Skeletal, Craniofacial & Oral Biology NIH/NIDCR T90 Training Program Symposium 2017

Friday, June 16, 2017

School of Dental Medicine

Academic Rotunda
UConn Health • Farmington, CT

Welcome

Welcome to all participants and guests to the 2017 NIH/NIDCR-supported T90 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, 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 our special guest is *Ophir Klein, MD, PhD, Professor of Orofacial Sciences and Pediatrics, the Larry L. Hillblom Distinguished Professor in Craniofacial Anomalies, and the Charles J. Epstein Professor of Human Genetics at the University of California, San Francisco (UCSF)*. In addition to his scientific presentation, and viewing of oral and poster presentation, Dr. Klein will spend informal time with trainees talking about his career decisions and life in academics.

Welcome Dr. Klein!

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

A. Jon Goldberg, PhD
Professor
Biomedical Engineering—UConn Health
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, 2T90 DE021989 and 2R90 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, we 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!

Cover photographic of the new Academic Rotunda taken by Tina Encarnacion of the UCH Communications Department.

Dr. William B. Upholt

For the first time in over 25 years the training program is missing the leadership of Dr. William B. Upholt. Bill's passing has left a void, and we want to acknowledge his many contributions to this Symposium, the training programs, the department and the School of Dental Medicine. Bill, we miss you at the leadership table.

Schedule

All events in the Academic Rotunda

Time	Presenter/Short Title	Page
8:15 – 10:00 am	**Registration and Continental Breakfast**	
8:45 – 9:30 am	**Informal session for trainees with Dr. Klein on academic life**	
9:30 – 9:45 am	**Opening Remarks** Dr. R. Lamont "Monty" MacNeil, Dean School of Dental Medicine Dr. Jon Goldberg, Co-Director, T90 Training Grant	
9:45 – 11:00 am	**Oral Session I: Progenitors, Gene Function, Regeneration** Session Chair: Matthew Zambrello Sierra Root/*Characterization of novel resident bone marrow population*…….....4 Aundrya Montgomery/*Nail tissue regeneration using stem cells and scaffold*.....6 Michelle Spoto/*Genome-wide guide designer for CRISPR interference*.....…..8 David Paglia/*Runx1 loss depletes callus mineralization following fracture*…….10	
11:00 – 11:50 am	**Break and Poster Session** Jumana Alhamdi/*Tuning delivery kinetics of multiple factors*……..12 David Manz/*Iron regulation and prostate cancer metastasis*……...14 Ninna Shuhaibar/*Odontoblast processes investigated by serial SEM*……..16 Veneta Qendro/*CD13/receptor agonist increases efficacy of cancer vaccine*…..18 Xi Wang/*PDGFRβ signaling regulates osteogenesis of periosteal MSC*…….20 Matthew Zambrello/*Receiver operating characteristics in NMR spectroscopy*...22	
12:00 – 1:00 pm	**Guest Speaker** **Ophir Klein, MD, PhD** Professor of Orofacial Sciences and Pediatrics, the Larry L. Hillblom Distinguished Professor in Craniofacial Anomalies, and the Charles J. Epstein Professor of Human Genetics at the University of California, San Francisco (UCSF) *Biting into Development and Regeneration: Insights into odontogenesis and dental stem cells*	
1:00 – 2:00 pm	**Luncheon open to all participants**	
2:00 – 2:15 pm	**Skeletal Biology and Regeneration Awards**	
2:15 – 3:30 pm	**Oral Session II: Signaling** Session Chair: David Manz Anu Vijaykumar/*Effects of FGF2 on Wnt/β-catenin signaling in dental pulp*….24 Ryan Russell/*Specification of sclerotome cells via axial skeletal lineage*……26 Patience Meo Burt/*Global and chondrocyte-specific deletion of FGF2*……...28 Henry Hrdlicka/*miR-29 regulation on E-cadherin expression*……..…30	

Characterization of a novel resident bone marrow population

Sierra Root[1], Brya Matthews[1], Ivo Kalajzic[1], and H. Leonardo Aguila[1]

[1]University of Connecticut

Abstract:

Objective: Primary hematopoiesis occurs within the bone marrow space in a highly organized microenvironment that define sites of hematopoietic progression. These microenvironments or niches are complex and contain multiple cell types. The development of these cells is interdependent and their functions are crucial to maintain the HSC pool and ensure their homeostatic differentiation and expansion. Even when the major populations involved have been studied, the identity of all the relevant components within hematopoietic niches has not been 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. Three types of murine bone marrow resident macrophages have been identified in the literature- the osteomac, HSC niche macrophage and erythroid island macrophage. Because of the phagocytic nature of the dmp1-tom positive cells and their cell surface expression, we propose that these tomato+ cells are a novel unidentified bone marrow resident macrophage that may share features of known resident macrophages.

Methods: We have used a combination of histology, 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, and their response to the myeloablative agent 5-Flurouracil and the HSC mobilizing agent GCSF were done. In order to obtain a clearer understanding on whether or not they localize with erythroid components within blood islands in the bone marrow, a specialized method of bone marrow flush, flow cytometry staining and analysis was done in order to capture these 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. DMP1/tdTomato+ CD11b+F480+ cells phagocytose latex beads and can be depleted *in vivo* when injected with clodronate loaded liposomes. These cells can be enriched and found within the blood island aggregates by costaining for the erythroid marker Ter119 and the macrophage marker F4/80. These cells also express a cohort of signature

markers for resident macrophages including CD169, VCAM1, Ly6G and ER-HR3. In conclusion, 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 tissue resident macrophages. The localization of this population is almost exclusive in bone marrow, where it forms a network. We propose that this population corresponds to a bone marrow resident myeloid cell playing important roles in the control of hematopoiesis.

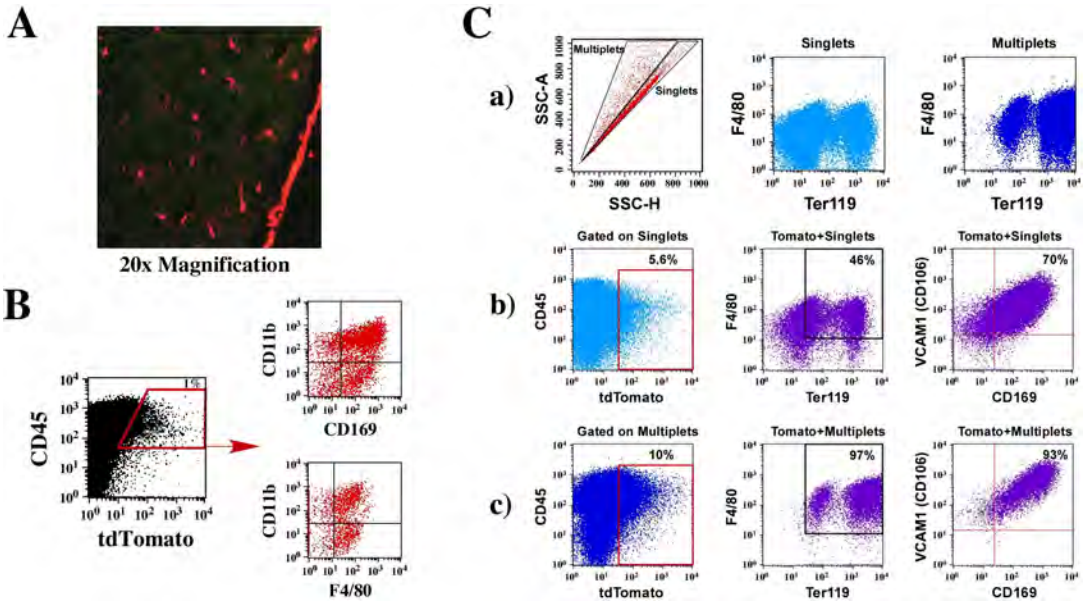


Figure 1. DMP1/TdTomato+ myeloid cells reside within the bone marrows erythroid island compartment
A) Femur histology of dmp1-tomato+ cells interspersed within the bone marrow. **B)** Standard bone marrow flush with RBC lysis reveals only 1% tomato+ cells containing cells with macrophage markers. **C) a-c** Bone marrow flushes without RBC lysis to maintain erythroid island macrophage compartment with specialized analysis of erythroid-marophage aggregates by flow cytometry **a)** Live cells were analyzed by side scatter area vs height in linear mode to identify single cells (singlets) and aggregates (multiplets). Gated populations were then analyzed for costaining of the macrophage marker F4/80 and the erythroid marker Ter119. **b and c)** dmp1-tomato+ cells within singlets and multiplets were analyzed for costaining of F4/80 and Ter119 and for the resident macrophage markers VCAM1 and CD169.

Acknowledgements: This work was supported by the NIH/NIDCR 2T90DE021989 and 1R01AR055607-01A2

Major Advisor: Dr. H. Leonardo Aguila

Biography:

BS Chemistry-Biology (University of Hartford, 2005)

PhD Biomedical Sciences-Genetics and Developmental Biology (UConn Health, 2014) Post-Doctoral Fellow (Year 3)

It is my career objective to advance the field of monocyte and osteoclast development, their supporting cell types and their role in pathological conditions. During my PhD, I focused 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.

Nail Tissue Regeneration Using Stem Cells and Nanostructured Scaffold

Aundrya Montgomery[1], Emmanuel Kuyinu[1], Lakshmi Nair[1], Cato T. Laurencin[1]

[1]Institute for Regenerative Engineering, UConn Health, Farmington, CT

INTRODUCTION

The nail plate of the distal phalanx, which functions in external sensation and protection of underlying soft tissue, regenerates continuously. Digit regeneration can occur after amputation (e.g., upon traumatic injury to a finger) distal to the nail bed. However, if the nail plate is removed, regeneration cannot occur without an external structure that facilitates interaction with the proximal nail fold and the underlying germinal matrix. As a potential remedy, engineering a biomaterial that interacts with stem cells will support efforts to regenerate a hard nail plate and ultimately an entire digit. The overall goal of the study is to develop a biodegradable scaffold that mimics the nail germinal matrix to support cell-mediated regeneration of hard nail tissue. The objective of the present study is to develop biodegradable and biocompatible extracellular matrix (ECM) mimic nanofiber scaffold to facilitate the interaction between the proximal nail fold and germinal matrix of the nail organ and to use a cell-delivery system to accelerate the regeneration process of hard nail tissue.

