Dual Genes of Muscle Cell Senescence: Unraveling the Radiation-Induced Roles of HDAC4 and MORC2a

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Introduction

During missions, modern-day astronauts on the ISS are exposed to galactic and solar radiation, impairing astronaut physiology for deep space missions to Mars and beyond. While radiation is known to be detrimental to skeletal muscle function, the molecular basis of these radiation-induced responses, beyond oxidative stress, has not been delineated. To investigate the role of radiation-induced oxidative stress on muscle damage, our proposed experiment uses transcriptomic dataset GLDS-426 for analysis via the *Mus musculus* C2C12 cell culture.

Background

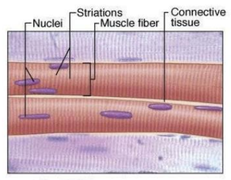


Figure 1. Depiction of skeletal muscle fibers

- Actin and myosin protein binding causes muscle fiber contraction.
- Muscular atrophy occurs when degradation of proteins exceeds muscle fiber formation via myoblasts (stem cells).

- Exposure to ionizing radiation causes an imbalance between reactive oxygen species and antioxidants, causing pathological dysfunctions.
- Genes associated with cell senescence and muscle dysfunction include:
  - p21
  - MORC2a (DNA damage response)
  - HDAC4 (muscle inactivity response)

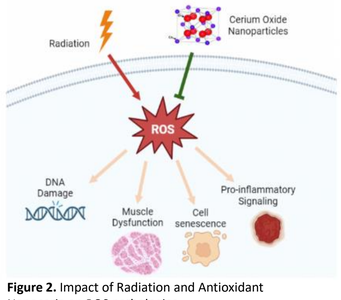


Figure 2. Impact of Radiation and Antioxidant Nanoceria on ROS pathologies

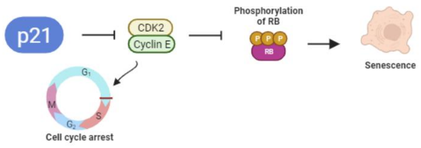


Figure 3. p21 Pathway.

- Increased expression in p21 is associated with cell senescence and skeletal dysfunction in mice and humans *(Englund et. al, 2021)*.
- The senescence-associated secretory phenotype (SASP) is a collection of biomarkers to detect cellular senescence.

Chromatin remodeling protein MORC2a recruits HDAC4

↓

Enhances the inhibition of p21/p53 transcription

↓

Increased cellular proliferation *(Ou et al., 2022)*

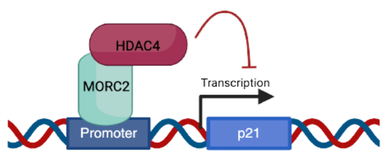


Figure 4. MORC2a and HDAC4 repression of p21 transcription

No reported studies have examined the relationship between MORC2A and HDAC4 in skeletal muscle proliferation under spaceflight conditions.

Metadata Analysis

GLDS-426, *Cerium oxide nanoparticle administration to skeletal muscle cells under different gravity and radiation conditions,* was accessed from the NASA GeneLab Data Repository.

- Proliferating commercial C2C12 mouse skeletal soleus muscle cell cultures were subjected to varying gravity, radiation levels, and nanoceria treatment.
- RNA sequencing analysis was performed on data for all frozen space samples via the NASA GeneLab Galaxy platform.

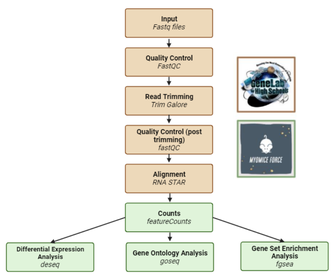


Figure 5. Data Analysis Process for GLDS-426.

	Nanoceria	Radiation	Microgravity (ug)
FLT_uG_Nonano	✗	✓	✓
FLT_uG_nano	✓	✓	✓
FLT_1G_Nonano	✗	✓	✗*
FLT_1G_nano	✓	✓	✗*
GC_1G_Nonano	✗	✗	✗
GC_1G_nano	✓	✗	✗

*centrifugation was applied to simulate 1G.

Table 1. GLDS-426 Conditions.

