Somatic Mutation in Mice on the International Space Station (ISS): Guanine Substitution Suggests Link to Cancer Risk

Viktor Stolc[1], Miloslav Karhanek[2], Friedemann Freund[3], Yuri Griko [1], David J. Loftus[1], Maurice M. Ohayon[4]
[1]Space Biosciences Research Branch, NASA Ames Research Center, Moffett Field
[2]Slovak Academy of Sciences, [3]SETI Institute, [4]Stanford University School of Medicine

ABSTRACT

We conducted comprehensive analysis of single nucleotide somatic mutations in mice exposed to microgravity and other factors aboard the International Space Station (ISS), using data archived in GeneLab. Animals in the experimental cohort consisted of mice that spent 37 days on the ISS within the Rodent Habitat. Ground control animals consisted of mice of identical age, sex, strain, in a terrestrial Rodent Habitat controlled for temperature, humidity and carbon dioxide levels, to match ISS conditions as closely as possible. RNA extracted from eye, liver, skeletal muscle, and kidney tissue specimens was subjected to next-generation sequencing to acquire primary data.

Our analysis employed cutting-edge software developed at NASA Ames Research Center, executed on the NASA Ames Supercomputer and on another high-performance computer, for accurate variant calling of single point mutations. ISS-flown mice exhibited a notably heightened level of somatic mutation compared to control mice. The degree of somatic mutation correlated with the degree of gene expression across the four tissue types, i.e., the greatest rate of mutation accumulation was seen in highly expressed genes.

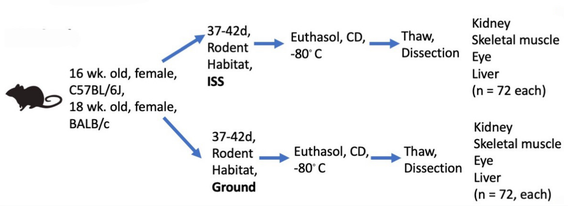
We discovered that guanine substitutions were the most common type of somatic mutation. This observation is consistent with the hypothesis that DNA mutation events stem from reactive oxygen/nitrogen/chlorine species-mediated guanine oxidation induced by the spaceflight environment. Since guanine oxidation is a prominent feature of the DNA mutation landscape that accompanies malignant transformation, our findings suggest a possible link between the spaceflight environment and cancer risk that is independent of radiation carcinogenesis.

BACKGROUND

- Somatic mutations (single nucleotide variations) are acquired point mutations in the DNA of somatic cells in the body.
- Unlike germline mutations, which can be passed on from one individual to another, somatic mutations are not heritable, although the progeny of mutated somatic cells are expected to maintain the somatic mutation.
- Somatic mutation has been recognized for decades as an important mechanism for initiating the development of cancer. A somatic mutation event (in a gene related to cell proliferation, survival advantage or some other phenotype that allows the cell to expand in numbers) is considered the critical first step toward carcinogenesis, with other processes such *promotion* and *progression* needed to fully manifest "cancer."
- Somatic mutation can be evaluated by direct DNA sequencing or by RNA sequencing. RNA editing, however, may introduce nucleotide changes that are not present in the DNA sequence, and thus may confuse the analysis.
- To differentiate between nucleotide changes induced by RNA editing ("apparent somatic mutations") versus nucleotide substitutions reflective of actual DNA sequence changes (true somatic mutations), we have performed a detailed analysis of the specific nucleotide alterations for all putative somatic mutations we identified in mice flown to the ISS.

