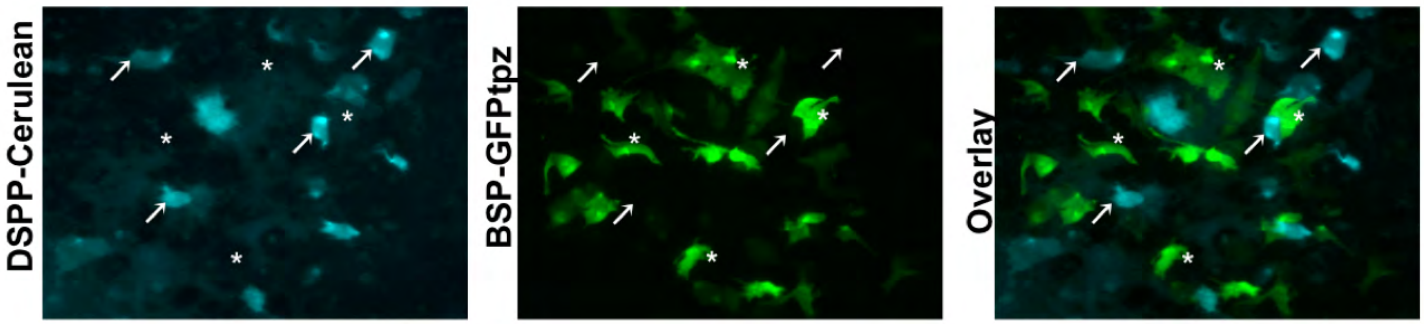
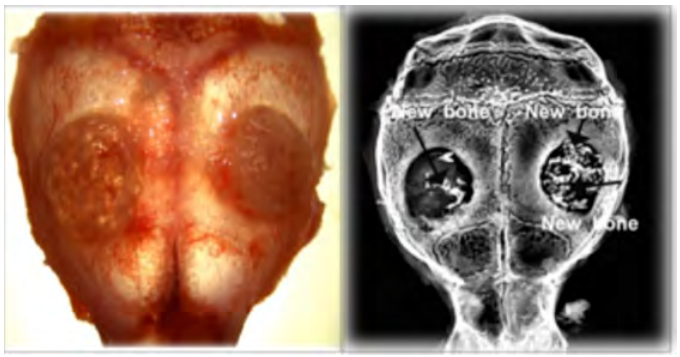
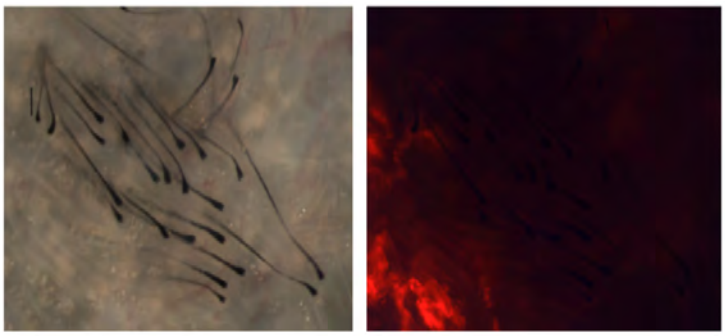
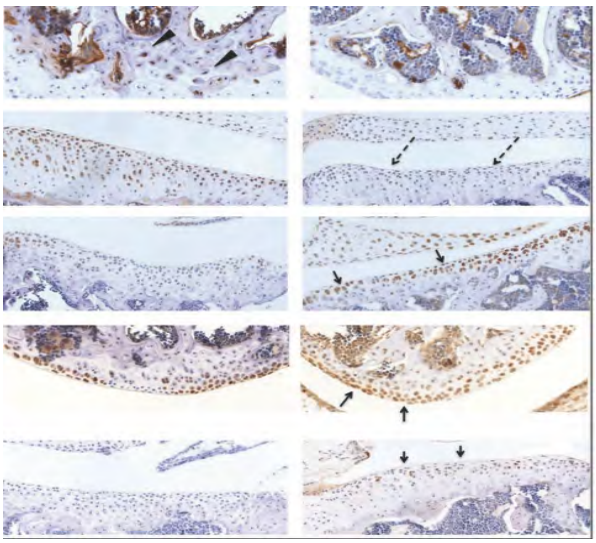
Skeletal, Craniofacial & Oral Biology Training Program--Symposium 2016

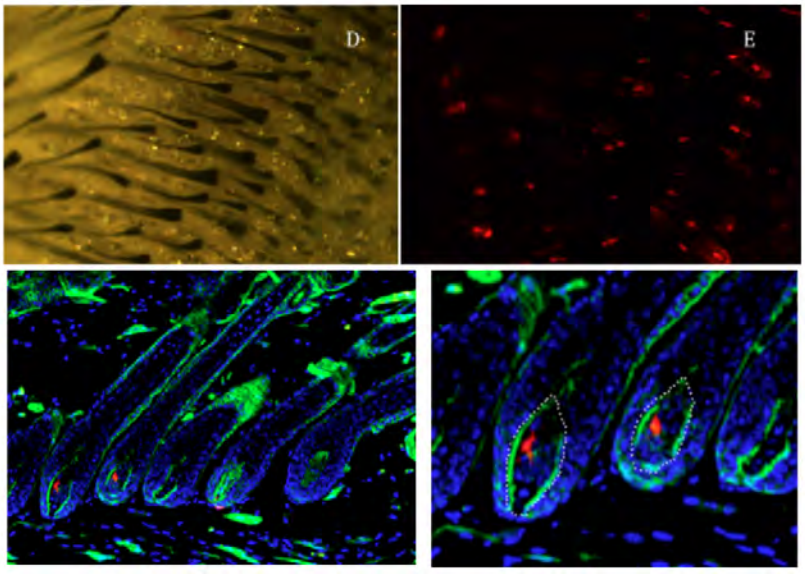


Monday, June 6, 2016 • Low Learning Center
UConn Health • Farmington, CT









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 2016 NIH/NIDCR-supported Skeletal, Craniofacial and Oral Biology Training Program Symposium.

The purpose of the Symposium is to provide trainees in the Skeletal Biology and Regeneration area of concentration and other trainees supported by this 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; 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 our special guest is *Dr. Sotirios Tetradis, Professor and Chair, Section of Oral and Maxillofacial Radiology, UCLA School of Dentistry*. Dr. Tetradis did his clinical specialty training and PhD at UConn Health. In addition to his scientific presentation and viewing of oral and poster presentations, Dr. Tetradis will spend informal time with trainees talking about his career decisions and life in academics.

Welcome back Dr. Tetradis!

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

The Symposium is supported by NIDCR/NIH "Skeletal, Craniofacial and Oral Biology" Institutional Training Grants 1T90 DE021989 and 1R90 DE022526. We gratefully acknowledge the support of the NIDCR/NIH.

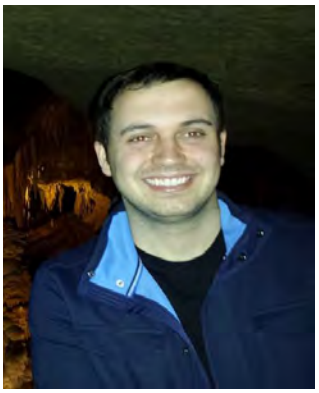
Acknowledgements

This Symposium is the result of much hard work by many people. In particular, the Symposium Planning Committee would like to thank Lisa Ramsdell who has done an outstanding job of organizing and managing all aspects of the Symposium, and Laura Didden, who helps to plan and manage during the Symposium. Lisa and Laura help our trainees and the Directors with many aspects of the training grant all year, so thanks to both of you!

This program brochure is based on previous designs by Cynthia Smith and we continue to benefit from her past efforts.

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* Dr. Rajesh V. Lalla, Dr. Jon Goldberg
8:30 – 9:45 am	**Oral Session I: Progenitors, Lineage** *Low Learning Center* Session Chair: David Manz Spenser Smith /4 Ivana Vidovic /6 Xi Wang/8 Sierra Root /10
9:45 – 10:30 am	**Break and Poster Session** *Keller Lobby* Anushree Banerjee/14 Laura Doherty/16 Nathaniel Dyment/18 Henry Hrdlicka/20 David Manz/22 Michelle Spoto/24 Matthew Zambrello/26
10:30 – 11:45 am	**Oral Session II: Signaling, Regeneration** *Low Learning Center* Session Chair: Matthew Zambrello Ryan Russell /28 David Paglia/30 Patience Meo Burt/34 Aja Aravamudhan/36
12:00 – 1:00 pm	**Guest Speaker** *Low Learning Center* Dr. Sotirios Tetradis, DDS, PhD/40 Professor and Chair, Section of Oral and Maxillofacial Radiology, UCLA School of Dentistry
1:00 – 2:15 pm	**Trainee and Advisor Luncheon** (SCOB, T90, R90 Trainees and Advisors, Dr. Tetradis) *Onyiuke Dining Room*
2:15 – 3:00 pm	**Informal Session for Trainees with Dr. Tetradis** *"Navigating an Academic Career and Life"* *Onyiuke Dining Room*



microRNA-433 Dampens TGBβ Signaling and Restrains Osteoblastic and Chondrogenic Differentiation

Spenser S. Smith, Neha S. Dole, Rosa Guzzo, Anne M. Delany

Center for Molecular Medicine and Department of Orthopaedics,
UConn Health, Farmington, CT

Lineage commitment and differentiation of skeletal cells requires coordinated regulation of multiple signaling systems by microRNAs (miRNAs). Transforming growth factor β (TGFβ) is important for osteoblastogenesis, chondrogenesis and adipogenesis. Here, we show that miR-433 limits TGFβ signaling and that miR-433 is a negative regulator of osteoblastogenesis and chondrogenesis.

In mouse bone marrow stromal cells (BMSCs), culture in osteogenic medium caused a progressive decline in miR-433. In contrast, miR-433 gradually increased in BMSCs cultured in micromass to induce chondrogenesis. Indeed, in late chondrogenesis miR-433 was 15 fold higher than in undifferentiated cells. Similar effects were observed in human induced pluripotent stem cells (iPSCs) undergoing chondrogenesis. Although miR-433 is expressed in adipocytes, its levels remained constant during adipogenesis. To determine the impact of miR-433 on these differentiation processes, multipotent C3H/10T1/2 cells expressing a miR-433 competitive inhibitor, or tough decoy, were examined. In monolayer cultures treated with BMP2, miR-433 inhibition induced alkaline phosphatase, Runx2, and osteocalcin mRNAs, and promoted calcium deposition. In micromass cultures treated with BMP2 and TGFβ, miR-433 inhibition promoted expression of chondrogenic mRNAs, Sox9 and Col2a1. In cells treated with an adipogenic cocktail, miR-433 inhibition failed to alter adipogenic gene markers or Oil-red O staining. These data suggest that miR-433 blunts osteogenic and chondrogenic differentiation.

