Examining Impacts of Spaceflight-Induced Cell Cycle Dysregulation on Skin Health and Wound Healing in Mice with Metabolic Profiling of Igf2

YASH BHUVA[1], PAKHI GUPTA[2], AZIMULLAH RIFAI[3], SHREYA SRIKANTH[4], JENNIFER CLAUDIO[5], ELIZABETH BLABER[6]

FREMONT HIGH SCHOOL (CA)[1], NOTRE DAME HIGH SCHOOL (CA)[2], POOLESVILLE HIGH SCHOOL (MD)[3], MIRA LOMA HIGH SCHOOL (CA)[4], BLUE MARBLE SPACE INSTITUTE OF SCIENCE (WA)[5], RENSSELAER POLYTECHNIC INSTITUTE (NY)[6]

INTRODUCTION & BACKGROUND

Since early ventures into space, it has been noticed that spaceflight has many impacts on the human body, particularly related to skin health and wound healing, where functions of wound healing in the broader immune system are suppressed in spaceflight. To identify how spaceflight impairs wound healing, we investigated dataset OSD-254 "Transcriptional analysis of dorsal skin from mice flown on the RR-7 mission," available via NASA Open Science Data Repository. This research aims to find potential solutions to improve skin processes whilst mitigating metabolic side effects of such solutions in order to support further space exploration.

HYPOTHESIS

We hypothesize that spaceflight factors hinder cell proliferation and differentiation through the downregulation of cell cycle genes, impairing wound healing and overall skin health.

STUDY METHODS & METADATA

OSDR's OSD-254, "Transcriptional analysis of dorsal skin from mice flown on the RR-7 mission" dataset was compiled using samples collected from the RR-7 mission launched on June 29, 2018, via SpaceX-15, carrying ten 11-week-old female C57BL/6J and C3H/HeJ mice to the ISS. The mice were housed in two Rodent Habitats, with half euthanized after 25 days and the remainder after 75 days using Ketamine/Xylazine/Acepromazine and cardiac puncture. Dorsal skin samples from these mice were processed for RNA extraction, library generation, and sequencing. **Our study focuses on five female C57BL/6J ground samples and five 75-day flight samples for comparative transcriptional analysis.**

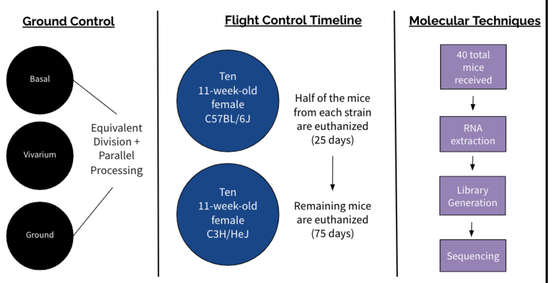


Figure 1: Metadata and breakdown for the OSD-254 Dataset

TRANSCRIPTOMIC ANALYSIS & RESULTS

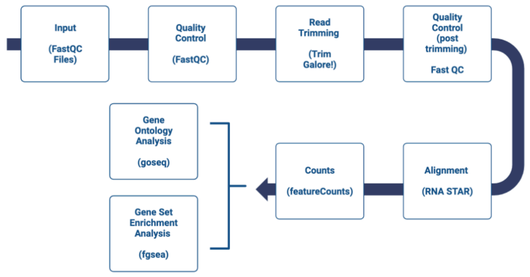


Figure 2: Pipeline for analysis on UseGalaxy platform

Volcano Plot: Genes of Interest
The volcano plot helps visualize differentially expressed genes (adj p-value <0.05, |log2 fold change| > 1). The plot shows that cell cycle genes, such as Cdk1, Ccnb1, and Ccnb2, were downregulated in spaceflight.

GoSeq Analysis
Gene ontology enrichment analysis was performed to analyze altered pathways and account for gene length bias. The analysis showed heavy dependence on cell cycle regulation.

ShinyGO Analysis
A combined gene set was inputted into ShinyGo, with a false discovery rate < 0.05, to identify processes relating these genes. *Cdk1, Ccnb1, Ccnb2* and *Igf2* shared cell growth, cell proliferation, wound repair, and metabolism genesets.

