MicroRNA Expression Profile and DNA Damage Response in Cultured Human Fibroblasts in Space

Micro-7 Flight Experiment

(Funded by the NASA Fundamental Space Biology Program)

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Objectives

Aim #1. Investigate changes of miRNA and RNA expressions in G1 human fibroblast cells in space

- Cells were fixed with RNALater II in space

Aim #2. Investigate the cellular response to bleomycin-induced DNA damage in G1 human fibroblast cells in space

- Cells were treated with bleomycin (1 μg/ml) to induce DNA damages in space for three hours before fixed with RNALater II and paraformaldehyde

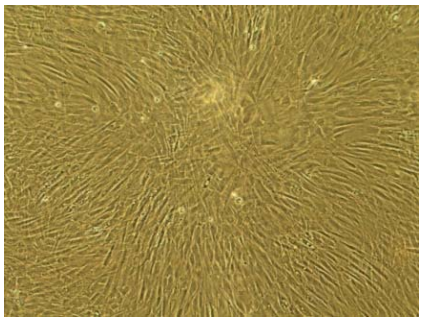
Aim #3. Detect DNA damage in the cells from direct exposure to space radiation

- Cells were fixed with paraformaldehyde and stained with γ-H2AX antibodies, a DNA damage marker

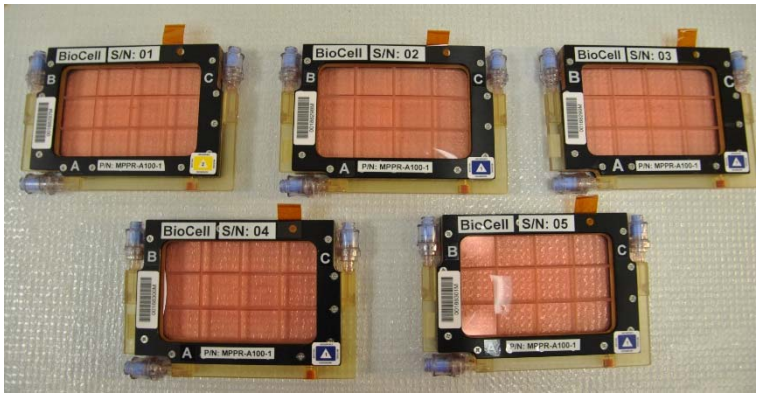
Cell culture and flight hardware

Confluent human fibroblast cells were cultured in BioCells. The cells were kept in CGBA on ISS at 37 C.

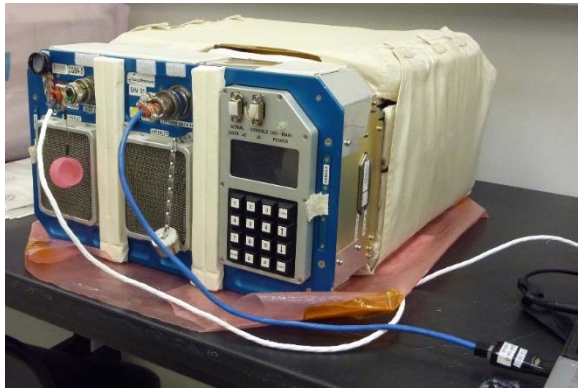
Human fibroblast cells



BioCell from BioServe



BioServe's CGBA incubator



Flight Schedule

4/18/14 – Cells were launched to ISS on board SpaceX-3

4/22/14 – Cells were transferred to a 37 C incubator

4/25/14 – Cells were fixed for RNA and miRNA analysis (Day 3)

4/25/14 – Cells were treated with bleomycin (1 μg/ml) (Day 3)

5/6/14 – Cells were fixed for RNA and miRNA analysis (Day 14)

5/20/14 – The fixed samples returned to JSC

Does spaceflight influence RNA and miRNA expressions in ***non-dividing*** cultured cells?

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Effects of Simulated Microgravity on Expression Profile of MicroRNA in Human Lymphoblastoid Cells*[S]

