 6, and 8 weeks of age) and assessed at 4 and 10 weeks of age. Repetitive imaging cryohistology. Frozen mineralized sections were prepared and each section went through up to 4 rounds of imaging: i) fluorescent GFP reporters and/or mineralization labels, ii) collagen SHG signal via two photon imaging, iii) alkaline phosphatase (AP) fluorescent staining, and iv) toluidine blue staining.

RESULTS: Gdf5-labeled progenitors clonally expand to contribute to axial growth of the enthesis. Labeled cells were vertically oriented between collagen fibers. Over 58% of labeled cells were in clones of 2 or more cells, with 12% being in clones of 4 or more cells. The spatial distribution of Col1a1, Col2a1, and Col10a1-expressing cells changes during maturation of the enthesis. Prior to mineralization, the enthesis consisted of Col1-YFP+ cells that abut Col2-CFP chondrocytes of the primary cartilage. At the onset of mineralization, cells at the base of the enthesis adjacent to the primary cartilage expressed ColX-RFP and high levels of AP. Hh-responsive Gli1+ cells matured from unmineralized to mineralized fibrochondrocytes during growth. At 3 days following tamoxifen/calcein injections, all Gli1-labeled cells were in unmineralized regions of the enthesis. Following the 4-week chase, a portion of cells closest to the calcein label (tidemark at t=0) had matured from unmineralized to mineralized fibrochondrocytes as they were between the 2 mineralization labels. ScxCre:Smof/- mice exhibited severely reduced mineralized fibrocartilage apposition during growth compared to littermate controls. Targeted deletion of Smo in tendons resulted in severely impaired mineralized fibrocartilage apposition with reduced mineral apposition rate in all tendons investigated.

DISCUSSION: Progenitor cells that contribute to the enthesis have a common origin that traces back to a Gdf5 progenitor pool during embryogenesis [2-3]. As illustrated in the current study (Fig. 1), progeny of these cells progress through several stages of differentiation during enthesis maturation. Enthesis progenitors amplify during growth and synthesize a collagen template that anchors to the underlying primary cartilage. At the onset of mineralization, cells at the base of the enthesis adjacent to the primary cartilage express AP and ColX and begin the production of mineralized fibrocartilage. The mineralization continues in an appositional manner from the base of the enthesis towards the midsubstance. Hh signaling drives the mineralization process, as ablation of Smo leads to severe reductions in mineralized fibrocartilage apposition. These 3 processes lead to the development of a mature zonal enthesis with a cell maturation gradient from the midsubstance (Col1+) to unmineralized fibrocartilage (Col1+, Col2+, Gli1+) to mineralizing fibrocartilage (AP+, ColX+) and finally mineralized fibrocartilage (ColX+).

REFERENCES: 1. Snippert et al, Cell, 2010, 2. Rountree et al, PLOS Biol, 2004, 3. Dyment et al, PLOS One, 2014.
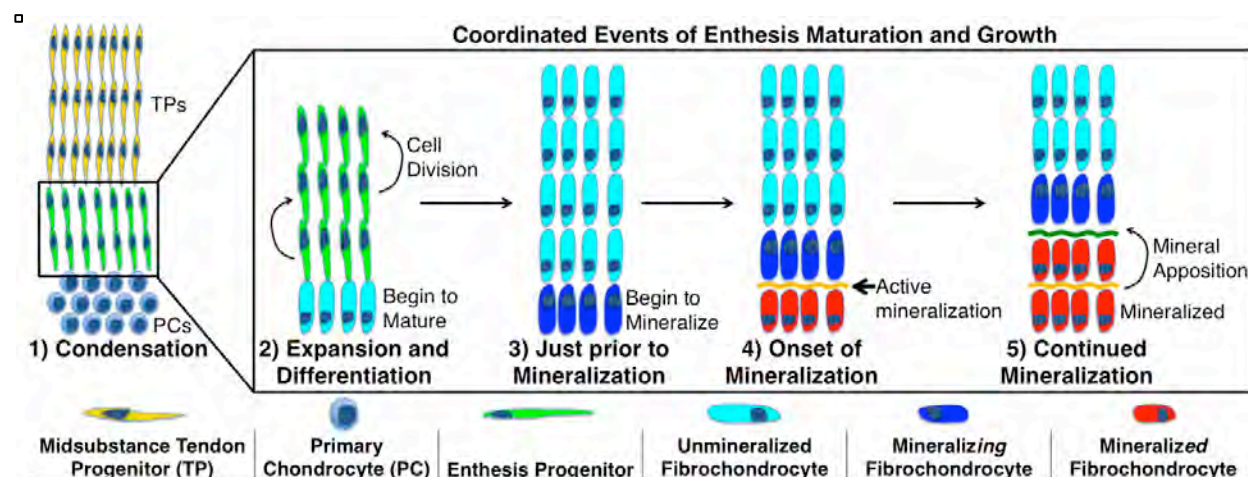

Fig. 1. Model for enthesis cellular expansion and maturation during growth.

BIOGRAPHY:
Education
University of Illinois at Urbana Champaign, B.S., Materials Science and Engineering, 2001-2005
University of Cincinnati, PhD, Biomedical Engineering, 2005-2011
Position: Postdoctoral Fellow
Year in Program: 4th
Career Aspirations: My goal is to remain in academia as a principal investigator with research focused on improving repair of musculoskeletal injuries.
Why UCHC: My PhD work focused on using tendon development as a guide for improving tissue engineering strategies to improve repair. This work was a part of an NIH Bioengineering Research Partnership grant for which Dr. David Rowe was a consultant. I came to Dr. Rowe's lab to improve our understanding of tendon differentiation. We are now using Cre lineage tracing models to characterize progenitors cells as they differentiate into mature tendon cells.

Characterization of Cellulose- Collagen based Micro- Nano structured scaffolds for Osteoinductivity *in vitro* and Biocompatibility *in vivo*

Aja Aravamudhan[1,2], Daisy, M Ramos[1,2,3], Matthew, D Harmon[1,2,3],

Sangamesh, G Kumbar[1,2,3,4,*]

1. Institute for Regenerative Engineering, 2. Department of Orthopeadic Surgery, University of Connecticut Health Center, Farmington, CT, 3. Department of Materials Science and Engineering, University of Connecticut, Storrs, CT, 4. Department of Biomedical Engineering, University of Connecticut, Storrs, CT, *kumbar@uchc.edu

The use of polymeric scaffolds is an alternative to traditional autografts and allografts to repair non-healing bone defects. Natural polymers, due to their chemical and structural similarity with native tissue components often present several qualities beneficial to applications of tissue regeneration. In this study, we present the characterization of mechanically stable[1,2] cellulose acetate and collagen based micro-nano structured scaffolds in terms of their ability to promote hMSC's progression into osteoblastic lineage *in vitro* and the biocompatibility of the scaffolds *in vivo*. Cellulose acetate (CA), poly (lactic-co-glycolic acid) (PLGA) and their collagen-functionalized scaffolds (CAc, PLGAc) were formulated as discs. Each scaffold was seed with 500,000 human bone marrow derived mesenchymal stem cells (hMSCs) and cultured using inductive media for 21 days. hMSC viability, proliferation and differentiation were evaluated temporally by Live Dead, Pico green (DNA) assays, alkaline phosphate activity (ALP), mineralization (Figure1.A.), changes in gene osteogenic expression (RUNX2, Coll1, ON, and BSP) and presentation of proteins (coll1 and BSP) (Figure1.B.). Further, CA, CAc and PLGA scaffolds were subcutaneously implanted into Sprague- Dawley rats. At set time points, the samples were recovered and evaluated histologically for immune responses and vascularization. Both the test CA and CAc scaffolds maintained higher levels of osteogenic gene and protein expression levels indicating greater long-term osteoinduction on these scaffolds. The test scaffolds also showed greater cellular infiltration and vascularization in the subcutaneous implants. These results indicate the osteogenic potential of CA and CAc scaffolds *in vitro* and their biocompatibility *in vivo*.
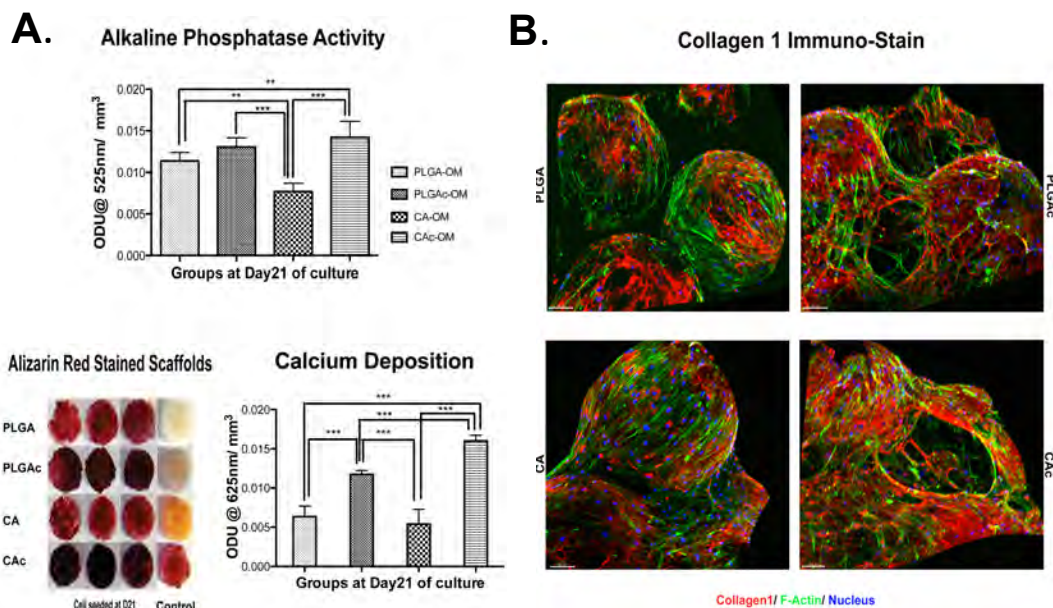
Figure 1:Greater osteogenic progression on CA and CAc scaffolds *in vitro*: (A) Greater alkaline phosphatase levels and mineralization on CAc than PLGA and PLGAc (B) Greatest uniformity in collagen (red) distribution, actin remodeling (F-actin- green) and cellular distribution (nuclei- blue) on CAc.

Biography:

      B.Tech., Biotechnology; Anna University, Chennai- 600025.
      Ph.D. in Biomedical Science; Skeletal, Craniofacial and Oral Biology - 6th year

I aspire to be in academia. I hope to teach and conduct research in the future.
Being one of the leading institutes in biomedical research, UCHC was my first choice among the schools I had applied to. The research atmosphere and the guidance provided by professors in my department have helped me evolve into a better student of science.

