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Special Issue: Biological effects of space radiation -Part II-

The First Life Science Experiments in ISS: Reports of "Rad Gene"-Space Radiation Effects on Human Cultured Cells-

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Abstract

To clarify the biological effects of space environment, especially space radiations, a proposal of" Rad Gene" was performed as the first life science experiment with two human lymphoblastoid cell lines bearing wild-type *p53* gene (wt*p53*) and mutated *p53* gene (m*p53*) in an International Space Station (ISS) for 133 days. We scheduled four projects: (1) DNA damage induced by space radiations including the high linear energy transfer (LET) particles was detected as a track of γH2AX foci in the nuclei of these frozen cells. (2) To examine the biological effects of microgravity and space radiations on gene and protein expression of *p53*-dependent regulated genes, these cells were grown under microgravity and 1 gravity in ISS, and on ground for 8 days and analyzed by DNA and protein arrays. (3) *p53*-Dependent regulated genes were analyzed in the cultured cells after spaceflight at frozen state exposed to space radiations. (4) To clarify the effects of space radiations on the radio-adaptive response, the space flown cells at frozen state were cultured, and then exposed to challenging X-ray-irradiation. All of the radio-adaptive responses of cell killing, apoptosis, chromosomal aberrations and mutations were found only in wt*p53* cells, but not in the m*p53* cells. ©2010 Jpn. Soc. Biol. Sci. Space; Article ID: 102401003

***Keywords*: space radiations, DNA damage, gene expression, radio-adaptive response, dosimetry**

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Introduction

Once astronauts venture beyond the Earth's protective atmosphere and magnetic field, they may be exposed to severe high energy charged particles originating from galactic cosmic rays, solar particle events, and secondary protons and neutrons encompassing a broad range of energies (Fig. 1) (Ohnishi *et al.*, 2009a). From a view of safety long term stay in space, biological effects of space radiations have been studied many times in these about 10 years by space shuttles. Space radiations have been reported to induce DNA damage (Ohnishi *et*

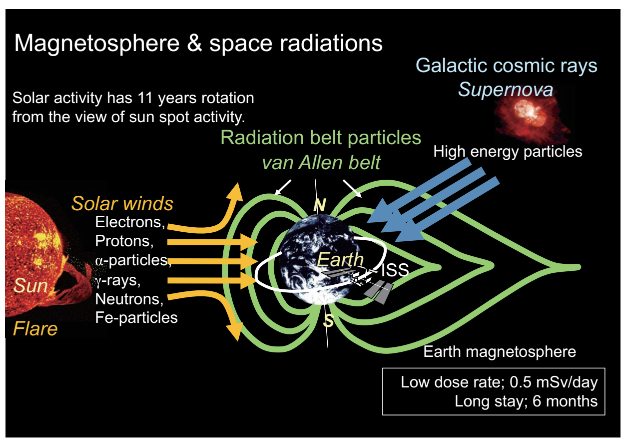


Fig. 1. Species and origin of space radiations. Space radiations include many kinds of radiations such as electrons, protons, γ-rays, neutrons Fe-particles and other heavy particles from solar winds, supernova and galaxy. Earth ground is protected from most of them by magnetosphere and air components. Space radiation environment in ISS is low dose and low dose-rate during long stay. To travel to moon and Mars for more long periods, we have to apply radiation protection for space crews.

al., 2001), mutation frequencies (Ikenaga *et al*., 1997), chromosomal aberrations (Obe *et al*., 1997; Yang *et al*., 1997; Fedorenko *et al*., 2001; Greco *et al*., 2003; George, 2005), abnormal differentiation (Bücker *et al*., 1986; Takahashi *et al*., 1997), light flashes (McNulty *et al*., 1977; Bidoli *et al*., 2002) and cataracts in the eye (Cucinotta *et al*., 2001; Jones *et al*., 2007). A proposal for the “Rad Gene” project was accepted by Japan Aerospace Exploration Agency (JAXA) in 2000 (Ohnishi *et al*., 2009a). This project is designed to examine the biological effect of space radiations on cultured human cells, and was scheduled as the first life science experiment to be conducted on the ”Kibo” facility of ISS (Ohnishi *et al*., 2009a).