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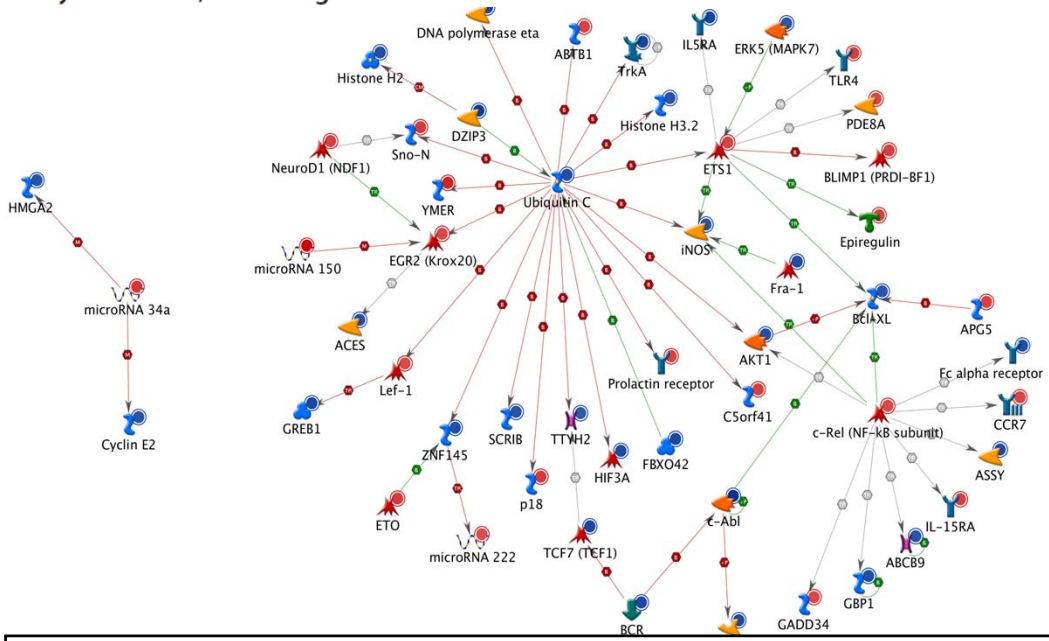
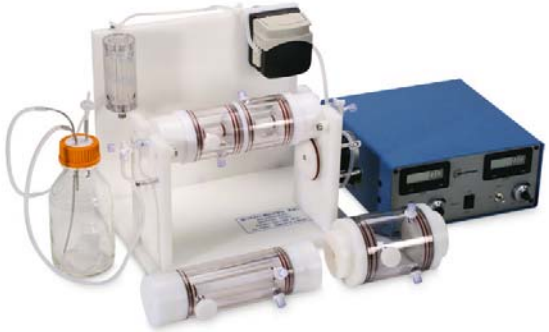


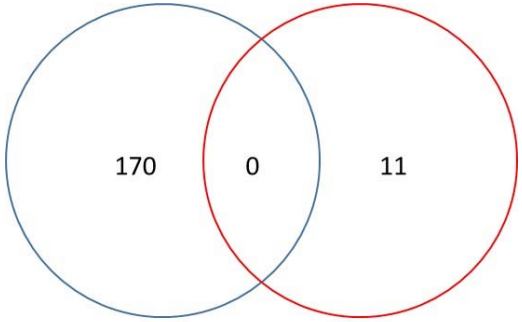


The direct interaction analysis showed several projected networks with c-Rel, ETS1 and Ubiquitin C as key factors. Several genes showed direct interactions with miRNAs that were found to be altered in simulated microgravity environment. Seven genes cyclin E2, HMGA2, EGR2, ZNF145, Ubiquitin C, ETS1 and c-Rel were subjected to validation analysis using Quantitative Real-time PCR.

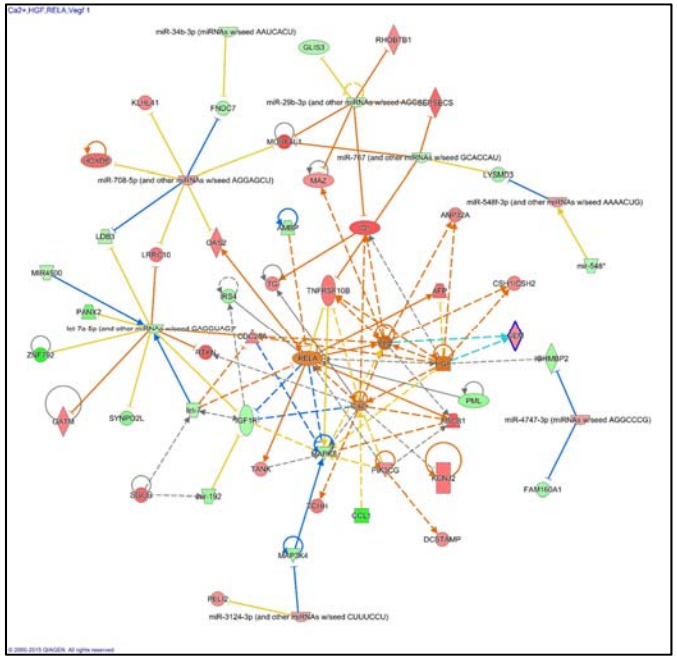
Spaceflight or simulated microgravity influences gene and miRNA expressions in proliferating cells

Microarray Results – Day 3 and Day 14

Number of genes having significant expression changes in the flight sample in comparison to the ground control (2 fold change with $p<0.05$) on Day 3 and Day 14

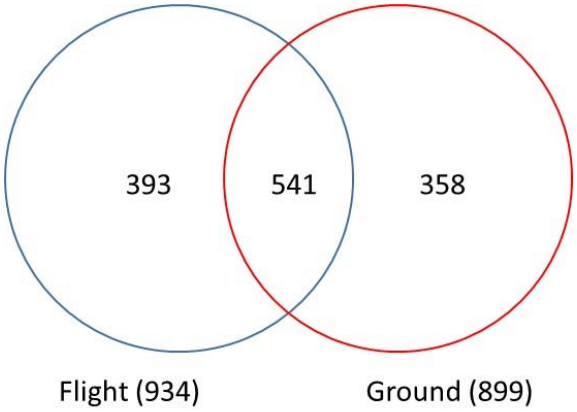


When the threshold of fold change of gene expressions is reduced to 1.5 ($p<0.05$), NFκB and TGF-β were identified as potential upstream regulators.