Contribution of αSMA+ Perivascular Cells in Dental Pulp to Dentin during Development

Banerjee A; Vidovic I, Kalajzic I, Matthews B, and Mina M
University of Connecticut Health center. School of Dental Medicine

Introduction: The origins of mesenchymal stem cells (MSC) in teeth have been the subject of considerable discussion. Currently it is thought that perivascular cells constitute MSC in most tissues. **Purpose:** The goal of our studies was to examine the contribution of perivascular cells expressing a smooth muscle α-actin promoter (αSMA) to dental MSCs in mice incisor and molars. **Methods:** We used Cre-mediated genetic lineage tracing, using a Tamoxifen (TM)-inducible αSMA-CreERT2 crossed with Cre-dependent Ai9 reporter mice (Rosa26-tdTomato). αSMA-Cre activity was induced in 5 days and 8-10 week old αSMACreERT2; Ai9 double transgenic mice by one intraperitoneal (IP) injection of TM. The untreated (vehicle-injected) αSMACreERT2 mice and αSMACreERT2 negative; Ai9 TM-treated mice served as controls. At various time points animals were sacrificed, and FACS and image analyses were used to compare the location, number and the contribution of αSMA-Tomato+ descendants to dentinogenesis in molars vs. incisors. **Results:** One day after TM injection in 5 days old pups, very few αSMA-Tomato+ cells were located around the blood vessels in pulp of the incisor and molars, in the bone marrow of the alveolar bones and muscles. FACS analysis showed that percentage of αSMA-Tomato+ in incisor pulps were higher than in molars. In vivo and in vitro FACS analysis showed about 12- 500 and 10-48 fold increases in the number of αSMA-Tomato+ cells in molar and incisor pulp respectively 4 days after, indicating high rate of proliferation. In adult mice, αSMA-tdTomato+ cells were detected in pulps of incisors and molars 2 and 7 days after TM injection. A few αSMA-tdTomato+ cells were detected underneath and within the odontoblast layer suggesting that αSMA-tdTomato+ descendants contribute to odontoblasts in primary and secondary dentinogenesis. **Conclusions:** In the dental pulp, the αSMA+ perivascular cells are one of potential MSC population. Supported by R01-DE016689 & R90-DE022526 grants.

Biography:
BDS, Mumbai India
MS, University of Medicine & Dentistry New Jersey (Rutgers School of Dental Medicine)
PhD in Biomedical Sciences, Skeletal Craniofacial and Oral Biology (SCOB)

I am currently in my 3rd year of PhD and would like to do a dental residency following completion. My long term goals are to incorporate research and clinical dentistry to make a career as a clinician-scientist. The PhD program at UCHC provides an excellent environment to train and work with accomplished minds from diverse backgrounds that will help me achieve my professional goals.

Localized Delivery of microRNAs from Nanofibers Enhances Extracellular Matrix Deposition: Post-transcriptional Regulation in Osteoblasts

Eric N. James[1], Anne M. Delany[2] and Lakshmi S. Nair[1]

University of Connecticut Health Center, CT 06030

Affiliations

[1] Department of Orthopaedic Surgery

[2] Center for Molecular Medicine

Background: Nanofiber scaffolds are attractive for bone tissue engineering, as they closely mimic the morphology of collagen fibrils in the natural extracellular matrix (ECM)[1]. MicroRNAs (miR RNAs, miRs) are important regulators of bone maintenance and have emerged as powerful new therapeutic molecules[2]. However, efficient tools to deliver miRNA mimics or antisense oligonucleotide inhibitors (antagomirs) to specific target tissues are limited[2].

The miR-29 family is well studied in bone. miR-29 inhibits the synthesis of ECM molecules, such as fibrillar collagens, as well as the non-collagen matrix protein, osteonectin. Osteonectin regulates collagen fibril assembly and is critical for normal bone remodeling[2]. Inhibiting miR-29 activity increases ECM synthesis. The objective of this study is to develop a localized therapy for bone regeneration by combining nanostructured scaffolds with miR-29a inhibitors to enhance the production of ECM. We evaluated the ability of these scaffolds to increase ECM production by quantifying osteonectin *in vitro*.

Methods: Gelatin was dissolved in trifluoroethanol to obtain a 7.5% solution. Scramble (control), miR 29a inhibitor with TKO transfection reagent was added to the gelatin solution, to yield concentrations of approximately 50nM/scaffold. Electrospinning was performed to fabricate miRNA-loaded nanofibers. Release kinetics of miRNAs were quantified by NanoDrop spectrometry. Delivery and bioactivity of miR-29a inhibitor was determined by Western Blot analysis of target gene osteonectin. Pico green assay was performed to quantify DNA content.

Results & Discussion: Dy547 labeled miRNA (scramble) encapsulated in electrospun gelatin nanofibers were uniform and bead-free **(Figure 1).** Sustained miR-29a inhibitor release from the gelatin nanofibers was observed over a period of 72hrs **(Figure 2)**. miR-29a negatively regulates osteonectin by binding to its mRNA, causing instability and disrupting translation. Thus, introducing a miR-29a inhibitor will enhance osteonectin expression. Western blot analysis revealed that MC3T3-E1 cells seeded on miR-29a inhibitor loaded gelatin fibers and scramble loaded nanofibers (3D) showed comparable transfectability to cells on miR-29a inhibitor or scramble on glass coverslips (2D) after 24h. Further, both 2D and 3D groups with miR-29a inhibitor had significantly increased expression of osteonectin compared to scramble controls (**Figure 3 A, B).** DNA quantification revealed no differences cell number.

Conclusions: The study demonstrated the feasibility of producing miR-29a inhibitor loaded nanofibers as an ECM stimulating scaffold. Gelatin nanofibers locally delivered bioactive miR-29a inhibitor in a sustained manner, inducing the expression of the critical ECM component, osteonectin. Applications for this novel technology include the ability to deliver transient RNA-based therapy, without potential for cell transformation. Further, this approach is flexible, with the potential to deliver any miRNA inhibitor or mimic. The unique bioactivity of miRNA-based therapeutics, combined with ECM mimicking nanostructured scaffolds serves as a novel

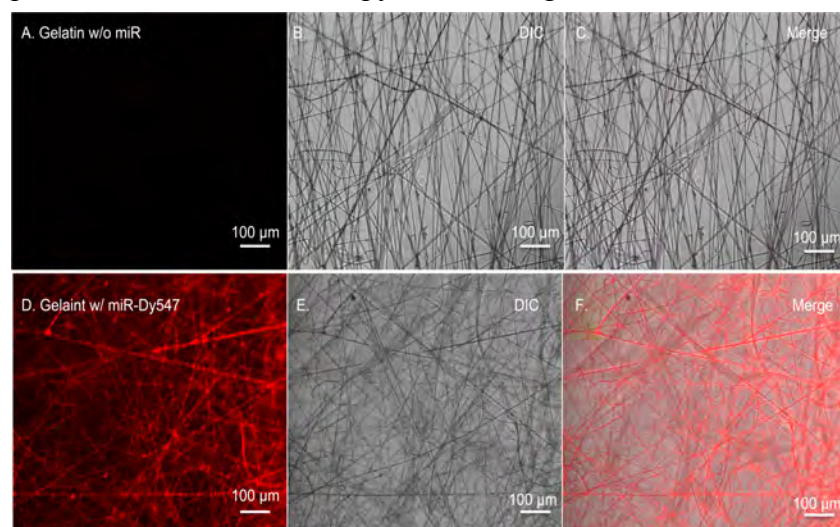platform for localized therapy for bone regeneration.

Figure 1. Fluorescence micrographs of Dy547 conjugated miRNA incorporated into gelatin nanofibers. A-C) Unloaded gelatin nanofibers and D-F) gelatin nanofibers loaded with fluorescently labeled miRNAs. Differential interference contrast (DIC) image, fluorescent miRNAs (red) (*scale 100 m*).
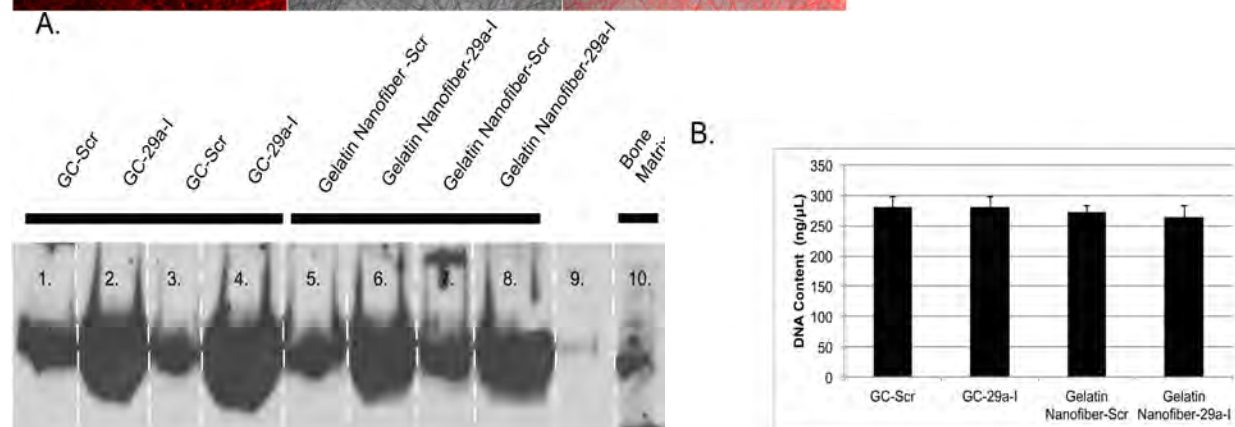

Figure 3. Osteonectin protein secreted from transfected MC3T3-E1 cells seeded on 2-D cover slips or miR-29a inhibitor loaded gelatin nanofibers A) Western blot analysis of osteonectin was performed 24h after cells were seeded on scaffolds, B) DNA content of cells cultured on glass coverslips and miRNA loaded nanofibers for 24h No statistical differences were found among groups DNA content.

