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Heat Shock Protein 40 and Immune Function in Altered Gravity

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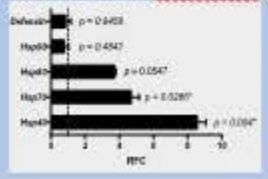
Abstract

During spaceflight, astronauts are more susceptible to immunosuppression, which poses limits to their health and the mission. During space flight, stress-inducible heat shock proteins (HSPs) are robustly induced, and the overexpression of HSPs have been implicated in immune dysregulation. Therefore, HSPs may be critically involved in regulating immune homeostasis. HSP40 plays a major role in proper protein translation and folding. To determine the role of HSP40 during stress-induced altered gravity conditions, wild type and Hsp40 mutant *Drosophila melanogaster* were exposed to ground-based chronic hypergravity conditions, followed by quantitative PCR analysis of immune gene expression. This data indicates a role of Hsp40 in strengthening immune function during stress-induced spaceflight in flies. A critical need to evaluate the relationship between HSPs and immune suppression during space flight is necessary.

Introduction

- Astronauts are immunocompromised during spaceflight. Understanding the mechanisms of this is vital for successful long-term spaceflight. (1)
- Heat shock proteins (HSPs) are a major class of proteins activated by stress of altered gravity. HSPs respond to oxidative stress induced by hypergravity. (2)
- Preliminary data shows that Hsp40 is upregulated in hypergravity conditions.

Figure 1. Chronic hypergravity (3g) flies, wild type male.



A.M. Paul, unpublished. qPCR results of various genes normalized to 1g conditions (dotted line). Hsp40 is particularly induced under chronic hypergravity conditions.

- So, further characterizing Hsp40 in hypergravity can prove useful to the stress response and immune response during hypergravity.
- *Drosophila melanogaster* is a useful model organism for this study because of its ease of genetic modification, large sample size, and genetic similarity to humans.

Hypothesis

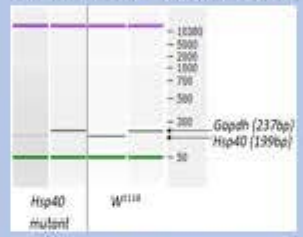
Loss of Hsp40 in stressful conditions of hypergravity will result in increased expression of innate immunity genes.

Methods

Figure 2. Methods.

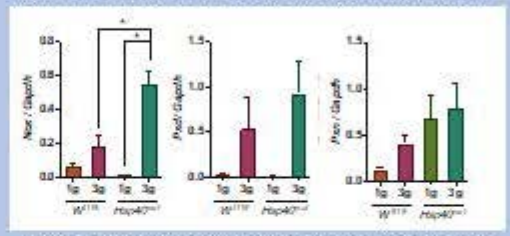
Results

Figure 3. Gel electrophoresis of *Hsp40* mutant and wild type.



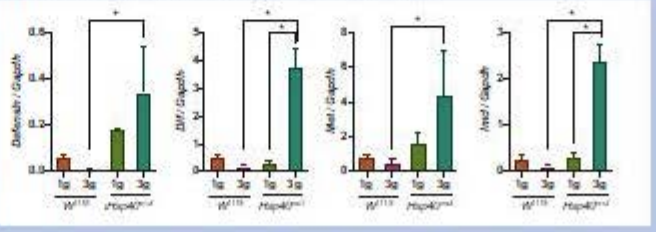
Comparison of *Hsp40* mutant to wild type. Fainter band on *Hsp40* mutant confirms the reduced *Hsp40* expression in mutants. Equally dark *Gapdh* bands on both samples indicates constitutive expression.

Figure 4. Oxidative stress response gene expression.



qPCR results for oxidative stress response genes. *Nox* expression significantly increased in *Hsp40* mutants compared to wild type flies, with similar trends in *Pxd* and *Pxn*. Data analysis performed by one-way ANOVA with post hoc Sidak's multiple comparisons test. Data represented as standard error of the mean (SEM), $p \leq 0.05$* *SEM*. *Nox*=NAPDH oxidase, *Pxd*=Drosophila peroxidase, *Pxn*=Peroxidasin precursor.

Figure 5. Innate immunity gene expression.



qPCR results for innate immunity genes. Significantly increased expression of all four genes. Data analysis performed by one-way ANOVA with post hoc Sidak's multiple comparisons test. Data represented as standard error of the mean (SEM), $p \leq 0.05$* *SEM*. *Dif*=Dorsal-related immunity factor, *Met*=Metchikowin, *Imd*=*Drosophila* immune deficiency gene.

Figure 6. Sample sizes.

Hsp40 1g	n= 40 flies, 4 samples
Hsp40 3g	n= 36 flies, 4 samples
W1118 1g	n= 88 flies, 9 samples
W1118 3g	n= 89 flies, 7 samples

Sample sizes for qPCR of oxidative stress genes and innate immunity genes. ~10 flies per sample.

Conclusions

- Defensin, Dif, Met, and Imd are involved in the antibacterial response, antifungal response, and neonatal immunity. Because of their increased expression in Hsp40 mutant samples, Hsp40 may have a regulatory effect on these immune response genes.
- *Hsp40* could also be involved in regulating the activity of oxidative stress response genes like *Nox* because of its increased expression in 3g Hsp40 knockdowns.

References

1. Sibonga, J. D., Spector, E. R., Johnston, S. L. & Tarver, W. J. Evaluating Bone Loss in ISS Astronauts. *Aerospace medicine and human performance* **86**, A38-44.
2. Feger, B. J. *et al.* Microgravity induces proteomics changes involved in endoplasmic reticulum stress and mitochondrial protection. *Sci Rep* **6**, 34091, doi:10.1038/srep34091 (2016).

Acknowledgements

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