Microarray Results – Day 3 vs. Day 14

Number of gene having significant expression changes in the Day 3 sample in comparison to the Day 14 sample (2 fold, $p<0.05$)

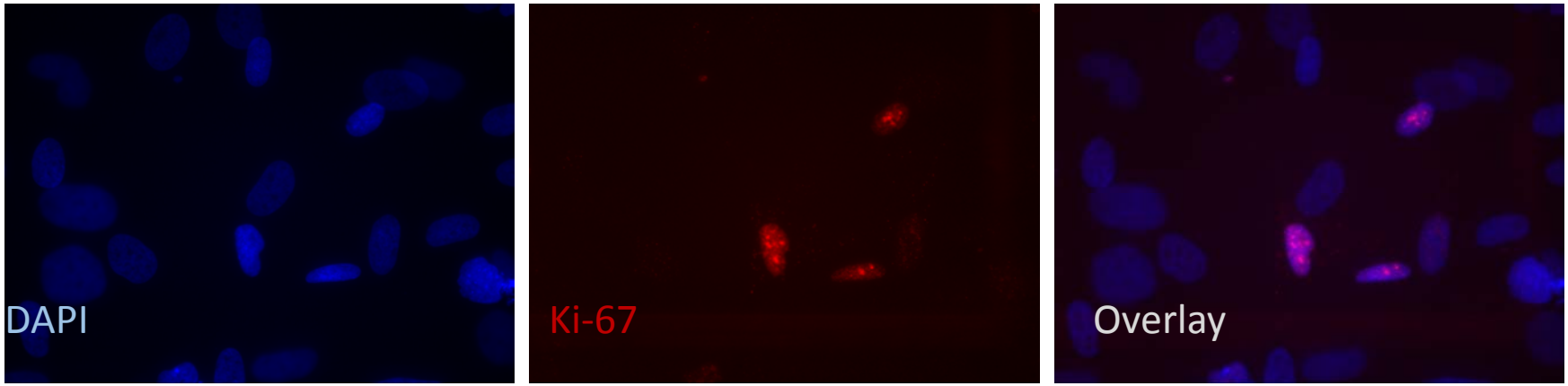


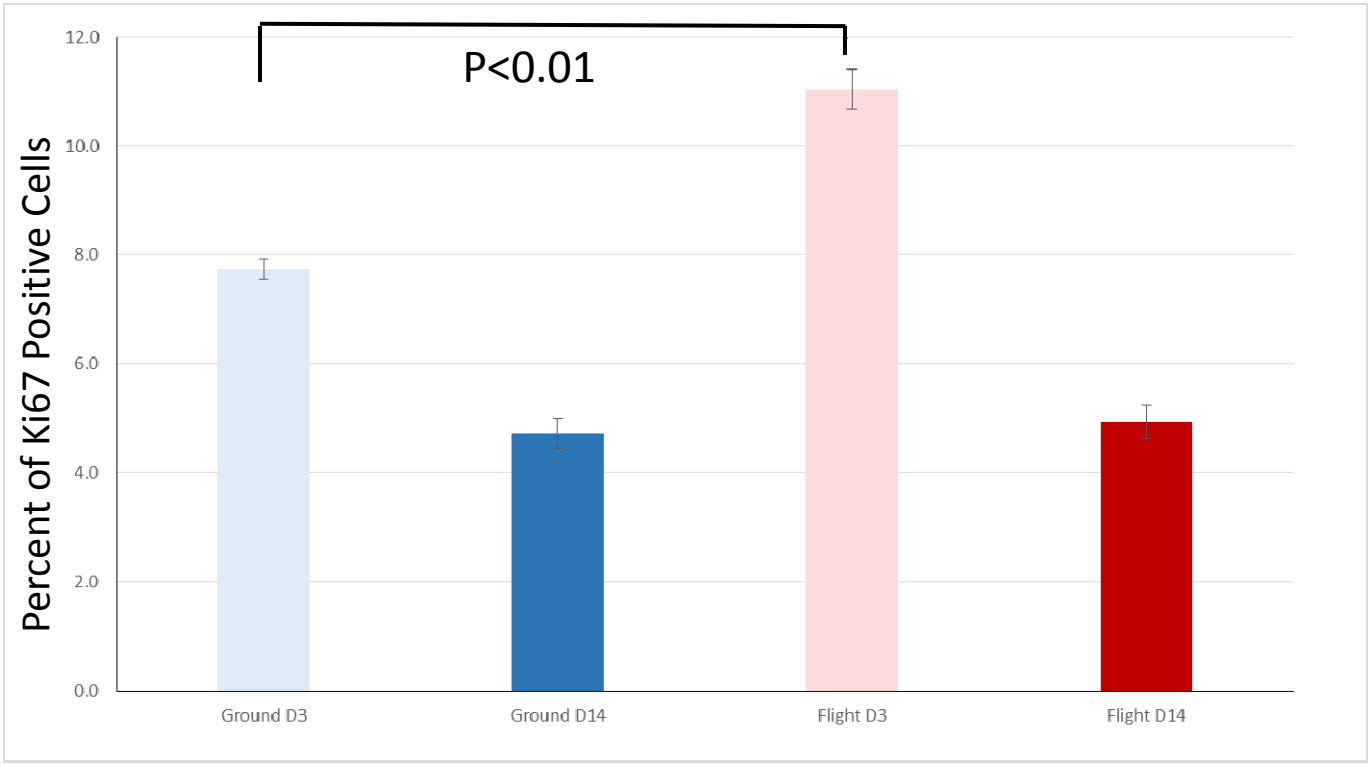
Top Upstream Regulators

Ground		Flight	
TP53	Inhibited	TP53	Inhibited
TGFB1		Vegf	Activated
dextran sulfate		HGF	Activated
CDKN1A	Inhibited	dextran sulfate	
CSF2	Activated	CDKN1A	Inhibited

The Day 3 cells still grew slowly even when the majority of the cells were in G1 phase.

Cell Proliferation Marker – Ki67





Summary 1

- On Day 3, both the flown and ground cells were still proliferating slowly even though they were confluent, as measured by the expression of ki-67 positive cells, and the cells in space grew slightly faster.

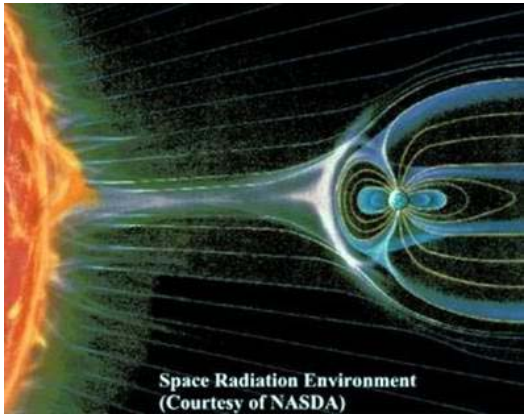
