CDMI
CENTER FOR DISRUPTIVE MUSCULOSKELETAL INNOVATIONS

Nerve/Bone Crosstalk: PEMF Control of Adrenergic and TGFβ signaling

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NEED: A major challenge in osteoporosis therapy is the shortage of anabolic agents for bone. A powerful anabolic signal is mechanical loading, which stimulates new bone formation through a TGFβ and SOST/Wnt/β-catenin-dependent mechanism ***(Figure 1)***. However, many questions remain about how this pathway might be exploited therapeutically to stimulate bone anabolism. Because mechanical loading and TGFβ are critical regulators of bone mass, bone quality, and cartilage degeneration, elucidation of these pathways has great potential to advance new musculoskeletal therapies.

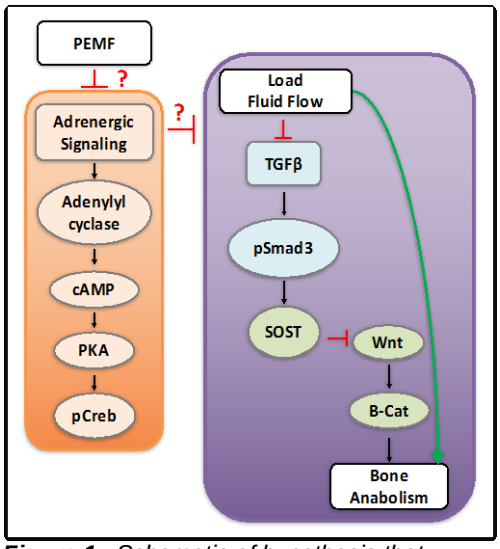

Figure 1. *Schematic of hypothesis that physical cues such as fluid flow or PEMF alter the epistatic control of SOST/Wnt/β-catenin function by adrenergic and TGFβ signaling.*

Our preliminary data suggests a novel mechanism of 'nerve/bone crosstalk' in mechanosensitive bone anabolism. Specifically, the mechanosensitive repression of TGFβ signaling and SOST expression that is required for anabolism was reduced or reversed by adrenergic signaling. This is consistent with other reports that adrenergic signaling antagonizes mechanosensitive bone anabolism. Our preliminary in vitro findings in osteocytes agree with reports in other cell types that crosstalk between adrenergic neurotransmitters and TGFβ signaling is cell-intrinsic and cAMP-dependent. Though many questions remain, our overall goal is to harness this TGFβ-dependent nerve/bone crosstalk to enhance the anabolic effects of mechanical loading in bone.

Pulsed electromagnetic fields (PEMF) stimulate bone formation and nerve regeneration through mechanisms that remain unclear. Several publications suggest that PEMF modulates cAMP signaling. Furthermore, PEMF has recently been shown to suppress adrenergic signaling in cardiomyocytes and to induce TGFβ signaling in osteogenic cells. The extent to which PEMF regulates TGFβ signaling through a neurotransmitter or cAMP-dependent mechanism remains to be determined.

Therefore, as a first step in investigating the role of PEMF in 'nerve/bone crosstalk' in bone anabolism, we will examine the epistatic regulation in osteocytes of adrenergic receptor and TGFβ signaling in the control of SOST expression ***(Figure 1)***. Furthermore, we will examine the extent to which physical cues, such as PEMF or fluid flow shear stress, modulate these regulatory relationships in vitro.

INDUSTRIAL RELEVANCE: PEMF exerts its clinically beneficial effects through mechanisms that remain unclear. Elucidation of these mechanisms will improve the tuning of PEMF for maximum therapeutic

effect and will clarify the clinical indications that can most benefit from PEMF-based intervention.

PROJECT AIMS AND HYPOTHESIS

As illustrated in Figure 1, we will test the **hypothesis** that adrenergic receptor signaling epistatically regulates TGFβ-inducible SOST expression in an osteocyte-intrinsic and cAMP-dependent manner. Furthermore, we will test the hypothesis that physical cues, such as PEMF or fluid flow shear stress, interfere with these regulatory relationships.

Aim 1: Determine the extent to which TGFβ-inducible SOST expression is sensitive to adrenergic signalling. Using OCY454 osteocyte cultures, we will evaluate the effect of adrenergic signalling on 1) cAMP signalling, 2) TGFβ signalling, and 3) SOST expression. Agonists and antagonists of the adrenergic and TGFβ pathways will be used to elucidate the implicated mechanisms.

Aim 2: Elucidate the mechanosensitivity of adrenergic effects on TGFβ-inducible SOST expression. Using a validated custom microfluidic flow system, we will examine the effect of fluid flow shear stress, a surrogate for mechanosensitive bone anabolism, on the adrenergic control of cAMP and TGFβ signalling, and SOST expression in cultured OCY454 cells.

Aim 3: Elucidate the PEMF-sensitivity of adrenergic effects on TGFβ-inducible SOST expression. Using an established custom PEMF system, we will examine the effect of PEMF on the adrenergic control of cAMP and TGFβ signalling, and SOST expression in cultured OCY454 cells.