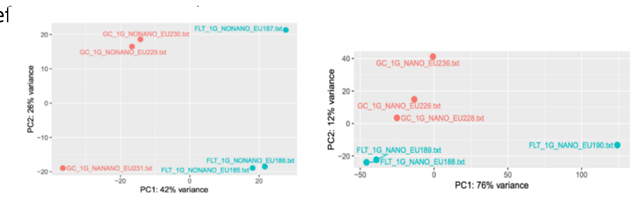
Transcriptomics Analysis & Results

Two separate differential gene analyses were conducted for the paired radiation and nanoceria conditions:

1. Group 4 (with nanoceria, in space with 1G) and Group 6 (with nanoceria, on land with 1G) to evaluate radiation conditions.
2. Group 3 (without nanoceria, in space with 1G) and Group 5 (without nanoceria, in space with 1G) to evaluate antioxidant effects on balancing ROS.

Principal Component Analysis Plots

Using the DESeq2 tool, we annotated each trial's top 40 most significant DEGs with a ref

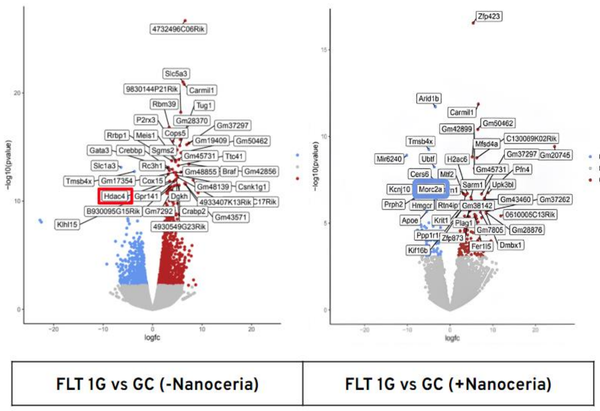


FLT 1G vs GC (-Nanoceria)	FLT 1G vs GC (+Nanoceria)

Figure 6: PCA plots for FLT 1G vs GC with/without Nanoceria. Clear divergence between the FLT and GC groups.

Volcano Plots: Genes of Interest

The volcano plots exhibit upregulation in HDAC4 (logFC=3.46, p=6.19×10^{-10}) and downregulation in MORC2a (logFC=-3.21, p=1.77×10^{-4}).



FLT 1G vs GC (-Nanoceria)	FLT 1G vs GC (+Nanoceria)

Figure 7. Volcano plots. Red indicates upregulation of a differentially expressed gene (DEG), whereas blue indicates downregulation. HDAC4 upregulated and MORC2a downregulated.

Relationship between HDAC4 and MORC2a

HDAC4- UPREGULATED
- Regulates condensation of heterochromatin
- Downregulation of HDAC4 impairs DNA repair pathways

Absence of nanoceria in Space Radiation → More DNA Damage and HDAC4 → Activation of DNA repair pathways

MORC2a- DOWNREGULATED
- Regulates condensation of heterochromatin + gene silencing
- Part of the DNA damage response

Presence of nanoceria → Less ROS and DNA Damage → Less MORC2a

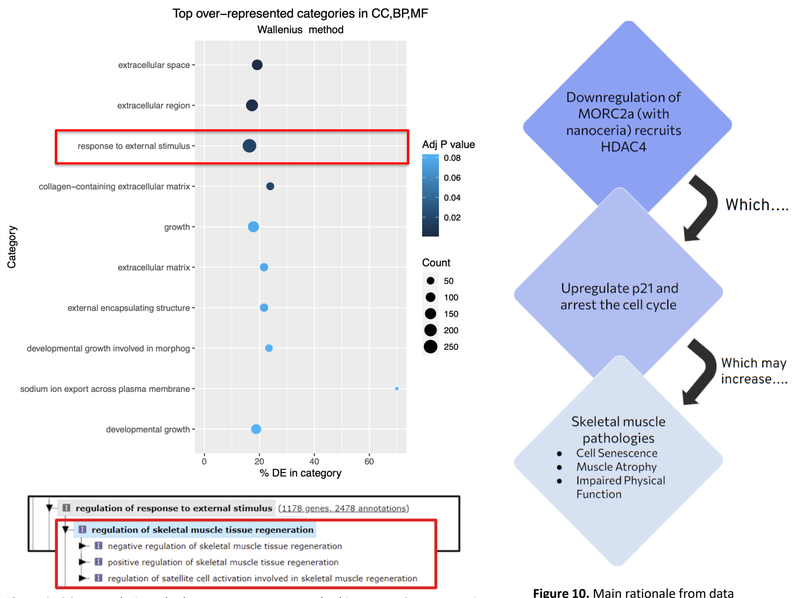


Figure 9. GOseq analysis and relevant processes researched in Mouse Genome Institute (MGI). Significant p-value of response to external stimulus, associated with skeletal muscle tissue regeneration