Bioinformatic analyses suggested that miR-433 might target critical components of the TGFβ signaling pathway. In C3H/10T1/2, inhibition of miR-433 amplified TGFβ signaling, evidenced by increased activity of a TGFβ-responsive SBE4 luciferase reporter and enhanced TGFβ-induced pSMAD2. To determine underlying mechanisms, we used Luciferase-3'UTR reporter assays, and experimentally validated SMAD2 and TGFBR1 as novel miR-433 targets, and showed that miR-433 does not target SMAD4 or TGFBR2. Lastly, miRNAs and their regulators often participate in regulatory loops, and we found TGFβ down regulates miR-433 after 24 hours, suggesting an indirect regulatory mechanism.

Overall, miR-433 attenuates TGFß signaling, at least in part by direct 3'UTR targeting of SMAD2 and TGFBR1. This effect likely contributes to the ability of miR-433 to restrain osteoblastic and chondrogenic differentiation, with potential implications for bone and cartilage repair.

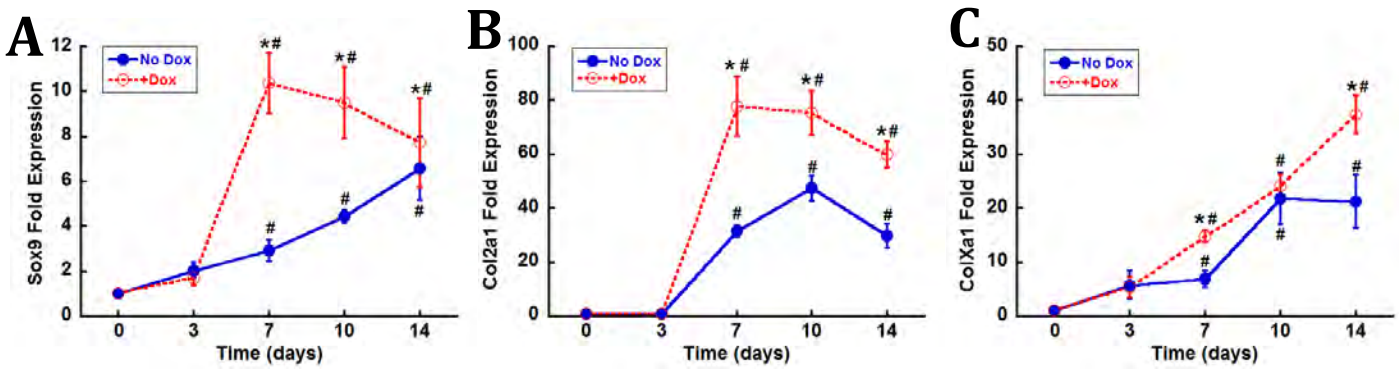


Figure 1. Disruption of miR-433 activity enhances expression of chondrogenic markers
C3H10T1/2 cells were stably transduced with a Doxycycline (Dox)-inducible miR-433 decoy construct, to inhibit miR-433 activity. Cells were cultured in the absence or presence of 1000 ng/ml doxycycline and differentiated with a chondrogenic cocktail including 10ng/ml TGFβ and 50ng/ml BMP. Data are represented as fold expression. (A) Sox9, (B) Col2a1, (C) ColXa1 mRNA expression. * = significantly different from corresponding time point with no Dox treatment, $p < 0.05$. # = significantly different from Day 0, $p < 0.05$. n=4.

Acknowledgements: This work was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health [AR44877]; the National Institutes for Dental and Craniofacial Research [5T90DE21989]; a Grant-in-Aid award from the American Society for Bone and Mineral Research; the UConn Health Center Research Advisory Council; and the Center for Molecular Medicine at UConn Health.

Major Advisor: Dr. Anne Delany

Biography: I graduated from The College of Idaho in 2008 with a B.A. in Biology and History. I am currently a fourth year Ph.D Biomedical Science student in Dr. Anne Delany's lab in the Skeletal Biology and Regeneration 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.

Lineage tracing of αSMA perivascular cells during reparative dentinogenesis

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

University of Connecticut Health, Farmington, CT

Objectives: The goal of our studies was to examine the contributions of perivascular cells expressing alpha-smooth muscle actin (αSMA) during reparative dentinogenesis.

Methods: We used an inducible Cre-loxP fate mapping approach to examine the contributions of descendants of cells expressing αSMA-CreERT2 transgene in reparative dentinogenesis. To induce αSMA-Cre activity, 4-5 week old αSMACreERT2;Ai9/Col2.3-GFP triple transgenic mice were injected intraperitoneally (IP) with oil (vehicle) or Tamoxifen for 2 days (one injection a day). Reparative dentinogenesis was induced by experimental pulp exposures (PE) of first maxillary molars following the 2nd injection of TM. The mineral deposition at the sites of injury was examined by fluorochrome labeling 4 weeks following PE. Animals were sacrificed at various time points after PE. Frozen sections were processed for various analyses to examine the contribution of αSMA-tdTomato^{+} descendants to repair in molars.

Results: In control samples and immediately after PE the αSMA-tdTomato^{+} cells were located around blood vessels. Two days after PE there were increases in the number of αSMA-tdTomato^{+} cells and migration of these cells to the site of injury. Distinct lines of fluorochrome labeling and histological analyses indicated the deposition of new mineralized tissue containing atubular and tubular matrix at the site of injury 4 weeks after PE. The newly mineralized tissues at the injury sites were lined with αSMA-tdTomato^{+} cells and 2.3-GFP^{+} cells. A small percentage of cells lining the mineralized tissue were αSMA-tdTomato^{+}/2.3-GFP^{+}.

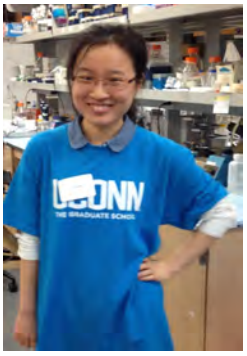
Conclusions: Our observations showed that a small fraction of cells associated with newly formed mineralized tissue (reparative dentin) *in vivo* are derived from the αSMA-tdTomato^{+} population suggesting the involvement of other progenitors to reparative mineralization.

Acknowledgements: This work was supported by R01- DE016689 & R90-DE022526 grants.

Major Advisor: Mina Mina

Biography: DMD, Dental School, Medical Faculty, University of Rijeka, Croatia
3rd year Post-Doc, Division of Pediatric Dentistry, UConn Health, Farmington, CT

I aspire to become a clinician-scientist and the R90 research training program at UConn Health provided me with a great opportunity to be involved in research conducted by Dr. Mina Mina. Work in this scientific environment has improved my research skills and I hope I will be able to implement this knowledge conducting my own research program when I return to Croatia. I am sure that the experience gained at UConn Health will allow me to become a well-rounded dental practitioner and researcher.



PDGF-BB mediated regulation of BMP2-Smad signaling during osteogenic differentiation of periosteal progenitor cells

Xi Wang, Brya Matthews, Ivo Kalajzic

Affiliations
[1] University of Connecticut Health Center, Farmington, CT

Objective: Increasing evidence has demonstrated a critical role of the periosteum during fracture healing, but the factors that influence the differentiation of progenitor cells are not well defined. Platelet derived growth factor (PDGF) is well known as a mitogenic and chemotactic molecule, but its osteogenic effects and underlying mechanisms of action need further investigation. We observed that PDGF blocked BMP2-induced osteogenic differentiation of periosteal progenitor cells (PPC), leading to our hypothesis that PDGF is a negative regulator of BMP2 signaling. We aimed to investigate the crosstalk between PDGF and BMP2 signaling in PPCs.

Methods: In order to culture PPCs, periosteum is harvested from mouse femurs and tibias, followed by enzymatic digestion and in vitro expansion. Primary cells were used for osteogenic differentiation and treated with PDGF (10ng/ml) and/or BMP2 (100ng/ml). Evaluation was based on mineralization, Col2.3GFP activity and expression of osteogenic genes. We also checked the gene expression of the components of BMP signaling pathway (bmpr1a, bmpr2, bmp2, noggin) by RT-PCR. Phosphorylation of Smad1/5/8 was determined by western blot.

Results: PDGF-BB (10ng/ml) prevented mineralized nodule formation and dramatically reduced the gene expression of Osteocalcin and Bone Sialoprotein in PPCs. In addition, while BMP2 (100ng/ml) significantly promoted the differentiation of PPCs, this effect was inhibited by PDGF-BB treatment. PDGF did not alter expression of BMP signaling pathway components Bmp2, Bmpr1a, Bmpr2 and Noggin in PPCs. We then further determined the effects of PDGF-BB on BMP2-induced downstream signaling in PPCs. Western blot results show that BMP2 clearly induced the phosphorylation of Smad1/5/8 in PPCs after 20 minutes, which is inhibited by the addition of PDGF-BB.

Conclusions: In conclusion, our data show that PDGF-BB blocks BMP2-induced osteogenic differentiation of periosteal progenitors in vitro. The expression of BMP signaling pathway components were not altered by PDGF-BB, but our data indicates a PDGF-mediated inhibition of BMP2-Smad signaling pathway as likely mechanism.

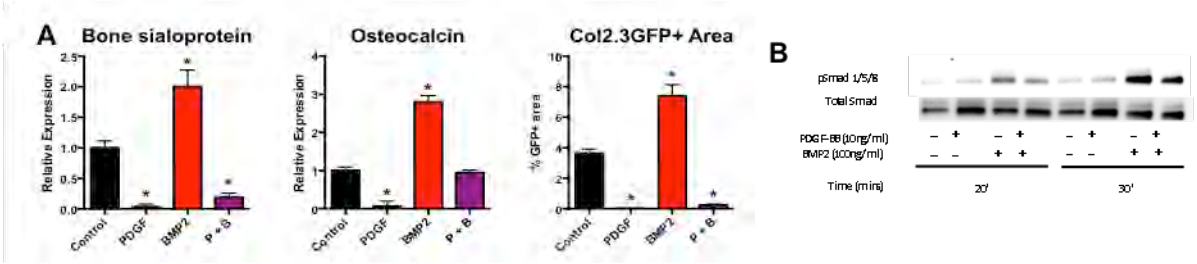


Figure 1. PDGF-BB inhibits BMP2-induced osteogenic differentiation of PPCs.
A. Differentiation was performed in primary cultures with addition of growth factors at day 7 and differentiation was assessed at Day 21 by Osteocalcin, BSP and Col2.3 GFP gene expression
B. Western Blots analysis of phosphorylated Smad and total Smad protein levels in PPCs under the treatment of growth factors after 20 and 30 mins.