METHODS

To compose a biomimetic ECM scaffold, poly(lactide-*co*-glycolide) (50:50) was dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol to compose a 24.5% (w/v) polymer solution for the electrospinning process. The optimal parameters 15 kV, 20 cm needle tip to collector distance, and flow rate of 2 mL/hour were used in nanofiber scaffold fabrication. The diameter and morphology of the electrospun PLGA nanofibers was characterized by scanning electron microscopy (SEM) and analyzed using ImageJ software. Adipose derived stem cells (ADSCs) were isolated from the inguinal region of Sprague Dawley rats according to standard protocol. Nail stem cells (NSCs) were isolated from the germinal matrix region of Sprague Dawley rat claws. The purity of the isolated stem cell populations were confirmed using flow cytometry (positive markers: CD 29 and CD 90; negative markers: CD 45, CD 11b, CD 34, and Lgr6).

RESULTS

Optimal parameters for the electrospinning process was established with the fabrication of bead-free fibers with fiber diameter distribution ranging between 143-1109 nm (Fig. 1). Studies also showed the ability of adsorbing growth factors, such as epidermal growth factor, on nanofibers matrices to modulate cell behavior. ADSCs were successfully isolated and positive markers, CD 90 and CD 29, confirmed the identity of the cells. NSCs were positive for mesenchymal stem cell markers CD 29 and CD 90 as well.

CONCLUSIONS

Bead-free nanofibers were successfully fabricated and will serve as the biomimetic structure facilitating the interaction between the proximal nail fold and germinal matrix to promote the

regeneration of hard nail tissue. ADSCs and NSCs have been isolated and properly identified for their use in the present study to stimulate the growth of the hard nail plate in a rat model. Future directions will evaluate the effect of PLGA nanofibrous matrices to serve as an ECM mimic epidermal growth factor delivery system to modulate ADSC and NSC function *in vitro* and *in vivo*.

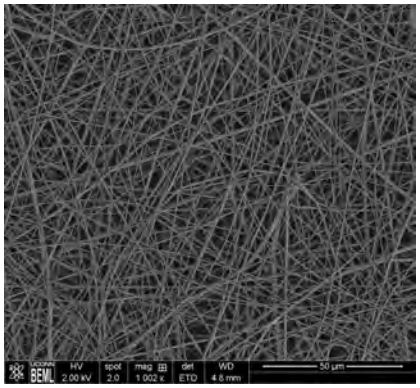
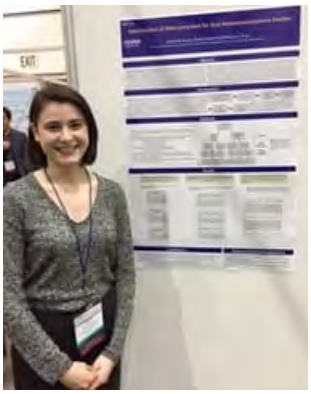


Figure 1. SEM image of 24.5% (w/v) electrospun bead-free nanofibers with an average diameter of 468 nm.

Acknowledgements
This work was supported by:
National Institutes of Health Grant #: DP1 AR068147
Biomedical Research Trust Fund Grant#: 600940-10301-20

Biography
Education:
Alabama State University, B.S., Biology Pre-Health
Position: Young Innovative Investigator Program (YIIP) Scholar
Year in Program: 1st
Career Aspirations: I aspire to build a career in academic medicine as a surgeon and continue to serve my country as a soldier in the United States Army. Upon earning my master's degree, I plan to earn a MD/PhD so that I may clinically translate bench research to those suffering with traumatic limb loss.
Why UConn Health: During my enrichment year, I wanted to participate in groundbreaking traumatic limb loss research. Being selected as 1 of 4 scholars to participate in YIIP has helped me accomplish this goal, and remains to facilitate my research experience on my journey to becoming a person of color in the healthcare profession.



Development and Implementation of a Genome-wide Guide Designer for CRISPR Interference

Michelle Spoto[1], Elizabeth Fleming[1], Julia Oh[1]
[1]*The Jackson Laboratory for Genomic Medicine*

INTRODUCTION

CRISPR interference (CRISPRi) is a modification of the CRISPR/Cas9 system which uses a catalytically inactive Cas9 protein for transcription repression. When applied genome-wide, CRISPRi has tremendous potential to systematically investigate gene function. Briefly, guides targeted to each gene in the genome are utilized to create a pool of mutants in which each mutant has one gene knocked down. This mutant pool can then be subject to a diverse range of phenotypic assays to assess morphology and growth under a variety of different conditions. However, despite the utility of CRISPRi for the interrogation of gene function, no pan-bacterial, genome-wide tools exist for guide discovery. We have created a customizable, user-friendly program that can design guides for any annotated microbial genome.

METHODS

Our automated guide finder designs guides from NGG PAM sites for any number of genes using an annotated genome and fasta file input by the user. Guides are filtered according to user-defined design parameters and removed if they contain any off-target matches. Iteration with lowered parameter thresholds allows the program to design guides for genes that did not produce guides with the more stringent parameters (Fig. 1). We have tested this guide designer *in silico* on a variety of diverse bacterial genomes to determine, on average, the proportion of genes that are suitable for guide creation.

To test the functionality of our guide designer, the program was utilized to design guides targeted to essential genes in *S. epidermidis*. Guides were selected using the automated guide designer, ligated into our CRISPR/dCas9 vector, and transformed into *S. epidermidis*. Growth assays were performed. It is expected that successfully designed guides lead to a reduction in essential gene product production and therefore cause a growth defect.

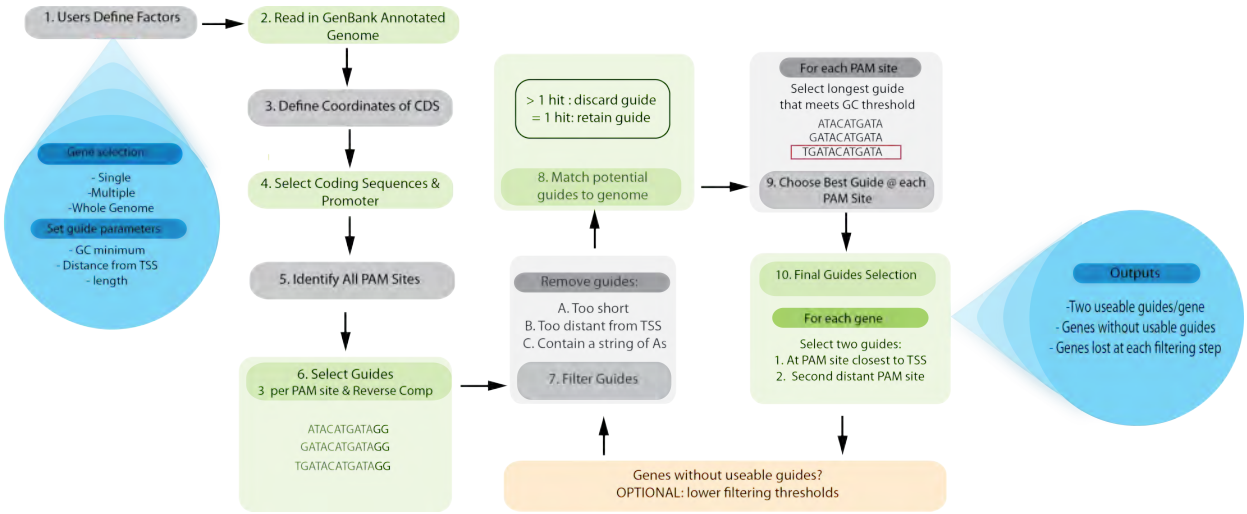
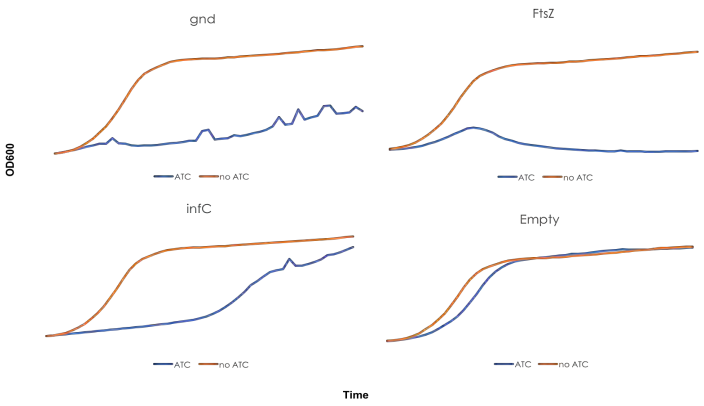


Fig. 1. To use the guide designer program, users select parameters, provide Genbank accession #, and input fasta file of microbe of interest. The program uses the annotated genome and fasta file to select coding and promoter regions for each gene. Within this sequence, NGG PAM sites are identified and guides selected adjacent to these sites. Guides are filtered according to user-defined thresholds and guides with off-target matches are discarded. For each gene, two guides are selected. For genes that did not produce guides, design parameters can be lowered and iteration through the program with these lowered thresholds can be utilized to recover more guides.

RESULTS

Preliminary *in silico* application of our guide designer demonstrated that the program was able to design guides for approximately 95% of all genes per genome. Application of the designer to essential genes in *S. epidermidis* resulted in significant growth defects (Fig. 2).

Fig. 2. Guides were selected via our automated guide designer to target essential genes in *S. epidermidis* (gnd, FtsZ, infC); successful knockdown produces a growth defect. As shown here, induction of dCas9 by ATC abates the growth of *S. epidermidis* carrying the CRISPR/dCas9 vector + guide (blue line). Non-induced bacteria (growth represented by orange line) also contain this construct but dCas9 is not expressed so knockdown of the essential gene does not occur. This demonstrates the ability of the program to successfully design guides. The CRISPR/dCas9 vector without a targeting guide ("Empty") acts as a control.