Figure 10. Main rationale from data about linkage between MORC2a, HDAC4, and p21

Proposed Experiment

The study will investigate the effects of MORC2a and HDAC4 on tumor suppressor p21 in varying knockout conditions and irradiation to determine whether the absence of dual inhibitors may enhance p21 transcription for skeletal myocyte proliferation.

We hypothesize that inhibition of DNA repair response genes MORC2a and HDAC4 causes upregulation of CDKN1a/p21, causing cell cycle arrest and muscle cell senescence, and ultimately, induction of skeletal muscle pathologies.

Aim 1: Measure the effects of knocking out transcriptional repressors MORC2a and HDAC4 on cell senescence.

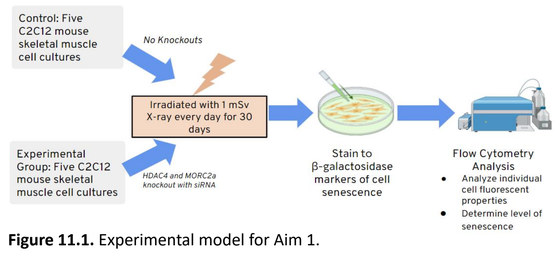


Figure 11.1. Experimental model for Aim 1.

We expect that knockout of MORC2a and HDAC4 will cause increased p21 levels, increasing levels of SASP. The knockout cultures will fluoresce more, indicating increased senescence and cell cycle arrest.

Aim 2: Measure the effects of knocking out tumor suppressor p21 on muscle cell proliferation.

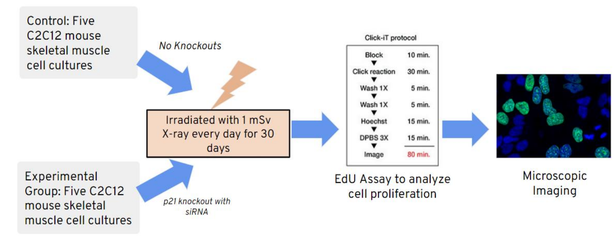


Figure 11.2. Experimental model for Aim 2.

We expect that knockout of p21 will promote cell cycle progression in response to radiation-induced DNA damage, increasing muscle cell proliferation. The ratio between Alexa Fluor™ 488 dye newly proliferated cells and Hoechst dye naming all cells will be greater in the knockout group than in the control.

Significance and Conclusion

Our study can help define the ability of radiation-damaged musculoskeletal tissues to recover function and possibly identify drug therapies. It has applications in space and for Earth treatments, including possible biomarkers, drug targets, and inhibitors for long-duration missions.

Space	Earth
Deep Space Radiation (DSR) Missions • Astronauts can lose 20% of their muscle mass within 5-11 days on the ISS. • DSR exposure is 50-2000 mSv vs. 1 mSv/day on the ISS.	Radiation Induced Injury • Therapeutic doses of radiation for cancer can cause muscle atrophy and irreversible fibrosis (RFS). • Identifying the under/overexpression of genes that mitigate muscle atrophy can lead to the development of altered radiation-resistant cells.
Astronaut Productivity • Exposure to gamma rays can cause reduced performance in motor tasks critical to astronaut activities including repair and research. • Astronauts spend 2-2.5 hours of precious time daily exercising to counteract muscle atrophy	Sarcopenia and Muscle Aging • Reduction in muscle cell proliferation at a cellular level enhances sarcopenia. • Aim 2 to knockout p21 and observe cell proliferation may be able to be directly applied to symptoms of sarcopenia

Table 2. Implications of the research study in Earth and space environments

Future Direction

Studying the effects of space radiation on muscle loss can offer insights into the broader understanding of muscle atrophy on Earth in concurrence with space travel safety. Future research can investigate therapeutics that include p21 inhibitors or overexpression of HDAC4 and MORC2 contribute to cell proliferation, cell senescence, and progression of muscle atrophy.

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