The Role of Nuclear Cytoskeleton in the Osteocytic Response to Simulated Weightlessness

National Aeronautics and Space Administration

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Background

While in space, astronauts exercise frequently to combat bone and muscle loss due to weightlessness. This loss in bone can lead to serious health problems, one of which is increased fracture risk. Cells known to play a vital role in bone formation and resorption are osteocytes. It is theorized that osteocytes act as the mechanosensors of bone, detecting mechanical stimulus and relaying these signals to surrounding cells. Previous research suggests that, during space flight, osteocytes exhibit matrix remodeling and resorption capabilities (osteocytic osteolysis) leading to enlarged lacunar size and contributing to overall bone degradation. The intercellular causes of these changes have not yet been determined. LINC or Linker of Nucleoskeleton and Cytoskeleton is a protein complex that forms a connection between the nucleus and cytoskeleton. LINC serves as a way to transmit mechanical signals to the nucleus and is also involved in the regulation of Wnt signaling via KASH proteins (Nesprin -1 and Nesprin -2). Wnt signaling in osteocytes assists with bone homeostasis and perturbed Wnt signaling can lead to bone loss. This suggests that LINC may play a role in the bone diminishing effects of microgravity.

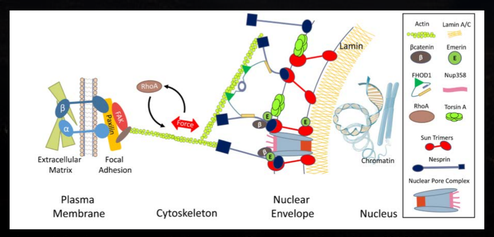

Figure 1.0. Visual representation of LINC complex (from Uzer 2016).

Objectives

1. Analyze past *in vivo* hindlimb unloading (HU) experiments using qPCR techniques to establish a valid time line for future studies.
2. Conduct an animal study to determine if LINC components are changing due to simulated microgravity (HU).

Hypothesis

Disruption of LINC components by simulated microgravity (HU) affects Wnt signaling in osteocytes leading to decreased bone formation and increased osteocyte lacunar size.

Methods: Experimental Design

Animal Study

Hindlimb Unloading
- Male C57BL6 mice
- 16 weeks of age
- Control, n = 5
- 7 day HU, n = 5
- 24 hour HU, n = 5

Analysis

Immunostaining
- Osteocyte Markers
- Osteolysis Markers

Gene Expression
- qPCR on LINC complex genes and Noncanonical Wnt ligands

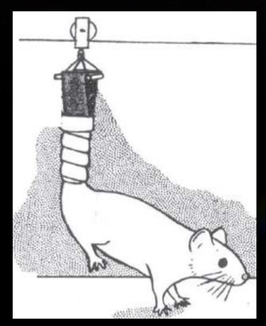

Figure 1.1. Mouse undergoing hindlimb unloading.

Group #	Treatment	Endpoints	# of mice
1	Control	7 days	5
2	HU	7 days	5
3	HU	24 hours	5

Genes of Interest
Sun – 1
Sun - 2
Nesprin - 1
Nesprin - 2
Emerin
Lamin A/C
Lamin B
Non-Canonical Wnt Ligands

Preliminary Results

Past Experiment Analysis

This experiment was conducted in 2015 with 16 week old male mice undergoing HU for 3 days. qPCR was ran on previously isolated RNA samples (femur) and showed no significant change in LINC complex components (Nesprin – 1, Sun – 1, Sun – 2 and Emerin). This suggests an earlier or later time point would render better gene expression results.

Sample Name	RIN #
D7.1*	5.70
D7.2	4.00
D7.3*	4.10
D7.4	2.30
D7.5*	2.30
D7.6	4.00
D7.7*	3.40
D7.8	2.30
D7.9*	3.60

Figure 1.2. RIN # of each RNA sample before analysis. These low RIN #s suggest RNA degradation which is to be expected after 2 years of storage. * are control samples.

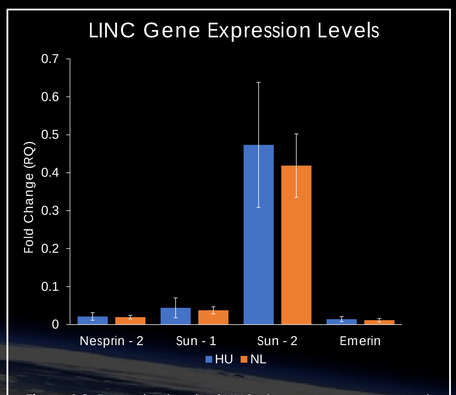

Figure 1.3. Expression levels of LINC elements were compared between control and HU groups via PCR, GAPDH was used as a house-keeping gene. HU resulted in no significant decrease or increase in all elements. ($p>0.05$, $n=4$).

Next Steps

Currently, we are conducting the 24 hour and 7 day HU animal study. After the desired time points, we will utilize quantitative real-time PCR techniques to determine the changes in gene expression of LINC complex genes and noncanonical Wnt ligands. To determine changes in osteocyte lacunar size and osteocytic remodeling activity, we will immunostain for osteocyte markers (Dmp-1 and Sost) and osteolysis markers (TRAP and MMPs).

References

[1] Uzer, G et al., Current Molecular Biology Reports, 2016; [2] Blaber, E et al., PLoS ONE, 2013; [3] Pajevic, P et al., Current Biotechnology, 2013.

Acknowledgments

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