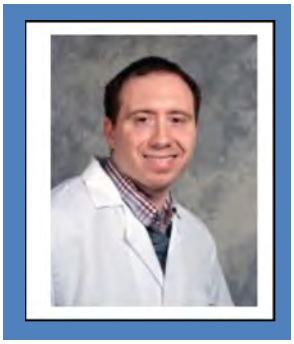
CONCLUSIONS

Our *in silico* analysis and application of the guide designer for essential gene knockdown in *S. epidermidis* provides evidence of the utility of the program as a versatile genome-wide guide designer. Moreover, we have applied it to a diverse set of genomes with broad success across different genome sizes and GC content. Through the generation of guides targeted to any number of microbial genes, our open-access software will improve accessibility to CRISPRi studies for a variety of microorganisms. Additional work is required for speed optimization and continued testing on a greater number of microbial genomes to further assess generalizability.

Acknowledgements:
This work was supported by the T90-DE021989 grant.

Biography:

Education
Rochester Institute of Technology, B.S., Biomedical Sciences
Position: DMD/PhD program
Year in Program: 3rd
Career Aspirations: In pursuing both a DMD and PhD, I want to better understand the field of dentistry from both a clinical and scientific standpoint. My goal is to work as a clinician and researcher at an academic dental institution.
Why UCHC: I chose UCHC for its emphasis on evidence-based dentistry and its significant research contributions. Over the past three years, UCHC has given me the skills to be an inquisitive self-learner. Looking forward to learning even more during my time here!



Runx1 conditional loss of function progressively depletes early callus mineralization following fracture through increased osteoclastogenesis

Paglia DN[1], Yang X[1], Kalinowski J[1], Jastrzebski S[1], Drissi H[2], Lorenzo J[1]

Affiliations
[1] UConn Health,
[2] Emory University

We previously established Runx1 as an inhibitor of osteoclast (OC) differentiation and function in vivo[1]. Here, we evaluated the impact of conditional deletion of Runx1 in OC precursor on fracture healing. We crossed Runx1 floxed mice ($Runx1^{F/F}$) with LysM-Cre mice to generate tissue specific deletion of Runx1 in OC precursors (Runx1 cKO). $Runx1^{F/+}$ were used as controls. A standard mid-diaphyseal fracture was created in 12-week-old mice of each genotype using a 3-point bending device. Fractured femurs were harvested at 7, 11, 14, and 18 days post-fracture for micro-CT, histomorphometric, and molecular analyses. Femurs were micro-CT scanned and analyzed for differences in callus bone density and connectivity (n=6 per group). Histologic images were captured to determine the gross morphometric composition of the callus. OsteoMeasure software was used to evaluate tartrate resistant acid phosphatase (TRAP)-stained sections to determine the number and perimeter of multinucleated TRAP+ cells on callus cartilage (chondroclasts) and bone (OCs) as healing progressed (n=5 per group). RNA was isolated from OCs at day 14 post-fracture via laser capture microdissection (LCM). It was then processed by QPCR to evaluate gene expression. Torsional testing at day 28 post-fracture (n=8 per group) was evaluated, to determine the impact of this conditional deletion on bone mechanical integrity following fracture.

Micro-CT and histomorphometric analyses of the fracture callus at 14 and 18 days post-fracture found a progressive decrease in callus bone density in Runx1 cKO mice, compared to controls ($p<0.05$). OsteoMeasure analyses found significantly more OCs, chondroclasts, and eroded surface ($p<0.05$) in Runx1 cKO calluses compared to control at day 11 post-fracture (Figure 1). By day 14 post-fracture, the Runx1 cKO mouse calluses demonstrated residual cartilage matrix associated with chondroclasts coinciding with pathological woven bone resorption. By day 18, the number of OCs in the Runx1 cKO mouse was significantly decreased ($p<0.05$) compared control, as most of the callus woven bone was already eroded. QPCR analysis of LCM-OC captured RNA identified critical genes for OC function that are regulated by Runx1 during fracture repair. In Runx1 cKO calluses we found an expected 90% decrease in Runx1 expression in OCs. This was associated with greater than 5-fold increases in *Nfatc1, DC-Stamp* and greater than 100-fold increases in *Ctsk and Calcr*.

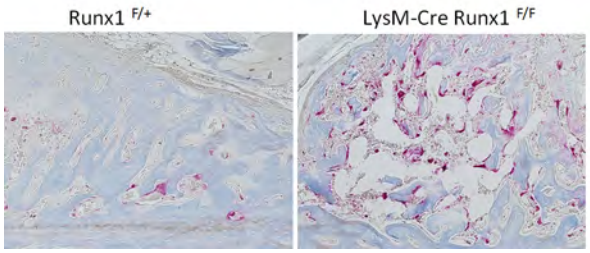


Figure1: TRAP staining at day 11 post-fracture for Runx1cKO and control mice shows early woven bone resorption.

Biomechanical testing found that Runx1 cKO calluses had significantly greater torsional rigidity, compared to controls ($p<0.05$), but torsional strength and work were not affected.
This study demonstrates for the first time, a role for Runx1 in cartilage matrix remodeling and in resorption in the context of bone repair.

Acknowledgements: Funding for this research comes from the National Institutes of Health Grant R01AR060867 to H.D. and J.L. Support of D.P. comes from NIDCR T90 funding (T90 DE021989).

Major Advisor: Joseph Lorenzo, M.D.

References:
1. Paglia DN, Yang X, Kalinowski J, Jastrzebski S, Drissi H, Lorenzo J. Runx1 Regulates Myeloid Precursor Differentiation Into Osteoclasts Without Affecting Differentiation Into Antigen Presenting or Phagocytic Cells in Both Males and Females. Endocrinology 157(8):3058-69, 2016.

Biography: David N. Paglia, Ph.D.
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 5th Year (T90 NIDCR Trainee-3nd Year), Farmington, CT

It is my career objective to promote the advancement of research in the fields of orthopaedics, cell biomechanics, 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.

Tuning Delivery Kinetic of Multiple Biological Factors from a Drug Delivery System

J. Alhamdi[1,2], E. Jacobs[1,2],M. Hurley[1], G. Gronowicz[1], L.T. Kuhn[1,2]

Affiliations

[1] University of Connecticut, Storrs
[2] University of Connecticut Health Center, Farmington

INTRODUCTION

Highly localized sequential delivery of multiple factors is needed to trigger multiple steps of tissue regeneration and to avoid conflicting messages to cells from co-delivery. A new system for localized, sequential, cell-mediated delivery of multiple factors was recently developed in our laboratory [1]. Two different bioactive factors were separated by a biomimetic calcium phosphate (bCaP) barrier layer and poly-L-Lysine/poly-L-Glutamic acid polyelectrolyte multilayer (PEM) film, (bCaP-PEM) to achieve sequential delivery of two different factors. This study focused on tuning the kinetics of delivery by modulating the layer thickness, cell type and layered structure to investigate if the kinetics of access to factors could be tuned.

MATERIALS AND METHODS

The sequential delivery of two factors was investigated using fibroblast growth factor-2 (FGF-2 or F) and cytotoxic antimycin-A (AntiA or A). First AntiA (213 µg/disk) was applied to the tissue culture plastic disks and then a layer of bCaP mineral crystals was deposited above the AntiA via immersion in simulated body fluid (SBFx5) [2]. Layer-by-layer build up of PEM was applied by automated dipping in PEM solutions [1]. FGF-2 (150 ng) was adsorbed into PEM. MC3T3-E1 mouse calvarial osteoprogenitors or RAW 264.7 macrophages were plated on coated disks. bCaP thickness, the number of PEM bilayers, bCaP layers and presence and absence of PEM were varied. To change bCaP thickness, time in SBFx5 solution was varied: 7, 24, 48h. LIVE® staining and imaging was used to quantify cell proliferation and death from. Scanning electron microscopy (SEM) was used to evaluate coating morphology before and after culture.

RESULTS

MC3T3-E1s initially proliferated and then abruptly accessed the embedded AntiA at day 3 on both bCaP(7h and 24h)-PEM, and on day 2 on bCaP(24) without PEM (Fig.1). Depositing a thicker bCaP(48h) with PEM slightly delayed the access to AntiA; however, bCaP(48) without PEM did not further delay access. Increasing the number of PEM bilayers to 102 did not further delay access. Depositing two layers of bCaP with two AntiA dose resulted in pulsatile profile.

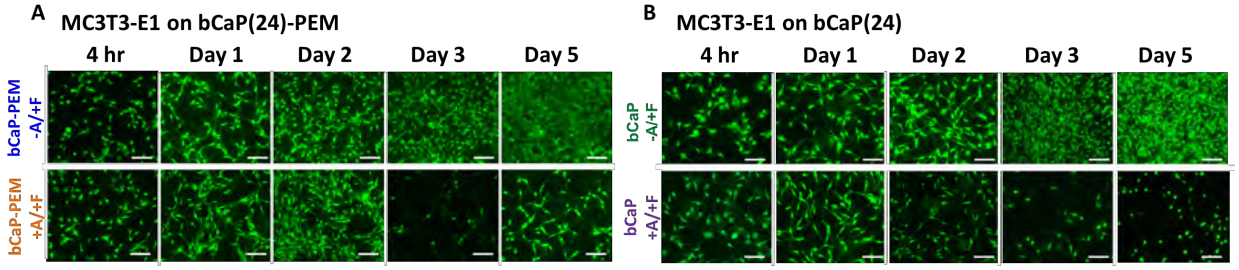


Figure1. Fluorescent LIVE® stained images of (A) MC3T3E1 cells on bCaP-PEM vs. (B) MC3T3E1 cells on bCaP

RAW264.7 cells immediately accessed the embedded AntiA on bCaP(24)-PEM but not until day 3 on bCaP(24) without PEM (Fig.2). SEM revealed a wider crack between the crystal clusters of bCaP after cell culture with aggressive coating degradation by RAW264.7 cells as compared to MC3T3-E1 cells (Fig.3,4).