RNA-sequencing analysis included the five female C57BL/6J ground samples and five 75-day flight samples. Data was analyzed on the Galaxy platform to analyze the data for altered genes and pathways from ground control to spaceflight.

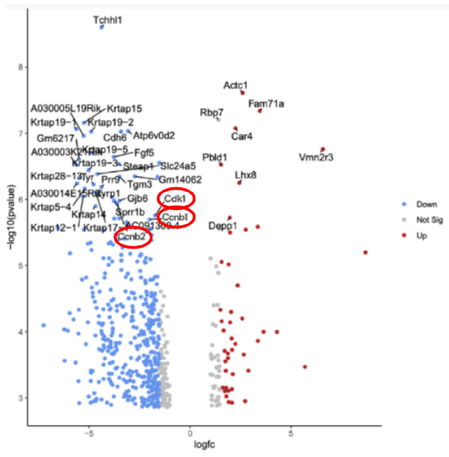


Figure 3: Distribution of genes of interest

16	Regulation of developmental process	BRCA1 FBXO5 CDK1 MSX2 LEF1 LTF MMD2 FANCD2 ASPM HMGB2 ADRA2B CCNB1 DMBT1 IGF2 LRP8 HMGB3
14	Positive regulation of developmental process	BRCA1 FBXO5 CDK1 MSX2 LEF1 LTF MMD2 ASPM HMGB2 ADRA2B CCNB1 DMBT1 IGF2 LRP8
9	Cell population proliferation	CDK1 MCM10 LEF1 E2F8 ASPM CKS2 DMBT1 IGF2 FIGNL1
5	Growth	MSX2 ASPM CCNB1 DMBT1 IGF2
5	Regulation of growth	CDK1 LEF1 CCNB2 CCNB1 IGF2

Figure 4: Truncated ShinyGo enrichment pathway table, highlighting genes of interest

KEY FINDINGS + SUPPORTING EVIDENCE

Through our studies, **Igf2 was found to be upregulated** (p-value < 0.05, log2FC > 1), where pathway analysis identified connections between *Cdk1, Ccnb1, Ccnb2*, and *Igf2* through the MAPK pathway. *Igf2*, known for its mitogenic and proliferative properties, may serve as a potential compensatory mechanism to mitigate the adverse effects of downregulated cell cycle genes, supporting skin thickness, cell differentiation, and wound healing. *Igf2*'s involvement in metabolic pathways, such as oxidative phosphorylation, also suggest spaceflight may induce unique metabolic profiles resulting from the gene's upregulation. The MAPK-ERK Pathway is a known part of the MAPK signaling Pathway, and although its mechanisms for regulation of proliferation are not well known, some of its targets have been identified.

Studies demonstrate that activation of ERK instigates transcription of cyclin D1, involved in the G1/S transition (Wang and Tournier, 2006). Others prove its role in regulating the G2/M transition, indicating regulation of mitotic entry/regulation (Cude et al., 2007). ERK can also modulate the activity of cyclin B1 and *Cdk1*, key regulators of the G2/M transition (Zheng, 2008). With this in mind, the downregulation of *Cdk1, Ccnb1,* and *Ccnb2,* genes involved in the MAPK pathway that are crucial for cell cycle regulation, can impair cell proliferation and thus wound healing as cells struggle to progress through the cell cycle. This can result in slower cell differentiation, which are significant health concerns for astronauts. Conversely, the upregulation of Igf2 can result in increased cell growth and development. Considering this information, we concluded that *Igf2* expression in space is a potential compensatory mechanism. We postulate that through the MAPK pathway, *Igf2* interacts with *Cdk1, Ccnb1,* and *Ccnb2* to potentially increase expression, thus impacting the G2/M transition.