- Gene and miRNA expression data for Day 3 indicated activation of NFkB and other growth related pathways involving HGF and VEGF in the flown cells.

- On Day 14 when the cells were mostly non-dividing, the gene and miRNA expression profiles between the flight and ground samples were indistinguishable.

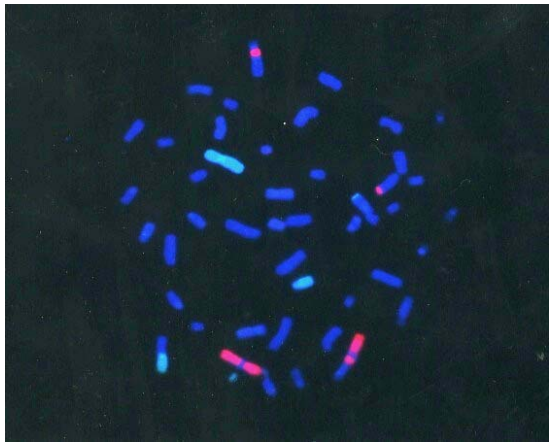
- Comparison of gene and miRNA expressions in the Day 3 samples in respect to Day 14 revealed that most of the changes on observed on Day 3 were related to cell growth for both the flown and ground cells.

- Does microgravity affect cellular response in living organisms to space radiation exposure?

- Do microgravity and other spaceflight factors affect cellular response to DNA damages?



Chromosome aberration frequencies in pre- and post-flight astronaut lymphocytes irradiated *in vitro* with low-LET radiation (Wu *et al.* Phys. Med. 2001)