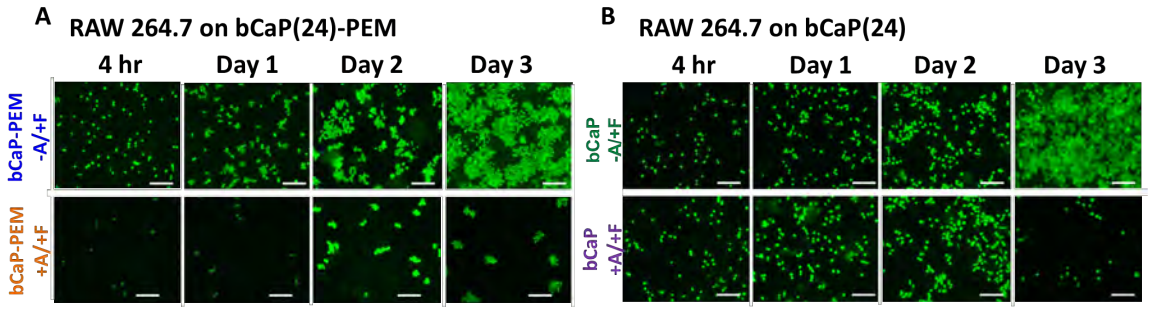


Figure2. Fluorescent LIVE® stained images of (A) RAW cells on bCaP-PEM vs. (B) RAW cells on bCaP.

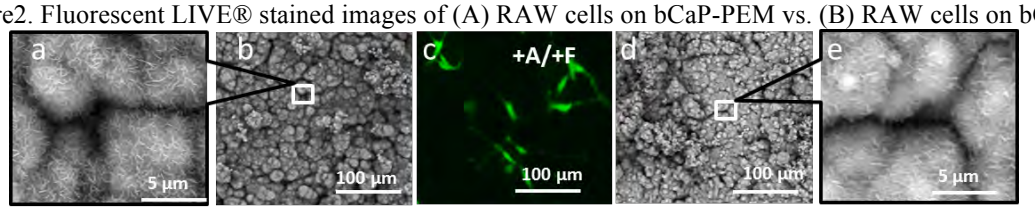


Figure3. a, b) bCap incubated 3 days in cell medium, c) day-3 LIVE staining +A/+F bCaP-MC3T3-E1, d, e) 3 days after culture.

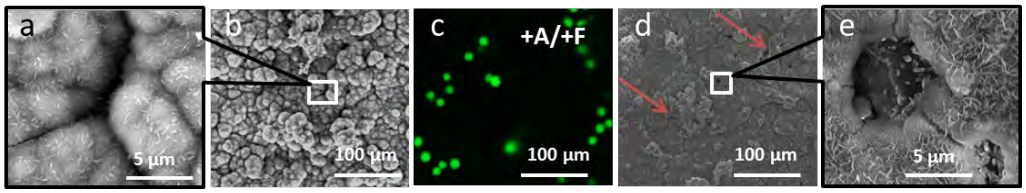


Figure4. a, b) bCap incubated 3 days in cell medium, c) day-3 LIVE staining +A/+F bCaP-RAW, d, e) 3 days after culture.

CONCLUSIONS

The drug delivery profile of bCaP-PEM system is cell mediated and governed by the interaction of cells with the biomaterial rather than typical drug diffusion. Delivery kinetics can be tuned by increasing bCaP coating thickness more effectively than by varying PEM coating thickness. Varying cell type profoundly affected the kinetics of delivery due to (i) the enhanced ability of macrophages over osteoprogenitors to degrade the coating, and (ii) reduced bCaP crystallinity after the PEM coating. A variety of cell types should be used when testing in vitro drug delivery release to better represent in vivo conditions.

Acknowledgements: This work was supported the National Institutes of Health, National Institute of Dental and Craniofacial Research (NIDCR) R01DE021103. The author would like to thank Henry Hrdlicka for providing RAW264.7 cells.

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2. Goldberg AJ et al. J Biomat Sci Polymer. 2010;21:1371-87.

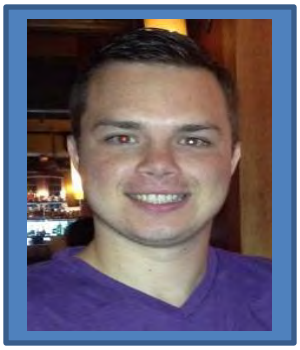
Major Advisor: Liisa Kuhn

Biography:

Education:

University of Baghdad, B.S., Biomedical Engineering, Medical Instrumentation.
University Of California San Diego, M.A., Biomedical Engineering.
Position: PhD Candidate
Year in Program: 2nd
Career Aspirations: My goal is to pursue a career in academia to work on innovative research area and to pass my knowledge to next generation students.

Why UConn Health: UCH have the most breakthrough research especially in the area of bone regeneration. I wanted to be trained by world leader scientist at UCH to further improve my research skills and to translate my research findings to the clinical setting.



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 transduced with an inducible ferroportin construct and assessed for changes in cellular motility. **Results:** Ferroportin overexpression may reduce 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.

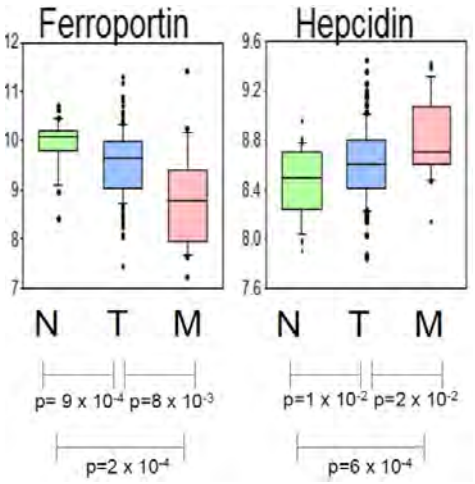


Figure 3. Ferroportin is reduced in prostate cancer patient samples and hepcidin is elevated
Shown are relative microarray transcript levels of normal prostate tissue (N, n=29), primary tumor tissue (T, n= 131), and metastatic tumor tissue (M, n = 19). Ferroportin is significantly reduced in primary prostate cancer and further reduced in metastatic prostate cancer while hepcidin is significantly elevated. 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.

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

Odontoblast processes in developing teeth investigated by serial section electron microscopy

Ninna Shuhaibar[1] and Mark Terasaki[2]

[1]*Department of Skeletal, Craniofacial and Oral Biology, UConn Health Center, Farmington, Connecticut*
[2]*Department of Cell Biology, UConn Health Center, Farmington, Connecticut*

Introduction

The tooth is composed mostly of dentin, the highly mineralized collagenous matrix that supports the enamel. In contrast to the non-living enamel, dentin undergoes growth throughout life and is essential for limiting damage to and protecting the tooth pulp. The odontoblasts are the cells that form and maintain the dentin. They are not located within the dentin, but instead form a layer at the boundary between the pulp and the dentin. The odontoblasts deposit the predentin and subsequently mineralize it around thin, branching cellular processes that extend from the distal end of the cell body throughout the dentin. These are called odontoblast processes, and are present within a dentinal tubule. In the Terasaki lab, we are investigating the three-dimensional structure of the odontoblast processes, using serial section electron microscopy.

Methods

For this study used incisor and molars dissected from 5 week old wildtype mice. We fixed the mice by cardiac perfusion, removed the hemimandibles, cleaned them, and stored them in a decalcifying solution for 4 weeks. After that time, we cross-sectioned the hemimandibles, and split them into 5 segments using the 1st, 2nd and 3rd molar as a reference (Smith and Nanci, 1989). Each segment was processed for 14 days with heavy metals, and then embedded in epoxy resin. We sectioned the block with an ultramicrotome (75 nm thick), collected the sections with a tape collector, and imaged them with a scanning electron microscope. The images were analyzed by 3D reconstruction software (Trak EM 2 /FIJI/ Image J). All procedures involving mice have been approved by the UConn Health Center animal care committee (protocol #100913-0717, Mark Terasaki PI).

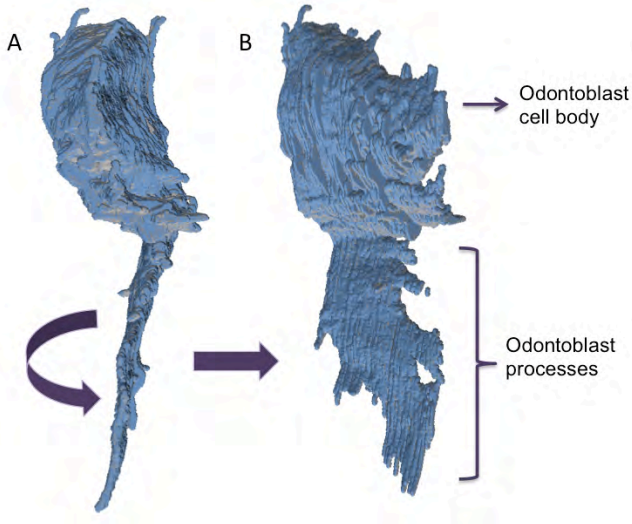


Figure 1. Odontoblast with its process in a 3D reconstruction.
A) Side view. B) Rotated 90 degrees.

Results

Our preliminary results indicate that the shape of the odontoblast process is a plate, and not a tubule as the textbook figures indicate. We found this in the incisor and we are now testing whether this is true in molars as well.

Acknowledgements
This work is supported by:
Training grant NIH/NIDCR 2R90DE022526.

Major advisor and co-advisor:
Dr. Mark Terasaki, PhD. and Dr. Arthur Hand, DDS.

Biography
Education:
University of Zulia, Maracaibo, Venezuela. Dentistry
University of Connecticut, Cell Biology Department, Post-doctoral Fellow.
Position:
R90 Post-doctoral Fellow.
Year in program:
1st year.
Career Aspiration:
After completing my degree in Dentistry in December 2015, I became interested in developing a career as a researcher to complement my clinical training. I have been working as a postdoctoral fellow at UConn Health since July 2016. In the future, I am interested in pursuing an orthodontist degree with the ultimate goal of applying my research skills to help develop new therapies for the improvement of dental health.