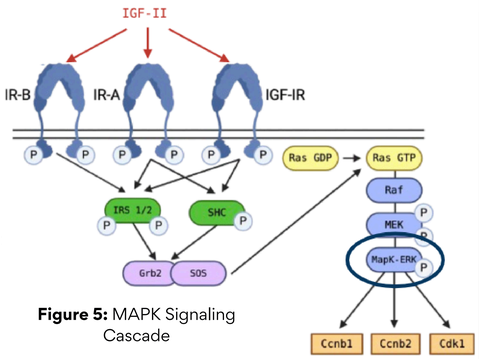
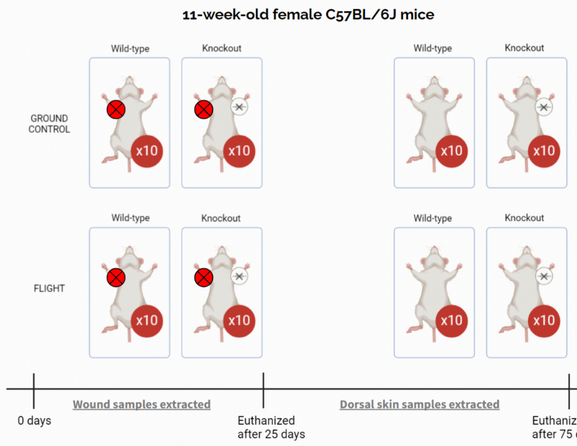


Figure 5: MAPK Signaling Cascade

PROPOSED EXPERIMENT & EXPECTED RESULTS



Our study will use 80 female C57BL/6J mice, stratified between two main factors: Wild Type vs. *Igf2* knockout (KO) and Ground Control vs. Flight.
These samples will be used to address 3 aims:

Aim 1: To analyze the impacts of the downregulation of cell cycle genes *Cdk1, Ccnb1,* and *Ccnb2* on skin in microgravity
Aim 2: To examine the impact of *Igf2* expression on skin health and wound healing
Aim 3: To identify metabolic/glycolytic impacts through *Igf2* upregulation on skin health and wound healing

	Spaceflight vs. Control (75 Day Dorsal Skin)	IGF2 Present vs. IGF2 KO (Wound-Healed Sections)	IGF2 Wild-type vs. IGF2 KO (General Skin Sections)	IGF2 Present (Flight Samples) vs. IGF2 Present (Ground Samples)
AO/PI Flow Cytometry Assay	Accumulation of cells in G0 phase (cell cycle arrest) in spaceflight			
Immuno fluorescence	Reduced fluorescence intensity of cyclin markers (e.g., CCNB1) in spaceflight			
Histological Analysis (Ki67 Density)		Higher Ki67 density in IGF2-present samples (increased cell proliferation)		
Histological Analysis (Skin Health)	Thinner skin, lower cell density, lower involucrin staining in spaceflight		Thicker skin, higher cell density, higher involucrin staining in IGF2 wild-type samples	
Metabolomic Assays (Glycolytic Intermediates)			Increased levels of glucose-6-phosphate, fructose-1,6-bisphosphate, and pyruvate in IGF2 wild-type samples	Increased levels of glucose-6-phosphate, fructose-1,6-bisphosphate, and pyruvate in IGF2 flight samples.
Targeted Metabolomics (Acetyl-CoA Levels/ mechanisms)			Increased Acetyl-CoA levels and standard Acetyl-CoA function in IGF2 wild-type (since they're on ground)	Increased Acetyl-CoA levels. Increase in lipogenesis/ decrease in β-Oxidation in IGF2 present flight samples

Aim 1: We hypothesize that the downregulation of cell cycle genes in spaceflight will lead to lower levels of cyclins, indicating that cells are being sent to G0 phase/stuck in G2 with lack of transition to M-phase, leading to less cell concentration and skin thickness. Impacts will be assessed with the use of immunofluorescence (IF) with respective cyclin antibodies of targets (ie. Ccnb1/Abcam ab72, Keratinocytes/K10) and AO/PI flow cytometry assay to identify if spaceflight is causing cells to halt at a certain stage of the cell cycle.