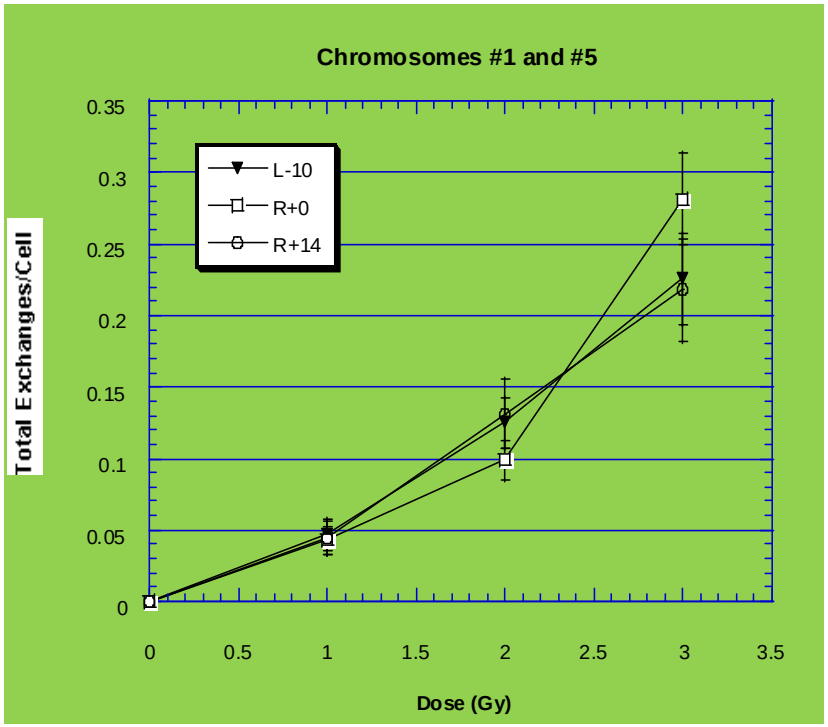
Mission: STS-103

Duration: 8 days

Blood draw schedule: L-10, R+0 and R+14

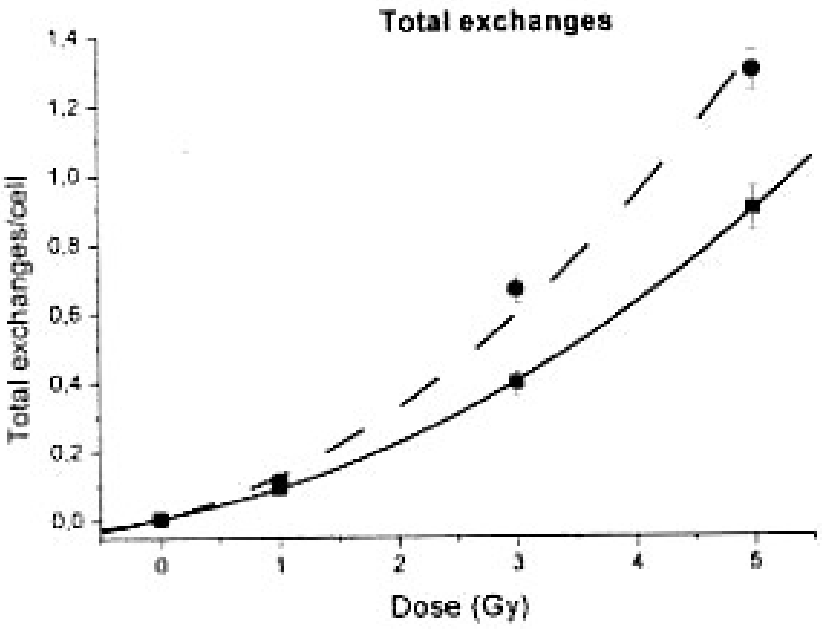
Irradiation: Whole blood was collected from the astronaut and irradiated with gamma rays

Chromosome analysis: Chromosomes #1 and #5 were painted.



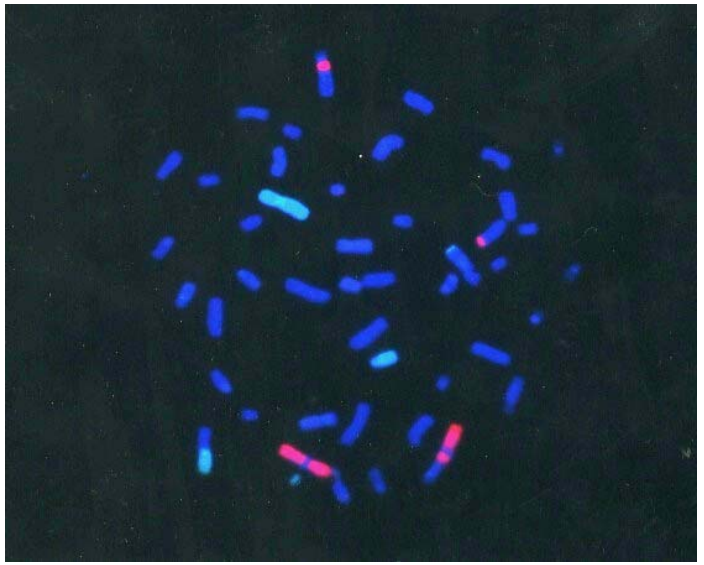
No changes in the chromosome aberration frequency were observed between the pre- and post-flight samples