Why UConn Health
Because it has a great dentistry department both in the clinical and research and it is also a welcoming environment for people all around the world.

Absent CD13 in combination with a toll like receptor agonist can increase the efficacy of cancer vaccine adjuvants

Veneta Qendro, Mallika Ghosh, Linda Shapiro
University of Connecticut Health
Department of Cell Biology

Introduction: Vaccines have been extraordinarily successful in the fight against human disease by exploiting the immune system to recognize and eliminate abnormal cells or pathogenic organisms. Recent advances in the research of cancer vaccines have led to the development of three FDA-approved anti-cancer vaccines for cervical, liver and prostate cancers . However, despite the great innovation these vaccines represent, they have proven limited to only prophylactic uses, with little to no therapeutic effects (extending patients' life by only a few months- prostate cancer).

Ongoing research in the field of cancer vaccines recognizes that poor immunogenicity is one of the main issues that prevents generation of reliable therapeutic cancer vaccines. Therefore major effort has been dedicated in the identification of efficient cancer adjuvants- the necessary vaccine components that enhance and activate the immune system.

Objective: This project aims to investigate the combination of two molecules, which have independently exhibited ideal individual properties for the generation of vaccine adjuvant; MPLA and CD13.

MPLA (monophosphorylated lipid A), a synthetic analog of LPS shown to vigorously activate the adaptive immune response without triggering deleterious inflammatory consequences, has been recently approved as a component of the prophylactic, HPV-driven (Human Papilloma Virus) cervical cancer vaccine, Cervarix.

On the other hand, absent CD13 – a multifunctional cell surface peptidase constitutively expressed on all lineages of myeloid cells – has been shown to preferentially increase TLR4 endocytosis towards the same endocytic-signaling pathway triggered by MPLA. Additionally, absent CD13 has been shown to increase tumor antigen uptake and presentation, resulting in enhanced activation of tumor-specific cytotoxic T cells. The combination of these facts has led to the intriguing possibility that blocking CD13 may amplify the efficacy of MPLA, and combination of the two could generate the "super adjuvant" needed in cancer vaccines. For this reason, the proposed project is designed to investigate the combinatorial adjuvant-effect of blocked CD13 and MPLA utilizing the HPV-driven cancer model.

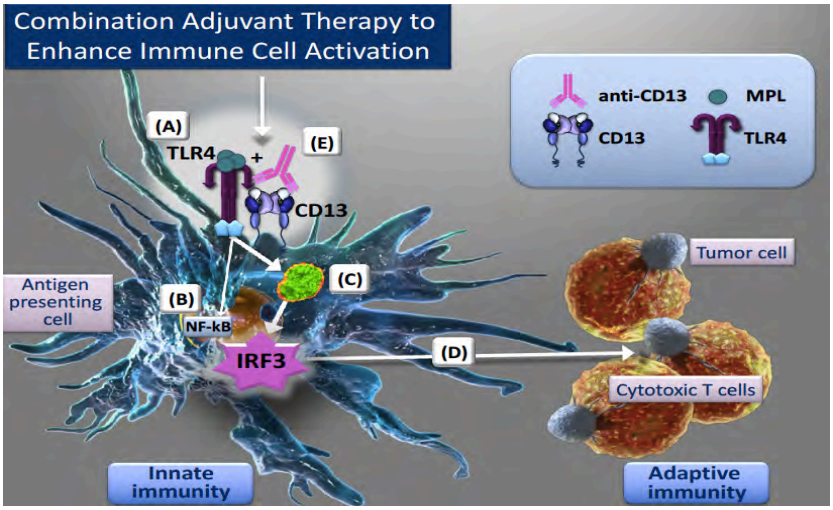
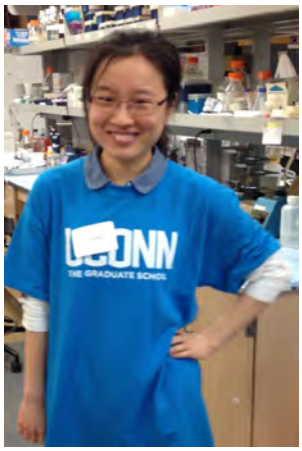


Figure 1. The combination of adjuvant MPLA+ anti-CD13 enhances anti-tumor immunity.
MPLA binding to TLR4 (**A**) does not increase production of (**B**) pro-inflammatory NF-kB dependent cytokines and favors the endocytic signaling pathway (**C**) while activating IRF3 production, which leads to activation of tumor-specific cytotoxic T cell proliferation and tumor cell death (**D**). We propose that treatment with anti-CD13 mAb (**E**) will agument this effect by enhancing TLR4 endocytosis, further boosting the endosomal signaling pathway (**C**) and cytotoxic T cell activation (**D**). In addition, blocking CD13 leads to increased tumor antigen presentation and increased cytotoxic T cell activation.

Acknowledgments: This work is supported by the Public Health Service grant HL127449-01A1 from the National Heart, Lung and Blood Institute and the NIH/NIDCR T90DE021989 training grant.

Major Advisor: Dr. Linda Shapiro

Biography: B. S., Biology **(**University of Natural Sciences in Tirana (Albania)
B. S., Biomolecular Sciences (Central Connecticut State University)
PhD Candidate, Biomedical Sciences, Cell Biology
In the near future I would like to incorporate the knowledge I have gained during my years of research into investigating new pathways of cancer therapy, in the academic or industrial settings.



PDGFRβ signaling regulates osteogenesis of periosteal mesenchymal stem cells.

Xi Wang[1], Brya G Matthews[1], Jungeon Yu[1], Archana Sanjay[1], Danka Grcevic[2], Ivo Kalajzic[1]

1. Department of Skeletal Biology and Regeneration UConn Health, Farmington, CT, USA
2. University of Zagreb, Zagreb, Croatia

Abstract: Fracture repair is a complex process involving the release of growth factors, induction of signaling pathways and their interplay with stem cells. As an important progenitor pool, the periosteum plays an essential role for fracture healing. To understand the mechanisms regulating differentiation of MSCs within periosteum, we isolated and phenotyped cells from the early phases of fracture callus formation. Four days after fracture, over 50% of $CD45^-$ non-hematopoietic cells were $PDGFR\beta^+$. Using mesenchymal stem cell marker αSMA, we found the expression of PDGFRβ was highly enriched in progenitors, with about 90% αSMA^+ cells expressing PDGFRβ. FACS sorted $PDGFR\beta^+$ cells from uninjured periosteum formed significantly more colonies than $PDGFR\beta^-$ cells, indicating enrichment of progenitor cells.

We further investigated the regulation of PDGF/PDGFRβ signaling on periosteal progenitor cells (PPCs). PPCs were isolated and treated with 10ng/ml PDGF-BB. We observed PPCs to be highly responsive to PDGF-BB with activation of multiple downstream signaling including the phosphorylation of Erk1/2, Akt, p38, and PLCγ1. This effect was mainly mediated through PDGFRβ, as cells with PDGFRβ knocked out, some of which still express PDGFRα, were not responsive to PDGF-BB.

Interestingly, PDGF-BB exerted an inhibitory effect on osteogenic differentiation, and dampened BMP2-induced osteogenesis of PPCs. Treatment of PPCs with BMP2 activated canonical Smad signaling but this effect was reduced in the presence of PDGF-BB. Additionally, inhibition of PDGFRβ, with a chemical inhibitor (su16f) rescued the inhibition of pSmad signaling as well as osteogenic differentiation of PPCs in vitro (Figure 1).

PDGFRβ is highly expressed in αSMA labeled periosteal progenitor cells, therefore we conditionally deleted PDGFRβ using αSMA-CreERT2 crossed with PDGFRβ flox/flox mice. We treated isolated PPCs with 4-OH Tamoxifen and induced the periosteal differentiation. ALP staining showed that PDGFRβ deficient cells exhibited significantly enhanced osteogenic differentiation in vitro.

In conclusion, we have demonstrated that PDGF, signaling through PDGFRβ, inhibits BMP2-induced differentiation of PPCs in vitro. In addition, PDGFRβ is widely expressed in progenitor cells that contribute to fracture healing in vivo. It would be interesting to further understand the regulation of PDGF-BB/PDGFRβ signaling on periosteum during bone regeneration.

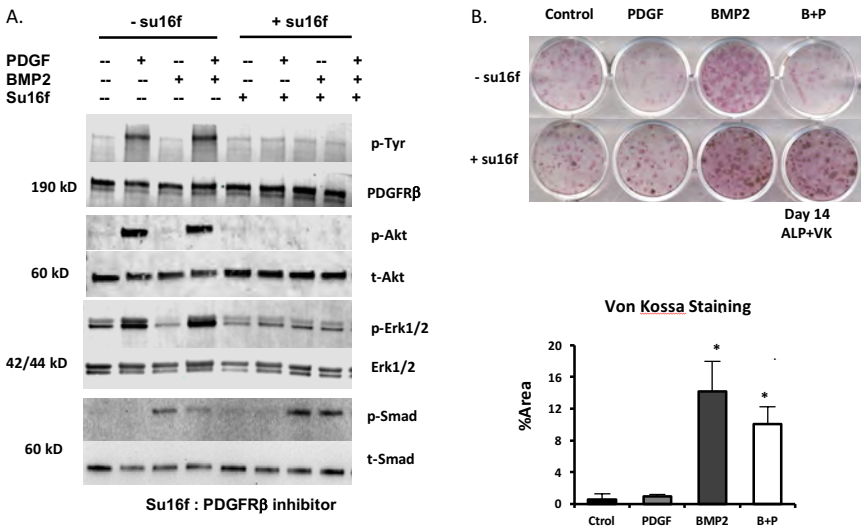


Figure 1. PDGFRβ inhibitor rescues PDGF-BB blocked BMP2-Smad signaling and periosteal differentiation.
A. Western Blots detection of multiple signaling pathways in periosteum derived cells under the treatment of growth factors for 30 mins. B. Osteogenic differentiation of periosteum derived cells after 14 days. PDGF-BB(10ng/ml), BMP2(100ng/ml), su16f (PDGFRβ inhibitor, 5uM)

Acknowledgements: This work was supported by grant R01AR070813.

