WRMISS - 2024

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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

Premkumar B. Saganti, Ph. D.

Regents Professor – Texas A&M University System Radiation Institute for Science and Engineering (RaISE) A Texas A&M Chancellor's Research Initiative (CRI) *Prairie View A&M University* **www.pvamu.edu/raise**

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

Authors and Principals

Kayo Yoshida¹, Megumi Hada², Masami Hayashi¹, Akane Kizu¹, Kohei Kitada¹, Kiyomi Eguchi-Kasai³, Toshiaki Kokubo³, Takeshi Teramura⁴, Hiromi Suzuki⁵, Hitomi Watanabe ⁶, Gen Kondoh ⁶, Aiko Nagamatsu ⁷, Sachiko Yno⁷, Premkumar Saganti ², Masafumi Muratani ⁸ **, Francis A. Cucinotta ⁹ and Takashi Morita ¹**

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Space

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¹Graduate School of Medicine, Osaka Metropolitan University, Osaka 545-8585, Japan; **KIBO** ²Radiation Institute for Science and Engineering, Prairie View A&M University, Prairie View, TX 77446, USA; ³QST National Institute of Radiation Sciences (NIRS), Chiba 263-0024, Japan; ⁴Faculty of Medicine, Kindai University, Osaka 577-8502, Japan; SS 5 Japan Space Forum (JSF), Tokyo 101-0062, Japan; ⁶Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8501, Japan; 7 Japan Aerospace Exploration Agency (JAXA), Tsukuba 305-8505, Japan; ⁸Department of Genome Biology, Faculty of Medicine, University of Tsukuba, Tsukuba 305-8575, Japan; PDepartment of Health Physics and Diagnostic Sciences, University of Nevada, Las Vegas, NV 89154, US. ^{Te} Sciences in SP City Univ.

BACKGROUND

Historical and Current

100+ Years of History: 1912-2012 Discovery of Cosmic Rays - **Victor 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"**
- Victor Francis Hess **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 l</u>iving 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"

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nasa.gov

September 4, 2024

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

Source: *Wikipedia*

Radiation Climate on Mars

Mars Exploration – NASA

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

Mars Radiation Predictions and Observations

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. The set of the

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

Fe-ion beam 500 MeV/n LET 218 keV/μm

Proton beam 230 MeV LET 0.415 keV /μm

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

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

Yoshida K. et al., *Int J Mol Sc*i; *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**

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

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

Research article

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

Kayo Yoshida^a, Megumi Hada^b, Akane Kizu^a, Kohei Kitada^a, Kiyomi Eguchi-Kasai^c, Toshiaki Kokubo^c, Takeshi Teramura^d, Sachiko Yano^e, Hiromi Hashizume Suzuki^f, Hitomi Watanabe⁸, Gen Kondoh⁸, Aiko Nagamatsu^e, Premkumar Saganti^b, Francis A. Cucinotta^h, Takashi Morita^{a,}

^b Radiation Institute for Science and Engineering, Prairie View A&M University, TX, USA

^a Osaka City University, Graduate School of Medicine, Osaka, Japan

^c QST National Institute of Radiation Sciences (NIRS), Chiba, Japan

^d Kindai University, Faculty of Medicine, Osaka, Japan

^e Japan Aerospace Exploration Agency (JAXA), Tsukuba, Japan

^t Japan Space Forum (JSF), Tokyo, Japan

⁸ Kyoto University, Institute for Frontier Medical Sciences, Kyoto, Japan

^h University of Nevada, Las Vegas, NV, USA

- 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: Morita et al., in PRESS
- 2024 : Yoshida et al., in PRESS
- 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

M. Hada^{a,b}, B. Gersey^c, P.B. Saganti^c, R. Wilkins^c, F.A. Cucinotta^a, H. Wu^{a,*}

^a NASA Johnson Space Center, Houston, TX 77058, USA

^b Universities Space Research Association, Houston, TX 77058, USA

^c Prairie View A&M University, Prairie View, TX 77446, USA

Chromosome painting with Multicolor fluorescence in Situ Hybridization

mFISH

Multicolor Fluorescence *in situ* Hybridization

- 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 y-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 -rays, and neutron induced more aberration than Fe ions.

Induction of terminal deletion in human chromosome 3 by neutron, Fe ions or -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)

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

Break in chromosome 4

Complex exchange (1 and 2 & other)

Normal Simple exchange (1 & 2) Simple exchange (1 & other)

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

Complex exchange (2, 4 & other)

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.

Both dose and gravity significantly contribute to total exchanges.

Yamanouchi S et al. Life 2020;10:0187

Chromosome aberration – Blood Lymphocytes

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

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

Both dose and gravity significantly contribute to total exchanges.

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

Simple and Complex Exchanges

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 **wi**th **N**eutron-irradiation and **G**ravity-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 ⁵¹

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)

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 ⁵⁸

Texas A&M University System (TAMUS) Chancellor's Research Initiative (CRI) @ Prairie View A&M University (PVAMU) 59

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 <http://srhp.jsc.nasa.gov/>

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