References:

1. James EN and Nair LS. J Biomed Nanotechnol 2014;10(8)500-507
2. Kapinas K, Kessler CB, Delany AM. J Cell Biochem 2009;108;216-24

Biography: I earned my B.S. in Biology at Stillman College, Tuscaloosa AL in 2006. I continued my education and earned a Professional Science Master's degree in the applied genetics, genomics and bioinformatics program in 2009 at the University of Connecticut, Storrs. I choose UConn Health based on multidisciplinary projects that were being performed in the Skeletal Craniofacial and Oral Biology program. My future milestone is to obtain a postdoctoral position at an institution in the U.S. that allows me to conduct quality molecular and cell biology and tissue engineering research while also serving as a part-time instructor to college students. My ultimate goal is to obtain a permanent position at a research institute and successfully secure grant funding for my own research goals.

Genetic requirement for progenitor cell responses in a mammalian limb regeneration model

Sandra Lopez[1], Melanie Fisher[1], Nickesha Anderson[1*], Tatiana Blanchard[1],
Ken Muneoka[2], and Caroline Dealy[1,3]

[1] *University of Connecticut Health Center, Department of Reconstructive Sciences, Center for Regenerative Medicine and Skeletal Development;* [3]*Department of Orthopedic Surgery*
[2] *Texas A&M University, Department of Veterinary Medicine and Biomedical Sciences*
[*]*Current address: Wesleyan University*

At least 2 million people in the United States live with limb loss due to trauma, birth defects or disease. Although some animals, like salamanders, are able to regenerate their entire limbs at any stage of life, regeneration capacity in mammals including humans is restricted to the digit tip. The Epidermal Growth Factor Receptor (EGFR) is family of tyrosine kinase receptors that plays a role in cell proliferation, migration, survival and cell differentiation. EGFR signaling is also required for differentiation of skeletal progenitors, and for development of cartilage and bone tissue. Studies in salamander and zebra fish systems suggest roles for EGFR signaling in limb/fin regeneration responses. In prior studies, we identified a genetic requirement for the EGFR-related receptor, ErbB3, for spontaneous regeneration of the mouse digit tip following digit tip loss. The goal of this project is to understand the cellular and molecular mechanisms underlying the requirement for ErbB3 in mammalian limb regeneration responses.

Methods: To examine the functional role of ErbB3 in limb regeneration responses, we evaluated spontaneous digit regeneration capacity in mice with conditional limb-specific ErbB3 loss. In this approach, two different strains of transgenic mice are crossed: one in which production of the bacterial enzyme Cre recombinase is controlled by the activity of the tissue-specific Prx1 limb-mesoderm promoter and the other in which the ErbB3 gene is flanked by *Cre recombinase* recognition sequences. A cross between this these transgenic mice will result in limb-targeted excision of the ErbB3 gene. The distal digit tips of newborn ErbB3-deficient mice and their littermate controls were amputated under anesthesia, and the mice were harvested at various times to assess the degree to which spontaneous digit tip regeneration occurred, and to evaluate critical limb regeneration responses.

Results: Our studies demonstrated a genetic requirement for the ErbB3 receptor in spontaneous digit tip regeneration in mice. We observed that progenitor cell proliferation, which is necessary for the formation of a mesodermal structure critical for successful limb regeneration called the blastema is impaired in the absence of ErbB3. Our observation that bone formation is also impaired in the absence of ErbB3 suggests that another potential role for ErbB3 may be to promote the differentiation of skeletal progenitors.
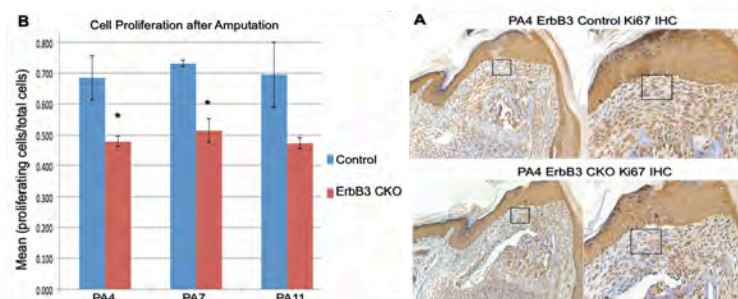

Fig 2. ErbB3 CKO mice show a decrease in cell proliferation in digits stumps collected 4, 7 and 11 days after amputation. A. Immunohistochemistry images (IHC). B. Quantification of labeled cells within the boxed area under the epithelial layer of digit stumps. *p values compared to control. PA4: 0.0494, PA7: 0.0066, PA11: 0.1728**.** Cell Proliferation Index in the blastemal region of the amputated digit stumps of control, normal mice was approximately two-fold higher than in the same region of the amputated digit stumps of ErbB3-CKO mice at each time point examined. These results indicate that progenitor cell proliferation, an important feature of the blastema, is impaired in the absence of ErbB3.

Conclusions: We observed that progenitor cell proliferation, an important feature of the blastema, is impaired in the absence of ErbB3. We propose that induction and/or maintenance of blastema progenitor cell proliferation is one mechanism by which ErbB3 normally functions to mediate the spontaneous regeneration of amputated digit tips in this system. Our observation that bone formation is also impaired in the absence of ErbB3 suggests that another potential role for ErbB3 may be to promote the differentiation of skeletal progenitors into cartilage or bone. Ongoing studies are further examining these and other potential functions for ErbB3 in digit tip regeneration.

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Biography:
B.S. Biological Sciences, University of Connecticut, Storrs, CT

I am a graduate student in a new Biomedical Science initiative created by The Connecticut Institute for Clinical and Translational Science and the University of Connecticut Health Center called the Young Innovative Investigator Program. This program provides minority students with intensive research training in the biomedical field in order to prepare for a future career in health care. I am currently on the first year and a candidate for entry into the Master's degree in Biomedical Science program. My long-term goal is to attend Dental School, and ultimately, to obtain a Master's degree in Public Health. At this institution I have been able to learn laboratory techniques and develop critical thinking and problem solving skills that will help me achieve my future goals.
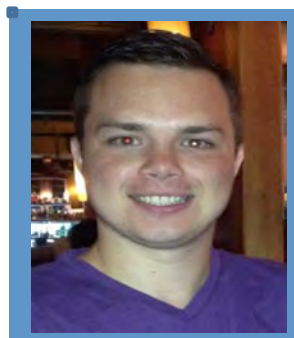
Investigating the role of BMP signalling on hepcidin expression in cancer cells

Manz D[1,3], Torti F.M[2] Torti S.V[3]

Affiliations
[1] University of Connecticut School of Dental Medicine, Farmington, CT
[2] University of Connecticut School of Medicine, Farmington, CT
[3] Department of Molecular Biology and Biophysics, The University of Connecticut Health Center, Farmington, CT

Abstract: Hepcidin is an important protein involved in the regulation of intracellular and systemic iron. Aberrantly overexpressed in prostate cancer (Figure 1), hepcidin promotes an increase in intracellular iron by targeting the iron export protein ferroportin for degradation. Increased intracellular iron is a characteristic of several types of cancer, including breast, liver and prostate, and has been shown to contribute to mutagenesis, growth, and metastasis. In liver cells it has been established that bone morphogenetic proteins (BMPs) play a significant role in inducing hepcidin expression. We hypothesize that BMPs, specifically BMP4 and BMP7, contribute to abnormal iron homeostasis in prostate cancer cells by transcriptionally inducing hepcidin. **Methods:** Prostate cancer cells grown *in vitro* were treated with recombinant BMPs and/or a small molecule BMP signaling inhibitor (compound LDN – 193189). RNA was isolated from harvested cells and RT-PCR was used to evaluate relative levels of hepcidin transcript. **Results:** Hepcidin transcripts were significantly induced in LNCaP prostate cancer cells treated with BMP4, BMP6, or BMP7; an effect inhibited by LDN-193189. Basal levels of hepcidin, however, were not affected by treatment with the BMP inhibitor. **Conclusion:** These results suggest that novel mechanisms may regulate basal hepcidin transcript levels in prostate cancer cells. Indeed, subsequent research has revealed that basal hepcidin in DU145 cells, another prostate cancer cell line, may depend on Wnt signaling. The role of Wnt signaling in regulating aberrant iron homeostasis in prostate cancer cells warrants further study.
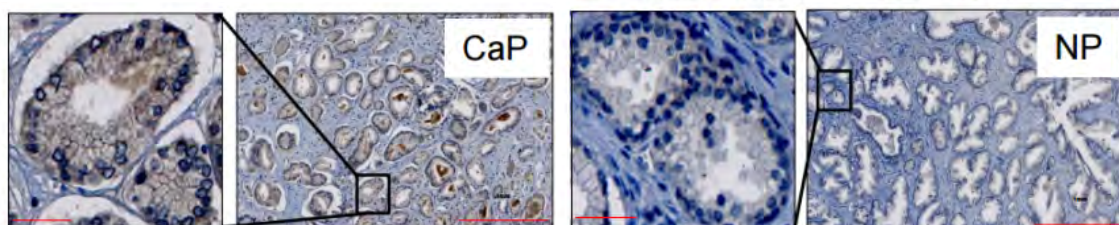
Figure 1: Hepcidin is elevated in Prostate Cancer
Immunohistochemical staining of hepcidin, brown, in patient derived prostate cancer (CaP) and normal prostate glands (NP). Scale bar equal to 1mm in wide view and 100μm in th magnified image. Modified from Tesfay, Lia, et al. "Hepcidin regulation in prostate and its disruption in prostate cancer." Cancer research (2015): canres-2465.

Biography: David graduated from the University of Connecticut with a major in biological Sciences and a minor in business. He is currently in his third year of the combined D.M.D./Ph.D.

program at the University of Connecticut Health Center. Being a former intern at the University of Connecticut Health Center, David felt very comfortable with the culture and opportunities available here. David aspires to integrate clinical practice with advancing knowledge to innovate, and ultimately improve, patient care.

The Role of Fibroblast Growth Factor 2 Isoforms in Osteoarthritis

Patience Meo Burt[1], Thomas Doetschman[2], and Marja Marie Hurley[1]

[1]*Department of Medicine/Endocrinology, School of Medicine, UCONN Health, Farmington, CT*
[2]*BI05 Institute and Dept. Cellular and Molecular Medicine, University of Arizona, Tucson, AZ*

Osteoarthritis (OA) is the leading cause of chronic disability in the U.S., characterized by the loss of articular cartilage and changes in underlying bone. There is no effective therapy for OA. Mice deficient in fibroblast growth factor 2 (FGF2) develop accelerated and more severe spontaneous OA. Thus, FGF2 plays a role in cartilage maintenance, but a comprehensive analysis is indicated since the *Fgf2* gene encodes multiple protein isoforms including high molecular weight isoforms (HMW) with nuclear localization sequences and low molecular weight isoform (LMW) that is exported from cells. We utilized mice in which all FGF2 isoforms are knocked-out ($Fgf2^{ALLKO}$), mice in which only the FGF2HMW isoforms are knocked-out ($Fgf2^{HMWKO}$) that have increased bone mineral density (BMD) compared to wild-type (WT) littermates, and mice in which the FGF2LMW isoform is knocked-out ($Fgf2^{LMWKO}$) that have decreased BMD compared to WT littermates. We hypothesized that $Fgf2^{LMWKO}$ mice will develop OA whereas $Fgf2^{HMWKO}$ mice will not develop OA compared with WT littermates.