Acknowledgements: This work was supported on R01AR055607 by NIH/NIAMS.

Major Advisor: Dr. Ivo Kalajzic

Biography:

3rd year PhD student in Skeletal Biology and Regeneration Program
B.S. Clinical Medicine: Shandong University, Jinan, China
M.S. Internal Medicine: Shandong University, Jinan, China

I would like to complete a post-doctoral fellowship after graduation and complete my medical training in the future to work as a clinician-scientist. This will allow me to incorporate my research experiences with clinical practice. I choose UCHC graduate program because of the excellent environment with dedicated faculties and more research opportunities. In the past three years, I learned the importance of critical thinking for research, and most importantly the commitment to the career.

Characterization of a novel 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

Objective: Primary hematopoiesis occurs within the bone marrow space in a highly structured microenvironment that define sites of hematopoietic progression. The components of these microenvironments or niches are variable, and apart from the hematopoietic progenitors include cells of the skeletal, vascular endothelial and peripheral nervous systems. The development of these cells is interdependent and their functions are crucial to maintain the hematopoietic stem cell pool and ensure their homeostatic differentiation and expansion. Even when major populations involved have been studied, the identity of all the relevant components within the hematopoietic niche in not yet completed. A visual reporter transgenic mouse model (DMP-1cre/Ai9) was generated crossing a strain expressing cre under the control of the osteocyte Dental Matrix Protein 1 (DMP-1) promoter, with the Ai9 strain bearing tdTomato under the control of the Rosa26 locus promoter containing a floxed STOP signal. Histological analyses of bone sections from DMP-1cre/Ai9 mice showed tdTomato expression in populations within the bone marrow space, reflective of activation of the DMP-1 promoter outside the osteocyte lineage. Initial phenotypic characterization showed that these cells are hematopoietic, and they are not present in circulation, spleen or liver. The restricted localization suggests that these cells could represent a novel population of bone marrow resident macrophages, cells that have been proposed as modulators of bone marrow microenvironments with functions associated to maintenance of hematopoietic and skeletogenic homeostasis.

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*. Because of their macrophage phenotype, functional assays for phagocytosis included *in vivo* depletion of macrophages using clodronate loaded liposomes and *ex vivo* phagocytic uptake of latex beads. In order to evaluate their interdependence on osteoblastogenesis, we evaluated hematopoietic DMP-1/tdTomato cells using a tamoxifen inducible DMP-1cre mouse crossed with Ai9 and a mouse expressing herpes virus thymidine kinase under the control of Collagen type I promoter (Col2.3ΔTK) which allow the ablation of osteoblasts upon treatment with ganciclovir (GCV). Animals were ablated for 17 days with GCV, and a single Tamoxifen injection was administered 2 days before the end of GCV treatment. Mice were analyzed 1 and 14 days after ablation for the presence of hematopoietic DMP-1/tdTomato expressing cells.

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+ 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. DMP1/tdTomato+ CD11b+F480+ cells phagocytos latex beads and can be depleted *in vivo* when injected with clodronate loaded liposomes. When osteoblastogenesis is ablated, the ability of these cells to develop is hindered when using an inducible DMP1-Cre reporter system but have the ability to recover in accordance with osteoblastogenesis.

Conclusions: We have identified a new population of hematopoietic cells that at some point during their development can activate the DMP-1 promoter. This novel population expresses markers associated to monocyte/macrophage lineage, more specific tissue resident macrophages. The localization of this population is almost exclusive in bone marrow, where it forms a network interspersed within the bone marrow space. Preliminary gene expression studies showed that these cells have the ability to produce important cytokines for hematopoietic lineage progression. This population appears early in ontogeny (before bone mineralization), and can be generated from adult progenitors. Functionally, at least a fraction of this population can be eliminated upon depletion of phagocytes and has the ability to perform phagocytosis. The generation of this population is dependent on osteoblastogenesis implying that its appearance is coordinated with skeletogenesis. We propose that this population corresponds to a bone marrow resident myeloid cell playing important roles in the control of hematopoiesis by directing hematopoietic lineage progression, and modulating bone remodeling through their ability to influence osteoclastogenesis.

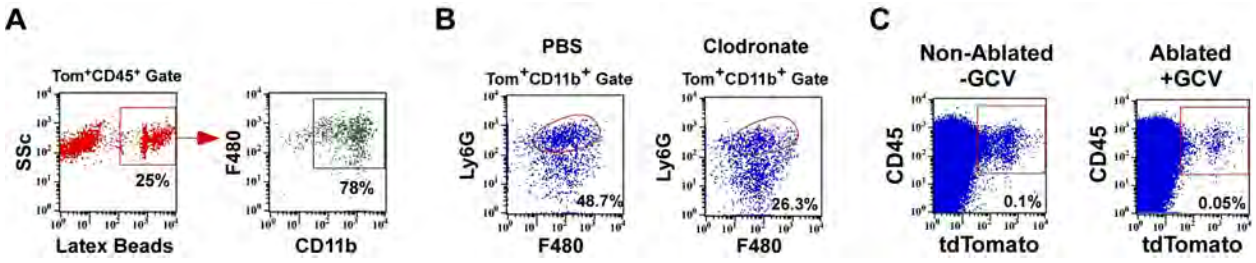


Figure 1. DMP1/TdTomato+ myeloid cells have characteristics of tissue resident macrophages, including phagocytic activity and their development is influenced by osteoblastogenesis.

A) Bone marrow from DMP1/TdTomato+ mice were incubated *ex vivo* with latex beads conjugated to FITC and then stained with antibodies and analyzed by flow cytometry for phagocytosis in the Tom+CD45+ gate. **B)** Mice injected IV with PBS or Clodronated loaded liposomes were evaluated 36 hrs post injection for the depletion of Tom+CD11b+Ly6G+F480+ cells in the bone marrow. **C)** iDMP1/CreTdTomato mice with and without osteoblast ablation were evaluated for the presence of CD45+tdTomato+ cells.

Acknowledgements: This work was supported by the NIDCR; 5T90DE21989-04 and 1R01AR055607-01A2

Biography:

BS Chemistry-Biology (University of Hartford, 2005)
PhD Biomedical Sciences-Genetics and Developmental Biology (UCONN Health, 2014)
Post-Doctoral Fellow (Year 2)

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.

Differential Effects of FGF2 on Osteogenesis and Dentinogenesis *in vitro*

Banerjee A; Dyrkacz P; Vidovic I; Rogers B; Maye P and Mina M

University of Connecticut Health Center, School of Dental Medicine

Introduction: Recent studies in our lab showed that the limited and early exposure of primary pulp cultures to FGF2 did not affect the extent of mineralization but increased the number of odontoblasts. **Objectives**: To determine if FGF2 alters the allocation of the dental pulp progenitor populations to odontogenic vs. osteogenic lineages using pulp cultures from Bone Sialoprotein (BSP)-GFPtpz and Dentin SialoPhosphoProtein (DSPP)-Cerulean reporter transgenic mice that display distinct expression during osteoblast and odontoblast differentiation respectively.

Materials and Methods: Primary pulp cultures were established from molars of 5-7 day old BSP-GFPtpz, DSPP-Cerulean and non-transgenic mice. Cultures were treated with 20 ng/ml FGF2 between days 3-7 prior to induction of mineralization at day 7. Cells were grown up to day 21 and processed for image analysis, qPCR, immunohistochemical staining and FACS analysis at various time points.

Results: *In vivo* studies showed that during molar development, BSP-GFPtpz similar to BSP was expressed exclusively by osteoblasts and osteocytes and not by odontoblasts. Image and FACS analyses of primary pulp cultures from BSP-GFPtpz animals showed that this transgene was first expressed at around day 10. At days 14 and 21, there were increases in the number of BSP-GFPtpz+ cells in mineralized nodules (detected by XO staining). During the mineralization of pulp cultures BSP-GFPtpz expression was similar to endogenous Bsp. Limited and early exposure of these cultures to FGF2 resulted in significant decrease in the number of BSP-GFPtpz+ cells, intensity of BSP-GFPtpz expression and levels of *Bsp* at D14 and D21 compared to controls. In cultures from DSPP-Cerulean animals this treatment resulted in significant increases in the levels of *Dspp* and in the percentage of DSPP-Cerulean+ cells.

Conclusions: Our observations provide evidence that the limited and early exposure of pulp cells to FGF2 altered the allocation of progenitor cells entering into the dentinogenic vs. osteogenic lineages.

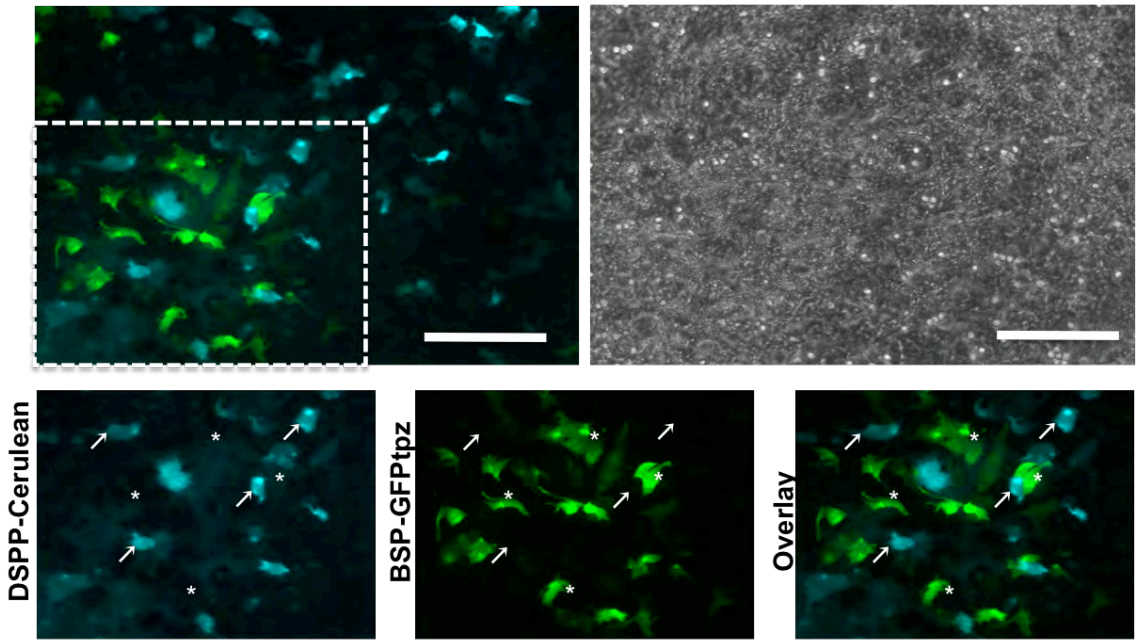


Fig 4: Expression of DSPP-Cerulean and BSP-GFPtpz transgenes in primary dental pulp cultures.
Representative images of primary dental pulp cultures from DSPP-Cerulean/BSP-GFPtpz transgenic animals at D14 showing cells expressing BSP-GFPtpz and DSPP-Cerulean. Note that the cells expressing DSPP-Cerulean (indicated by arrows) do not express BSP-GFPtpz, while cells expressing BSP-GFPtpz (indicated by asterix*) do not show any DSPP-Cerulean expression. Scale bar 200um

Supported by R01-DE016689 and T90-DE022526.