Aim 2: We hypothesize that skin samples from *Igf2* wild-type mice will yield greater Ki67 intensity (indicating better wound healing) and increased Keratin 10/involucrin levels (indicating more keratinocyte maturation/cell differentiation and better skin health) compared to *Igf2* -/- mice. Wound healing will be assessed with hematoxylin and eosin (H&E) staining for general histological assessment (pattern, shape, and structure of cells). Ki67 immunohistochemical (IHC) staining will be done to measure rates of wound healing, (Ki67 marks dividing cells) Skin health will be assessed with H&E staining for histological assessment, and keratin 10 (K10)/IHC staining will be done to measure keratinocyte concentration (indicative of skin health). IHC staining for involucrin will be done to evaluate differentiation statuses of skin cells.

Aim 3: We hypothesize that *Igf2* positive mice in spaceflight will have enhanced metabolic activity that increases production of glycolytic intermediates like lactate/Acetyl-CoA, where Acetyl-CoA will also have alternative metabolic mechanisms (due to mitochondrial dysregulation) compared to *Igf2* -/- and wild-type mice. Metabolomic impacts will be assessed through tandem mass spectrometry to identify changes in metabolite levels between *Igf2* positive and knockout groups. Targeted metabolomics will be performed (via ultra-performance liquid chromatography and mass spectrometry) to analyze Acetyl-CoA's mechanistic changes resulting from *Igf2* expression and spaceflight-induced mitochondrial dysregulation, where we expect changes to lipogenesis and β-Oxidation.

SIGNIFICANCE & CONCLUSION

Spaceflight is known to inhibit various cell proliferation and differentiation processes by downregulating cell cycle genes (Mao, 2024), of which we focus on *Cdk1, Ccnb1,* and *Ccnb2*, impacting skin health and wound healing. We found that *Igf2* may counteract this effect via the MAPK pathway, though other factors like *Erbb2* complicate the outcome. The role of *Igf2* has **not been reported in current literature** in conjunction with *Cdk1, Ccnb1,* and *Ccnb2*, emphasizing the importance of this study, where these insights into cell cycle regulation under microgravity are crucial for improving astronaut health. This holds broader implications for pharmaceutical interventions. **Therefore, it is of utmost importance to investigate the therapeutic properties of *Igf2* both on wound healing and skin health while researching metabolic implications to minimize the risks astronauts face, combating cell cycle dysregulation and issues alike.**

HIGHLIGHTED REFERENCES & ACKNOWLEDGMENTS

1. Cope, H., Elsborg, J., Demharter, S. et al. Transcriptomics analysis reveals molecular alterations underpinning spaceflight dermatology. Commun Med 4, 106 (2024). https://doi.org/10.1038/s43856-024-00532-9
2. Safa, A., Abak, A., Shoorei, H., Taheri, M., & Ghafouri-Fard, S. (2020). MicroRNAs as regulators of Erk/MAPK pathway: A comprehensive review. Biomedicine & Pharmacotherapy, 132, 110853. https://doi.org/10.1016/j.biopha.2020.110853
3. Dangi, S., Chen, F. M., & Shapiro, P. (2006). Activation of extracellular signal-regulated kinase (ERK) in G2 phase delays mitotic entry through p21. Cell Proliferation, 39(4), 261–279. https://doi.org/10.1111/j.1365-2184.2006.00388.x
4. Safa, A., Abak, A., Shoorei, H., Taheri, M., & Ghafouri-Fard, S. (2020). MicroRNAs as regulators of Erk/MAPK pathway: A comprehensive review. *Biomedicine & Pharmacotherapy*, *132*, 110853. https://doi.org/10.1016/j.biopha.2020.110853
5. Xie, B., Wang, S., Jiang, N., & Li, J. J. (2019). Cyclin B1/CDK1-regulated mitochondrial bioenergetics in cell cycle progression and tumor resistance. Cancer letters, 443, 56–66. https://doi.org/10.1016/j.canlet.2018.11.019

We would like to take this opportunity to thank Dr. Blaber, Ms. Claudio, Ms. Stallmith, and the GL4HS faculty, teachers, alumni, and fellow student interns for their pivotal roles in supporting our proposal. A special thanks to our subject matter experts (SMEs) Dr. Michael Pecaut and Dr. Tarun Mamidi for their insight into our project.