Impact Of Microgravity on Osteoblast Cells Treated by Osteoinductive Agents: A Multi-Omics Analysis

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Introduction

- Musculoskeletal disuse, driven by chronic unloading, is most likely the major contributing factor to bone loss in the spaceflight environment.
- This hypothesis is supported by our previous space studies that linked spaceflight-induced stress to several degenerative mechanisms including bioenergetic deprivation, immunodeficiency, and inhibition of growth factors.
- We aimed to characterize cell specific molecular profiles that would untangle the roles of individual bone cells in responding to spaceflight-induced stress.
- Available literature suggests that the impact of osteoinductive agents would differ between Space and Ground.
- Bone morphogenetic protein-2 (BMP-2) is an FDA approved osteoinductive agent, which was recently linked to carcinogenesis. Therefore, our group was interested in examining an alternative osteoinductive agent, Thrombopoietin (TPO), the main growth factor of megakaryocyte cells.

Methods

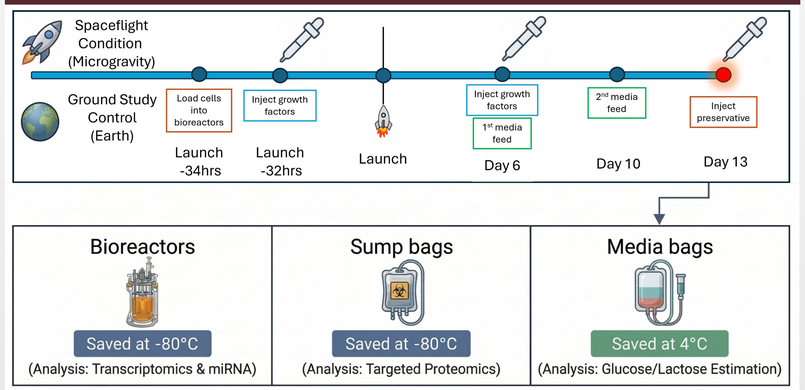


Figure 1. Cell Science-02 (CS-02) Study design. Before launch, cells were seeded into the bioreactors followed by an injection of growth factors (BMP-2, TPO, or saline solution (SAL, no treatment control)). The flight bioreactors were launched into space and were transferred to the International Space Station (ISS). The cells were harvested on Day 13 and shipped to Ground. RNA and miRNA from the bioreactors were extracted and integrative functional analyses were conducted. An equal number of bioreactors were operated either in spaceflight (FLT) or on the ground (G), resulting in six experimental groups: G-SAL (n=4), FLT-SAL (n=4), G-BMP (n=3), FLT-BMP (n=3), G-TPO (n=3), and FLT-TPO (n=3).

BMP treatment as the leading factor behind molecular perturbations

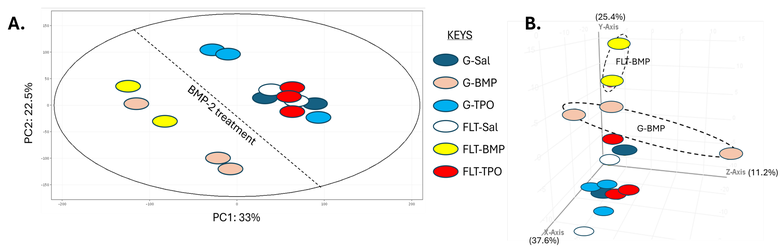
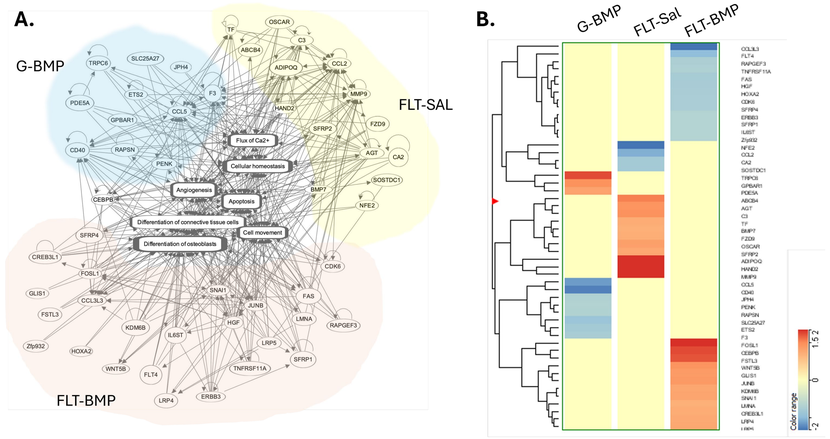


Figure 2. Principal Component Analysis (PCA). (A) PCA of mRNA transcriptomic expression values. **(B)** PCA of microRNA expression profile. (Flight (FLT); Ground (G); Saline (SAL), Bone morphogenetic protein-2 (BMP); Thrombopoietin (TPO)).