Among a variety of radiations induced DNA damage events, double strand breaks (DSBs) are the most critical threat (Bryant, 1985; Downs and Cote, 2005). In particular, an immuno-cytochemical method capable of specifically recognizing γH2AX has become the gold standard for the detection of DSB (Rogakou *et al*., 1998; Fernandez-Capetillo *et al*., 2004; Takahashi and Ohnishi, 2005). This assay is currently considered to be an extremely sensitive and specific indicator for the existence of one DSB; specifically, one γH2AX focus correlates to one DSB (Rogakou *et al*., 1999; Rothkamm and Lobrich, 2003). High linear energy transfer (LET) radiations, such as heavy ion particles, are believed to produce high yields of clustered DNA damage including DSB (Goodhead, 1994; Lobrich *et al*., 1996; Rydberg, 1996; Terato and Ide, 2004). From the indirect biological evidence, it is well speculated that space radiations induce DSBs. Physical dosimetry data have also indicated that the radiation doses acquired in space are sufficient to lead to the induction of DNA damage in space. In the ground experiments, it was reported that clusters of grains were observed near large tracks from high LET radiations, especially from Fe-ion beams which are one of the components of space radiations. In contrast, smaller grain numbers were seen scattered in cells exposed to low LET radiations such as γ-rays or X-rays (Takahashi *et al*., 2008a). Clear tracks were observed in nuclei and their appearance occurred in a dose-dependent manner (Takahashi *et al*., 2008a). High LET heavy particles also resulted in larger numbers of DSBs along the particle tracks (Goodhead, 1994).

In view of these ground experiment observations, DSBs induced by space radiations in the present space experiments will be measured. Frozen samples maintained in a freezer in space will be compared with control samples simultaneously maintained in a ground freezer. It is expected that the frozen cells in space will acquire more DSBs than the ground samples during a 4 month period. In addition, we applied physical dosimetry by use of plastic plates such as CR30. These results should provide information and the quantity and nature of DNA damage induced by space radiations.

Interestingly, the accumulation of p53 tumor suppressor protein was reported in the skin and muscle of rats after spaceflight (Ohnishi *et al*., 1996a, 1999a). *p53*-Centered signal transduction contributes to apoptosis, cell cycle arrest and DNA repair after exposure to environmental stresses such as radiations, UV, heat, cold, oxidative stresses, and low pH in cultured human cells (Wang and Ohnishi, 1997; Ohnishi *et al*., 1996b; Ohnishi *et al*., 1998a; Ohtsubo *et al*., 1997). The activation of p53 molecules by phosphorylation induces *p53*-regulated genes (Riley *et al*., 2008). The p53 is generally thought to contribute to the genetic stability of cells against DNA damage through *p53*-centered signal transduction pathways (Lane, 1992). We designed to investigate gene expression of *p53*-regulated genes in two human cell lines bearing wt*p53* and m*p53* after spaceflight in a frozen state and during spaceflight under microgravity and 1 gravity (1*G*) in ISS. Systematic study and detailed molecular mechanisms of the adverse effect of space radiations and/or microgravity on living cells are still lacking. Therefore, we applied the DNA and protein array for the gene and protein expression in sapce and after spaceflight in these two cell lines. We also compared with ground control to analysis the effects of microgravity, space radiations and space environment by using of microgravity and 1*G* in cell biology experiment facility (CBEF) of ISS (Fig. 2).

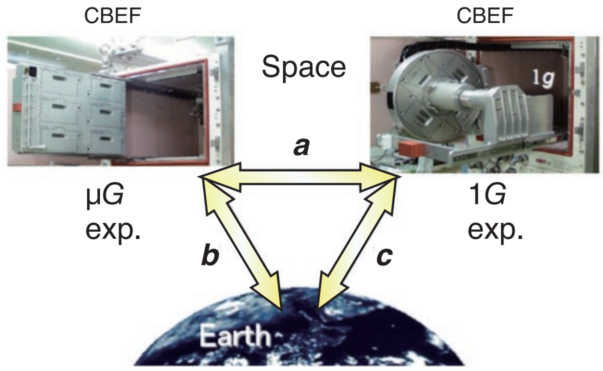


Fig. 2. Comparison of different environmental factors among microgravity (μ*G*) and 1 gravity (1*G*) in ISS CBEF and Earth. ***a***, effect of microgravity; ***b***, effect of microgravity and space radiations; ***c***, effect of space radiations.

