CDMI

CENTER FOR DISRUPTIVE MUSCULOSKELETAL INNOVATIONS

Integrated in vivo and in vitro high-throughput analyses of osteocyte-mediated bone remodeling

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Key Findings and Recommendations for Future Studies

We have completed the proposed studies and now have a suite of in vitro and in vivo assays for the qualitative and quantitative analysis of osteocyte-mediated bone remodeling (OMBRE) available in the UCSF Skeletal Biology and Biomechanics Core. These services are available on a recharge basis for CDMI members. In addition, our validated protocols are available to CDMI members upon request.

Motivation: Need and Industrial Relevance

Need: Understanding the control of bone mass has yielded essential therapies to treat bone fragility, including those that target parathyroid hormone or sclerostin ***(Figure 1)***. However, *at least half of people with fragility fractures do not have clinically low bone mass, pointing to the importance of bone quality*.

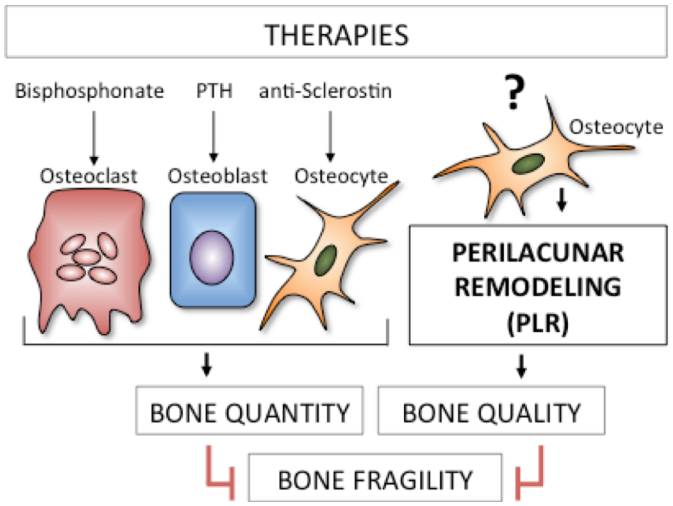

Figure 1: *Schematic of therapies targeting cellular participants in bone remodeling to improve skeletal health.*

A key aspect of bone quality is the material quality of bone extracellular matrix (ECM). Defects in the material quality of bone contribute to bone fragility in aging, diabetes, and other conditions. The deterioration of bone quality has significant orthopaedic and dental implications, including the integration of implants and osteonecrosis. Currently, *therapies for bone quality simply do not exist*.

Agents that control bone quality have great and untapped therapeutic potential for treating skeletal disease or for improving orthopaedic implant fixation. Because the protection and improvement of bone quality could be clinically transformative, *our long-term goal is to discover new diagnostic markers for bone quality and new therapies to treat it.*

Industrial Relevance: Toward the long-term goal of identifying novel therapies to improve bone quality and prevent bone fragility, this project aims to develop a novel, integrated panel of in vivo and in vitro assays to monitor the cellular control of bone quality by osteocytes, called OMBRE.

When implemented, OMBRE will support industry and basic science in the implementation of high-throughput screens (HTS) and performance of pre-clinical analyses to identify compounds that improve bone quality systemically or locally near orthopaedic devices.

Background

Bone fragility is frequently associated with a low bone mass phenotype, such as osteoporosis. Current therapies for preventing bone fragility aim to increase bone quantity by targeting osteoblasts, bone forming cells, and/or osteoclast, bone resorbing cells. However, bone fragility can occur in individuals with normal bone mass, suggesting that bone quality also contributes to bone fragility.

Osteocytes, mature osteoblasts embedded within the bone matrix, sense and respond to a variety of biological and physical cues in a number of ways. These cells have an endocrine function, releasing factors such as FGF23 to control mineral homeostasis, and are also central to mechanosensation and the anabolic response of bone to mechanical load. Osteocytes control the bone remodeling activity of osteoblasts and osteoclasts by the expression of SOST and RANKL, respectively. In addition, osteocytes

have more recently been shown to directly remodel the bone perilacunar-canalicular matrix. Osteoctyes release proteases and acid in response to a variety of biological cues, including parathyroid hormone and TGFβ. Through this process, called perilacunar-canalicular remodeling (PLR), osteocytes resorb and then replace local bone matrix. PLR is essential for the maintenance of mineral homeostasis and the canalicular network. We found that through PLR, osteocytes regulate bone quality. Thus, PLR is a central homeostatic mechanism by which bone maintains biological and mechanical homeostasis. Consequently, PLR is disrupted in various skeletal diseases, including osteonecrosis, and likely others. We anticipate that therapeutically targeting PLR will become a powerful approach to restore skeletal health in a variety of conditions in which deregulation of PLR plays a causal role in disease progression. To that end, appropriate outcome measures of PLR are needed to determine the role of osteocyte-mediated bone remodeling in skeletal health and in disease. We therefore aimed to identify appropriate outcomes to measure PLR.