Major Advisor: Dr. Ivo Kalajzic

Biography:

4th 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 my future career goal is to become a professor in Medical School where I can incorporate my research experiences with clinical practice as a clinician-scientist. I choose the UConn Health graduate program because of the ideal environment with caring faculties and more research opportunities. In the past years, I learned the importance of critical thinking for research, and most importantly the commitment to the career.

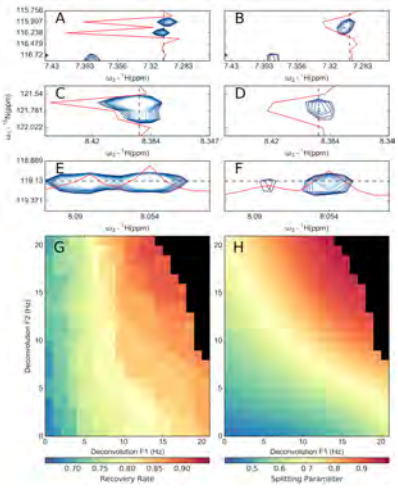
Applications of Receiver Operating Characteristic Analysis in NMR Spectroscopy

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 useful 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. Quantification of sensitivity 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, which scale noise differently from resonances, are becoming popular due to their versatility. One such technique is Maximum Entropy Reconstruction (MaxEnt). MaxEnt is an iterative process that builds a spectrum from scratch, finding the unique spectrum that is consistent with the data and can 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 not applicable making it challenging to assess the quality of MaxEnt spectra MaxEnt. The goal of this work is to develop alternative 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. 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 NIH grants R21GM104517 and P41GM111135, and an NIH predoctoral fellowship to Matt Zambrello (F30DE026353).

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2. Hoch, J. C. & Stern, A. S. Maximum Entropy Reconstruction, Spectrum Analysis and Deconvolution in Multidimensional Nuclear Magnetic Resonance. Methods Enzym. 338, 159-178 (2001).
3. Fawcett T. "An Introduction to ROC Analysis" Pattern Recognition Letters 27, 861-874 (2006)
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 6

Effects of FGF2 on Wnt/β-catenin Signaling in Dental Pulp

Vijaykumar A*; Vidovic I; Rodgers B and Mina M
Department of Pediatric Dentistry, UConn Health, School of Dental Medicine, Farmington, CT

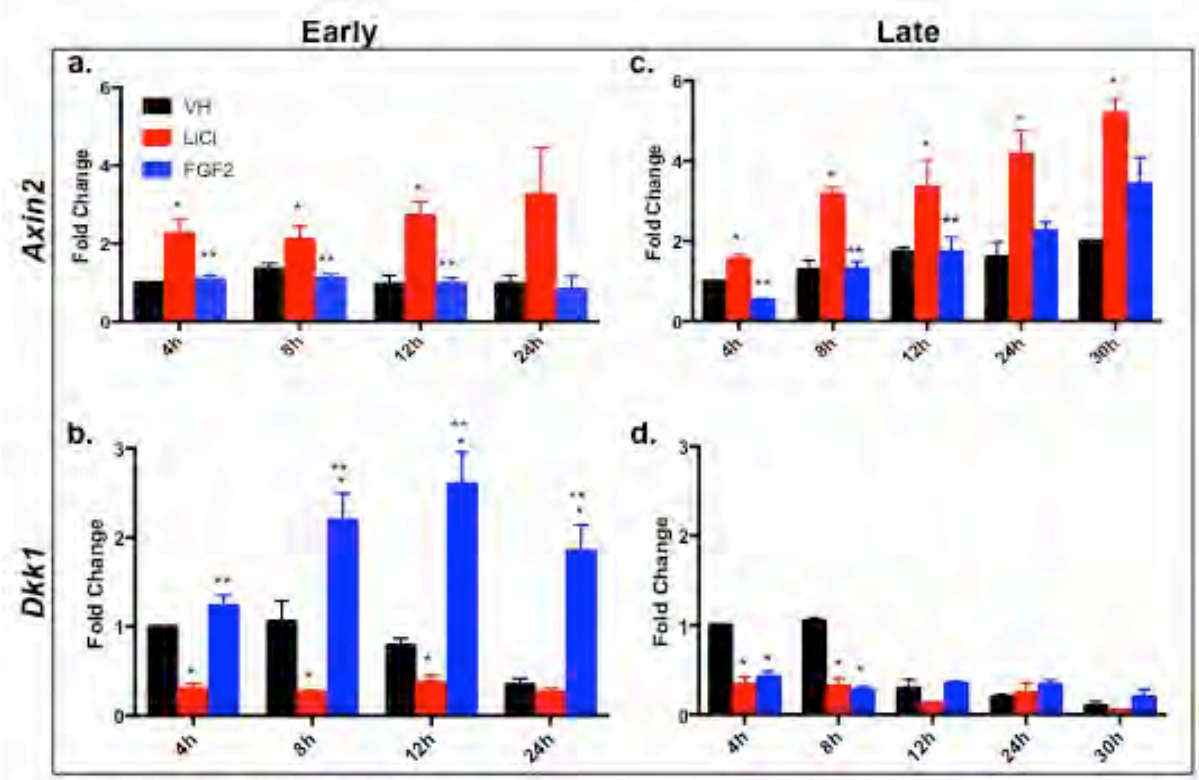
Introduction: We have recently reported that FGF2 promoted the formation of functional odontoblasts from early progenitors but inhibited their differentiation into fully differentiated odontoblasts. These observations indicated that effects of FGF2 on differentiation of dental pulp cells are stage-specific and depended on the stage of cell differentiation/maturity. There is plenty of evidence in literature suggesting a positive as well as negative role of canonical Wnt/β-catenin signaling during odontoblast differentiation. Also, several studies have shown that interaction of FGF and Wnt/β-catenin signaling regulates many aspects of tooth development.

Objectives: To examine if the stage specific effects of FGF2 on dental pulp are mediated through modulating canonical Wnt/β-catenin signaling.

Methods:
We investigated the effects of FGF2 on the canonical Wnt/β-catenin signaling using primary pulp cultures from a novel Wnt/β-catenin transgenic reporter mouse line TCF/Lef:H2B-GFP in which GFP expression acts as a single cell readout for pathway activation. In addition, we analyzed the effects of FGF2 on the expression of *Axin2* and *Dkk1*, a canonical Wnt target gene and antagonist respectively. Cultures were treated with 20ng/ml FGF2 and 10mM Lithium chloride (LiCl) during proliferation (days 3-7) and mineralization (days 7-10) phases of growth. Cells were processed for image analysis, qPCR, and FACS analysis at various time points.

Results:
Our observation showed that addition of LiCl during the proliferation and differentiation phase of culture increased the number of GFP cells, increased the expression of *Axin2* and decreased the expression of *Dkk1* as compared to control. Similar analysis showed that addition of FGF2 during proliferation phase of growth showed no increase in GFP cells and *Axin2* expression, while significantly increasing *Dkk1* expression. During differentiation phase however, FGF2 significantly decreased *Dkk1* expression similar to LiCl while increasing the number of GFP cells and *Axin2* compared to control.

Conclusion:
Our observations provide evidence that the effects of FGF2 on differentiation of dental pulp may be mediated through interaction with Wnt/β-catenin signaling pathway.



Effect of FGF2 treatment on Wnt response genes.
Primary pulp cultures were grown in the presence of VH, 10mM LiCl and 20ng/ml FGF2 during proliferation or Early (a-b) and differentiation or Late (c-d) stages of growth. At various time points cultures were harvested and processed for qPCR analysis. Histograms showing fold changes in expression levels of *Axin2* and *Dkk1* normalized to *Gapdh*. Note that, Early and Late LiCl treatment increased *Axin2* (a,c) while decreasing *Dkk1* expression (b,d) significantly compared to VH as early as 4h after treatment. Early FGF2 treatment, however shows no changes in *Axin2* expression (a) compared to control while significantly increasing *Dkk1* expression (b) compared to VH and LiCl. Late FGF2 treatment on the other hand, shows increased *Axin2* expression (c) compared to control 24h onwards (not significant) and shows significant decrease in *Dkk1* to levels comparable with LiCl (d). n≥3; $p<0.05$ *compared to VH **compared to LiCl

Supported by R01-DE016689 and T90-DE021989.

Major Advisor: Dr. Mina Mina

Biography:
BDS, Mumbai India
MS, University of Medicine & Dentistry New Jersey (Rutgers School of Dental Medicine)
5th 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.

Specification of Sclerotome Cells via Axial Skeletal Lineage Differentiation of Pluripotent Stem Cells

Ryan P. Russell[1] and Peter Maye[1]

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

INTRODUCTION: Pluripotent stem cell technologies are continually advancing the 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 stem cells into mature, functional skeletal cell types remains a necessity. Our research employs a stepwise, embryonic differentiation strategy to produce skeletal progenitors via paraxial mesoderm and sclerotome intermediates. Such axial skeletal lineage derivatives possess vast therapeutic utility owing to a capacity to form mature cells including chondrocytes, osteoblasts and tenocytes. We have directed human ESCs and iPSCs into paraxial mesoderm and sclerotome by targeting signaling pathways critical for embryonic lineage specification. This approach, coupled with diagnostic readouts from transgenic reporter cell lines, including that for TBX6, a key regulator of paraxial mesoderm specification, and MEOX1, a transcription factor critical for somitogenesis and sclerotome specification, demonstrates the initial stages in our axial skeletal differentiation protocol.

METHODS: In-vitro differentiation was performed using WNT3a and CHIR99021 (WNT pathway stimulation), FGF2 (FGF stimulation), AGN193109 (retinoic acid receptor inhibition), and LDN193189 (BMP inhibition) to promote paraxial mesoderm formation, and with SAG (Hedgehog agonist) and LDN to promote subsequent sclerotome maturation. These strategies were tested on an H9 ESC TBX6-mCherry/UbiquitinC-Citrine reporter line and an HDFa-YK27 iPSC MEOX1-Citrine reporter line to facilitate diagnostic analyses using fluorescent reporter expression.

