Simulated Microgravity Induces SOST/Sclerostin upregulation in osteocytes

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ABSTRACT

Osteocytes are theorized to be the mechanosensors and transducers of mechanical forces in bone, yet the biological mechanisms of this action remains elusive. Recent evidence suggests that SOST/sclerostin is an important regulator of mechanotransduction.

To investigate the molecular mechanisms of SOST/Sclerostin regulation under in vitro and ex vivo unloading we used the NASA Rotating Wall Vessel (RWV) Bioreactor. For in vitro experiments, MG63 osteoblastic cells were seeded at a concentration of 250,000 cells onto 3D collagen scaffold (BCS). Scaffolds (4 per condition) were either rotated in a vertical 50ml NASA/bioreactor vessel at 18 rpm (unloaded), cultured in a horizontal 50 ml NASA-bioreactor vessel at 18 rpm (control for the shear environment of vertical rotating vessel), or cultured in a static 7.75 cm dish (static condition) for 7 days. For ex vivo experiments, calvaria bones were harvested from 12-week old C57/Bl6 mice and subjected to 6 collagenase digestions.

Simulated unloading, as achieved in the NASA RWV, resulted in increased, round osteocytes, as assessed by H&E staining, that was reminiscent of prior reports of unloading causing loss of osteocyte morphology and dendritic network connectivity. Semiquantitative realtime qPCR and immunohistochemistry from both in-vitro and ex-vivo conditions showed a four-fold up-regulation of SOST/Sclerostin. Furthermore, mRNA of the transcriptional SOST enhancer Mef2C was upregulated 1.4 fold in ex-vivo calvaria subjected to unloading conditions as compared to static conditions described above.

Future work is focused on validating results with additional osteocytic cell lines our lab is developing in preparation for an International Space Station flight experiment.

MATERIAL & METHODS

Cells: MG63 cells were grown on 3D collagen scaffold and subjected to static (A and C) or simulated microgravity (B and D) culture conditions. H&E staining (A and B) showed increase in cell proliferation in MG63 cells cultured under simulated microgravity condition (rotating bioreactor, panel B). Immunohistochemistry for Sclerostin (C and D) showed an increase in Sclerostin expression (black staining) in MG63 cells grown under simulated microgravity (panel D).

RESULTS

Figure 2: MLO-Y4 Osteocytic cells subjected to simulated microgravity: MLO-Y4 cells were grown on 3D collagen scaffold and subjected to static (A and C) or simulated microgravity (B and D) culture conditions. H&E staining (A and B) showed increase in cell proliferation in MLO-Y4 cultured under simulated microgravity condition (rotating bioreactor, panel B). Immunohistochemistry for Sclerostin (C and D) showed an increase in Sclerostin expression (black staining) in MLO-Y4 grown under simulated microgravity (panel D).

Figure 3: Unloading causes characteristic osteocyte morphologic changes: Osteocytes from immobilized rat (A and C, Krempien, 1976) and MLO-Y4 cultured on 3D collagen scaffold subjected under simulated microgravity (B and D, H&E staining). Loaded osteocytes are spindle-shaped with small nuclei; unloaded osteocytes have enlarged round nuclei and cytoplasmic.

Figure 4: Simulated Microgravity increases SOST & Mef2C expression @ 7 days: Real-time qPCR for SOST and Mef2C mRNA in MLO-Y4 cells (A1 and calvaria (A2 and B) placed in simulated microgravity. Simulated microgravity induced a 4-to-6 fold increase in SOST and mRNA expression comparable to response in hindlimb unloaded mice. Results are expressed as relative RNA and are normalized by RPL13. Data are expressed as mean ± SD of triplicates. *: student unpaired two-tail t-test p<0.05

Figure 5: Sclerostin protein expression up-regulated @ 7 days: Sclerostin expression in MLO-Y4 cultured on 3D scaffold for 7 days under static (A), rotational control (B), and simulated microgravity (C).

CONCLUSIONS & FUTURE WORK

- Simulated Microgravity induces:
  - Osteocytic morphologic changes similar to immobilization unloading
  - Increase in SOST/Sclerostin & anti-sclerostin antibody expression relative to rotational control
- Future work is focused on validating results with additional osteocytic cell lines our lab is developing in preparation for a International Space Station flight experiment.