Integrated Analysis of Bionetworks and Biofunctions



C.

Gene networks linked to Bone health	FLT-SAL	FLT-BMP	G-BMP	Gene #
Angiogenesis	up	down	down	26
Apoptosis	down	up	up	37
Cell movement	up	down	down	34
Cellular homeostasis	up	down	xx	31
Differentiation of connective tissue	up	down	down	41
Differentiation of osteoblast	up	up	down	25
Flux of Ca+2	up	down	xx	19

Figure 3. Gene cluster linked to bone health. Fifty-one differentially expressed (DE) genes were linked to bone health. **(A)** Gene network. The genes perturbed by the three co-factors, G-BMP, FLT-SAL and FLT-BMP clustered separately. **(B)** Hierarchical cluster of the DE genes. **(C)** Regulation profile of the sub-networks co-enriched with bone health as depicted in Figure 3A. (Z score > 1.0 and < 1.0 were considered activated (up) and inhibited (down) networks, respectively. The gene numbers linked to each sub-network were noted under "Gene #").

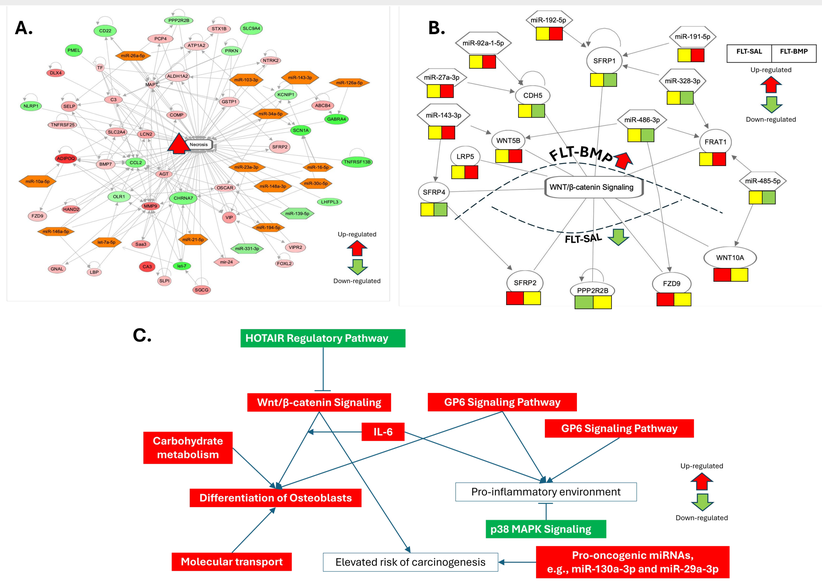
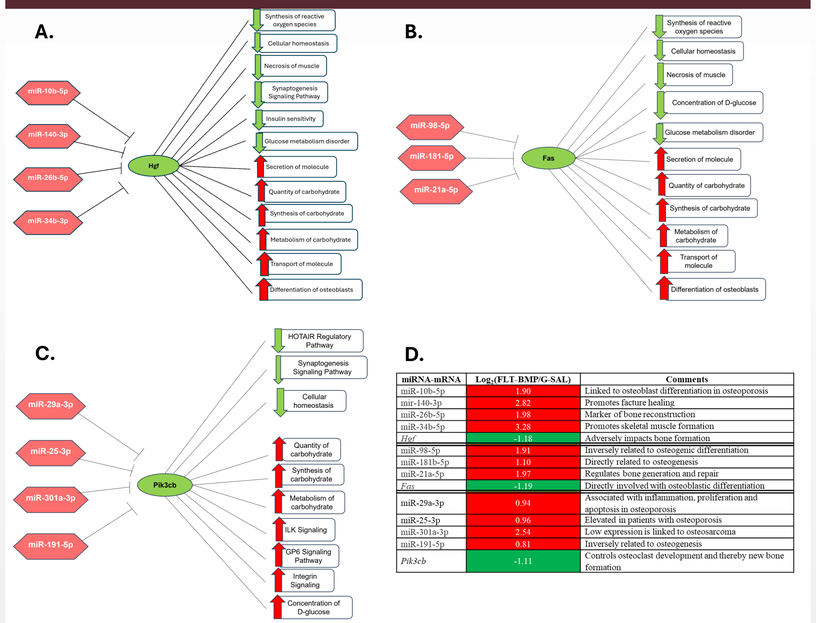