Major Advisor: Dr. Mina Mina

Biography:
BDS, Mumbai India
MS, University of Medicine & Dentistry New Jersey (Rutgers School of Dental Medicine)
4th year PhD in Biomedical Sciences, Skeletal Biology and Regeneration (SBR)

My long-term career objectives 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.

Analysis of dermal papilla cell origin in hair follicle neogenesis after wounding

Laura Doherty[1], Denise Gay[2], Ying Zheng[2] and George Cotsarelis[2]

[1] University of Connecticut School of Dental Medicine, University of Pennsylvania Department of Biology
[2] Perelman School of Medicine at the University of Pennsylvania Department of Dermatology

Objectives

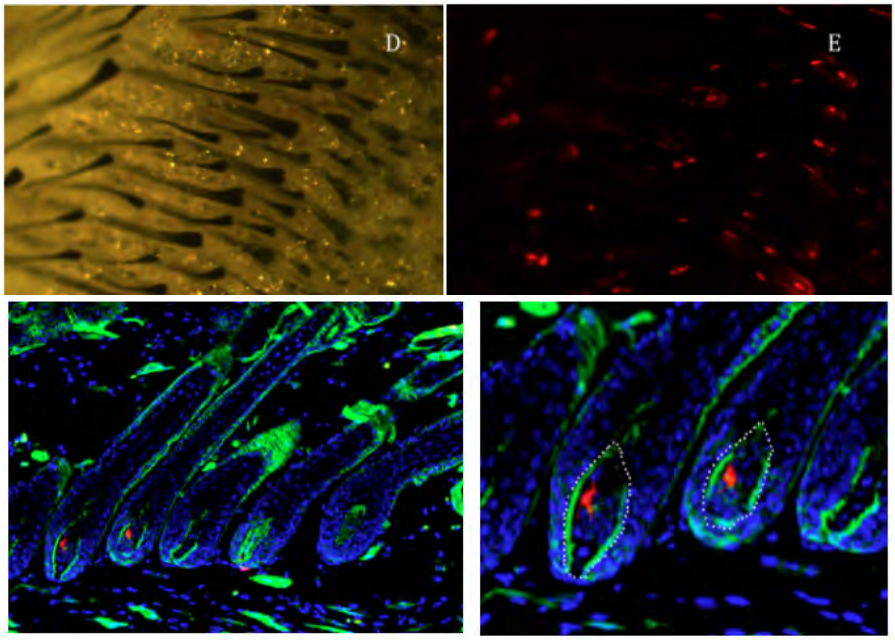
This study aimed to identify the source of dermal papilla cells in hair follicles formed de novo after wounding in mice.

Methods

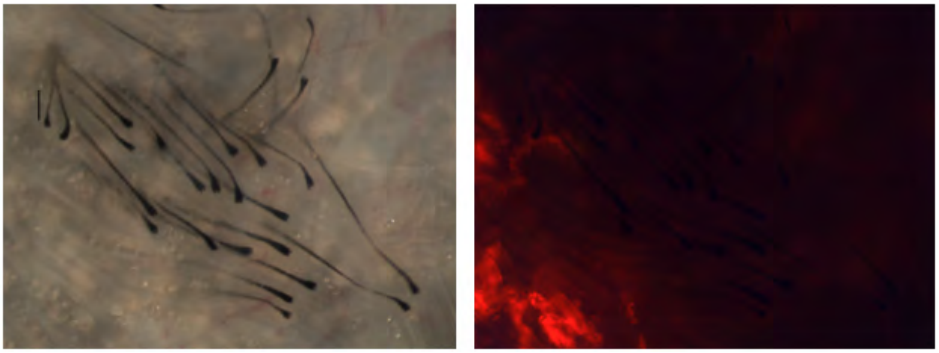
The mice in this experiment contain a 'knock in' creER^{T2} fusion protein and an internal ribosome entry site (IRES)-β-galactosidase (lacZ), which replaces the first ATG codon of CD133 expression. The creER^{T2} enzyme is a Cre recombinase fused to a mutated form of the estrogen receptor, which can be activated by tamoxifen, an agonist of the estrogen receptor. After proper tamoxifen induction, creER^{T2} can gain access to the nucleus of the cells expressing CD133. CD133 is a known marker of dermal papilla cells.

Results

Tamoxifen induction successfully marked DP cells before wounding. CD133 is an effective marker of most DP cells when mice are positive for both the CD133 and Tomato genes. After tamoxifen induction from P0-P7, mice were allowed to develop for approximately 4 to 6 weeks after birth, and were then wounded. We genotyped these mice and analyzed the back-skins that were taken during wounding, focusing on those that were positive for both of the target genes. A sample of these double positive skins is found in photographs below.



After new HFs had formed after wounding, these skins were analyzed under a microscope to check for red fluorescence. No fluorescence of DP cells was observed in any of the post-wounding skins. This indicates that DP cells may not migrate into the wound area, but this is not a definitive answer.



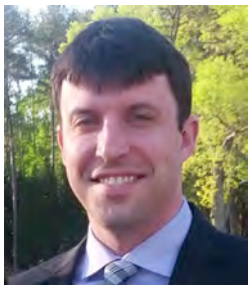
Conclusions

Initial data shows that DP cells in HFs formed de novo after wounding are not derivative of surrounding DP sources, indicating the possibility of an embryonic-like state of HF development post-wounding in mice. More experimentation is needed in order to induce all subpopulations of the DP with a fluorescent marker to confirm lack of DP cell mobility. Understanding the molecular pathways of HF formation can lead to therapies for skin disorders including alopecia and male pattern baldness.

Acknowledgements: Funded by grants from the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Number R01AR055309 and supported by awards from the College House Research Fellow Program at the University of Pennsylvania.

Biography: B.A. Biology, Religious Studies (University of Pennsylvania, 2015)
D.M.D./Ph.D. Candidate (University of Connecticut School of Dental Medicine)

I aspire to become a dental scientist and use my research experience to advance the field of dental medicine. I chose UCHC for my graduate training for the opportunities to pursue both degrees, and because of the strong research here related to bone and skeletal regeneration.



High Throughput, Multi-Image Cryohistology of Joint Tissues

[1]Nathaniel A Dyment, [1]Xi Jiang, [2]Yusuke Hagiwara, [1]David W Rowe

Affiliations
[1] University of Connecticut Health Center
[2] Nippon Medical School Hospital, Tokyo, Japan

OBJECTIVE: High-quality histological assessment of tendon/ligament research is becoming more prominent with the advent of animal models containing fluorescent proteins such as GFP. Maintaining GFP fluorescence through paraffin processing is challenging, therefore frozen sectioning is the preferred method in these animal models. However, producing frozen sections with minimal sectioning artifact is difficult, especially in adult tissues. Our lab has developed a cryosectioning method using a unique cryotape that adheres to the tissue during sectioning, maintaining the tissue morphology. Consequently, this method can be used to produce high-quality sections with minimal artifact in adult, mineralized tissues of GFP animals. These sections can then be adhered to microscope slides and subsequently taken through multiple rounds of staining and imaging on the same section. As a result, the multiple rounds can be aligned allowing for colocalization measurements of several response measures. This abstract will outline the general workflow for this technique and provide examples from previous tendon/ligament development and repair studies.

METHODS: Experimental Design. *Normal Growth*. The Achilles tendon from triple transgenic reporter mice containing Col2a1-CFP, Col1a1(3.6kb)-GFPTpz, and Col10a1-mcherry transgenes were assessed at 2 weeks of age (n=4). *Joint Destabilization*. The ACL was transected (ACLT) in 10-week-old Col1a1(3.6kb)-CFP and Col10a1-mcherry mice. Mineralization labels were delivered on the day before surgery (demeclocycline), 2 weeks post-surgery (calcein), and 4 weeks post-surgery (alizarin complexone). MCL entheses were assessed at 4 weeks post-surgery and compared to intact or sham controls. Sample Preparation. Following CO2 asphyxiation, limbs were fixed in 10% neutral buffered formalin for 1-3 days at 4°C and then transferred to 30% sucrose for 12-24 hours at 4°C. The limbs were then oriented in either sagittal or coronal planes and embedded in cryomatrix. Sections (7-8μm) were made using cryofilm type-2C [1] and temporarily stored on plastic microscope slides at 4°C. The captured sections were adhered to glass microscope slides using UV-activated glue. The slides were hydrated in 1X PBS and then processed through several rounds of imaging, staining, re-imaging, re-staining, etc. These rounds included 1) endogenous fluorescent signals (fluorescent proteins and/or mineralization labels), 2) collagen structure (two photon second harmonic generation), 3) immunostaining (anti-IHH), 4) enzyme activity staining (tartrate-resistant acid phosphatase and alkaline phosphatase), and 5) chromogenic staining (toluidine blue O). Imaging. Two photon second harmonic generation (SHG) for collagen was imaged on the Prairie Ultima IV multiphoton microscope while all other images were acquired using the Zeiss Axio Scan.Z1 digital slide scanner. Images from each round were aligned and constructed into multi-layer composites in Photoshop.