When living organisms such as cells, organs and whole bodies were exposed to radiation environments with low dose or low dose-rates, they acquired a radio-adaptive response which was manifested as a depression of cell killing, gene mutations, micronuclei, chromosome aberrations, and malignant transformation (Takahashi and Ohnishi, 2009a). Particular interest in the radio-adaptive response is the dose window which is a specific range of doses and/or dose rates which can serve as pre-irradiation exposures prior to challenging radiation (Yonezawa *et al*., 1990, 1996; Takahashi and Ohnishi, 2009a). It has been reported that a pre-irradiation with low-dose and low dose-rates can induce a tumor suppressor gene, *p53*-dependent radio-adaptive response in mammalian cells (Sasaki, 1995; Takahashi, 2001; Takahashi *et al*., 2001, 2008b; Matsumoto *et al*., 2007; Takahashi and Ohnishi, 2009b). Radiation environment in space includes low doses and low dose-rates. From our previous reports, this value was suitable dose within window doses for radio-adaptive response (Sasaki, 1995; Takahashi *et al.*, 2008b). Therefore, the aim of this study was to examine the radio-adaptive response after spaceflight in these two cell lines. The frozen samples maintained in a freezer in space will be compared with the control samples simultaneously maintained in a ground freezer. The measuring parameters of radio-adaptive response were the number of viable cells, the frequency of apoptosis, and the frequency of chromosome aberrations and mutations after a challenging irradiation in cultured condition after thawing.

Materials and methods

Cells

Experiments were performed with two human lymphoblastoid cell lines; TSCE5 and WTK1. The TSCE5 cell line was established from TK6 cells having a wt*p53* status (Honma *et al.*, 2003), whereas WTK1 cells overexpress an m*p53* with a single base-pair substitution in codon 237 of exon 7 resulting in a change from Met (TAC) to Ile (TAT) (Little *et al.*, 1995). The cells are grown at 37˚C in suspension cultures in a humidified 95% air/5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% heat-inactivated horse serum (JRH Biosciences, Lenexa, KS, USA), penicillin (100 U/ml), streptomycin (100 µg/ml) and sodium pyruvate (200 µg/ml). These exponentially growing cells were immediately washed and resuspended at a concentration of 2×10^6 cells/ml in medium containing 10% dimethyl sulfoxide at 4˚C.

Spaceflight

Ten ml of suspension cells were placed into a bag (*ca* 4 cm × 7 cm) which was constructed from a sheet of polypropylene (Hybrid MekkinBag HM-1304, HOGY, Osaka, Japan) (Ohnishi *et al.*, 2009a), and the samples were then frozen at –80˚C (Fig. 3***a***). The two compartments were separated by a double temporary partition. The under compartment contained 10 ml of cell culture medium and frozen cells, and the upper compartment contained 10 ml of a cryo-protectant solution such as medium containing 20% DMSO to stop cell growth. Before activation, the bags were frozen at –80˚C. The frozen samples were taken into space on the space shuttle (Endeavor, STS-126) which was launched at 9:55 am Nov. 15th, 2008 (Japanese time) from the Kennedy Space Center (KSC; Florida, FA, USA), and were stored in General Laboratory Active Cryogenic ISS Experiment Refrigerator (GLACIER) at –80˚C in the space shuttle. At Nov. 22th, 2008 (Japanese time), the frozen samples were moved from GLACIER to the Minus Eighty degree Celsius Laboratory Freezer in the ISS (MELFI) at –80˚C in ISS. The cell culture was performed from February 20th to 28th, 2009 in space. The cell culture work was performed with excellent technique by a member of the space crews, Dr. Sandra H. Magnus. To start culture growth, the bags were moved into CBEF at 37˚C in a humidified 95% air/5% CO_2 atmosphere (Fig. 3***b***). To stop culture growth, the partition was broken (Fig. 3***c***). Immediately after mixing the contents of the two compartments, the cells were frozen again at –80˚C. Experiments were designed to obtain information concerning the microgravity effects on biological processes in the presence of space radiations by comparing results between cells grown in µ*G* and cells grown under 1*G* in CBEF (Ishioka *et al.*, 2004) of the ISS.