To assess for signs of OA, knee joints from 2, 6 and 9 month old $Fgf2^{ALLKO}$, $Fgf2^{HMWKO}$ and $Fgf2^{LMWKO}$, and their WT littermate mice underwent digital x-ray imaging and microCT analysis. To determine articular cartilage integrity and proteoglycan content, Safranin-O staining was performed. Immunohistochemistry was used to examine expression of cartilage degrading metalloproteinases MMP-13 and ADAMTS-5.

Digital x-rays and Safranin-O staining revealed no evidence of OA in 2 month-old mice of all genotypes. As shown in Figure 1, x-rays of 6 month-old mice confirmed OA in knees of $Fgf2^{ALLKO}$. Interestingly osteophyte formation and thinning of the subchondral bone was observed at 6 months in $Fgf2^{LMWKO}$ knees. Similar changes were observed, including thickening of the subchondral bone at 9 months in $Fgf2^{LMWKO}$ knees, which was not present in $Fgf2^{HMWKO}$ or WT mice (data not shown). MicroCT analysis of 6 and 9 month old $Fgf2^{LMWKO}$ knees revealed changes in trabecular architecture indicative of OA compared to WT, while changes observed in $Fgf2^{HMWKO}$ mice were minor compared to their WT controls (this was also observed at 2 years of age). At 6 months old Safranin-O staining was decreased and evidence of fibrillation of articular cartilage was observed in $Fgf2^{ALLKO}$ and $Fgf2^{LMWKO}$ mice (Figure 2). Metalloproteinase labeling was increased within the joint of $Fgf2^{LMWKO}$ mice (data not shown).

Overall, these results suggest that lack of the LMW isoform of FGF2 contributes to the progression of OA, and they offer insight into a factor that causes the disease, while being a potential therapeutic target for OA.

Fig. 1: Digital x-rays of knee joints of 6 month old *Fgf2^ALLKO*, *Fgf2^HMWKO* and *Fgf2^LMWKO* mice and their littermate WT control. *Fgf2^ALLKO* and *Fgf2^LMWKO* mice show osteophyte formation (arrow) and flattening of the tibial plateau. There are no structural changes in the bone of the knee joint between the *Fgf2^HMWKO* mice and its WT control.
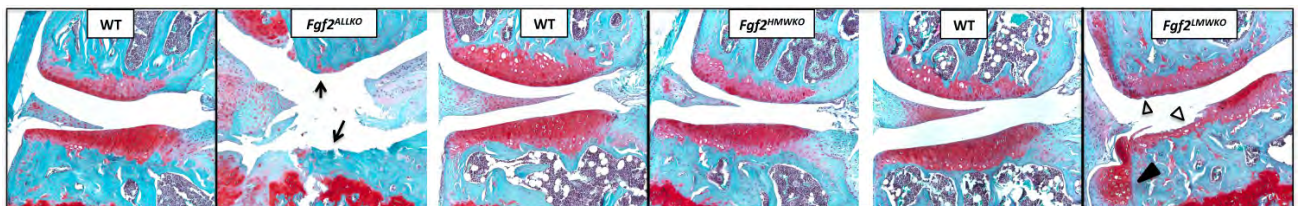

Fig. 2: Safranin-O staining comparing knees of 6 month old *Fgf2^ALLKO*, *Fgf2^LMWKO*, and *Fgf2^HMWKO* and their littermate WT control. *Fgf2^ALLKO* and *Fgf2^LMWKO* shows decreased proteoglycan content. *FGF2^ALLKO* has a complete loss of articular cartilage (arrow). *Fgf2^LMWKO* shows fibrillation of the articular cartilage (open arrowhead) and osteophyte development (closed arrowhead) compared to its WT control. There is no difference in proteoglycan content and joint integrity between *Fgf2^HMWKO* and its WT control at 6 months.

Biography:

      B.S. Biology: Post University, Waterbury, CT
      M.A. Biomolecular Sciences: Central CT State University, New Britain, CT

      Ph.D. in Biomedical Science; Skeletal, Craniofacial and Oral Biology – 2nd year

As a second year student, I am still exploring future career options, but I am interested in pursuing translational research and science policy. I chose UCHC due to the excellent skeletal biology program and research opportunities that combine basic science and human health.

PI3K Signaling Regulates SDF-1-Mediated Recruitment Of Osteoclast Precursors In Homeostasis And During Fracture

Scanlon V, Soung D, Adapala NS, Hansen M, Drissi H and Sanjay A.

Introduction: The two major functions of osteoblasts (OBs) are to make bone and to coordinate bone resorption through recruitment of osteoclast precursors (OCPs). We have previously reported that a point mutation in mice, which abrogates the interaction between Cbl an E3 ligase and PI3K a lipid kinase (YF mice), have increased osteoblast numbers and bone formation [1]. Cells of the osteoblast lineage are one of the major sources of Stromal derived factor-1 (SDF-1) in bone, which is required for the initiation and maintenance of hematopoietic niches [2]. Moreover, the interaction between SDF-1 and its receptor CXCR4 in OBs regulates the OCP pool [3] and its recruitment [4]. We have shown that, in the absence of Cbl-PI3K interaction during normal growth and fracture repair, there is an increase in OC numbers. These observations, led us to **hypothesize that in concert with Cbl-PI3K signaling, osteoblast derived SDF-1 regulates OC recruitment during bone homeostasis and injury repair.** To test this premise, we examined expression of SDF-1, migration of OCPs towards SDF-1 gradient, and number of OCs during homeostasis and fracture repair in YF and wild type (WT) mice.

Results: In BM, cells expressing $CD45^-Ter119^-CD31^-Sca1^-VCAM^+PDGFbR^+$ are known as reticular cells, which are responsible for SDF-1 synthesis [5]. By flow analysis we found that in the absence of Cbl-PI3K interaction there is a 50% increase in the number of the reticular cells. Moreover, expression of SDF-1 protein in reticular cells was also increased by 30% in YF mice. SDF-1-CXCR4 signaling in stromal cells regulates the OCP pool [3]. By flow analysis we found that YF mice had 1.82-fold (p<0.05) more OCPs than the WT mice. By using the transwell migration assay we found that that more numbers of YF derived OCPs migrated towards an SDF-1 gradient. Moreover, treatment of cells with CXCR4 inhibitor significantly reduced migration in response to SDF-1. To recapitulate the SDF-1-mediated recruitment of OCPs in absence of the Cbl-PI3K interaction we performed fracture experiments. We found that 7 days post-fracture expression of SDF-1 and CXCR4 was significantly upregulated in YF calluses (Panel A). Examination of the cellular composition of the fracture callus revealed that there was a more than 2-fold (p<0.05) increase in the number of TRAP+ cells at 14 d.p.f. (Panel B). Molecular analysis of callus by LCM followed by qRT-PCR supported this cellular finding by showing a 1.7-fold (p<0.05) increase in expression of TRAP, as well as a 3.2-fold (p<0.05) increase in Cathepsin K, and a 1.5-fold (p<0.05) increase in Calcitonin Receptor.

Discussion: Our results demonstrate that the regulation of PI3K activity by E3 ubiquitin ligase and adaptor protein, Cbl, controls SDF-1 production by reticular cells. We also found that in YF mice, enhanced SDF-1 expression in fracture callus may be responsible for increased recruitment of OCPs resulting in increased OC numbers.

A. Expression of SDF-1 and CXCR4 is up-regulated in YF cells forming fracture callus
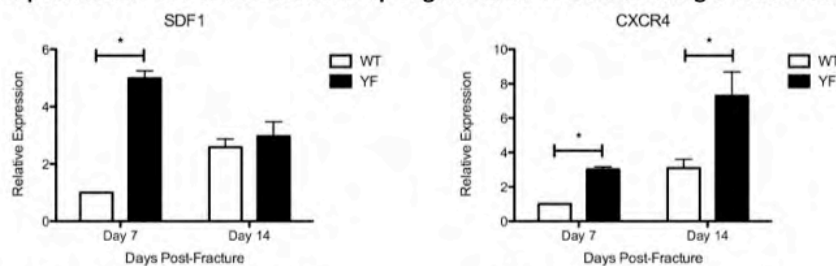
B. Lack of Cbl-PI3K Interaction increases OC numbers in remodeling fracture callus
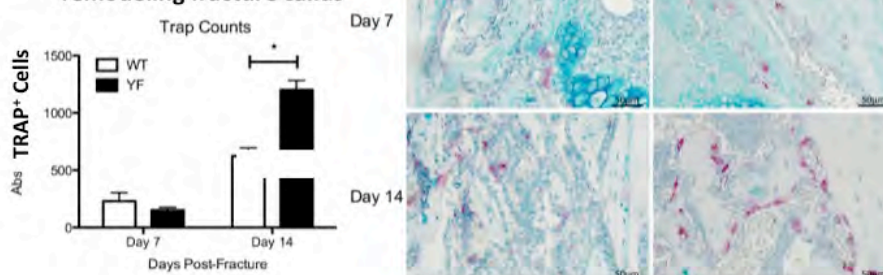
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Biography:

B.S. Molecular Cell Biology, UConn, Storrs, CT, 2006
B.S. Diagnostic Genetic Sciences, UConn, Storrs, CT, 2006
PhD-7th year
After graduation, I plan to complete a post-doctoral fellowship in clinical cytogenetics, and return to working in the cytogenetic diagnostic field. In the future, I'd also like to initiate clinical research that will aim to understand how different genetic variants affect the phenotype of patients with specific genetic disorders. I came to UConn Health to obtain my doctorate and further my career. I chose UConn Health specifically because of its reputation in clinical and translational research, as well as its proximity to my family and friends, without whose support I could not have accomplished my goals and aspirations. During my time in the graduate program, I have learned the importance of determination, keeping an open mind, accepting help when it's needed, and most of all, that there is always more to learn. I can never express my gratitude to all the people who have played such a significant role in shaping who I am today.
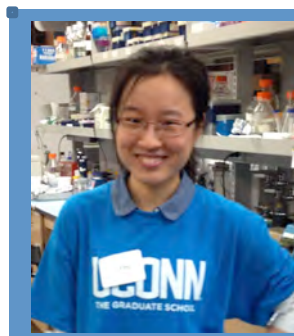
PDGF-BB and BMP2 Enhance Proliferation, Migration and Differentiation of Periosteal Progenitor Cells

Xi Wang[1], Brya Matthews[1], Ivo Kalajzic[1]

[1] Department of Skeletal Biology and Regeneration
University of Connecticut Health Center, USA

Objectives : BMP2 has been widely used in orthopaedics but supraphysiological doses lead to side effects. A growth factor which could potentially reduce BMP2 doses is Platelet Derived Growth Factor-BB (PDGF-BB). It is involved in the early stages of bone repair but its effect on periosteal cells is unknown. This study aims to evaluate the effects of PDGF-BB and BMP2 on periosteal progenitor cells (PPCs).

