The Role of H2AC21 and H2AC11 in Reducing Thymic Epithelial Cell Proliferation

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INTRODUCTION

The thymus is a primary lymphoid organ that plays an important role in **T cell maturation and adaptive immune system function.** Over time, the structure and function of the thymus degrades in a process known as **thymic involution,** resulting in a decreased output of naive T lymphocytes. Involution can be accelerated by numerous stressors, many of which are associated with spaceflight. Moreover, as Thymic Epithelial Cells (TECs) are crucial to regulating thymopoiesis, it is imperative to understand the complications associated with **aberrant TEC proliferation,** especially under **spaceflight conditions**.

BACKGROUND

Our research investigates **GLDS-289**, a study conducted by the Japanese Aerospace Exploration Agency (JAXA). Over two missions (MHU1 and MHU2), the team measured thymic weight and thymus/bodyweight ratio of mice in three experimental groups: ground control, microgravity, and artificial gravity as shown in **Figure 1**. Thymic atrophy was significantly present in the microgravity group, indicating **reduced thymic epithelial cell proliferation.**

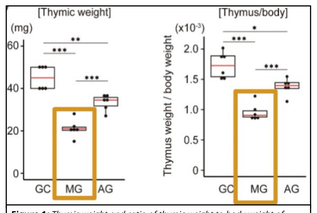


Figure 1: *Thymic weight and ratio of thymic weight to body weight of ground control (GC), microgravity (MG), and artificial gravity (AG) mice.*

Literature review allowed for a more in-depth understanding of histones and cyclins. Cyclin and histone interactions are crucial for normal cell cycle function. The **expression of histone genes** at the G1/S phase transition in the cell cycle is induced by **cyclin-mediated phosphorylation** of a scaffolding protein as seen in **Figure 2**.

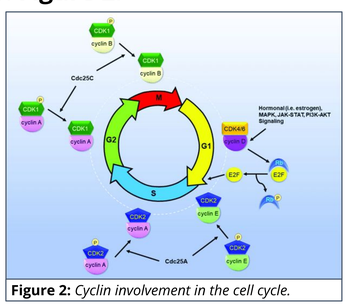


Figure 2: *Cyclin involvement in the cell cycle.*

DATA PROCESSING

In **Figure 3**, blue dots represent significantly downregulated genes and red dots represent significantly upregulated genes. We noted the **down-regulation of histone genes** and the **upregulation of stress response genes.**

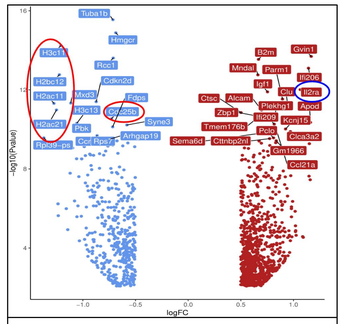


Figure 3: *Team-generated Volcano plot.*

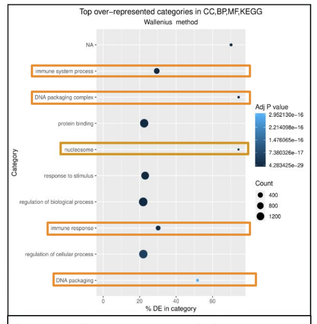


Figure 4: *Team-generated GoSeq plot.*

In **Figure 4**, we took note of the **overexpression of nucleosomes/packaged DNA** as it related to histones. We also noted **overexpression of genes in the immune response** and immune system process pathways.

TRANSCRIPTOMICS ANALYSIS

SIGNIFICANT GENES

In examining Gene Set Enrichment Analysis of downregulated genes in the microgravity group (**Figure 5**) of the GLDS-289 study, we noticed many genes involved in cell cycle progression pathways, especially the **G2-M checkpoint and mitosis.**

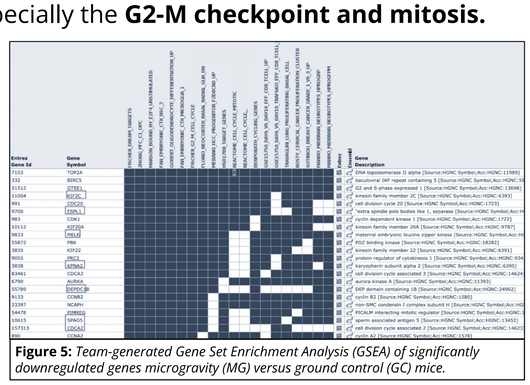


Figure 5: *Team-generated Gene Set Enrichment Analysis (GSEA) of significantly downregulated genes microgravity (MG) versus ground control (GC) mice.*

The following heatmaps of histone gene expression (**Figure 6**) in both missions for all three experimental groups- Ground Control, Microgravity, and Artificial Gravity- show **significant downregulation of histones** in the microgravity group alone.

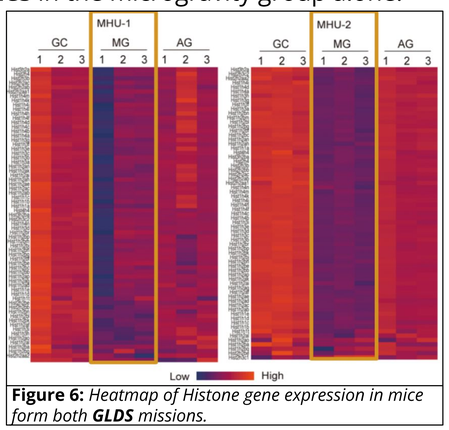


Figure 6: *Heatmap of Histone gene expression in mice form both **GLDS** missions.*

PATHWAY ANALYSIS

Literature strongly suggests that CDC25 isoforms regulate the Cyclin B/CDK1 complex, which is necessary to trigger mitosis as shown in **Figure 7**. **Inhibition of CDC25 isoforms leads to cell cycle arrest,** as the cyclin B/CDK1 complex remains inactive.

