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Variations in the Radiation Environment and Observed Biological Consequences on the Long-Term Stored Embryonic Stem Cells in the Kibo Module of the ISS

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This talk is dedicated to the memory of **Prof. Richard Wilkins (1960-2024)** Prairie View A&M University



Authors and Principals

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BACKGROUND

Historical and Current

100+ Years of History: 1912-2012 Discovery of Cosmic Rays - Victor Hess



Victor Francis Hess B 1883 Peggau, Austria D 1964 New York, USA

- Victor Hess 1912
 - Investigated sources of radiation – a balloon trip to 5300 m (risking life)
 - Radiation levels to be higher after 2500 m
 - Attributed to the fact that there was less atmosphere above to shield from radiation – source outside
- Concluded radiation is coming from space ...
 "cosmic radiation"
- Won Nobel Prize 1936



Readings on ionization chamber Victor Hess carried aloft in the Böhmen. Above four kilometers the ionization rose rapidly indicating "that rays of very great penetrating power are entering our atmosphere from above". These cosmic rays contain the only modern samples of matter from outside our solar system which can be investigated directly.



September 27, 2023: Astronaut **Frank Rubio** lands on Earth after **371 days on ISS** – longest US record with 5,963 orbits around the Earth, travelling 157.4 million miles (or 253.3 million km).



Nasa astronaut Frank Rubio has just returned from a record-breaking 371 days in space onboard the ISS, but the trip **may have** <u>altered his muscles, brain and even the bacteria</u> living in his gut

https://www.bbc.com/future/article/20230927-



nasa.gov





Dr. Peggy Whitson

More time in space by any American / woman ~ 666 days (over 3 expeditions) 10 EVAs (~ 60 hrs outside ISS)

Private – Axiom-02 (2023) + 9 days

"She is still the oldest woman to orbit the Earth, a record she broke in 2023, at 63"



nasa.gov

September 4, 2024

Rank	Name	Days	Flights	Status	Nationality
1	Oleg Kononenko	1092	5	(on ISS now)	Russia
2	Gennady Padalka	878	5	Retired	Russia
3	Yuri Malenchenko	827	6	Retired	Russia
4	Sergei Krikalev	803	6	Retired	Soviet Union / Russia
5	Aleksandr Kaleri	769	5	Retired	Russia
6	Sergei Avdeyev	748	3	Retired	Soviet Union / Russia
7	Anton Shkaplerov	709	4	Retired	Russia
8	Valeri Polyakov	679	2	Deceased	Soviet Union / Russia
9	Peggy Whitson	675	4	Active	United States
10	Fyodor Yurchikhin	673	5	Retired	Russia

As of today (Sept 4, 2024) **50** people spend more than **365** days in space

Source: Wikipedia

Radiation Climate on Mars



Mars Exploration – NASA



Mars Cosmic Ray Environment Dose Equivalent Values (rem/yr)



Mars Radiation Predictions and Observations

Comparison	GCR Dose Rate (mGy/day)	GCR Dose Equiv. Rate (mSv/day)
RAD Mars Surface Measurements	0.205 <u>+</u> 0.05	0.70 <u>+</u> 0.17
(Hassler / Zeitlin 2014)		
Model Calculations Saganti / Cucinotta 2002 (Based on Solar Cycle # 23)	0.212	0.65
Model Calculations Saganti / Cucinotta 2015 (updated for Solar Cycle # 24)	0.232	0.74

Overview

- A joint collaborative space project between JAXA and NASA
 - "Stem Cells Study on the Effect of Space Environment to Embryonic Stem Cells to their Development" in the Kibo Module.
- Frozen Embryonic Stem (ES) cells were launched to ISS in
 - Stored in the Minus Eighty Degree Laboratory Freezer for ISS (MELFI) March 2013 through July 2017.
- Four different sets of cell samples were retrieved back to earth
 - After periods of 443 days, 711 days, 1167 days, and 1584 days on the ISS
 - Along with the PADLES (Passive Dosimeter for Life-science Experiments in Space)
 - PADLES consisting of CR-39 and TLDs) dosimeters attached to the tubes (ES cells).
- After these ES cell stacks were brought back to the ground, they were thawed and cultured, and their gene expressions were comprehensively analyzed to elucidate the early response of the cells to long-time exposure to space radiation in microgravity conditions.