RESULTS: Postnatal Growth [2]. Mineralized fibrocartilage begins to form at 2 weeks of age in the Achilles tendon. The nucleation of mineral coincides with expression of Col10a1-mcherry, IHH, and alkaline phosphatase by fibrocartilage cells at the base of the collagen fibers of the enthesis (Fig. 1). All of these measures from four rounds of staining/imaging were colocalized to the same cells on the same section using this method. Joint Destabilization [3]. Unmineralized fibrochondrocytes adjacent to the original tidemark (demeclocycline) displayed increased Col1a1-CFP and Col10a1-mcherry with a strong calcein label, indicating active mineral deposition. These cells were also AP+ and a subset was TRAP+. There was continued mineral apposition at 4 weeks, demonstrated by the alizarin complexone label that was advanced from the prior calcein label. All of these measures were colocalized to the same cells on the same section using this method.

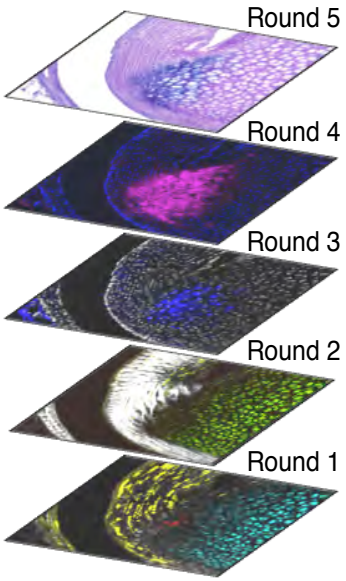


Figure 1. Imaging rounds. Round 1: endogenous fluorescent signals, Round 2: collagen SHG, Round 3: anti-IHH immunostaining, Round 4: enzyme activity, and Round 5: toluidine blue.

DISCUSSION: Using the cryotape sectioning in combination with multiple rounds of high-throughput staining and imaging, several response measures were acquired in high-quality sections of mineralized joint tissues. Not only does this method produce high quality sections and images, but the entire process from initial fixation through several rounds of imaging can be conducted in the time (<1 week) it takes to decalcify a specimen prior to paraffin embedding.

ACKNOWLEDGEMENTS: This work was supported by NIH grants NIH T90-DE021989, R01-AR54713, R01-AR052374, and K99-AR067283.

REFERENCES: 1. Kawamoto et al Arch Histol Cytol, 2003; 2. Dyment et al, Osteoarthr Cartil, 2015; 3. Dyment et al, Dev Biol, 2015.

BIOGRAPHY:

Education

University of Illinois at Urbana Champaign, B.S., Materials Science and Engineering, 2001-2005

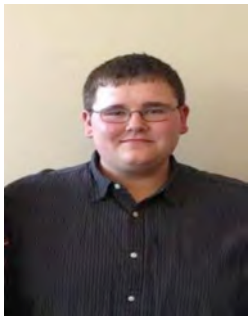
University of Cincinnati, PhD, Biomedical Engineering, 2005-2011

Position: Postdoctoral Fellow

Year in Program: 5th

Career Aspirations: My goal is to remain in academia as a principal investigator with research focused on improving repair of musculoskeletal injuries. I am moving on to the University of Pennsylvania to become a faculty member in the Orthopaedic Surgery Department in January 2017.

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, healing, and cell-assisted repair. We are now using Cre lineage tracing models to characterize progenitors cells as they differentiate into mature tendon cells.



microRNA regulation of GTPase expression and activation during osteoclast differentiation

Henry C. Hrdlicka[1], Bongjin Shin[2], Sun-Kyeong Lee[2], Anne M. Delany[1]

[1] Center for Molecular Medicine, UConn Health
[2] Center on Aging, UConn Health

Cell motility is critical for osteoclast differentiation. The Rho family of GTPases regulates actin remodeling and cell motility, and is important for osteoclast differentiation and function. GTPases are small molecular switch proteins that are active in their GTP-bound form, but inactive in their GDP-bound form. These proteins fine tune cellular effects through their unique capacity to interact with different effector molecules, depending if the GTPase is active or inactive. The aim of my project is delineate the role of the microRNA-29 family in the regulation of Cdc42 and Rac1 GTPases. Specifically, we are working to evaluate the expression and activation of Cdc42, which is directly targeted by miR-29, and Rac1, a GTPase whose activity is negatively regulated by SRGAP2, which is a miR-29 target.

To examine miR-29 regulation of Cdc42, we are using qRT-PCR and Western blot analysis to determine the overall expression of Cdc42 during osteoclastic differentiation, with future plans to evaluate Cdc42 activity via immunoprecipitation assays for the active molecule. To examine Rac1, we are currently optimizing the conditions to use the Dora-Rac1-FRET sensor (a generous gift provided by the Yi Wu lab) (**Figure 1**). This sensor, when appropriately incorporated into the cell, will allow us to determine the spatiotemporal regulation of Rac1 throughout osteoclast differentiation. Furthermore we have future plans to corroborate data obtained with the FRET sensor with specialized immunoprecipitation assays for active, GTP-bound, Rac1.

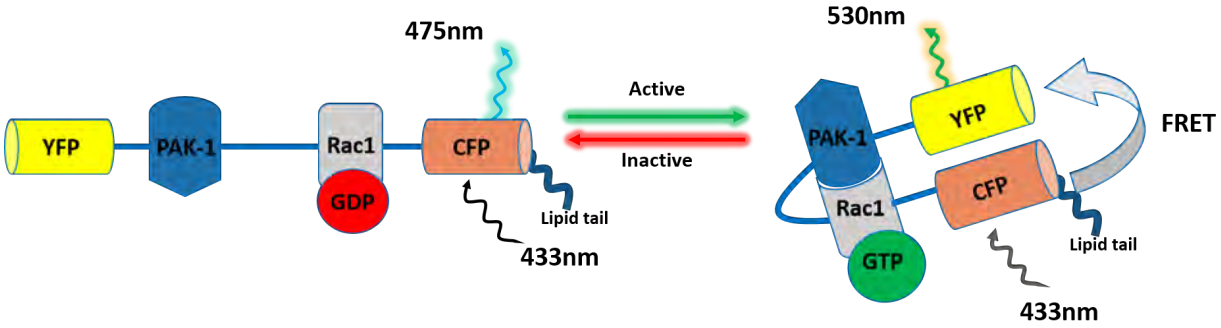


Figure 1. Förster resonance energy transfer (FRET) is a phenomena which occurs when two fluorophores come in close enough proximity to allow the higher energy fluorophore (i.e. CFP;Cerulean) to excite a lower energy fluorophore with its emission wavelength (i.e. YFP;Venus). The ratio of FRET fluorescence to CFP fluorescence can allow us to quantify the state of Rac1 activation as determined by the closed conformation of the molecule.

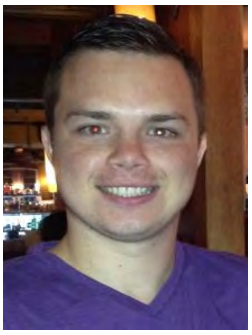
Acknowledgements: This work was supported by the NIH grant number R01-AR064867

Major Advisors: Anne M. Delany and Sun-Kyeong Lee

Biography:

B.S. Biochemistry and Molecular Biology (Nebraska Wesleyan University, 2014)

Currently, I am second year PhD student in the Skeletal Biology and Regeneration Area of Concentration. Upon completion of the program, I aspire to continue my studies with small ncRNAs, i.e. microRNAs, and apply my accumulated skills to other research areas such as osteoimmunology or plant biology. I chose to perform my PhD studies at UConn Health due to its umbrella program. Without this unique feature, I would never have been able to rotate in a variety of labs across different Areas of Concentration, which eventually led me to Skeletal Biology and Regeneration and my thesis lab and mentors.



Iron Regulation and Prostate Cancer Metastasis

Manz D[1,3], Deng Z[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: Increased intracellular iron is a characteristic of several types of cancer, including prostate cancer, and has been shown to contribute to mutagenesis, growth, and more recently, metastasis. Emerging evidence suggests that iron can promote metastasis by enhancing cellular motility and epithelial–mesenchymal transition. A key physiological regulator of intracellular iron levels is ferroportin, the only known mammalian iron export protein. Interestingly, ferroportin is aberrantly reduced in prostate cancer cells (Figure 1) and further reduced in metastatic prostate cancers. Consistent with its role in regulating intracellular iron levels, we hypothesize that ferroportin expression regulates the metastatic spread of prostate cancer.

Methods: Prostate cancer cells lines were transfected with an inducible ferroportin construct and assessed for changes in cellular motility.

Results: Ferroportin overexpression reduces prostate cancer cell motility, possibly though induction of the metastatic suppressor N-myc downstream regulated gene 1 (NDRG1).

Conclusion: These results suggest a novel role for ferroportin in prostate cancer progression and identify a new target for metastatic prostate cancer treatment.

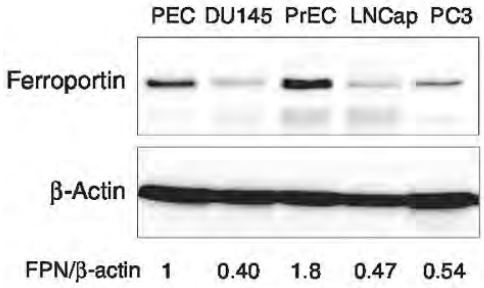
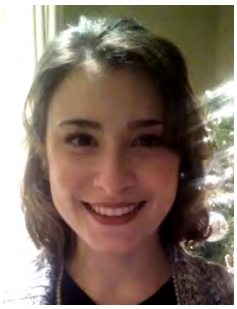


Figure 1: Ferroportin is Reduced in Prostate Cancer
Western blot analysis of ferroportin protein in normal prostate cells (PEC; PrEC) and prostate cancer cell lines (DU145; LNCaP; PC-3). Tesfay, Lia, et al. "Hepcidin regulation in prostate and its disruption in prostate cancer." Cancer research (2015): canres-2465.

Acknowledgements: This work was supported by grant R01-CA171101 from the National Cancer Institute, National Institutes of Health. David was supported by grant NIH T90-DE021989-02.

Biography: David graduated from the University of Connecticut with a major in biological Sciences and a minor in business. He is currently in his fourth 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.



Optimization of RNA Extraction for Oral Metatranscriptome Studies

Michelle Spoto[1], Anilei Hoare[1], Patricia I. Diaz[1]

[1]Division of Periodontology, Department of Oral Health and Diagnostic Sciences, UConn Health, Farmington, CT.

Objective: Characterization of the oral metatranscriptome through high throughput RNA sequencing (RNA-seq) is an indispensable tool to understand the functionality of the oral microbiome. However, RNA extraction from polymicrobial communities poses certain challenges such as the presence of species with different cell wall characteristics and low quantities of starting material. The aim of this study was to optimize RNA isolation from oral microorganisms for the purposes of RNA seq.