Greco *et al.* Adv. Space Res. 2003



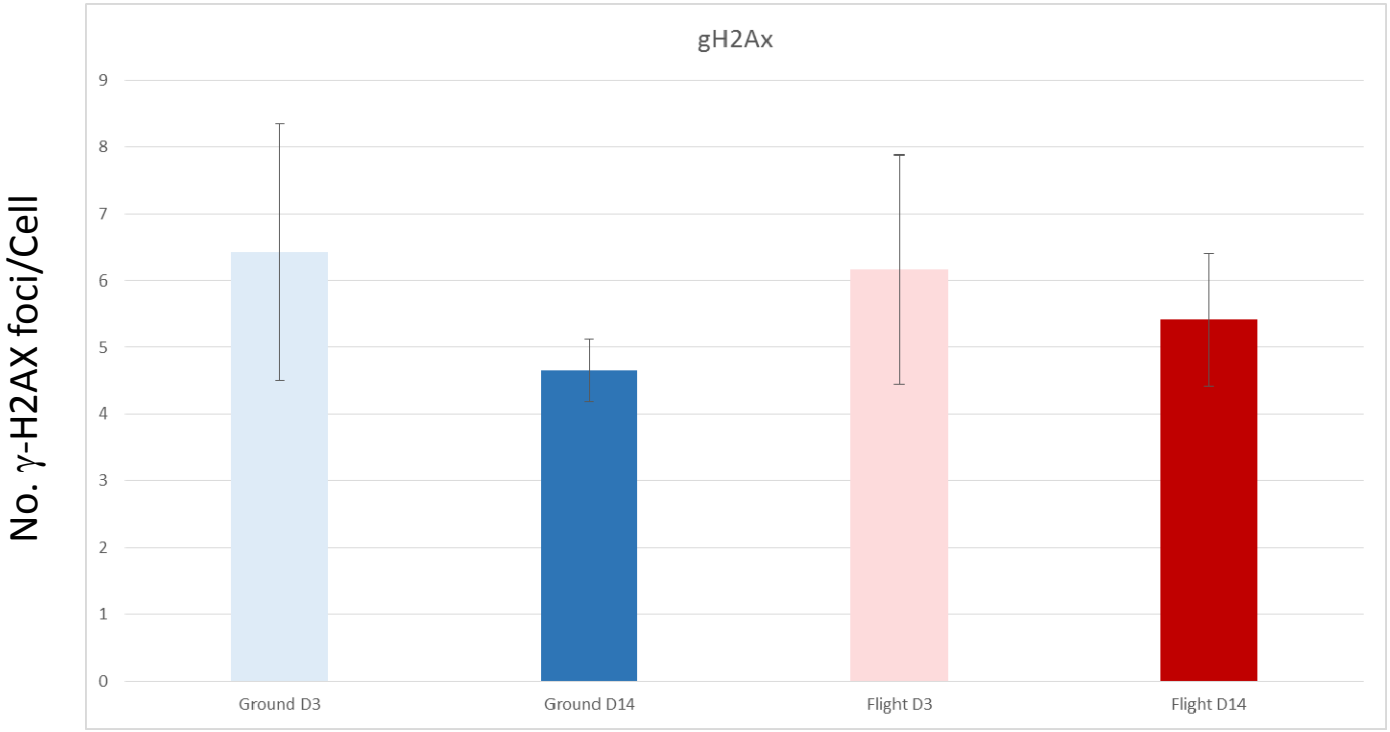
Significant difference was found in the dose response curve between the pre- and post-flight samples.

- Cosmonaut's blood samples were collected 3 days after landing
- Samples were exposed to X-rays



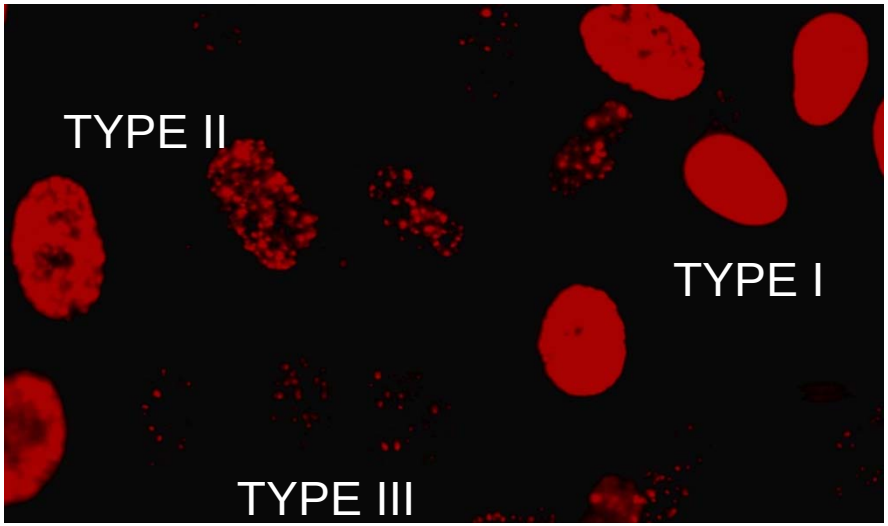
Do microgravity and other spaceflight factors affect cellular response to DNA damages?
Answer: Inconclusive