Observations

- The comparisons of gene expression involved in double-stranded break (DSB):
 - Also, the expressions of most of the genes that were involved in homologous recombination (HR) and non-homologous end joining (NHEJ) were studied and compared between the ISSstocked cells and ground-stocked control cells.
- It was noted that the transcription of Trp53inp1 (tumor protein 53 induced nuclear protein-1), Cdkn1a (p21), and Mdm2 genes increased in ISS-stocked cells.
 - This phenomenon is similar to the cells that were exposed to the Fe ions in ground-based experiments at HIMAC Facility.
 - This suggests that accumulated DNA damage caused by space radiation exposure would activate these genes.
- The PADLES measured dose-equivalent values on ISS:
 - 250 mSv (for 443 days),
 - 375 mSv (for 711 days),
 - 575 mSv (for 1167 days), and
 - 830 mSv (for 1584 days) between 2013 and 2017



Comparison of the incidence of chromosomal aberrations of wild-type and histone H2AX gene heterozygous-deficient ES cells on the ground (BU) and on the ISS (MELFI) chromosomes were analyzed by FISH method.

The wild-type embryonic stem (ES) cells showed no differences in chromosomal aberrations between the ground control and ISS exposures. However, we detected an increase of chromosome aberrations in radio-sensitized histone H2AX heterozygous-deficient mouse ES cells and found that the rate of increase against the absorbed dose was 1.54-fold of proton irradiation. 19

LET Distribution of Particles Detected by PADLES

Total 545 mGy (96%) 25 mGy (4.4%)



LET distribution of particles as measured by PADLES

Lower LET particles (< 10 keV /micron) contributed to 96% of the dose and

Higher LET particles (> 10 keV/micron) contributed to 4% of the total dose.

Morita et al., 2021 doi/10.24544/ocu.20210401-001



Yoshida, K. et al. *Heliyon* 8(8), e10266. 2022

Fe-ion beam 500 MeV/n LET 218 keV/μm

Proton beam 230 MeV LET 0.415 keV/μm

Expression of DNA Repair Genes on ISS and Ground(BU)



Yoshida K. et al., Int J Mol Sci; 25 (6)

Total Absorbed Dose [mGy in water]



Nagamatsu et al., JAXA - 2020

Chromosome Aberration Studies on ISS: 2012-2022

Morita (JAXA) / Cucinotta (NASA) (PIs) A CELL Press 2022 Publication *Heliyon* 8, no. 8 (2022) e10266 Π



Dr. Morita (JAXA) and Dr. Cucinotta (NASA) – A Study on ISS

Published in 2022 and Recognized as a NASA Science Discovery on ISS

Heliyon 8 (2022) e10266



Research article

Comparison of biological measurement and physical estimates of space radiation in the International Space Station

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- ISS Study This work is a result of experiments on board the ISS from 2012-2022 spanning over 10 years and 10 Expeditions
 - Dr. Morita, JAXA-Japan and Dr. Cucinotta, NASA-USA as the PIs
 - From 2016, through our CRI / RaISE, we analyzed samples and data from ISS and contributed to this unique work.
- ISS Discovery Included for 2022 •
 - Results from the ISS provide new contributions to the body of scientific knowledge in the physical sciences, life sciences, and Earth and space sciences to advance scientific discoveries in multidisciplinary ways.
- ISS Publication This publication made its presence on ISS Research Explorer's Page of the NASA Website (2022)
 - As part of the ongoing radiation biology / biotechnology research work
 - Stem Cells (nasa.gov)

Few Thoughts

- From these results, in contrast to the expression of homologous recombination repair genes such as RecA in prokaryotic cells, and Rad51 in yeast, and unicellular eukaryote (Ascomycete) which are induced in response to radiation.
- In higher eukaryotes such as mouse ES cells, DNA damage does not immediately induce the transcription of repair genes, but rather activates proteins such as ATM, followed by phosphorylation of p53 protein.
- Recently, it has been reported that the strong radiation resistance of tardigrades, which are classified as an invertebrate Panarthropod, is due to the dramatic induction of DNA repair genes such as Rad51 and ERCC6 (Ku70).