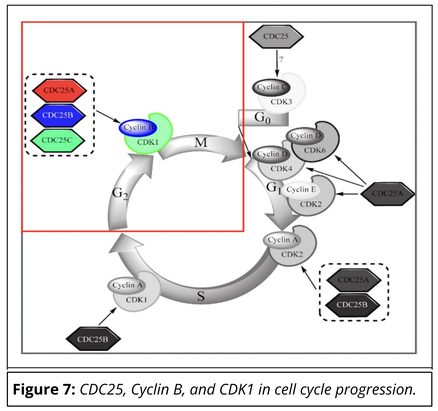


Figure 7: *CDC25, Cyclin B, and CDK1 in cell cycle progression.*

Günesdogan *et al.* (2014) investigated the relationship between histones and cell cycle progression using *Drosophila* embryos lacking histone genes (Hist^{C}). The study concluded that **downregulation of histone genes** prevents activation of the ATR/Chk checkpoint (**Figure 8**), which induces **cell cycle arrest** at the G2 checkpoint, and consequently, **decreases cell proliferation.**

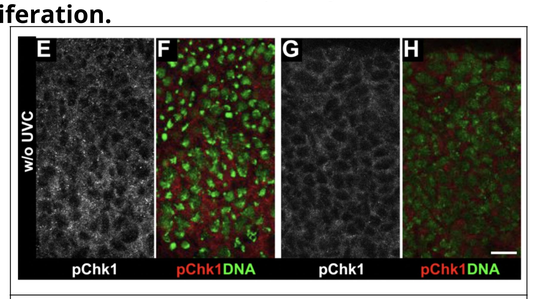


Figure 8: *Staining for pChk1 in wild type (E, G) and* Hist^{C} *mutant (F, H) (Günesdogan et al., 2014)*

HYPOTHESIS

We hypothesize that the downregulation of histone genes H2AC21 and H2AC11 in TECs contributes to **decreased TEC proliferation** and **lower T cell maturation rates**, accelerating thymic involution.

PROPOSED EXPERIMENT

Developing thymic lobe organoids
- kept in Air-Liquid interface system
- six male mice ranging from 5-6 weeks old
- cultured in standard cell medium for 14 days

Three organoids with histone genes H2AC21 and H2AC11 silenced using siRNA

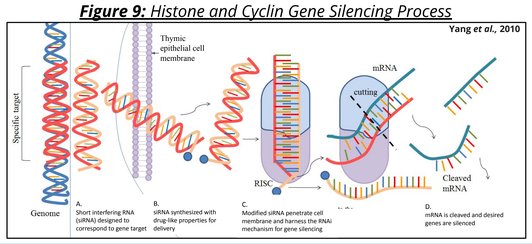
Aim 1: To measure the effects of silenced histone genes H2AC21 and H2AC11 on the proliferation of thymic epithelial cells (TECs).
- TEC proliferation measured through monitoring metabolism of TECs
- alamarBlue treated cells and spectrophotometry

Three organoids with cyclin genes CDK25 and CDK1 silenced using siRNA

Aim 2: To determine the effects of decreased TEC proliferation as a result of silenced CDC25 and CDK1 on the maturation of T cells.
- T cell maturation measured using production of effector cytokines and RNA sequencing on T cell activation markers
- TEC proliferation measured using methods mentioned in Aim 1

Aim 3: To determine the effects of silenced histone genes on CDC25 and CDK1 gene expression and protein levels in TECs.
- CDC25 isoform and CDK1 protein levels measured through antibody staining and counterstaining
- RNA sequencing to ensure gene expression is consistent with protein function

Figure 9: *Histone and Cyclin Gene Silencing Process*



ANTICIPATED OUTCOMES

Aim 1: We expect to observe **reduced thymic epithelial cell proliferation.**

Aim 2: We expect to observe reduced proliferation of TECs, and as a result, **lower rates of maturation for naive T cells.**

Aim 3: We expect to see the **downregulated expression of CDK1 & CDC25,** as well as lower levels of CDK1 & CDC25 proteins in TECs.

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REFERENCES

Figure 1,6: "Impact of spaceflight on the murine thymus and mitigation by exposure to artificial gravity during spaceflight" by Horie *et al.* (2019) used under CC BY 4.0 / Inserted yellow boxes around MG data points from the original

Figure 2: "Targeting Cyclin-Dependent Kinases for Treatment of Gynecologic Cancers" by Z Ping Lin, Yong-Lian Zhu, and Elena S Ratner (2018) used under CC BY 4.0

Figure 7: "Therapeutic Targeting the Cell Division Cycle 25 (CDC25) Phosphatases in Human Acute Myeloid Leukemia — The Possibility to Target Several Kinases through Inhibition of the Various CDC25 Isoforms" by Brenner *et al.* (2014), used under CC BY 4.0 / desaturated G1 and S genes, inserted red box around G2/M genes from the original

Figure 8: "Histone supply regulates S phase timing and cell cycle progression" by Ufuk Günesdogan, Herbert Jäckle, and Alf Herzig (2014), used under CC BY 4.0 / Cropped from the original

Figure 9: "Gene Therapy Using RNAi" by Yang *et al.* (2010), used under CC BY-NC-SA 3.0 / original labels replaced with spelling corrections