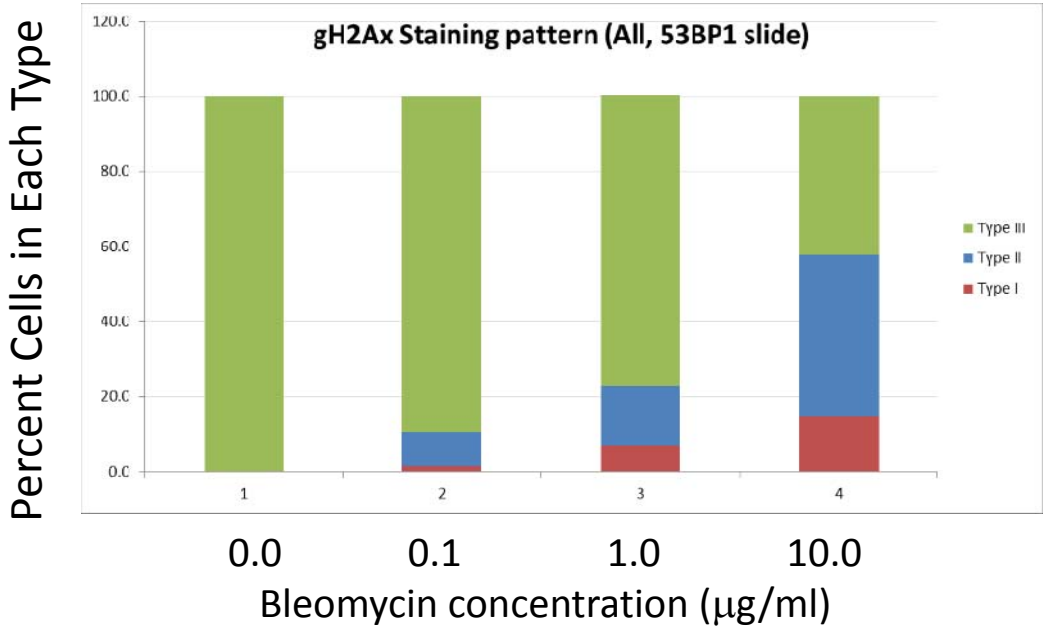
γ-H2AX Foci in Day 3 and Day 14 Cells



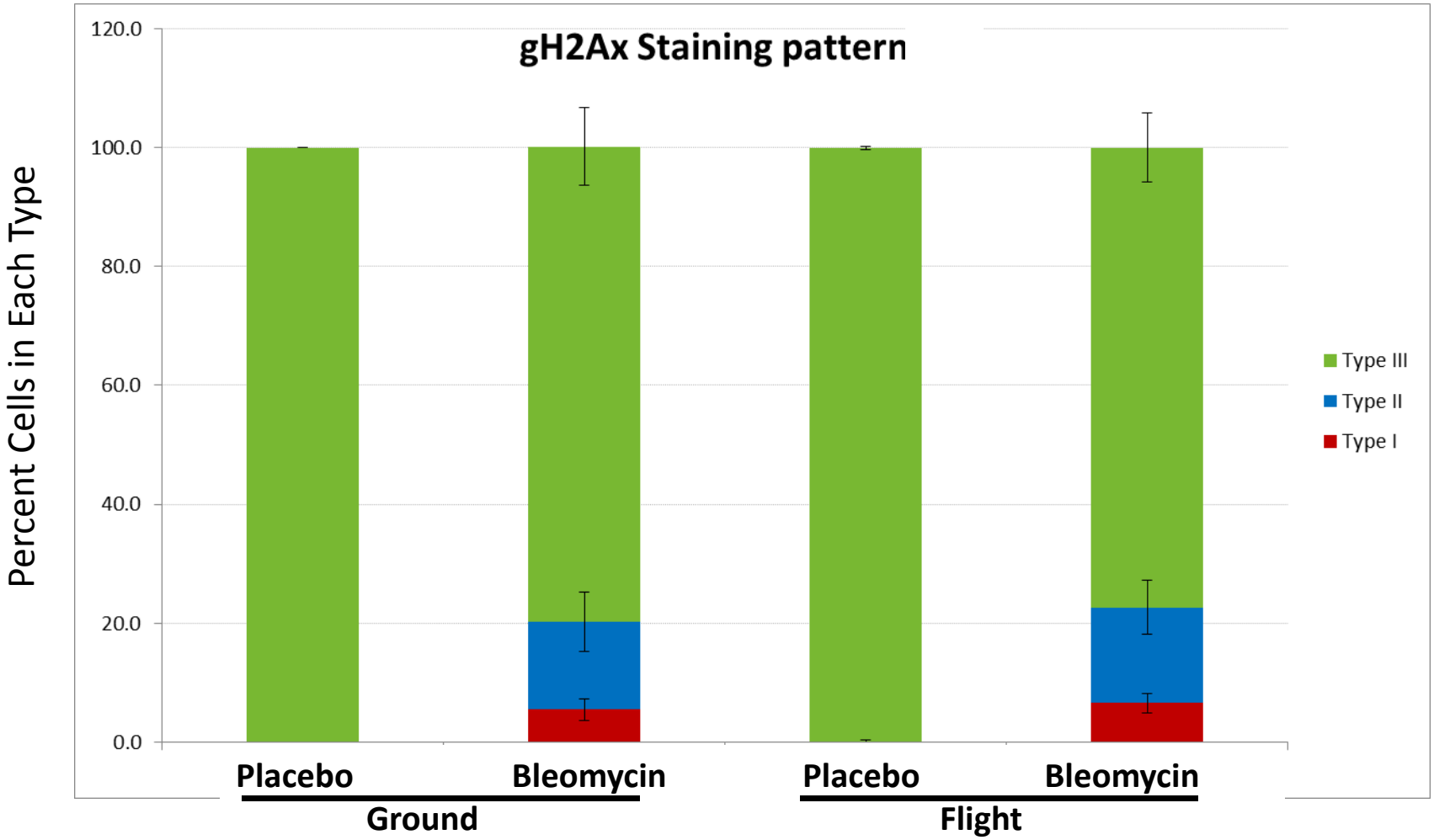
- The number of γ-H2AX foci appeared to be higher in Day 3 Cells in comparison to Day 14 cells for both the flight and ground samples.
- On Day 14, the number of γ-H2AX foci appeared to be higher in the flight sample in comparison to the ground, in consistent with the reported studies with cells exposed to low doses of radiation chronically.