Aims

This project promotes the development of new therapies to improve bone quality and prevent bone fragility by streamlining the analysis of perilacunar remodeling by osteocytes ***(Figure 1)***. **Perilacunar remodeling (PLR)** is a dynamic process by which osteocytes dynamically resorb and then replace the local bone matrix. Recent studies by our group and others reveal that PLR maintains bone quality, systemic mineral homeostasis, and the lacunocanalicular network. Our ongoing studies strengthen the causal link between PLR, bone quality, and diseases ranging from osteoarthritis and diabetes to osteonecrosis and aging. Nonetheless, many questions remain about the regulation and role of PLR in skeletal health and disease, in part, because reliable outcome measures to effectively study PLR are still needed.

Here we developed a comprehensive approach to evaluate PLR in vivo and in vitro, ultimately to advance the development of therapies to improve bone quality. Specifically, we aim to:

Aim 1: Develop in vitro measures of PLR function for high throughput screening.

Based on PLR-dependent changes in osteocyte pH and bone matrix resorption, we will develop and validate in vitro fluorescent PLR assays that can be used in high-throughput screens to identify novel PLR-regulatory compounds.

Aim 2: Establish the Osteocyte-Mediated Bone Remodeling ECM (OMBRE) Core.

The OMBRE Core will provide novel, streamlined in vitro and in vivo analysis of PLR for basic, pre-clinical, or clinical studies.

When implemented, the OMBRE Core will support scientists, including those affiliated with the CDMI, by providing PLR analyses as they seek to identify new therapies to improve bone quality systemically or locally near orthopaedic devices.

Methods

Aim 1: Develop in vitro measures of PLR function for high throughput screening

We developed a novel high-throughput screen to identify compounds that regulate osteocyte-mediated PLR. We will now work in partnership with the UCSF Small Molecule Development Center (SMDC), led by Dr. Michelle Arkin, to validate, and ultimately, perform this screen.

Based on their utility in our previous studies, we have used MLO-Y4 osteocyte-like cells for our primary screens. We have developed two functional in vitro PLR outcomes, namely intracellular pH and the expression of a prototypical PLR genes, namely MMP2, MMP13, MMP14, and Cathepsin K. Intracellular pH is measured using a fluorescent SNARF dye, which shows a drop in pH as osteocytes acidify their microenvironment to induce PLR.

Aim 2: Establish the Osteocyte-Mediated Bone Remodeling ECM (OMBRE) Core.

The OMBRE Core has developed a series of protocols to provide a comprehensive approach to evaluate the role and regulation of osteocyte-mediated perilacunar remodeling in bone health and disease. This Core has integrated this suite of rigorous histologic, cellular and molecular analyses for in vitro studies and for in vivo analysis of specimens from mouse, rat, and human bone. Each of the protocols was validated in a system of PLR deregulation.

Results

Aim 1: Develop in vitro measures of PLR function for high throughput screening.

We have completed five final protocols for the OMBRE core, which are provided as a core service through recharges available on the updated website on the UCSF CCMBM website. The five finalized OMBRE protocols are: I. Collagen Organization II. Lacunocanalicular Analysis III. PLR Gene Expression IV. In Vitro PLR Taqman Assay V. In Vitro Functional pH Assay. These OMBRE protocols are optimized towards developing a high throughput screening method for measuring PLR.

The first OMBRE core protocol, I. Collagen Organization, reveals birefringences of collagen matrix under polarized light microscopy and collagen orientation quantitative analysis can be obtained, which was validated in TGF Beta Receptor II (TBRII) osteocyte specific cell knockout mice. The Lacunocanalicular analysis protocol provides a histological analysis on the lacunae and canaliculi within the bone. Quantification on lacunocanalicular area and canalicular length was used to reveal decreased percent lacunocanalicular area and canalicular length in TBRII osteocyte specific cell knockout mice. Using the TBRII osteocyte specific cell knockout mice, we validated the PLR gene expression protocol, which revealed down regulated PLR gene expression. This suggests that PLR genes (Mmp2, Mmp13, Cathepsin K, Acid phosphatase 5, MMP14) are involved in PLR in vivo. The genes involved in PLR in vivo were also validated using OCY454 cells, an osteocyte cell line. When treated with TGF beta, PLR gene expression was significantly increased. We can therefore use taqman array plates to screen genes involved in PLR. In the fifth OMBRE core protocol, In Vitro Functional pH Assay, we showed that PLR is associated with acidification that can also be detected colorimetrically, revealing both quantitative and qualitative methods in our assay.

Aim 2: Establish the Osteocyte-Mediated Bone Remodeling ECM (OMBRE) Core.

Our established OMBRE protocols are incorporated in the OMBRE core and are provided as an OMBRE service accessible for CDMI members through the UCSF Skeletal Biology and Biomechanics Core and external users. Recharges for the core services were approved and are available on the updated

website for internal and external users. OMBRE core protocols are also available on Airtable for easy access to anyone who wants to use them.

Discussion and Conclusions

The OMBRE protocols were validated and established using in vivo and in vitro methods. These protocols were incorporated in our OMBRE core services and provided as recharge services available for internal and external users. Currently, some users that have utilized our OMBRE core services are from UC Davis, Buck Institute, Cleveland Clinic, and industry users such as Orthofix.