Methods : The periosteum was scraped from femurs and tibias of 6-8 week old mice and enzymatically digested for 1 hour. Primary periosteal cells were cultured in 5% oxygen for the first 4 days. For differentiation,cells were treated with 10ng/ml rhPDGF-BB and/or 100 ng/ml BMP2 beginning on Day 3, and cultured in osteogenic medium from Day 7-21. Gene expression and mineralized nodule formation was evaluated by expression of Col2.3GFP transgene and von Kossa staining. EdU and TUNEL assay was completed to evaluate the effects of PDGF-BB and BMP2 on the proliferation and apoptosis of PPCs. Migration was evaluated by measuring closure of a scratch wound.

Results: Flow cytometric analysis showed that a large proportion of CD45- PPCs express mesenchymal stem cell markers: 81.3% are Sca-1+ and 75.6% express CD105. 30.5% PPCs express PDGF Receptor-α, 71.8% cells are PDGFR-β+. During osteogenic differentiation, continuous PDGF-BB treatment inhibited the formation of mineralized nodules while pretreatment with PDGF-BB at days 3-7 of culture promoted mineralization. Sequential combination of PDGF-BB and BMP2 resulted in the highest level of mineralization and expression of osteocalcin and bone sialoprotein.
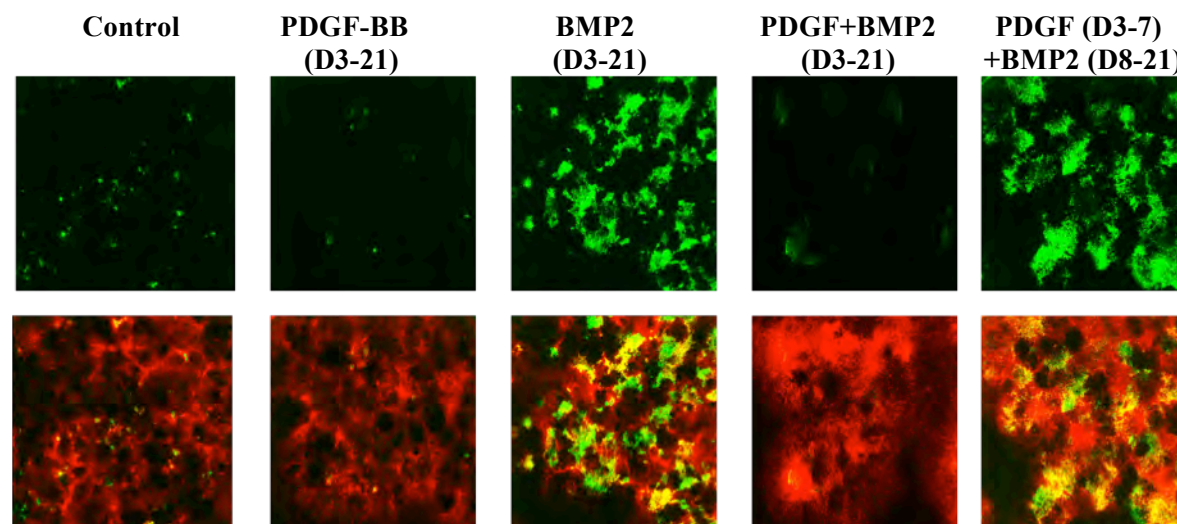

Figure 1. Effects of PDGF-BB and BMP2 on osteogenic differentiation.
(Green: Col 2.3 GFP; Red: α-SMA-tdTomato)

In addition, PDGF-BB significantly increased the proportion of EdU+ cells from 3.7% to 20.5% and prevented apoptosis of periosteal cells. PDGF also accelerated the migration of PPCs in a scratch assay, while the combination of PDGF-BB and BMP2 further increased speed of scratch healing in vitro.

Conclusions: Under temporal control, PDGF-BB and BMP-2 can significantly promote osteogenic differentiation of PPCs. They also jointly accelerate in vitro wound healing, suggesting therapeutic potential for combination treatment.

Biography: B.S. Clinical Medicine: Shandong University, Jinan, China
M.S. Internal Medicine: Shandong University, Jinan, China
PhD in Biomedical Science; Skeletal Biology and Regeneration---2nd year

I am interested in identifying and understanding the biology of skeletal progenitor cells. Transgenic mice provide us a good model and I would like to continue study on the molecular mechanisms of clinical related bone diseases in the future. I chose the SCOB department at UCHC because of the good academic environment and more training opportunities. I believe the systemic training from SCOB will be very helpful for my future career.

Non-Fourier Methods in NMR Data Processing

Matthew A. Zambrello,[1] Mark W. Maciejewski[1], Adam D. Schuyler[1], and Jeffrey C. Hoch[1]

Affiliations

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Abstract: Nuclear magnetic resonance spectroscopy (NMR) ranks among the most informative and versatile techniques used by structural biologists. Processing NMR data using conventional methods invariably forces spectroscopists to compromise sensitivity to attain higher resolution or vice versa. In the context of NMR, sensitivity is the ability to distinguish real signals from the baseline noise of the spectrum and resolution is the ability to distinguish signals that are in close proximity or overlapping. For a high quality spectrum it is important to have an appropriate combination of both. Data collected from a spectrometer is typically processed using the Fourier Transform, which converts the raw NMR data to the spectrum of peaks that we are familiar with. Raw data can be weighted to improve sensitivity or resolution, but it cannot be modified to improve both simultaneously. This limits the size and scope of proteins that can be investigated using NMR. For example, larger, more complex proteins produce spectra with a high degree of peak overlap[1]. To resolve these areas of overlap weaker signals that are embedded within noise are sacrificed. This can lead to incomplete peak data, making it difficult find solution structures to the proteins of interest. Despite these limitations, there are numerous non-Fourier methods that in principle can overcome this resolution/sensitivity tradeoff. Perhaps the most versatile among these methods is Maximum Entropy Reconstruction (MaxEnt). MaxEnt has been previously used for enhancement of images captured by deep space telescopes but it is now also used in spectrum analysis. It is an iterative process that builds multiple spectra from scratch, finding the unique spectrum that is consistent with the data. Because MaxEnt treats spectrum analysis as an inverse problem, it is also a compelling method for deconvolution, which is essentially the modification of the raw data to achieve both sensitivity and resolution enhancement while still finding a spectrum that is consistent[2]. The utility of MaxEnt for deconvolution has previously been demonstrated in a number of 1D NMR experiments, but it is not widely used today. Deconvolution of the 1D signal envelope was shown to enable resolution enhancement without amplifying noise [3,4]. In this work we systematically investigate the use of MaxEnt to deconvolve the signals in 2D experiments, the type used for investigation of protein structures. Our data suggests that MaxEnt deconvolution may be capable of overcoming the resolution/sensitivity tradeoff. The advantages to overcoming this limitation include the ability to use NMR for structural studies of larger, more complex proteins that naturally have more signals because they contain more amino acid residues.

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4 Ni, F., Levy, G. C. & Scheraga, H. A. Simultaneous resolution enhancement and noise suppression in NMR signal processing by combined use of maximum entropy and Fourier self-deconvolution methods. J Magn Reson 66, 385-390 (1986).

Biography:

Undergraduate Degree- Molecular and Cell Biology, University of Connecticut
DMD/PhD student, year 3

After I complete the DMD/PhD program, I aspire to attend a residency program, perhaps specializing in Oral and Maxillofacial Radiology. Over the longer term, I would like obtain a position as faculty at a dental school so that I can teach, conduct research, and see patients.

I chose to attend UConn because of the high quality of School of Dental Medicine and the unique opportunities it offers for clinicians and scientists. During my time here, I have learned how important it is to get immersed in the community and to get involved in ways outside of strictly science and dental medicine.
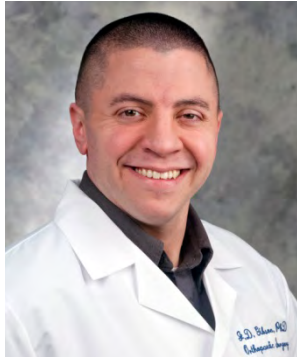
Wnt5a treatment of embryonic stem cell progenitors promotes cartilage repair in a rat chondral defect model

Jason D. Gibson, Farhang Alaee, David N. Paglia, Ryu Yoshida,
Thomas M. DeBerardino, Rosa M. Guzzo, and Hicham Drissi

University of Connecticut Health Center, Farmington, CT

Department of Orthopaedic Surgery, UConn Musculoskeletal Institute,
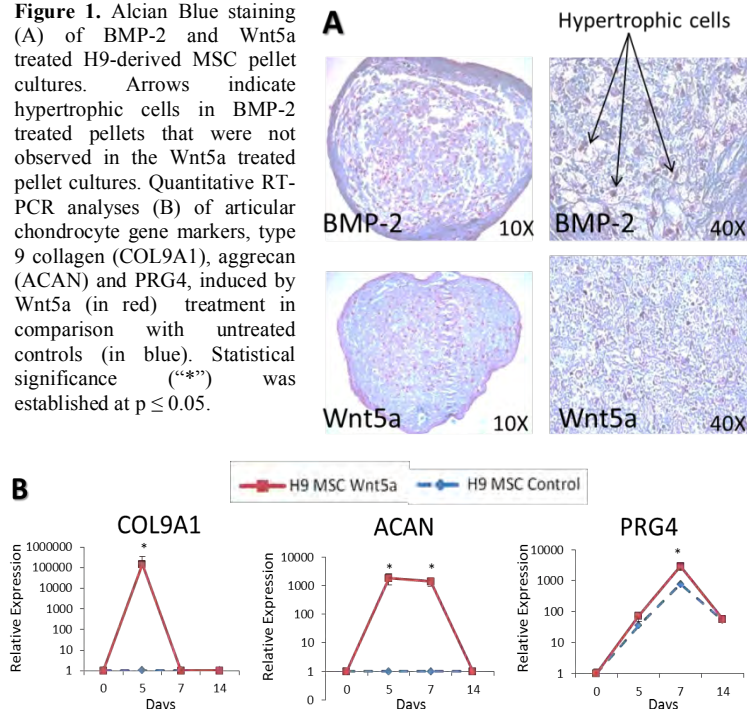UConn Stem Cell Institute