Figure 4. Integrative network analysis. The oval, hexagonal, rhombus and rectangular nodes represent mRNA genes, miRNA, proteins and biofunctions, respectively. The edges represent the relationship between two nodes. The red and green nodes represented up- and down-regulated features, respectively. **(A)** Necrosis network activated in FLT-SAL. BMP and TPO treatment brought this network to the baseline level. **(B)** Wnt/β-catenin Signaling network activated by FLT-BMP (z-score 2.2) and FLT-SAL (z-score 1.0). **(C)** Cluster of networks regulated by FLT-BMP. Colored boxes indicate networks directly regulated by FLT-BMP (red: upregulated; green: inhibited), while white boxes represent networks indirectly regulated by FLT-BMP.

Hub Genes and miRNA-mRNA Axes



D.

miRNA-mRNA	Log2(FLT-BMP/G-SAL)	Comments
miR-16b-5p	1.90	Linked to osteoblast differentiation in osteoporosis
mir-140-3p	2.82	Promotes facture healing
miR-26b-5p	1.98	Marker of bone reconstruction
miR-34b-5p	3.28	Promotes skeletal muscle formation
Hgf	-1.18	Adversely impacts bone formation
miR-98-5p	1.91	Inversely related to osteogenic differentiation
miR-181b-5p	1.10	Directly related to osteogenesis
miR-21a-5p	1.97	Regulates bone generation and repair
Fas	-1.19	Directly involved with osteoblastic differentiation
miR-29a-3p	0.94	Associated with inflammation, proliferation and apoptosis in osteoporosis
miR-25-3p	0.96	Elevated in patients with osteoporosis
miR-301a-3p	2.54	Low expression is linked to osteosarcoma
miR-191-5p	0.81	Inversely related to osteogenesis
Pik3cb	-1.11	Controls osteoclast development and thereby new bone formation

Figure 5. Hub genes of FLT-BMP and corresponding miRNA-mRNA axes. These clusters are enriched by the hub genes, its up-stream miRNA regulators, and biofunctions. **(A)** *Hgf* and its regulators and functions. **(B)** *Fas* and its regulators and functions. **(C)** *Pik3cb* and its regulators and functions. **(D)** miRNA-mRNA axes significantly altered by FLT-BMP and targeting a major hub gene, namely Hgf, Fas, Pik3cb. None of these molecules showed change in G-BMP and FLT-SAL with respect to G-SAL.

Conclusion

- Osteoinductive treatment emerged as the primary factor influencing the osteoblast gene profiles, surpassing the effects of altered gravitational force.
  - BMP-2 exerted distinct effects, separating its influence from that of TPO and spaceflight.
  - FLT-SAL, FLT-BMP and G-BMP triggered a mutually exclusive set of genes linked to osteogenesis and skeletal fitness. FLT-BMP displayed a relatively poor performance in comparison to G-BMP in maintaining homeostasis, differentiating osteoblast cell, and the flux of calcium ions.
  - The three hub molecules (*Hgf*, *Fas*, and *Pik3cb)* were inhibited in FLT-BMP samples and are critically linked to bone health. Analysis of upstream regulators revealed that activated miRNAs in FLT-BMP may form potential miRNA-gene axes responsible for osteoblast function in spaceflight.
- CS-02 may not have fully captured the impact of TPO, a comparatively slow-acting drug, since the mission was terminated before TPO could fully manifest its effects on osteoblast cells.
- Further probing of putative osteoinductive agents using this 3D cell culture model can deliver the next generation therapy with high clinical precision.

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