Methods: Different cell lysis methods, commercially available kits for RNA extraction and DNA removal strategies were tested on cultures of two common oral species, the Gram-positive Actinomyces oris and the Gram-negative Porphyromonas gingivalis. Microorganisms were grown under appropriate conditions, diluted to concentrations equivalent to 10^7 cells/mL or 10^9 cells/mL cell mL-1 and frozen in RNA Cell protect (Qiagen). Cell lysis was performed via membrane disruption with 0.1 mm silica beads for 40 or 80 seconds in a bead beater or by 10 min vortexing. Two RNA isolation kits, the PowerMicrobiome RNA Isolation kit (MoBio) and the RNeasy Isolation kit (Qiagen), and two DNA removal methods, the Turbo-free DNAse (Life technologies) and in-column DNAse digestion (Qiagen), were tested. RNA quantity was measured using Qubit RNA HS Assay Kit and quality was measured using the Agilent BioAnalyzer, which assigns a RNA Integrity Number (RIN) to each sample. A RIN of 7 was set as the minimum quality measure.

Results: Bead vortexing in combination with the Qiagen RNeasy kit and the Qiagen in-column DNAse digestion provided the best combination of high yield and good quality RNA for both species.

Conclusions: By using two microorganisms with different cell wall characteristics, we have optimized and RNA extraction protocol yielding RNA of sufficient quantity and quality for downstream RNA-seq applications.

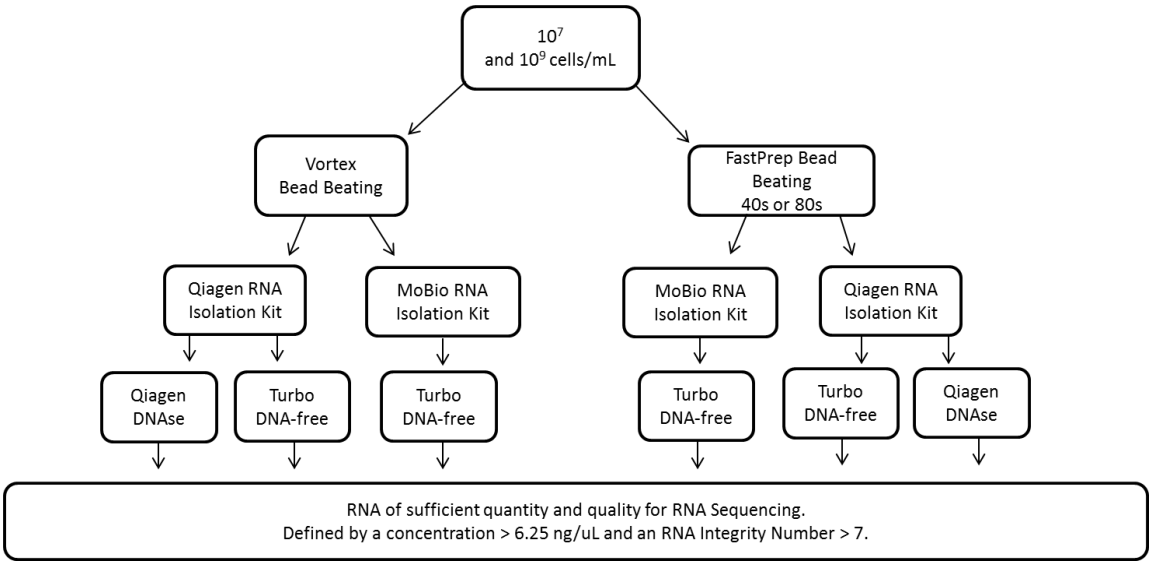


Figure 1: Diagram detailing different bead beating, RNA isolation, and DNA removal methods tested in this study

Acknowledgements:

This study was supported by grants R01DE021578 and R21DE023967 from NIH.

Biography:

BS Biomedical Sciences (Rochester Institute of Technology, 2014)
DMD/PhD program (Year 2)

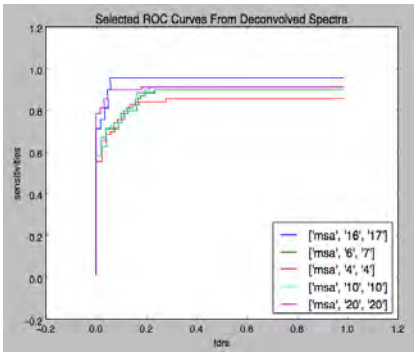
In pursuing both a DMD and PhD, I want to better understand the field of dentistry from both a clinical and scientific standpoint. I hope to balance both of these important aspects in order to contribute to the advancement of the field. I chose UCHC for its emphasis on evidence-based dentistry and its significant research contributions. Over the past two years, UCHC has given me the skills to be an inquisitive self-learner. Looking forward to learning even more during my time here!

Applications of Receiver Operating Characteristic Analysis in NMR Spectroscopy

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

Affiliations:
[1]UConn Health, Department of Molecular Biology and Biophysics, Farmington, CT 06030-0305 USA

Abstract: Nuclear magnetic resonance spectroscopy (NMR) ranks among the most informative and versatile techniques used by structural biologists. However, NMR is an intrinsically insensitive technique necessitating sophisticated multidimensional experiments, isotope labeling strategies, and exotic data processing algorithms to resolve the individual resonances in complex biological macromolecules[1]. Raw data collected from a spectrometer is typically processed using the Fourier Transform for conversion to the more familiar spectrum of peaks. Various operations are commonly applied to the data for improvement of sensitivity. Quantification of sensitivity gains can be accomplished by measurement of the signal-to-noise ratio (SNR). This metric is only appropriate for understanding conventionally processed spectra in which all portions of the raw data are scaled identically upon conversion to the spectrum. However, this presents a problem as non-Fourier techniques become more prominent due to their ability to extend the range of suitable proteins that can be investigated by NMR. 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 a nonlinear technique known as deconvolution, which modifies the raw data to achieve both sensitivity and resolution enhancement while still finding a consistent spectrum[2]. Unfortunately, due to the non-linear scaling of the spectra, SNR is inappropriate and it is difficult to assess the quality of spectra generated through MaxEnt. Therefore, the goal of this work is to develop more sophisticated approaches for assessing spectral quality. Here, we describe an efficient approach for assessing spectral quality that is based on Receiver Operating Characteristic (ROC) analysis[3]. This novel approach comprises the addition of mock signals with known properties to an existing dataset. The data is subsequently processed with the algorithm of choice and recovery of the corresponding peaks after spectral processing is evaluated via ROC analysis. The process yields a characteristic ROC curve for a spectrum, which can be used to identify the optimal threshold setting in the spectrum for discrimination of signal from noise, and provides a versatile and robust measure of sensitivity. Multiple metrics can be used to evaluate ROC curve, and by proxy the spectrum, including the area under the curve. An additional advantage to ROC analysis is that it can be used to compare between classes of processing algorithms. For example, SNR does not provide an



unbiased metric for comparing Fourier Transform spectra with reconstructed spectra, however ROC analysis can be used for this purpose. Through our approach, we have been able to find optimal processing parameters for both linear and non-linear techniques and also optimize sample schedule construction for use in nonuniform sampling approaches[4].

Acknowledgements:

This work was supported by NIDCR grant: T90DE021989

References:

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4. Schuyler A, Maciejewski M, Arthanari H, Hoch J. "Knowledge-base nonuniform sampling in multidimensional NRM" Journal of Biomolecular NMR

Biography:

After I complete the DMD/PhD program, I intend to integrate my NMR skills with a better understanding of healthy and diseased periodontium through completion of a residency program in periodontology. This will allow me to identify the most important questions that affect periodontal health and to apply my skillset to them through multiple avenues including NMR-based metabolomics studies of the oral flora, structural studies of inflammatory proteins, and studies of disordered proteins that are involved with biomineralization. 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. The School of Dental Medicine is renowned in Connecticut for being an outstanding institution. This status reflects the excellent training that it offers to its students. In particular, the strong emphasis on basic science is what makes the curriculum well designed. During my time here, I have also learned how important it is to get immersed in the community and to get involved in ways outside of strictly science and dental medicine.

Undergraduate Degree- Molecular and Cell Biology, University of Connecticut
DMD/PhD student, year 5

Generation of Paraxial Mesoderm in 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 as small patches or single cells, 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, FACS analyzed for surface marker expression, and analyzed for gene expression via qPCR.

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. Staining for CD184 (CXCR4) expression on Day 4 indicates a majority (78.3%) of the TBX6+ population is also positive for CXCR4 expression whereas ESC controls and cells not subjected to differentiation factors show no TBX6 reporter expression, but are also negative for CXCR4.

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.

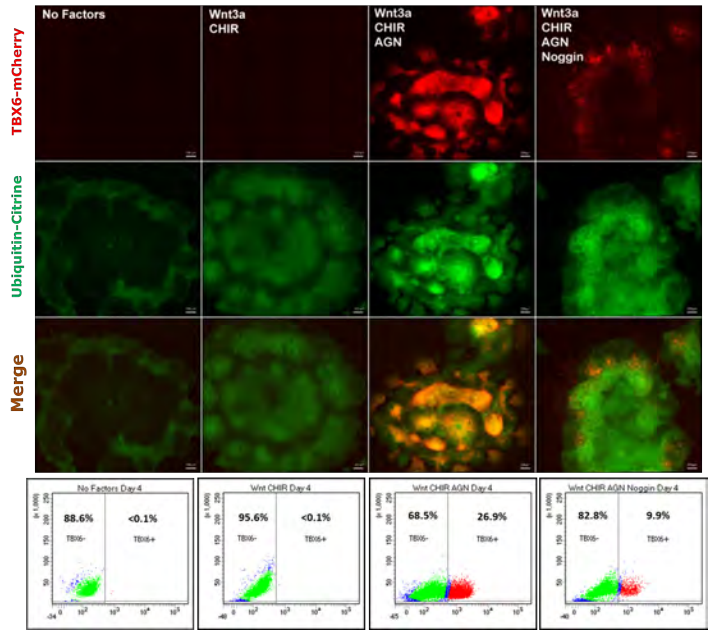


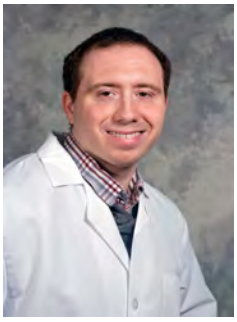
Figure 1. Retinoic Acid Inhibition Increases TBX6 Reporter Expression [Top] Fluorescent reporter expression in cultures treated for 4 days with the indicated combinations of Wnt3a, CHIR99021, AGN193109, and Noggin showing TBX6-mCherry (red) or UbiquitinC-Citrine (green) reporter expression. [Bottom] 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.