RESULTS: Evaluation of paraxial mesoderm formation in H9-TBX6 cells revealed WNT3a +CHIR combined with AGN for 4 days resulted in a considerable increase in TBX6 reporter expression confirmed by FACS sorting: 26.9% TBX6+ to <1% without AGN. Increased endogenous TBX6 (26-fold), Mesogenin (30-fold), and MEOX1 (18-fold) expression in the TBX6+ sorted population was shown by RT-PCR, indicating strong paraxial mesoderm induction with comparable reporter and endogenous gene expression. YK27-MEOX cells were used to assess ensuing sclerotome specification. Primary factors (FGF2, WNT3a, CHIR, AGN) with delayed LDN, followed by SAG+LDN treatment were tested. Gene expression revealed an initial FGF +WNT+CHIR+AGN strategy resulted in a 10-fold increase in PAX1 and 4-fold increase in PAX9, both key sclerotome markers, compared to FGF+CHIR alone, while including delayed LDN resulted in further enhancement of PAX1 (23-fold) and PAX9 (7-fold).

CONCLUSIONS : Specification of sclerotome from human stem cells was optimized by combined FGF and WNT stimulation with retinoic acid receptor and BMP inhibition during the paraxial mesoderm differentiation phase. Initial differentiation conditions appear to have a tremendous influence on downstream cell fate and may ultimately dictate therapeutic functionality. Thus, simulating embryonic lineage determination of the axial skeleton may afford a reproducible roadmap for generating several clinically relevant skeletal progenitor cell types in the future.

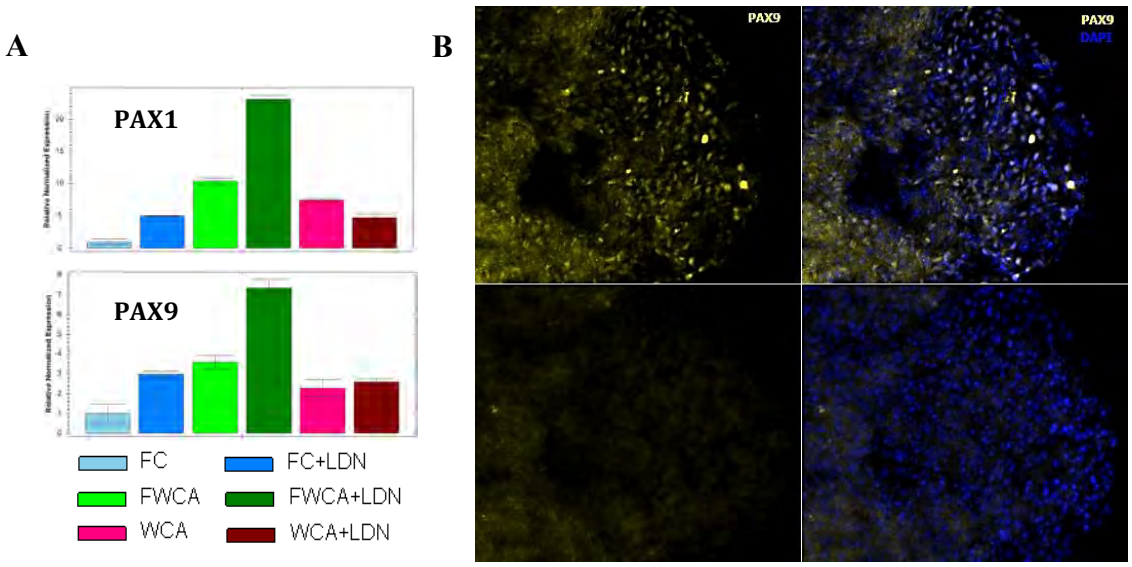


Figure 1. PAX expression in iPSCs differentiated towards sclerotome. [A] Day 14 *PAX1* and *PAX9* gene expression. Cultures were treated with the indicated factors for 7 days, with or without BMP inhibition via LDN193189 from day 4. All cultures were treated with SAG (Hedgehog agonist) and LDN from days 7 to 14. F, FGF2; W, Wnt3a; C, CHIR99021; A, AGN193109. [B] PAX9 staining in iPSC condensations. Sclerotome-like cells were passaged in SAG+LDN and treated with FGF2 and BMP4 to promote chondrogenic maturation. Spontaneous cell condensations were sectioned and stained with PAX9 antibody and DAPI. Lower panels show adjacent section as control with only secondary antibody staining and DAPI.

Acknowledgements
This work was supported by:
State of Connecticut Grant #: 13-SCA-UCHC-02 Maye
National Institutes of Health/NIAMS Grant#: R21AR056391
NIH/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: 4th

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 UConn Health: 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 UCH. 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.

Global and Chondrocyte-specific Deletion of FGF2 Contribute to Trauma-induced Osteoarthritis

Patience Meo Burt[1], Liping Xiao[1], Siu-Pok Yee[2], Brya G. Matthews[3] and Marja Marie Hurley[1]

[1]Department of Medicine/Endocrinology, School of Medicine, [2]Department of Cell Biology, [3]Department of Reconstructive Sciences, School of Dental Medicine, UConn Health, Farmington, CT

Osteoarthritis (OA) is the most common degenerative joint disease, but the molecular mechanisms that contribute to its initiation and progression are not well defined. Studies showed that global Fgf2 knockout (*Fgf2*$^{-/-}$) mice spontaneously develop severe and accelerated OA by 6 months of age. However, it is unclear whether it is the absence of FGF2 in cartilage or bone that contributes to OA development. Since FGF2 may be chondroprotective, we posit that global *Fgf2*$^{-/-}$ mice and chondrocyte-specific FGF2 knockout mice will develop OA following tibial compression loading compared to their WT littermates, and joints from *Fgf2*$^{-/-}$ mice will have modifications of gene expression of pathways involved in hypertrophic chondrocyte signaling before the OA phenotype occurs. To examine if changes in these genes are occurring before *Fgf2*$^{-/-}$ mice develop OA, total RNA was isolated from the knees at 2 months old and qPCR analysis showed significant increases in genes involved in chondrocyte hypertrophy including Adamts5, Elk1, Ihh, Il-1b, Nfkb1, Bmp2, Bmp4, BMPR1a, Hif2a, and Igf-1 in *Fgf2*$^{-/-}$ mice compared to WT. We also generated a novel inducible chondrocyte-specific Fgf2 knockout mouse (Col2creERT2$^{Tg/+}$**:**Fgf2$^{fl/fl}$), which were subjected to tamoxifen induction at 2 months old, followed by tibial loading 2 weeks later using our novel noninvasive mechanical loading model. Mice were sacrificed 2 weeks after loading, and x-ray, microCT, Safranin-O staining, and immunohistochemistry were performed. Chondrocyte-specific Fgf2 knockout mice expressed FGF2 protein in bone but not articular cartilage. Loaded right knees of Col2creERT2$^{Tg/+}$**:**Fgf2$^{fl/fl}$ mice showed osteophyte formation, joint space narrowing, sclerotic bone, eroded surface, fraying of cartilage, decreased superficial layer of cartilage and loss of proteoglycan content compared to the controls (Fig. 1). Expression of the degradative enzyme, MMP13, was enhanced in loaded right knees of Col2creERT2$^{Tg/+}$**:**Fgf2$^{fl/fl}$ mice compared to left and control mice (Col2creERT2$^{+/+}$**:**Fgf2$^{fl/fl}$). These results indicate that signaling pathways involved in hypertrophic chondrocytes are enhanced in phenotypically normal joints from young *Fgf2*$^{-/-}$ mice, implying that these pathways are initiating OA. Chondrocyte-specific deletion of Fgf2 *in vivo* leads to OA after mechanical loading. This data suggests that the presence of FGF2 in cartilage is vital for joint homeostasis and its absence leads to chondrocyte hypertrophy and OA development.

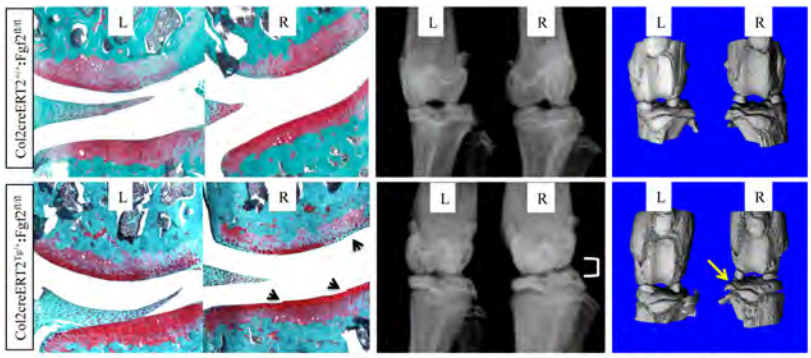


Fig. 1: Chondrocyte-specific FGF2 knockout mice develop an OA phenotype following tibial loading.
L=left non-loaded control knee, R=right tibial loaded knee; arrows=fibrillated cartilage; bracket=joint space narrowing; yellow arrow=erosion/pitting.

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Major Advisor: Dr. Marja Hurley

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

Currently, I am a 4th year Ph.D. candidate in the Biomedical Science program. I chose UConn Health due to the outstanding skeletal biology program and research opportunities that combine basic science and human health. In the future, I hope to continue to incorporate this translational component in my scientific career.