Quantification of Bleomycin-induced Damages with γ-H2AX Immunofluorescence Staining Patterns and Foci Counts

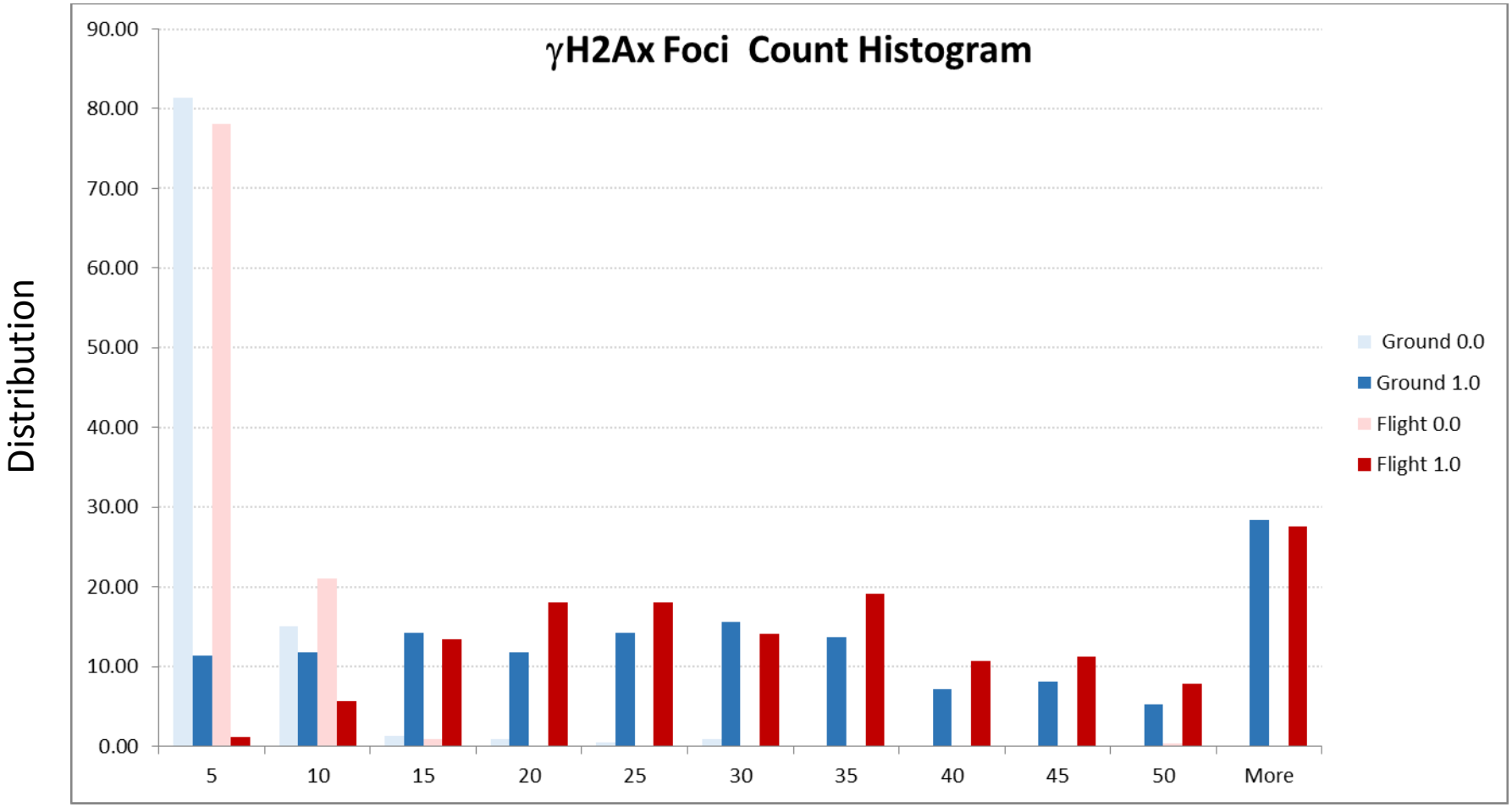




Types of γ-H2AX patterns in the ground and flight samples treated with bleomycin



Distribution of γ-H2AX foci in a Type III cells after bleomycin treatment



Summary 2

- The 3-dimensional g-H2AX foci were captured with a laser confocal microscope. Quantitative analysis revealed a small fraction of foci that were larger and displayed a track pattern in the flight samples in comparison to the ground control.
- Damage in the DNA was measured by the phosphorylation of a histone protein H2AX (g-H2AX), which showed slightly more foci in the cells on ISS than in the ground control.
- Although a number of the genes, including CDKN1A and PCNA, were significantly altered in the cells after bleomycin treatment, no significant difference of the expression profile of DNA damage response genes was found between the flight and ground samples.

Conclusions

- Results of our study suggest that in true non-dividing human fibroblast cells, microgravity in space has little effect on the gene and miRNA expression. Gene and miRNA expression changes were observed in cells that were confluent, but still proliferating slowly. The faster growth in the flown cells were associated with the activation of NFkB pathways which triggers the expression of several growth factors and the suppression of the cell cycle checkpoint.

- Our results suggested that the difference in γ-H2AX between flight and ground was due to the faster growth rate of the cells in space, but spaceflight did not affect the response of the DNA damage response genes to bleomycin treatment.

Acknowledgement

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