Timeline: Aim 1 will be completed in the first half of the project period to identify the most sensitive variables, outcome measures, time points, and doses for subsequent studies. Aims 2 and 3 will be pursued in parallel in the second half of the project period. Independently of the results of Aim 1, we will evaluate the effects of fluid flow and PEMF on cAMP and TGFβ signaling and SOST expression. These in vitro studies will provide foundational information needed to guide subsequent in vivo studies.

METHODS*:*

Aim 1: Determine the extent to which TGFβ-inducible SOST expression is sensitive to adrenergic signalling. OCY454 osteocytes are a powerful new model system for these studies because they faithfully express mature osteocyte markers and exhibit mechanosensitive control of SOST expression. Furthermore, we have efficiently transfected these cells and find that they are highly sensitive to TGFβ signalling. Therefore, we will use OCY454 cells to test the ***hypothesis*** *that adrenergic signaling regulates TGFβ-inducible SOST expression in an osteocyte-intrinsic and cAMP-dependent manner.*

Guided by our preliminary studies, OCY454 cultures will be treated with or without TGFβ, in the presence or absence of broad-spectrum agonists (epinephrine) and selective antagonists (atipamezole (α2), butaxamine (β2)) of adrenergic signalling, as well as agonists of adenylyl cyclase (forskolin), a common downstream target of α2 and β2 adrenergic receptors. The effect of each treatment will be assessed as follows: cAMP signalling: phospho-CREB and CREB Western; TGFβ signalling: phospho-Smad3 and Smad3 Western, Serpin1 gene expression; SOST: SOST gene expression. Based on these results, additional gain and loss of function studies may be conducted using available pharmacologic and genetic (i.e. siRNA) reagents to identify the adrenergic receptor type(s) and effectors that regulate TGFβ signalling and SOST expression in osteocytes.

Aim 2: Elucidate the mechanosensitivity of adrenergic effects on TGFβ-inducible SOST expression. To test the ***hypothesis*** *that fluid flow shear stress and adrenergic signaling antagonistically control osteocytic TGFβ signaling and SOST expression,* OCY454 cells will be cultured in a custom microfluidic flow system that we have extensively validated for cultured MLOY4 osteocytes. Specifically, we find that

a 30-minute exposure to fluid flow (5 dynes/cm^2) is sufficient to induce mechanoresponsive changes in Cox2 gene expression and Smad3 phosophorylation in MLOY4 cells. The system will first be validated for the analysis of OCY454 cells, since MLOY4 cells do not express SOST.

Since our preliminary in vivo data suggest that adrenergic signalling impairs the mechanosensitive control of TGFβ signalling, we will evaluate the outcomes from Aim 1, as well as Cox2 mRNA expression, in the presence or absence of fluid flow shear stress in OCY454 cells. Pending the outcomes of Aim 1, we will evaluate other time points in the presence or absence of agonists and antagonists of specific receptors to uncover the epistatic regulatory mechanisms.

Aim 3: Elucidate the PEMF-sensitivity of adrenergic effects on TGFβ-inducible SOST expression.
To test the ***hypothesis*** *that PEMF and adrenergic signaling antagonistically control osteocytic TGFβ signaling and SOST expression,* OCY454 cells cultured as in Aims 1 and 2 (i.e. +/- TGFβ, adrenergic agonists and antagonists) will be exposed to PEMF (triangular wave, 25% duty cycle, 3,850 Hz pulse frequency, 15 Hz burst frequency, maximum 10 T/s rate of change) using a custom system. The effect of PEMF will be evaluated using outcome measures described in Aim 1 (cAMP, TGFβ signalling, and SOST expression).

MILESTONES*:*

- Aim 1: Determined the epistatic regulation of adrenergic, TGFβ, and SOST signaling in osteocytes *(orange, blue, and green factors in Figure 1)* – 4/02/2018
- Aim 2: Determined the effect of fluid flow on epistatic regulation *(colored factors in Figure 1)* – 9/02/2018
- Aim 3: Determined the effect of PEMF on epistatic regulation *(colored factors in Figure 1)* – 11/02/18

DELIVERABLES*:*

- *December 2017 – Conference call*
- *Spring 2018 – Spring Symposium @ UT (conference call)*
- *April 2018 – Updated Figure 1 illustrating epistasis among TGFβ and adrenergic signaling*
- *June 2018 – Conference call*
- *August 2018 – ORS abstract submission*
- *September 2018 – Fall Symposium @ UCSF*
- *September 2018 – Updated Figure 1 illustrating mechanosensitive control of TGFβ and adrenergic epistasis*
- *November 2, 2018 - Final written report including an updated Figure 1 illustrating PEMF control of TGFβ and adrenergic epistasis, and a plan for subsequent in vitro and in vivo studies, abstracts, and manuscripts.*

General Budget Outline:

Personnel	$	24,000
Tissue Culture Supplies	$	6,000
Chemicals and Supplies	$	6,364
Total Direct	$	36,364
Indirects (10%)	$	3,636
Total	$	40,000

Start Date:	**End Date**:
October 2, 2017	September 30, 2018