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- 2024: Hada et al., in PRESS

NEUTRONS

Studies at LANL



Mutation Research 701 (2010) 67-74



Contents lists available at ScienceDirect Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox Community address: www.elsevier.com/locate/mutres



mBAND analysis of chromosome aberrations in human epithelial cells induced by γ -rays and secondary neutrons of low dose rate

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Chromosome painting with Multicolor fluorescence in Situ Hybridization



mFISH



<u>Multicolor Fluorescence</u> <u>in situ Hybridization</u>

- simultaneous presentation of all 24 different human
 - chromosomes with one single hybridization
- analysis of hidden or complex chromosome aberrations
- composition of marker chromosomes

mBAND



Multicolor Banding in situ Hybridization

- color banding pattern along one chromosome
- higher level of precision within one chromosome
- detection of intrachromosomal rearrangements
- detection of break points











Induction of chromosome 3 aberration in human cells by Neutron, Fe-ions or γ -rays



RBE for 20% of cells with damaged chromosome 3 Fe: 4.5 Neutron: 14.7

Fe-ions induced more aberrations in chromosome 3 compared to γ -rays, and neutron induced more aberration than Fe ions.





Induction of terminal deletion in human chromosome 3 by neutron, Fe ions or γ-rays irradiation



Neutron induced more terminal deletion compare to Fe ions and γ -rays irradiation.

Microgravity + Radiation

SYNEGESTIC EFFECTS 2023

ICRR 2023 Montréal, Quebec August 29, 2023 Hada / Saganti

S25-05





3D clinostat with C-ion system







290 MeV/n, 50 keV/m, ca 0.03 Gy/min (Gunma University Heavy Ion Medical Center, Maebashi, Japan)

Ikeda H et al. Life Sci Space Res 2017; 12:51-60

3D clinostat with X-ray system





200 kV, 14.6 mA, 0.3 mm aluminum filter ca 0.03 Gy/min (MultiRad225: Faxitron Bioptics, LLC, Tucson, AZ, USA)

Ikeda H et al. Biol Sci Space 2016; 30:8-16

3-color fluorescent in situ hybridization method



Normal



Simple exchange (1 & 2)



Break in chromosome 4



Complex exchange (1 and 2 & other)



Simple exchange (1 & other)



Chromosomes 1: red Chromosomes 2: green Chromosomes 4: yellow

Complex exchanges: An exchange involved a minimum of three breaks in two or more chromosomes Simple exchanges: Two breaks in two chromosomes (dicentrics and translocations)

Cell survival and chromosome aberration - Fibroblasts



Cell survival and chromosome aberration – Lymphoblast TK6



Yamanouchi S et al. Life 2020;10:0187

Statistical analysis – CA Lymphoblast TK6

Total Chromosome aberrations



The logistic regression analysis of the effect of radiation and gravity on total exchanges.

Radiation Type		Coefficient	Standard error of coefficient	<i>p</i> -value	Odd ratio -	95% CI for OR	
						Lower	Upper
	Dose	1.906	0.136	2.12 x 10 ⁻⁴⁴	6.727	5.150	8.789
X-ray	Gravity	0.491	0.132	2.04 x 10 ⁻⁴	1.635	1.261	2.118
	Constant	-4.826	0.171	3.30 x 10 ⁻¹⁷⁴			
	Dose	5.218	0.447	1.85 x 10 ⁻³¹	184.559	76.821	443.396
C-ion	Gravity	0.397	0.156	1.1 x 10 ⁻²	1.487	1.487	2.018
	Constant	-4.787	0.193	4.75 x 10 ⁻¹³⁶			

Both dose and gravity significantly contribute to total exchanges.