Objectives: The poor capacity of native cartilage tissue to self-repair remains a significant clinical challenge. However, no effective treatments have yet been developed to repair large cartilage defects following traumatic joint injuries. Therefore, identifying the appropriate source of progenitors as well as the necessary signals to control their differentiation into articular-like chondrocytes is prerequisite for an effective cell-based strategy to repair articular cartilage damage. We postulate that the source of multipotent stem cells as well as the ability to direct their differentiation is critical for achieving joint cartilage repair in vivo. Multipotent chondroprogenitors generated from human embryonic stem cells (hESC) have been increasingly reported to mediate cartilage tissue regeneration in vitro and in vivo. However, little evidence is available about the ability of these cells to adopt a permanent articular-like cartilage phenotype. Based on the genetic evidence demonstrating the chondrogenic potential of BMP-2 combined with the anti-hypertrophic effects of the non-canonical Wnt5a, we hypothesized that Wnt5a treatment is sufficient to induce the maturational arrest of BMP-2 mediated chondrocyte differentiation of hESC-derived MSC. Moreover, we hypothesized that sequential treatment with BMP-2 followed by recombinant Wnt5a is necessary to promote articular cartilage repair in vivo.

Methods: The human H9 embryonic stem cell line was used to derive mesenchymal stem cell (H9-MSC) progenitors using a direct plating method. The mesenchymal properties of the H9-MSC progenitor cells were evaluated by flow analyses and compared with somatic cells from bone marrow as well as human articular cartilage. In vitro differentiation assays were performed to validate the ability of the H9-MSC progenitors to differentiate into osteoblasts, chondrocytes, and adipocytes. Chondrogenic differentiation was induced in H9-MSC pellet cultures upon treatment with human recombinant BMP-2 (100ng/mL), a widely used chondrogenic factor known to promote chondrocyte maturation, Wnt5a (50ng/mL), or sequential combination of BMP-2 followed by Wnt5a treatment. Histological assessments of proteoglycan matrix deposition were compared across treatment groups and quantitative RT-PCR analyses were performed for the comparison of transcriptional profiles in vitro.

Distal femoral chondral defects were created in athymic rats in accordance with our approved animal protocol. The experimental groups were as follows: (I) empty defects; (II) defects filled with untreated H9-derived MSC pellets; and (III) H9-derived MSC pellets pretreated for 2 days with rhBMP-2, then rhWnt5a for 12 days. Rat subjects were euthanized at 2 and 4 weeks post-surgery and rat knees were harvested for histology and molecular analyses. Histological images were captured and analyzed to determine the extent of cartilage repair (n=5 per group). Immunohistochemical (IHC) staining for type II collagen and type X collagen were used to evaluate the extent of chondrogenic matrix production and chondrocyte hypertrophy, respectively (n=5 per group). IHC staining for human-specific mitochondrial antigen was used to distinguish human implants from rat host tissue in the defected regions (n=5 per group). Cartilage repair was graded using a modification of a widely published grading scale specific for cartilage regeneration. All data were given as means ± standard error of the mean. Statistical significance was established at $p \leq 0.05$. This study was approved by the CT State Department of Public Health and was conducted in accordance with federal and institutional guidelines.

Results: The H9 hESC line was used to derive MSC-like progenitors (H9-MSC). We demonstrated that these multipotent stem cells shared common mesenchymal features with the human articular chondrocytes and bone marrow derived MSC by flow analyses. Furthermore, the ability of the H9-MSC cells to differentiate into the chondrogenic as well as the osteogenic and adipogenic lineages was also established. H9-MSC progenitors were induced to differentiate in vitro into chondrocytes upon treatment with BMP-2 or Wnt5a. While BMP-2 treatment induced their terminal maturation, Wnt5a prevented their hypertrophy in pellet cultures and transiently induced the expression of hyaline cartilage gene markers (Fig. 1). We further examined whether Wnt5a can suppress the terminal maturation induced by BMP-2 in these cultures. Pellet cultures pretreated with BMP-2 for 5 days, and then exposed to Wnt5a for up to 14 days showed enhanced expression of chondrocyte markers and abrogation of chondrocyte terminal maturation compared to cultures treated with BMP-2 alone (Fig. 2). Wnt5a also limited fibrocartilage formation in pellet cultures as reflected by increased type II/type I collagen expression ratio. Collectively, the in vitro data suggest

Figure 1. Alcian Blue staining (A) of BMP-2 and Wnt5a treated H9-derived MSC pellet cultures. Arrows indicate hypertrophic cells in BMP-2 treated pellets that were not observed in the Wnt5a treated pellet cultures. Quantitative RT-PCR analyses (B) of articular chondrocyte gene markers, type 9 collagen (COL9A1), aggrecan (ACAN) and PRG4, induced by Wnt5a (in red) treatment in comparison with untreated controls (in blue). Statistical significance ("*") was established at $p \leq 0.05$.

that Wnt5a can mediate stem cell differentiation into articular-like chondrocytes while inhibiting their terminal maturation. The in vivo correlate of these in vitro studies showed that sequential pre-treatment of the H9-MSC cells with BMP-2 followed by Wnt5a resulted in cartilage regeneration of a rat chondral defect model (Fig. 3). The defected regions of the rat femoral condyles retained the pre-treated H9-MSC tissue, and successful integration with the host tissue was observed on both edges of the defect. At 4 weeks post-surgery, the H9-derived MSC progenitors were shown to generate type II collagen positive cartilage structures at the defect site while untreated controls and defects without implanted cells did not. The integration of the transplanted H9-MSC progenitor cells to the repair site was also verified using human mitochondrial antigen staining and quantitative species-specific RT-PCR.
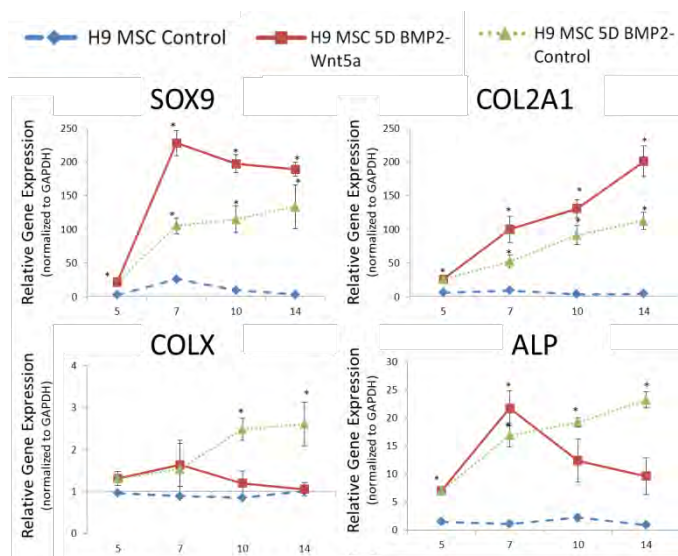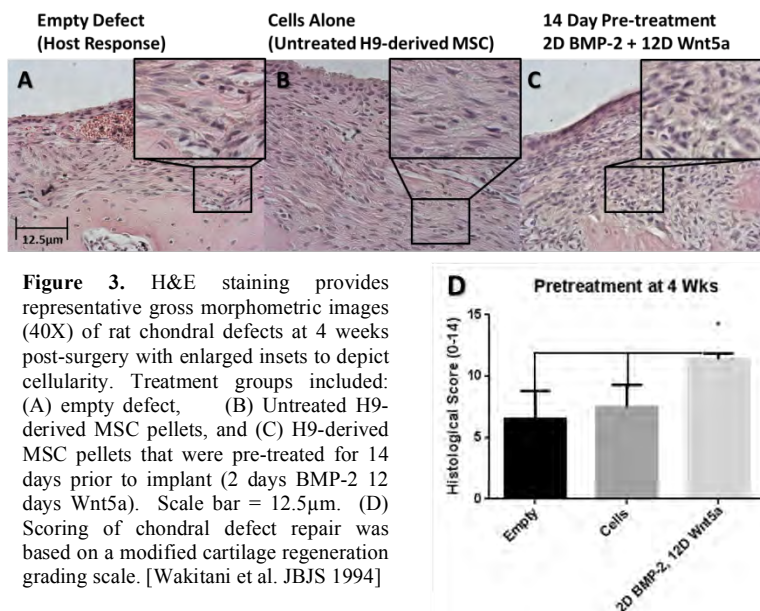

Figure 2. Quantitative gene expression of chondrogenic markers (SOX9 and COL2A1) and hypertrophic chondrocyte markers (COLX and ALP) in H9-derived MSC progenitor cell pellets cultured in basal chondrogenic media (H9 MSC Control: blue) for up to 14 days, or treated first with BMP-2 for 5 days then with Wnt5a (H9-MSC 5D BMP2-Wnt5a: red) or basal media alone (H9-MSC 5D BMP2-Control: green). Statistical significance ("*") was established at $p \leq 0.05$.

Significance: This is the first demonstration of a pluripotent stem cell repair of articular cartilage by the non-canonical Wnt5a in the absence of any exogenous matrix. The data indicate that Wnt5a alone can restrict the differentiation of committed stem cells to immature chondrocytes, a feature of hyaline cartilage and a rate-limiting factor for joint cartilage regeneration. The molecular analyses revealed that in addition to its anti-hypertrophic effects, Wnt5a appears to also trigger the expression of markers of hyaline cartilage. These findings set the stage for the development of successful cell-based approaches to repair large cartilage defects in the joint, potentially using a novel Wnt5a biomimetic combined with optimal biomaterials to enhance the efficacy of clinically relevant patient-specific stem cells.