Timeline (for those that have yet to finish)

Not applicable.

Recommendations for Future Studies

We have developed OMBRE protocols that are accessible as a core service and are further adapting some of these assays towards a high throughput screen. We next aim to develop and validate this high throughput screen with our collaboration with the UCSF Small Molecule Development Center. We aim to screen current FDA-approved drugs that can be repurposed as OMBRE-regulators for treating skeletal disease.

References

List of Presentations and Publications

List all presentations (formal or informal – other than the CDMI updates) and publications (abstracts and manuscripts) that have come out of this CDMI project

Publications

Fowler TW, Acevedo C, Mazur CM, Hall-Glenn F, Fields AJ, Bale HA, Ritchie RO, Lotz JC, Vail TP, **Alliston T**. (2017) Glucocorticoid suppression of osteocyte perilacunar remodeling is associated with subchondral bone degeneration in osteonecrosis. *Scientific Reports*. 7:44618. PMICD: PMC5361115

Dole NS, Mazur CA, Acevedo C, Lopez JP, Monteiro DA, Fowler TA, Gludovatz B, Walsh F, Regan JN, Messina S, Evans DS, Lang TF, Zhang B, Ritchie RO, Mohammad KS, **Alliston T**. (2017) Osteocyte intrinsic TGFβ signaling regulates bone quality through perilacunar remodeling. *Cell Reports*. 21(():2585-2596. PMID: 29186693

Abstracts

Fowler TW, Acevedo C, Mazur CA, Hall-Glenn F, Fields A, Bale H, Ritchie RO, Lotz J, Vail T, **Alliston T.** Osteocyte-Driven Perilacunar Remodeling is Impaired in Glucocorticoid Induced Osteonecrosis. *American Society for Bone and Mineral Research 2016 Annual Meeting*; 2016 September 16-19; Atlanta, GA, Abstract 182

*****Dole NS, Mazur CA, Acevedo C, Fowler TW, Lopez J, Monteiro DA, Woo JJ, Mohammad KS, **Alliston T**. (2017) Novel Role of TGFβ in Osteocytes: Regulation of Perilacunar Remodeling and Bone Quality. *Orthopaedic Research Society 2017 Annual Meeting*; 2017 March 19-22; San Diego, CA, Abstract 32, New Investigator Recognition Award.

*****Mazur CA, Dole NS, Acevedo C, Gludovatz B, Ritchie RO, **Alliston T.** (2017) TGF-beta Regulates Fracture Toughness via Osteocytes. *Orthopaedic Research Society 2017 Annual Meeting*; 2017 March 19-22; San Diego, CA, Abstract 270.

*****Dole NS, Mazur CA, Acevedo C, Fowler TW, Lopez J, Monteiro DA, Woo JJ, Mohammad KS, **Alliston T**. (2017) Osteocyte intrinsic TGFβ signaling in regulates bone quality through perilacunar remodeling. *American Society for Bone and Mineral Research 2017 Annual Meeting*; 2017 September 8-11; Denver, CO, Abstract 1141, Young Investigator Award.

*****Dole NS, Mazur CM, Acevedo C, Lopez J, Monteiro DA, Gludovatz B, Ritchie RO, Mohammad KS, **Alliston T**. (2017) TGFβ regulation of osteocytic perilacunar remodeling is crucial for maintaining bone quality. *Orthopaedic Research Society 47th International Musculoskeletal Biology Workshop*; 2017 August 6-9; Sun Valley, ID, ASBMR Harold M. Frost Young Investigator Award.

*****Acevedo C, Stadelmann VA, Pioletti DP, Ritchie RO, **Alliston T**. (2017) The connection between fatigue and fragility fracture in bone. *Orthopaedic Research Society 47th International Musculoskeletal Biology Workshop*; 2017 August 6-9; Sun Valley, ID, ASBMR Harold M. Frost Young Investigator Award.

Mazur CA, Dole NS, Acevedo C, Gludovatz B, Ritchie RO, **Alliston T.** (2017) TGF-beta Regulates Fracture Toughness via Osteocytes. *CCMBM Department of Orthopaedic Surgery Scientific Retreat*; 2017 September 13; San Francisco, CA, Best Poster Award.

Woo JJ, Fields A, Lotz J, Kuo A, Vail T, Alliston T. (2017) Evidence of Disrupted Subchondral Bone Osteocyte Function in Post-Traumatic Osteoarthritis. *Military Health Services Research Symposium*; 2017 August 27-30; Kimmissee, FL.

- Represents Oral Presentations.

Intellectual Property

Title(s) of IP Disclosure(s)

Not applicable.

Patent Title(s), Stage of Submission, and Patent # (if applicable)

Not applicable.

Appendix

Please contact Tamara Alliston for a link to the Airtable with detailed OMBRE protocols, or for service in analyzing OMBRE outcomes using the UCSF Skeletal Biology and Biomechanics Core.