Investigating the impact of miR-29 regulation on E-cadherin expression during osteoclastogenesis

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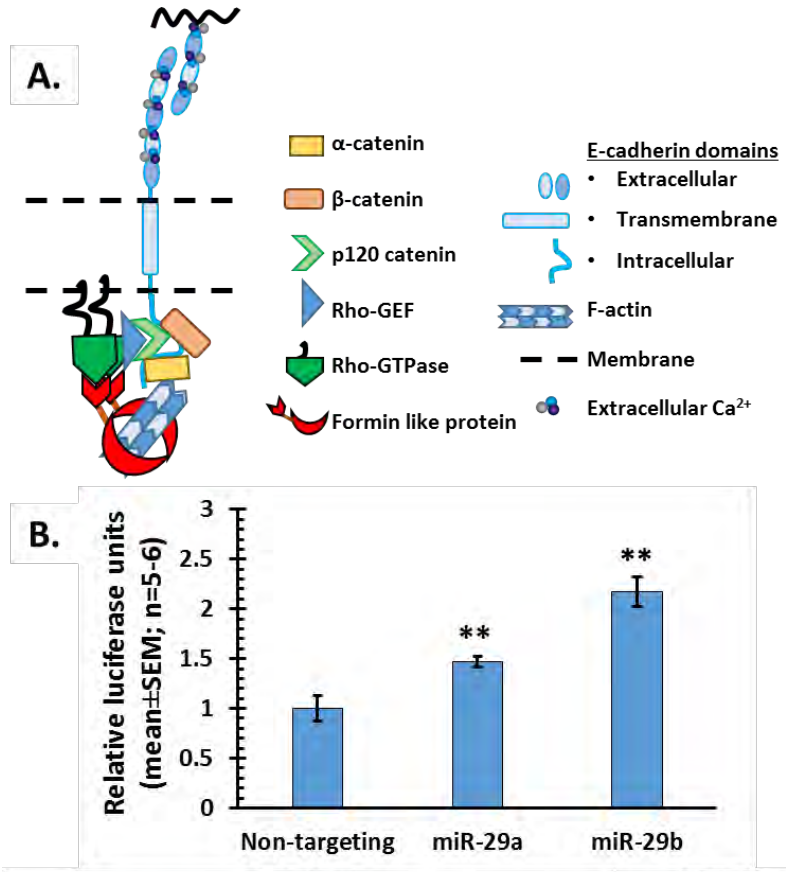
Abstract: miR-29 family members are positive regulators of osteoclastogenesis. Their inhibition results in decreased pre-osteoclast motility, and the formation of smaller and fewer osteoclasts. Two members of the Rho-GTPase family have been validated to be either direct (Cdc42) or indirect (Rac1 via srGAP2) miR-29 targets. These molecules positively regulate actin remodeling needed for osteoclast motility, polarization, and function as osteoclasts migrate, adhere to the bone surface, and resorb the bone matrix. In other cell-types, i.e. epithelial cells, Rac1 and Cdc42 facilitate E-cadherin function leading to strong cell-cell interactions.

E-cadherin signaling is mediated through α-, β-, and p120 catenins. The catenins attract growing F-actin chains by recruiting actin remodeling proteins, such as Formin-like protein 1 (Fmnl1) and Fmnl2, and their activators, the Rho-GTPases, to the sites of cell-cell contact (**Figure 1A**). In osteoclasts, E-cadherin is known to promote early stages of osteoclastogenesis by facilitating pre-osteoclast fusion, supposedly by creating strong cell-cell interactions between precursors. Using bioinformatics analysis, we observed that several molecules involved in E-cadherin signaling are either putative miR-29 targets, or previously validated targets. These include: Cdc42, srGAP2, E-cadherin, β-catenin, p120 catenin, and Fmnl2. The purpose of our current study is to determine the impact of miR-29 regulation on E-cadherin signaling.

E-cadherin itself is a predicted miR-29 target. To determine whether E-cadherin is a true miR-29 target, we cloned the E-cadherin 3' UTR, containing two predicted miR-29 binding sites, into the pMIR-REPORT luciferase construct. When the E-cadherin luciferase construct was co-transfected into RAW264.7 cells, along with a β-galactosidase control construct and miR-29a or miR-29b inhibitor, we observed a 2 fold increase in luciferase activity ($p<0.001$; $n=5$-6) (**Figure 1B**). This suggests that the E-cadherin 3' UTR is targeted by miR-29 family members.

Expression of miR-29 family members increases during the course of osteoclast differentiation *in vitro*. Using cultures of mouse bone marrow macrophages, we found that E-cadherin protein levels decreased during osteoclastogenesis. This demonstrates an inverse correlation between miR-29 and its target, E-cadherin. It is possible miR-29 family plays a role in regulating E-cadherin levels during osteoclast differentiation.

miRNAs are known to regulate multiple molecules within a pathway, to modify cell phenotypes. While our present data validate E-cadherin as a novel miR-29 target, other components of the E-cadherin signaling network may also be miR-29 targets. Our future studies will be focused on validating additional novel miR-29 targets and evaluating the impact of miR-29 regulation on E-cadherin signaling. Overall, we hypothesize the miR-29 family fine-tunes E-cadherin signaling and its dysregulation may contribute, in part, to the decreased osteoclast number and size associated with miR-29 inhibition.



ALIGNMENTS	ALIGNMENT SCORES
3' auuGGCUAAAGUCUACCA-CGAu 5' mmu-miR-29a : |:| | |: ||||| ||| 1004:5' ugcCUGCUCUU-GAUGGUAGCUa 3' Cdh1	mirSVR score: -0.5429 PhastCons score: 0.5557
3' auuggcuaaagucuaCCACGAu 5' mmu-miR-29a :: || |||||| 1511:5' gaguguaugugugugGGUGCUg 3' Cdh1	mirSVR score: -0.0757 PhastCons score: 0.6486

Figure 1. E-cadherin (Cdh1) is a miR-29 target.
A. **Model of the E-cadherin signaling complex, depicting the accumulation of F-actin and actin remodeling proteins at sites of E-cadherin interactions. GEF, Guanine nucleotide exchange factor.**
B. **Activity of the E-cadherin 3' UTR luciferase construct is derepressed by miR-29 inhibitors. **, significantly different from non-targeting, $p < 0.01$, (representative experiment). The Table shows predicted miR-29 binding sites in the E-cadherin 3' UTR.**

Acknowledgements: NIH R01-AR064867 and NIDCR T90-DE021989
Major Advisors: Anne M. Delany and Sun-Kyeong Lee
Biography:
Education
Nebraska Wesleyan University, 2014, B.S., Biochemistry and Molecular Biology
Position
Third year, Skeletal Biology and Regeneration PhD candidate
Why UConn Health
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.
Career aspiration
Upon completion of the program, I aspire to stay in academia where I will divide my time between teaching and training the next generation of students and pursuing my own research interests in skeletal autoimmune conditions, such as rheumatoid arthritis.

Skeletal Biology & Regeneration Student Awards

Thanks to the generosity of the Skeletal Biology and Regeneration program's faculty, students and alumni, and the support of Dean MacNeil and the School of Dental Medicine, two Student Awards have been established by the Skeletal Biology and Regeneration Alumni Committee.

William B. Upholt Student Achievement Award

On July 30, 2016 we lost an outstanding scientist, and one of our most impactful mentors and leaders in the Skeletal Biology and Regeneration PhD program, when Dr. Bill Upholt, Emeritus Professor, passed away from illness. Bill received his B.S. in Chemistry from Pomona College, and his Ph.D. in Chemistry from the California Institute of Technology. After two postdoctoral fellowships and a faculty post at the University of Chicago, in 1985, Bill joined UConn Health's Department of BioStructure and Function in the School of Dental Medicine (which he subsequently led as Chair from 1999-2004).

The research program Bill established and led for nearly 30 years at UConn Health focused on the genetic regulation of cartilage development and skeletal patterning. Bill was a pioneer in bringing molecular biology into this field, and his seminal work identified the intron/exon arrangement and key functional elements of the type II collagen promoter, which drives expression of the most important protein in cartilage tissue. Subsequently, he tackled the mystery of how genes are regulated during development and evolution to control the position and formation of the distinct skeletal elements of the limbs and face.

Bill's legacy includes his contributions and leadership in graduate and post-graduate education. He was the Director of the Developmental Biology PhD Program from 1991-1995, and the Director of the Skeletal, Craniofacial and Oral Biology PhD Program (now renamed the Skeletal Biology and Regeneration program) from 1996-2008. He was also the Program Director of the UConn Dentist Scientist Program from 1993-2004, and was co-Director of UConn's NIDCR-funded Skeletal, Craniofacial and Oral Biology Training Grant from 2001-2011.

It is possible however, that Bill might most prefer to be remembered for his extraordinary humanitarian and conservation activities. As a tireless advocate for social justice and for the environment, his efforts have lasting impact. Bill's commitment to science, knowledge, mentorship and a life of service sets both an example, and a lofty bar.

The first recipient of the *William B. Upholt Achievement Award* will be named in 2018.

Student Travel Award.

Two Travel Awards will be available per year, details to be finalized.

Please note that the recent donations to the Skeletal Biology and Regeneration program have also made possible continuation of the program's Seminar Series throughout the year. The Skeletal Biology and Regeneration Program and Alumni Committee are grateful for this support.

Ophir Klein is a Professor of Orofacial Sciences and Pediatrics, the Larry L. Hillblom Distinguished Professor in Craniofacial Anomalies, and the Charles J. Epstein Professor of Human Genetics at the University of California, San Francisco (UCSF). He serves as Chief of the Division of Medical Genetics, Chair of the Division of Craniofacial Anomalies, and Director of the Program in Craniofacial Biology. Dr. Klein was educated at the University of California, Berkeley, where he earned a B.A. in Spanish Literature. He subsequently attended Yale University School of Medicine, where he received a Ph.D. in Genetics and an M.D. He then completed residencies at Yale-New Haven Hospital in Pediatrics and at UCSF in Clinical Genetics. Dr. Klein has received several honors, including a New Innovator Award from the NIH, the E. Mead Johnson Award from the Society for Pediatric Research, and the Craniofacial Biology Distinguished Scientist Award from the International Association for Dental Research. Dr. Klein was elected to the American Society for Clinical Investigation, and he is a Fellow of the American Association for the Advancement of Science. Dr. Klein's research focuses on understanding how organs form in the embryo and how they regenerate in the adult, with a particular emphasis on the processes underlying craniofacial and dental development and renewal as well as understanding how stem cells in the intestinal epithelium enable renewal and regeneration.