Acknowledgements

This work was supported by:
State of Connecticut Grant #: 13-SCA-UCHC-02 Maye
National Institutes of Health/NIAMS Grant#: R21AR056391
UConn/NIDCR training grant T90-DE021989

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.



The role of Runx3 in bone repair

David N. Paglia[1], Do Yu Soung[1], Xiaochuan Yang[1], Hani Awad[2], Joseph Lorenzo[1], Hicham Drissi[1]

UConn Health[1], University of Rochester[2]

Introduction: Understanding the molecular mechanisms that govern fracture healing is paramount for the development of effective therapies to treat impaired bone repair. The *runt* homology-related family of transcription factors Runx1, 2 &3 have pleiotropic roles in organogenesis, tissue homeostasis and malignancy. Emerging evidence established a role for Runx3 in cartilage as well as bone development and disease. Moreover, we and others demonstrated a role for Runx3 in chondrogenic differentiation. A recent report indicated that conditional deletion of Runx3 in osteoblasts resulted in pathologic bone loss. However, whether Runx3 also regulates bone repair remains unknown. Thus, we postulated that conditional deletion of Runx3 in the periosteum will dose-dependently impair fracture healing, while conditional deletion of Runx3 in osteocytes would result in osteopenia.

Methods:

Animals: In this study, we crossed Runx3 floxed mice ($Runx3^{F/F}$) with Prx1-Cre transgenic mice to generate mice in which Runx3 expression was conditionally abrogated in limb mesenchymal progenitors (Prx1-Cre $Runx3^{F/F}$). We also crossed $Runx3^{F/F}$ mice with DMP1-Cre transgenic mice to generate mice in which Runx3 expression was conditionally abrogated in osteocytes (DMP1-Cre $Runx3^{F/F}$). Mutant mice were compared to control mice with 100% Runx3 activity ($Runx3^{F/+}$).

Fracture Experiments: Twelve week old mice were divided into groups and a standard mid-diaphyseal femoral fracture surgery was performed on mutant and control mice. Mice were euthanized at 3, 7, 14, 21, and 35 days post-fracture and femurs were harvested for histology, biomechanical and molecular analyses.

Statistical analyses: 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 local governmental institutional review board and was conducted in accordance with federal and institutional guidelines for the security of protected health information and the safety of all involved.

Results: When Runx3 was conditionally deleted in either the periosteum (Prx1-Cre $Runx3^{F/F}$) or in osteocytes (DMP1-Cre $Runx3^{F/F}$), there was no resulting developmental limb phenotype. However, bone mineral density of adult mutant mice displayed increased bone mineral density and content compared to control mice in both conditional knockouts. We therefore challenged the $Runx3^{F/+}$, and Prx1-Cre $Runx3^{F/F}$ mice using our femoral fracture model. The conditional deletion of Runx3 in the periosteum resulted in a reduced cartilaginous callus (day 7). Interestingly, we observed enhanced bone formation in bony calluses of conditional mutants compared to controls (day 21). We quantified the number of blood vessels and found that

conditional abrogation of Runx3 in the periosteum significantly increased callus vascular invasion (days 7-21). The data suggested enhanced fracture healing, which was evidenced by increased torsional strength of femora following bony union (day 35) in Prx1-Cre Runx3$^{F/F}$ calluses compared to Runx3$^{F/+}$ controls ($p<0.05$). To evaluate the mechanism underlying the osteogenic phenotype following loss of Runx3 expression in the periosteum, we induced periosteal cells from both genotypes (Runx3$^{F/+}$, and Prx1-Cre Runx3$^{F/F}$) into the osteogenic lineage. The number of colony forming units, mineralized tissue area, and osteogenic gene expression were significantly increased (days 7-21) for cells isolated from Prx1-Cre Runx3$^{F/F}$ compared to Runx3$^{F/+}$ mice, confirming the osteogenic phenotype we observed in vivo. We also evaluated gene expression from tissue sections for osteogenic, chondrogenic, and angiogenic genes Gene expression analyses of mRNAs isolated from histological callus sections from each genotype revealed significantly increased osteogenic gene expression and decreased chondrogenic gene expression for the Prx1-Cre Runx3$^{F/F}$ mice. Interestingly we found significant induction of angiogenic genes (days 14, 21) in these mice.

Conclusions: The results of this study suggest that Runx3 plays a critical role in the progression of fracture healing, particularly in mediating osteogenesis and angiogenesis in a periosteal precursor population. We found that conditional deletion of Runx3 in a Prx1-specific population induced bone formation following fracture, while inhibiting chondrogenesis. Osteogenic cultures confirmed that while there was no increase in mineralization for bone marrow cultures between groups, periosteal cultures from Prx1-Cre Runx3$^{F/F}$ cells demonstrated significantly more colony forming units and ALP staining than controls. Angiogenic gene expression and blood vessel formation were also significantly increased in Prx1-Cre Runx3$^{F/F}$ mice, suggesting both an osteogenic and angiogenic phenotype. Conditional deletion of Runx3 in osteocytes did not result in osteopenia, but rather increased bone mineral density, similar to what we observed following conditional deletion of Runx3 in periosteal cells.

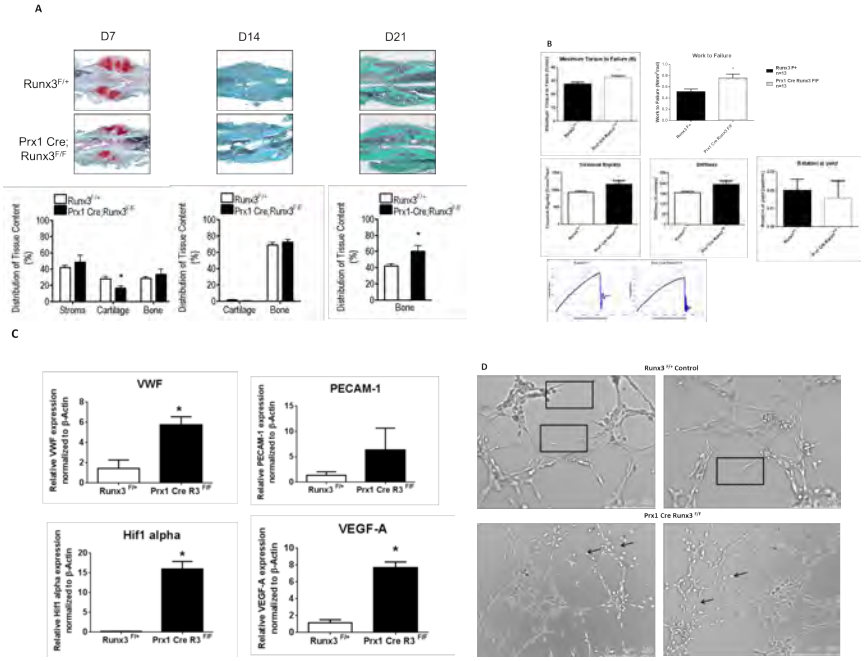


Figure 1: Evaluation of Prx1-Cre Runx3$^{F/F}$ compared to Runx3F$^{/+}$mice following fracture via (A) histology and static histomorphometry at days 7-21 post-fracture, (B) torsional biomechanics

at day 35 post-fracture, (C) gene expression at day 14 post-fracture, and (D) matrigel cultures of periosteal cells under angiogenic conditions from day 3 post-fracture.

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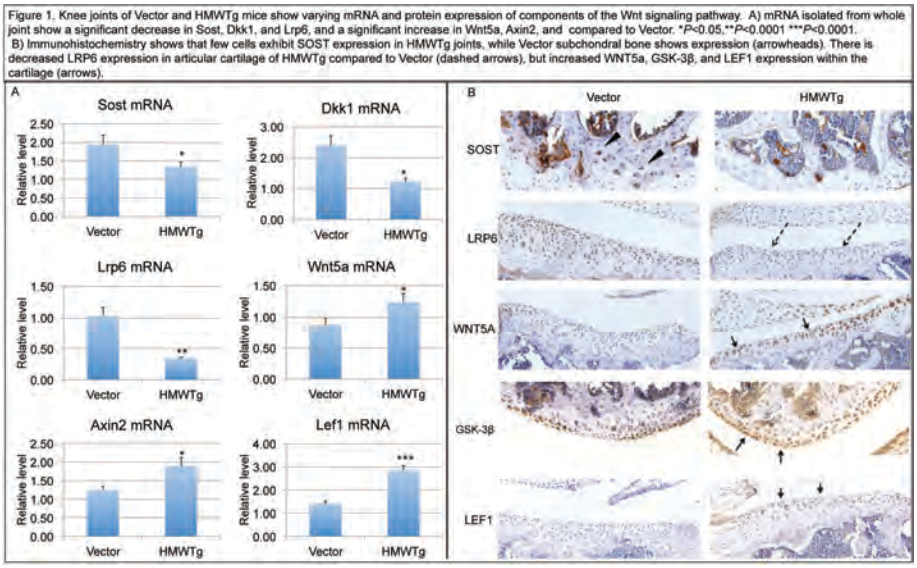
B.S. Mechanical Engineering: (Manhattan College, Riverdale, NY, 2006)
M.S. Biomedical Engineering: (New Jersey Institute of Technology, Newark, NJ, 2008)
PhD Biomedical Engineering; (Rutgers University and NJIT Joint Program, Newark, NJ, 2011)
Post-Doctoral Fellowship: Rutgers, Newark, NJ (2011-2012)
Post-Doctoral Trainee: UConn Health 4th Year (T90 NIDCR Trainee-2nd 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 strong foundation for collaborative research and mechanistic approach towards musculoskeletal research. As a T90 Trainee, I am investigating the role of Runx transcription factors in homeostasis and trauma. I have been privileged to work in a well-respected group with supportive mentors and to have the support of the T90 committee mentors.