Figure 3. H&E staining provides representative gross morphometric images (40X) of rat chondral defects at 4 weeks post-surgery with enlarged insets to depict cellularity. Treatment groups included: (A) empty defect, (B) Untreated H9-derived MSC pellets, and (C) H9-derived MSC pellets that were pre-treated for 14 days prior to implant (2 days BMP-2 12 days Wnt5a). Scale bar = 12.5μm. (D) Scoring of chondral defect repair was based on a modified cartilage regeneration grading scale. [Wakitani et al. JBJS 1994]

Biography: Post-doctoral Fellow in Orthopaedic Surgery – 4th year
Ph.D. in Genetics and Genomics, University of Connecticut, Storrs, CT
B.S. in Molecular and Cell Biology, University of Connecticut, Storrs, CT

It is my career objective to promote the advancement of research and education in the fields of molecular and cell biology, genetics and genomics, and translational stem cell biology for improving human health and disease management, and to serve as a mentor and role model for students in higher education to enhance the instruction of rising scientists. I chose a post-doctoral fellowship at UCHC based on our institution's advanced pursuit of medical technologies in the musculoskeletal field, as well as an exciting opportunity to perform translational research using embryonic stem cells to investigate potential cellular therapeutics for the treatment of musculoskeletal disorders. In my time here at UCHC I have learned a variety of techniques for the histological assessment of tissues and the characterization of cell types, and I have been privileged to train with orthopaedic residents to learn surgical models of cartilage defect repair.

PTH Stimulation of RANKL in Primary Osteoblasts Is Independent of PTH-Stimulated cAMP

T. Estus, S. Choudhary, T. Kwarko, C. Pilbeam
University of Connecticut

Parathyroid hormone (PTH) can stimulate both bone formation and resorption. When PTH is given continuously, resorption is greater than formation and bone is lost. We showed that the osteogenic actions of continuous PTH in vitro are suppressed by a factor that blocks PTH-stimulated cAMP production in osteoblastic cells. The production of this factor is dependent on the expression of cyclooxygenase 2 (Cox2), the major enzyme regulating prostaglandin production. When we treated wild type (WT) and Cox2 knockout (KO) mice with PTH infusion for 12-21 days, we found that anabolic actions of PTH were suppressed in WT, but not KO mice. However, the PTH stimulation of bone resorption was the same in both WT and KO mice. We have identified the inhibitory factor in vitro as serum amyloid A 3 (Saa3) and shown that Saa3 is secreted by bone marrow macrophages (BMMs) treated with RANKL. In this study we use the conditioned media (CM) from RANKL-treated WT BMMs or a recombinant, human-homolog of murine Saa3 (SAA) to study the involvement of cAMP-activated signaling in the PTH induction of Rankl. We cultured primary osteoblasts (POBs) from neonatal calvaria to confluence (5 days), treated with PTH (10 nM) in the presence or absence of CM or SAA (10 µg/mL) for 3 h and measured gene expression by qPCR. SAA concentration was determined by dose response as measured by cAMP signaling inhibition. As expected, both CM and SAA blocked the PTH-stimulated gene expression of cAMP-regulated receptor activity modifying protein 3 (Ramp3). However, PTH stimulated Rankl expression was not decreased by CM or SAA. The protein kinase A (PKA) inhibitor, H-89, blocked PTH-stimulated Ramp3 expression but had no effect on PTH-stimulated Rankl expression. PTH has also been shown to activate the protein kinase C (PKC) pathway, which subsequently signals via extracellular-signal-regulated kinases (ERKs). The PKC inhibitor GF109203X and the ERK inhibitor PD98059 both blocked PTH-stimulated Rankl expression but did not decrease PTH-stimulated Ramp3. The calcium chelation agent BAPTA also blocked PTH-stimulated Rankl expression. In conclusion, our results indicate that PTH-stimulated Rankl in POBs is independent of cAMP signaling and likely to depend upon the PKC pathway. These data support our PTH infusion study, which indicated that the anabolic and catabolic effects of PTH occur via different signaling pathways.
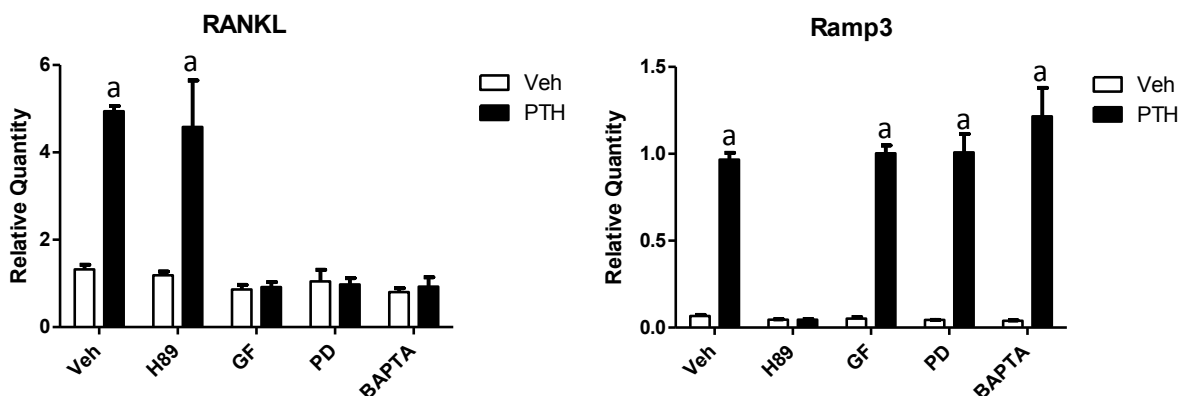

Fig. 1. PTH stimulation of RANKL is dependent on Ca^{2+}/PKC signaling not cAMP/PKA signaling. POBs were treated with 10 nM PTH following 2 hour pretreatment with either the PKA inhibitor H89 (30 µM), the PKC inhibitor GF (0.6 µM), the MEK/ERK inhibitor PD (50 µM), or the calcium chelation agent BAPTA (50 µM). Gene expression for RANKL and the cAMP response gene Ramp3 were analyzed. n=3. Bars are means ± SEM, aSignificant effect of treatment compared to vehicle control, $p < 0.0001$.

Biography: B.S. Bioengineering
                  PhD Student of Biomedical Engineering 5th year

Upon completion of my degree, I plan on joining the United States Navy as a Nuclear Propulsion Officer on a submarine. I came to the University of Connecticut due to the unique interdisciplinary research that the health center environment provides.

Runx1 mediates regulation of osteoclast formation and function in myeloid osteoclast precursors

David N. Paglia, Do Yu Soung, Sandra Jastrebski, Judy Kalinowski, Joseph Lorenzo, Hicham Drissi
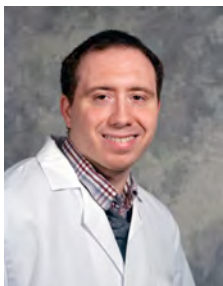
Affiliations: University of Connecticut

Introduction: Pathologic bone resorption is the cause of many bone disorders including osteoporosis. Identifying factors that can inhibit osteoclast formation and/or activity may help to define new therapeutic targets is of paramount importance. Anti-resorpative drugs such as bisphosphonates and anti-RANKL antibodies have been developed to inhibit bone resorption and the incidence of fractures, but their use has been linked to atypical fractures and osteonecrosis. Recent studies have suggested that the master-regulator of hematopoiesis, Runx1, is expressed in preosteoclasts and has the potential to influence skeletal health. We have previously demonstrated that conditional loss of Runx1 in the myeloid osteoclast precursors, through crossing Runx1$^{F/F}$ mice with CD11b Cre trangenic mice, resulted in a significant osteoclastogenic phenoype. Only male mice were used for this investigation because the CD11b-Cre transgene was inserted into the Y chromosome and as such it is unclear if this phenotype is altered by gender differences. For these reasons, we chose to evaluate a Lysm Cre; Runx1$^{F/F}$ mouse which should have altered osteoclast formation and function in myeloid osteoclast precursors for both male and female mice.

Methods: In this study, we crossed Runx1 floxed mice (Runx1$^{F/F}$) with Lysm-Cre transgenic mice to generate mice in which Runx1 expression was conditionally abrogated in osteoclast precursors (Lysm-Cre Runx1$^{F/F}$). After institutional animal care committee approval, mice were divided into groups to that were used for micro-CT scans, histomorphometry, and TRAP staining. Whole bone marrow was isolated from other long bones of these mice and enriched for bone marrow macrophages. The ability of these cells to differentiate into osteoclasts was measured following exposure to MCSF and RANKL. To examine if Runx1 plays a role during early osteoclastogenesis femora were harvested from both male and female Lysm-Cre Runx1$^{F/F}$ mice (greater than 50% deletion of Runx1 function in bone marrow macrophages), compared to control mice with 100% Runx1 activity (Runx1$^{F/+}$). TRAP staining was used to quantify the extent of osteoclastogenesis during bone homeostasis. Gene expression analysis following from captured tissue sections was used to evaluate effects of conditional loss of Runx1 on genes associated with osteoclast formation, fusion, and function expression. All data were given as means ± standard error of the mean. Parametric data were tested using a student t-test followed by an F-test to determine normality and differences between groups. Statistical significance was established at $p \leq 0.05$. This study was approved by the institutional review board and was conducted in accordance with federal and institutional guidelines.

Results: Similar to the phenotype we observed with the CD11b Cre; Runx1$^{F/F}$ in male mice, both male and female Lysm Cre; Runx1$^{F/F}$ mice demonstrated a significant decrease in trabecular bone mass, and increases in expression of genes associated with osteoclast formation, fusion, and function, compared to controls. Osteoclasts that were formed both from BMM cultures and in vivo for Lysm Cre; Runx1$^{F/F}$ mice, were noticeably larger with a greater number of nuclei than for controls. In contrast to the CD11b Cre; Runx1$^{F/F}$ phenotype, which demonstrated significant decreases in cortical bone mass, there were no observed differences in cortical bone mass following abrogation of Runx1 expression in Lysm-Cre positive cells.

Discussion: The results of this study suggest that abrogation of Runx1 in osteoclast precursors significantly induces osteoclast formation and fusion, resulting in a loss in trabecular bone mineral density and increased production of larger multinucleated osteoclasts. The confirmation of our previous study in CD11b Cre; Runx1$^{F/F}$ male mice with both genders for the Lysm Cre; Runx1$^{F/F}$ mice, suggests that loss of Runx1 expression in osteoclast precursors significantly induces osteoclastogenesis and implicates Runx1 in regulation of osteoclastogenesis. Further research will aim to evaluate the role of Runx1 during osteoclastogenesis in a mature osteoclast population.