METHODS

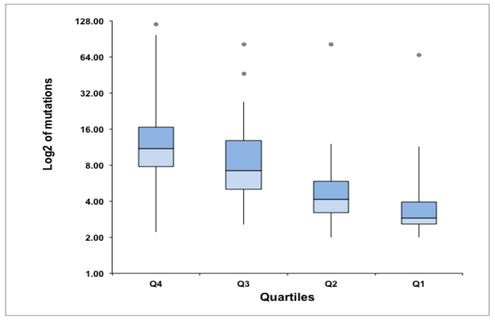
Mice Flown Aboard the ISS



Somatic Mutation Identification

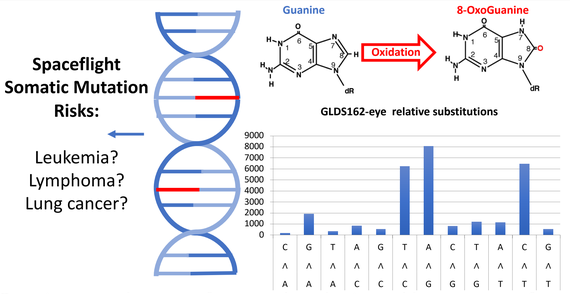
The core technology is a *variant call algorithm* derived from the Genetic Analysis Tool Kit 4.1 (GATK4), with additional tools based on the Python management software "Toil," and the programming language "R." Software is designed to work on Dell Servers as well as on systems with high-performance compute power, such as the NASA Ames Supercomputer.

Somatic Mutation Correlates with RNA Transcription



The highest quartile of gene expression is associated with the highest rate of somatic mutation (data shown for liver, as an example).

Oxidative DNA Damage: Guanine Substitutions Link to Cancer Risk

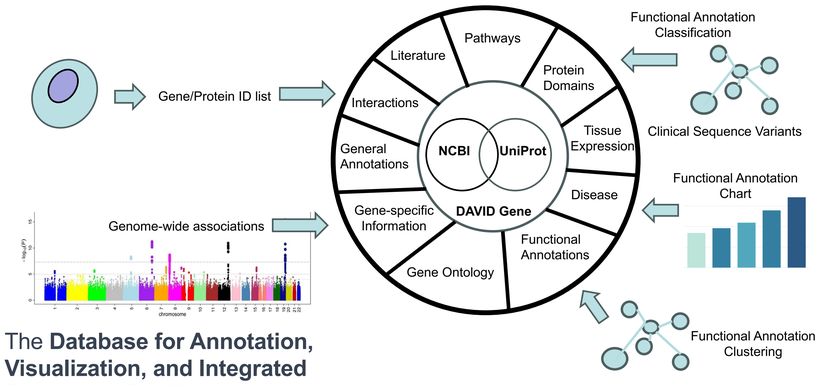


Guanine was the most frequently substituted base, most often involving conversion to adenine. This suggests that guanine oxidation may be playing a significant role in the process of somatic mutation on board the ISS. Of all the nucleotide bases, guanine is the most readily oxidized because of endogenous production of reactive oxygen species (ROS) inside cells.

RESULTS

Implications for Astronaut Health

Biological meaning behind the large list of sequence variants



The **Database for Annotation, Visualization, and Integrated Discovery (DAVID)** encompasses a suite of functional annotation tools tailored to elucidate the biological relevance of gene sequence variants. Built upon the comprehensive DAVID Knowledgebase and the DAVID Gene concept, which integrates diverse functional annotations from multiple sources, DAVID's tools excel in establishing connections between gene sequence variants and diseases. DAVID's tools can unravel crucial associations between specific gene sequence variants and diseases, thereby gaining deeper insights into their biological implications.

CONCLUSIONS

- **Rapid accumulation of somatic mutation occurs in mice flown aboard the ISS.**
- **The genome instability in space occurs to a much higher degree than previously expected, and this phenomenon may contribute significantly to some of the known biological consequences of spaceflight and can increase cancer risk.**

FUTURE WORK

The next step will be to perform an analysis of somatic mutations in humans flown aboard the ISS, using already-obtained data from the NASA Twin Study.

REFERENCE

Stolc, V., Karhanek, M., Freund, F., Griko, Y., Loftus, D.J., Ohayon, M.M. (2023) SPACEFLIGHT-INDUCED HYPERMUTATION IN MICE: IMPLICATIONS FOR ASTRONAUT HEALTH (submitted).

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