Wnt Signaling Contributes to Osteoarthritis in Mice Overexpressing the High Molecular Weight Isoforms of Fibroblast Growth Factor 2

Patience Meo Burt , Liping Xiao, and Marja Marie Hurley

Osteoarthritis (OA) is a debilitating joint disease that affects over 27 million adults in the U.S., characterized by loss of articular cartilage and changes in underlying bone with no effective therapy. Fibroblast growth factor 2 (FGF2) is known to play a role in OA, but the involvement of FGF2 isoforms has not been investigated. Mice that overexpress the high molecular weight FGF2 isoforms in pre-osteoblasts (HMWTg mice) display dwarfism, decreased bone mineral density, osteomalacia, hypophosphatemia, and increased FGF23 in serum and bone and phenocopy the Hyp mouse, a homologue of human X-linked hypophosphatemia (XLH). Hyp mice and XLH patients develop osteoarthropathies, so we investigated whether HMWTg mice have OA and increased FGF23 expression within the joint. Wnt/β-catenin signaling has been found to be upregulated within areas of osteoarthritic joints in humans/mice and Hyp mice have increased expression of Wnt signaling components. Also, FGF23 is upregulated in human OA chondrocytes and Wnt signaling modulates FGF23 promoter activity. Since HMWTg mice phenocopy Hyp mice and have an increase in FGF23 within the joint, we posit that the OA phenotype in HMWTg mice is due in part to enhanced Wnt signaling. We observed an OA phenotype in 18-month-old HMWTg mice. Time course studies demonstrated evidence of OA starting at 2 months. Examination of joints of 2-month-old Vector control and HMWTg mice by x-ray, microCT, Safranin-O staining, qPCR, and immunohistochemistry were used to assess for signs of OA. qPCR analysis of mRNA and immunohistochemistry were used to examine components of Wnt signaling pathway. We confirmed that 2-month-old HMWTg mice developed signs of OA, including flattening of tibial plateau, thinning of subchondral bone, decreased articular cartilage thickness, and increased expression of cartilage degrading enzymes. The mRNA and protein levels of Wnt inhibitors Dkk1 and Sost were decreased in HMWTg. Lrp-5 was unchanged but Lrp-6 mRNA and protein were decreased. The ligand Wnt5a mRNA and protein were increased and phospho GSK-3β and Axin-2 were also increased. There was a small increase in nuclear staining for β-catenin (data not shown) and Lef-1 mRNA and protein were increased (Fig.1). Overall, these results suggest that enhanced Wnt signaling contributes to the OA phenotype found in HMWTg mice and offer potential insight into a pathway that could be targeted to treat OA.



Acknowledgements: This work was supported in part by DK098566 and NIH/NIDCR T90DE021989.

Major Advisor: Dr. Marja Hurley

Biography:
B.S. Biology (Post University, 2009)
M.A. Biomolecular Sciences (Central CT State University, 2010)

Currently, I am in my 3rd year of the Ph.D. in Biomedical Sciences program. I chose UCONN Health due to the excellent skeletal biology program and research opportunities that combine basic science and human health. In the future, I hope to continue to incorporate a translational component in my scientific career.

Cellulose based Micro- Nano structured scaffolds for Bone Regeneration

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Introduction: Several polymer based scaffold systems have been used as an alternative to traditional autografts and allografts to repair non-healing defects of the bone. These materials fail to provide required osteoconductive, osteoinductive, and osteointegrative properties in line with autografts. Natural polymers have the advantage of being similar to biological macromolecules and elicit favorable tissue healing responses, in contrast to synthetic polymers that are used conventionally in scaffolds for bone regeneration. In this study, we present the characterization of cellulose acetate (CA) based scaffolds in comparison to synthetic poly (lactic-co-glycolic acid) (PLGA) scaffolds with respect to their ability to promote human Mesenchymal Stem Cell (hMSC) progression into osteoblastic lineage *in vitro,* biocompatibility in a rat subcutaneous implantation model and bone healing capability in a mouse calvarial defect model.

Materials and Methods: Cellulose acetate (CA) (Sigma-Aldrich, 30kD) and (PLGA) microparticles were prepared using emulsion solvent evaporation method. Solvent sintering technique[1,2] was employed to prepare CA scaffolds and heat sintering was used for PLGA. Composite scaffolds of CA (CAc) PLGA (PLGAc) were prepared by coating 0.1 % collagen solution on the scaffolds. *In vitro* studies, using hMSCs in basal media (BM) and osteogenic media (OM), were conducted by seeding 500,000 cells per tablet type scaffold. Osteoblastic differentiation was monitored by alkaline phosphate activity assay and mineralization along with change in osteogenic gene expression (RUNX2, Col1, Col3, and BSP after 21 days). Further, CA, CAc and PLGA scaffolds were implanted subcutaneously in rats to determine the biocompatibility of the materials by contrasting the immune responses between the groups using histological staining. The scaffolds seeded with bone marrow stomal cells from donor mice (Col 3.6-cyan) were implanted in critical sized (3.5mm dia) calvarial defect in mice (Col 3.6-GFP) and its ability to heal bone was evaluated by histological staining.

Results and Discussion: Osteogenic genes such as RUNX2, collagen1 and BSP showed greater expression on the CA and CAc groups in contrast to the PLGA groups (Figure 1.A.). The CA and CAc scaffolds showed greater progression of osteogenic phenotype as seen by greater mineralization of osteoinduced hMSCs on these scaffolds (Figure 1.B., C.). Finally, CA scaffolds seeded with MSCs showed uniform bone formation in contrast to PLGA scaffolds that had islands of new bone formation (Figure 1.D.). The collagen content (Figure 1.E.) and radioopacity of bone formed (Figure 1.D.) in the CA groups exceeded the bone formation in the PLGA groups, indicative of the higher quality of regenerated bone with the use of CA. The greater hydrophilic and biomimetic nature of CA may be attributed to these differences.

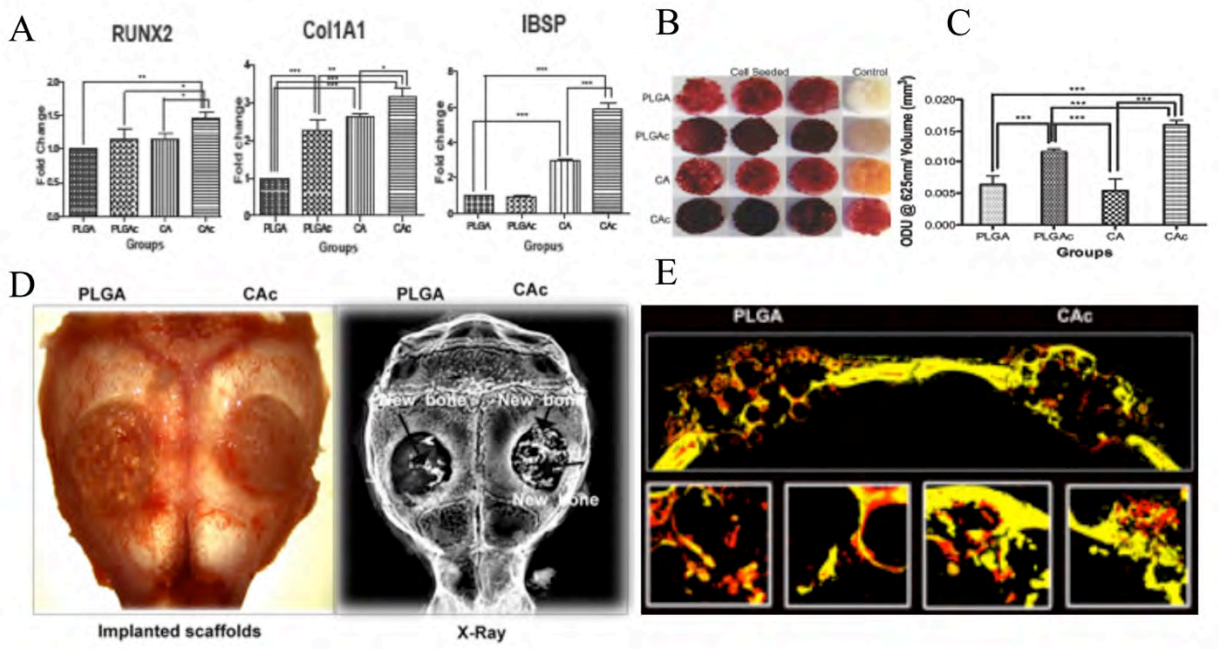

Figure 1: Characterization osteoinductive ability of Natural polymeric micro-nanostructured scaffolds (A) Osteoblastic gene expression on seeded and induced hMSCs (B) Mineralization at 21 days of culture by hMSCs. (C) Quantification of mineral deposition (D) Head and X-ray images of bone formation by scaffolds seeded with cells (E) Collagen/ Bonesialoprotein staining of histological sections of the implanted scaffolds.

Conclusions: The CA and CAc scaffolds induced and maintained osteoblastic differentiation of the seeded hMSCs. The biocompatibility and cellularity of CA increased over time, while that of PLGA decreased over time. The stable and more hydrophilic properties of CA could be attributed to these results. Finally the bone formation by CA scaffolds was denser, with higher collagen density and it was uniformly well distributed through the scaffold than PLGA, reflecting the superior osteogenic performance of the polysaccharide-based scaffolds. Hence CA based polysaccharide platform may be a viable alternative to synthetic polymers like PLGA in bone tissue engineering.

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Major Advisor: Dr. Sangamesh G. Kumbar

References: 1. Kumbar,S.G., *Biomedical Material*, 2011,6, 065005.
2. Aravamudhan,A., *Journal of Biomed Nanotechnology*, 2013, 9, 1-13.

Biography: B.S., Biotechnology; Anna University, Chennai- 600025.
Ph.D. in Biomedical Science; Skeletal, Craniofacial and Oral Biology - 7th 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.

Sotirios Tetradis, DDS, PhD
Professor and Chair, Section of Oral and Maxillofacial Radiology, UCLA School of Dentistry

Dr. Tetradis received his DDS from the University of Athens, Greece in 1990, and a certificate in OMF Radiology and a PhD in Biomedical Sciences at the University of Connecticut in 1996. He then pursued postdoctoral studies at the Harvard Institutes of Medicine. In 1998, Dr. Tetradis joined the UCLA School of Dentistry as an Assistant Professor. Currently, he is a professor and the Chair of the Section of Oral and Maxillofacial Radiology, and serves as the Advisor of the DDS/PhD combined degree program. Dr. Tetradis is a board certified Oral and Maxillofacial Radiologist, and practices at the state-of-the-art imaging facility at the UCLA Oral Radiology clinic. Since he joined UCLA, Dr. Tetradis leads an active NIH-funded research group that investigates the effects of growth factors, hormones and cytokines on the function and differentiation of bone cells, as well as the cellular and molecular mechanisms of antiresorptive–related osteonecrosis of the jaw. He has published more than 100 peer-reviewed articles in the fields of bone biology and maxillofacial imaging. Dr. Tetradis is active in several professional organizations, including the American Academy of Oral & Maxillofacial Radiology, the American Association for Dental Research, and the American Society for Bone and Mineral Research.