References: if needed

Biography: David N. Paglia, Ph.D.
      B.S. Mechanical Engineering: Manhattan College, Riverdale, NY
   M.S. Biomedical Engineering: New Jersey Institute of Technology, Newark, NJ
      PhD Biomedical Engineering; Rutgers University and NJIT Joint Program, Newark, NJ
      Post-Doctoral Fellowship: Rutgers, Newark, NJ
      Post-Doctoral Trainee: UConn Health 3rd Year (T90 NIDCR Trainee-1st Year),
      Farmington, CT

It is my career objective to promote the advancement of research in the fields of orthopaedics, translational orthobiologic therapeutics, cell biomechanics, musculoskeletal biology, and musculoskeletal biomechanics. Through my research endeavors I plan to mentor rising scientists and pursue teaching opportunities. I chose a post-doctoral fellowship at UConn Health based on the New England Musculoskeletal Institute's strong foundation for collaborative research and mechanistic approach towards musculoskeletal research. As a T90 Trainee, I am investigating the influence of temporal regulation of abrogation of a specific gene in limb mesenchymal progenitors on the progression and extent of osteoclastogenesis during fracture healing. I have been privileged to work in a well-respected group with supportive mentors and to have the support of the T90 committee mentors.
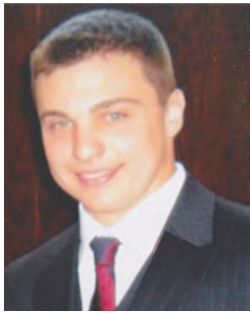
Inhibition of Retinoic Acid Signaling and Stimulation of Wnt Signaling Allows Efficient Paraxial Mesoderm Formation from Human Embryonic Stem Cells

Ryan P. Russell[1], Yaling Liu[1], Peter Maye[1]

[1]*Department of Reconstructive Sciences, UConn Health, Farmington, CT*

INTRODUCTION

Embryonic stem cell (ESC) technologies are continually advancing research and development of treatment strategies for various human diseases, including those that impact the human skeleton. However, a more comprehensive understanding of how to direct ESCs into mature, functional skeletal cell types remains a necessity as the debate persists regarding the most appropriate differentiation strategy. Our work has focused on a stepwise, embryonic differentiation program in which differentiation progresses from ESCs to paraxial mesoderm to sclerotome and eventually skeletal progenitors. The axial skeletal lineage pathway is advantageous as its derivatives are capable of forming multiple skeletal cell lineages including chondrocytes, osteoblasts and tenocytes. We have directed human ESCs into paraxial mesoderm through activation of Wnt signaling along with inhibition of the retinoic acid pathway. This approach, coupled with diagnostic readouts from transgenic reporter cell lines, including that for TBX6, a key regulator of paraxial mesoderm specification, demonstrates the first stage in our axial skeletal differentiation protocol.

METHODS

In-vitro differentiation was performed using a *TBX6-mCherry/UbiquitinC-Citrine* human H9 ESC reporter line. ESCs were plated on Matrigel at low density, and then switched to a base differentiation media containing N2, B27, MTG, and ascorbic acid the following day. On day 2, cultures were treated with combinations of Wnt3a (50ng/ml), CHIR99021 (3uM), AGN193109 (1uM), and Noggin (100ng/ml) over 4 days, with one media change. After 4 days of treatment, cultures were imaged and FACS sorted for TBX6^{+} reporter populations. RT-PCR was performed on sorted samples.

RESULTS

Wnt pathway stimulation with Wnt3a and CHIR99021 for 4 days resulted in nominal TBX6 reporter expression in comparison to untreated cells, however treated colonies were more robust and showed stronger Ubiquitin reporter expression. Addition of the retinoic acid receptor antagonist AGN193109 along with Wnt3a and CHIR99021 resulted in a strong increase in TBX6 reporter expression confirmed by FACS analysis, 26.9% TBX6^{+} compared to <1% without AGN. Addition of the BMP antagonist Noggin to the differentiation cocktail resulted in a more limited TBX6 reporter expression pattern (9.9% TBX6^{+}) compared to Wnt3a, CHIR and AGN. FACS sorting of the Wnt3a, CHIR, AGN treated samples resulted in a TBX6^{+} population of 22.8% of viable cells. RT-PCR was performed to compare gene expression between the sorted and unsorted populations. For the paraxial mesoderm markers MEOX1 and Mesogenin, the TBX6^{+} population showed an 18- and 30-fold increase over the unsorted population, respectively. There was a 26-fold difference in TBX6 expression between the positive and negative sorted populations, confirming the accuracy of the reporter construct in matching endogenous gene expression.

CONCLUSIONS

Wnt pathway stimulation combined with retinoic acid inhibition strongly promotes ESC differentiation into paraxial mesoderm. Key paraxial mesoderm regulatory genes are significantly upregulated in our reporter positive populations, indicating efficient paraxial mesoderm induction as well as reliable reporter function. The ability to isolate a uniform population from this primary differentiation stage will allow for more effective generation of sclerotome populations and ultimately, functional skeletal progenitors for therapeutic use derived via a differentiation scheme comparable to embryonic development.
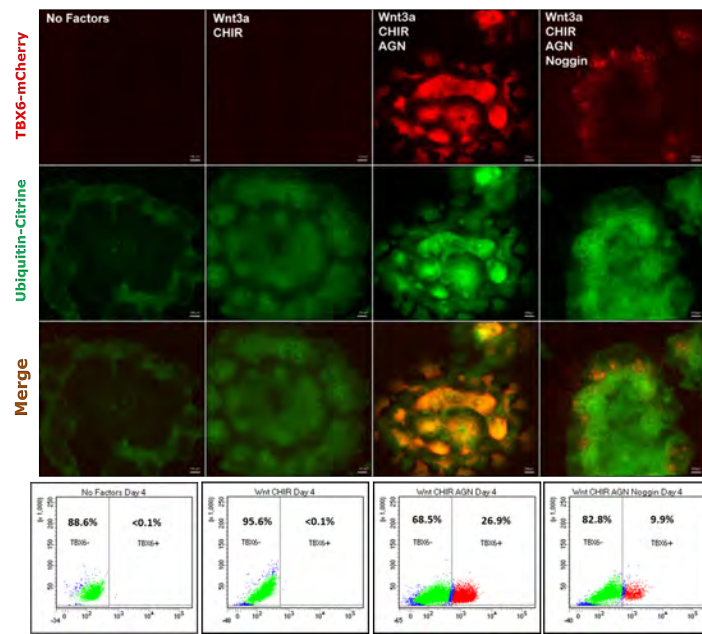
Figure 1. Retinoic Acid Inhibition Increases TBX6 Reporter Expression [Top] Fluorescent reporter expression in cultures treated for 4 days with the indicated combinations of Wnt3a, CHIR99021, AGN193109, and Noggin showing TBX6-mCherry (red) or UbiquitinC-Citrine (green) reporter expression. [Bottom] Corresponding FACS analysis results showing percentages of TBX6+ populations after each treatment. Wnt pathway stimulation with Wnt3a and CHIR99021 coupled with retinoic acid pathway inhibition by AGN193109 resulted in the formation of more robust and organized colonies. Cells subjected to this treatment combination also showed a strong increase in TBX6-mCherry reporter expression confirmed by FACS analysis, 26.9% TBX6+ compared to <1% without AGN. Addition of the BMP antagonist Noggin to the differentiation cocktail resulted in a more limited TBX6 reporter expression pattern (9.9% TBX6+) compared to Wnt3a, CHIR and AGN.

Biography
Education:
University of Connecticut, B.S., Physiology and Neurobiology
Central Connecticut State University, M.A., Biomolecular Sciences
Position: PhD Candidate
Year in Program: 3rd
Career Aspirations: My goal is to remain in academia in order to teach the next generation of scientists while pursuing my own research interests and fostering outside collaborations with industry to improve musculoskeletal repair outcomes.
Why UCHC: After completing my master's program, I did an internship in the Department of Orthopaedic Surgery and held a position in clinical research here at UCHC. I wanted to further my basic skeletal biology research knowledge to translate basic science breakthroughs to the clinic to improve the quality of life for individual patients.

Martha J. Somerman, D.D.S., Ph.D.
Director, National Institute of Dental and Craniofacial Research

Dr. Martha J. Somerman is the Director of the National Institute for Dental and Craniofacial Research, a position she has held since August 2011. Also, she is Chief of the Laboratory for Oral Connective Tissue Biology, National Institute of Arthritis and Musculoskeletal and Skin Diseases.

Dr. Somerman was the Dean of the University of Washington School of Dentistry and Professor in Periodontics, from 2002 to 2011, Associate Dean for Research at the University of Michigan, Ann Arbor, from 2001-2002, and chair of the Department of Periodontics/Prevention/Geriatrics (1995-2000) and professor in the Department of Pharmacology at the University of Michigan Medical School (1995-2002). From 1984 to 1990, she was on faculty at the University of Maryland College of Dentistry in the departments of Periodontics and Pharmacology. In the early 1980s, she was a Staff Fellow at the National Institutes of Health/National Institute of Dental Research (NIH/NCDR), serving first in the Developmental Biology and Anomalies Laboratory and then in the Laboratory of the Clinical Investigations and Patient Care Branch.

Dr. Somerman earned her B.A. in biology from New York University and her D.D.S. degree from NYU in 1975, and then earned a certificate in periodontology (1978) and PhD in pharmacology (1980) at the Eastman Dental Center and University in Rochester, NY, respectively.

Dr. Somerman's research focuses on defining the key regulators controlling development and regeneration of tissues that form the dental-oral-craniofacial complex and applying the knowledge gained to design therapies to regenerate tissues lost as a consequence of periodontal diseases and conditions. Dr. Somerman has published more than 150 peer-reviewed articles and prior to joining NIDCR/NIH served on several editorial boards. In the past, she has reviewed grants submitted to NIH/NIDCR and the NCRR, and has served on committees for the American Association for the Advancement of Science (AAAS), the IADR/AADR (International/American Association for Dental Research) and American Dental Association. In addition, she served on the Government Advisory Committee of the AADR, advising on public policy in the areas of

dental and biomedical research, education and training, access and disparities in care, and infrastructure and workforce.

Among Dr. Somerman's awards and honors include the William K. and Mary Anne Najjar Professorship at the University of Michigan (1992 to 2002). She was named a diplomate of the American Board of Periodontology in 1990 and was president of AADR in 2001. Dr. Somerman received the Geis Award from the American Academy of Periodontology (2003), IADR's Distinguished Scientist Award for Research in Oral Biology (2005), the IADR /Straumann Award in Regenerative Periodontal Medicine (2010), the Paul Goldhaber Award, Harvard School of Medicine (2011), and NYU, College of Dentistry Distinguished Scientist Award (2012). From 1999 to 2002, she was a member of the National Advisory Dental Research Council of NIH/NIDCR. In addition, Dr. Somerman is a Fellow of AAAS, the International College of Dentists, and the American College of Dentists.