Yamanouchi S et al. Life 2020;10:0187



Simple and Complex Exchanges



The logistic regression analysis of the effect of radiation dose and gravity on total exchanges

Radiation	Factor	Coefficient	SE* of coefficient	p-value	OR**	95%CI*** for OR	
						Lower	Lower
	Dose	2.732	0.203	3.65×10 ⁻⁴¹	15.370	10.318	22.895
X-ray	Gravity	0.760	0.150	4.00×10 ⁻⁷	2.138	1.594	2.868
	Constant	-5.247	0.178	1.85×10 ⁻¹⁹¹			
C-ion beam	Dose	6.284	0.355	3.13×10-70	535.886	267.397	1073.961
	Gravity	0.466	0.124	1.66×10 ⁻⁴	1.594	1.250	2.031
	Constant	-5.490	0.146	<1.00×10 ⁻¹⁹¹			

*SE, standard error; **OR, odds ratio; ***CI, confidence interval.

Both dose and gravity significantly contribute to total exchanges.

Yamanouchi S et al. Biol Sci Space 2021; 35:15-23

Comparison of cells



2019; 20:43

2020; 10:187

Biol Sci Space 2021; 35:15-23

SwiNG Simulator of the environments on the Moon and Mars with Neutron-irradiation and Gravity-change



Takahashi A, et al. Life 10: 274, 2020

The X:Y ratios of clino-rotation were set at 11:13 rpm. The rotary speed of motor 1 was 0-133 rpm (0-2*G*). Chromosome aberrations with low dose neutron exposure



Total Exchanges

Simple and Complex Exchanges

N: Neutron 0.5mGy/day x 5 days

Frequencies of CA induced by neutron exposure is depending on the gravity condition

OTHER BIOLOGICAL APPLICATIONS

Radiation Track Structure Detector

Track Structure Detector



Sensor Dimensions: 0.644 cm (H) x 0.461 cm (V) Active Pixels: 3664(H) x 2748(V); 10,068,672 Pixel Size: 1.67 x 1.67 μm



Example - DNA damage foci and pixel image (Wang / Saganti)

- DNA damage foci
 - Live imaging mCherry -53BP1 in mouse hippocampal neuronal cells
 - Staining 53BP1 in mouse hippocampal neuronal cells
 - Zeiss Fluorescent microscope at NSRL
 - Leica Confocal microscope at RaISE

- Pixel image
 - 1.67 um/pixel
 - 10 um each pixel spot and 100 um pixel track

- Foci image
 - 0.16 um/pixel
 - about 1 um each focal spot and 10 um foci track

EXPERIMENTS WITH CARBON IONS

BNL (USA) HIMAC (JAPAN)

C-300 MeV: Detector along the beam line (90° alignment) Beam: Dose-Rate = 1 cGy/min, Total Dose = 2cGy



© Wang-Saganti-Holland 2016

C-300 MeV: Detector along the beam line (0° alignment) Beam: Dose-Rate = 1 cGy/min, Total Dose = 2cGy



Mouse Hippocampal neuronal cells (HT22) and Radiation Particle Trajectory (C ions, LET = 50 keV/um) (GFP-LC3)



© Inage Credit – Leica SP8 Confocal System at CRI / RaISE

Mouse Hippocampal neuronal cells (HT22) and Radiation Particle Trajectory (C ions, LET = 50 keV/um) (mCherry-53BP1)



© Inage Credit – Leica SP8 Confocal System at CRI / RaISE

Mouse Hippocampal neuronal cells (HT22) and Radiation Particle Trajectory (C ions, LET = 50 keV/um) [(GFP-LC3) + (mCherry-53BP1)]



© Inage Credit – Leica SP8 Confocal System at CRI / RaISE

Individual Tracks (13 vs 25 keV/μm)



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Radiation Track Structure at Micron Level of Carbon Ions

© Saganti - 2016



Radiation Track at 1.67 micron per pixel resolution for carbon ion with 300 MeV/n and LET 50 keV/μm (approximately 100x50 pixels are shown from about 3600x2700 pixel image)



ACE: Example of Carbon data (~ 25 years) with prediction for solar cycle # 25

© Kallur / Erickson / Saganti -2021



Texas A&M University System (TAMUS) Chancellor's Research Initiative (CRI) @ Prairie View A&M University (PVAMU)

Backup Charts

© Cucinotta / Saganti: National Geographic January-2001



This illustration of DNA image (including radiation damage part) has been used and referenced in several books, numerous NASA websites, National Labs, major universities, and recruitment brochures of TAMU and others.



Cucinotta / Saganti: 2001

62 http://srhp.jsc.nasa.gov/