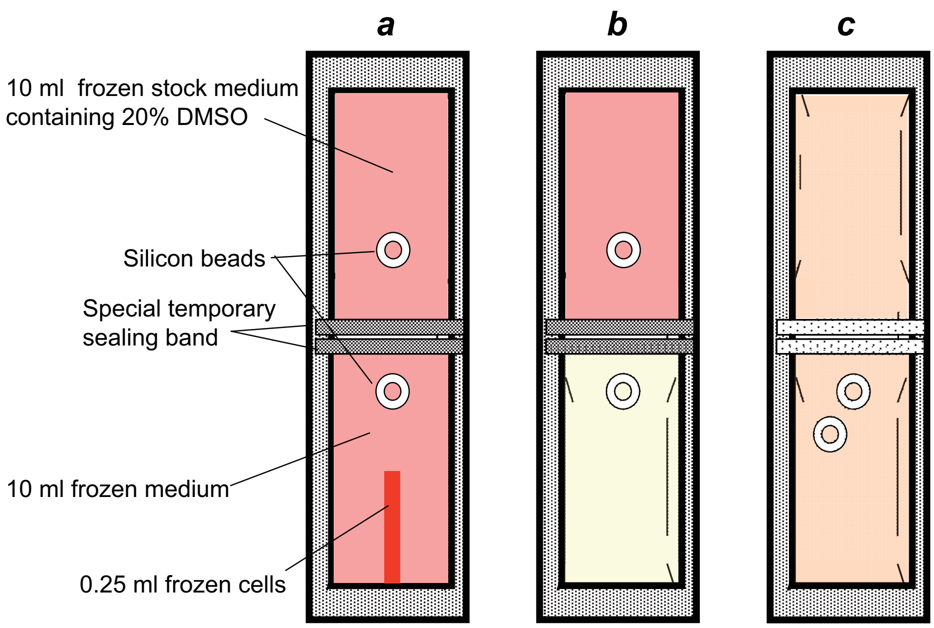


Fig. 3. Cell culture kit. ***a***, before culture condition, the samples were kept in a freezer at –80˚C. Red bar, 0.25 ml frozen cells; two circles, glass beads. ***b***, culturing condition in ISS CBEF. When cells grow to several generation, the color of culture medium turns to yellow from pink. ***c***, deactivation of cell culture by breakage of separation film. It is clear that upper beads moved to bottom area.

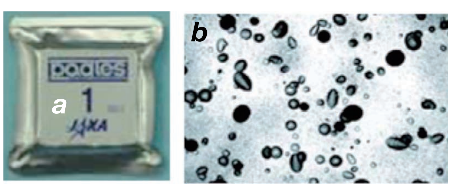
Data were obtained daily from the ISS which indicated that the culture conditions (temperature and CO_2 levels) were maintained in normal conditions. The deactivation time were decided by obtaining information from the ground control experiment which was performed three days later at JAXA Tsukuba facility. The growth rate of the cells was excellent, even though the experiment was delayed by about 3 weeks (data not shown). The cells were then re-frozen on the ISS by the astronauts. The Space Shuttle (Discovery, STS-119) returned at 4:13 pm on March 29th, 2009 (Japanese time) to the KSC.

Irradiation

Exponentially growing AG1522 cells were grown on Lab-Tek® Chamber Slide™ (177437, Nalge Nunc Int., Rochester, NY, USA) and irradiated with X-rays or Fe-ions. For X-ray irradiation, a 200-kVp X-ray generator (PANTAK-320S, Shimadzu, Kyoto, Japan) was used with a total filtration of 0.5 mm aluminum plus 0.5 mm copper. Cells were grown on glass slides for irradiation. X-ray dose rates were measured with a thimble ionization chamber (PTW FREIBURG, Freiburg, Germany) at the sample position and the dose rate was about 1 Gy/min. For Fe-ions (500 MeV/u, 200 keV/µm at the target entrance), the HIMAC at the NIRS in Chiba, Japan was used with a low-angle (5 degrees) between the axis of the ion beam and the plane of the cell monolayer. Irradiation was conducted using horizontal heavy-ion beams with a dose rate of 3 Gy/min, and not using a binary filter for a mono-energetic beam with a narrow Bragg Peak. It was measured exposures under these conditions using a calibrated parallel plate ionization chamber and/or a plastic scintillation counter at the sample position (Kanai *et al*., 1997).

Space radiation dosimetry with Bio Passive dosimeter for lifescience experiments in space (PADLES)

Each Bio PADLES package comprised four plates of CR-39 plastic nuclear track detectors (PNTDs; HARZLAS TD-1, Fukuvi Chemical Industry, Co. Ltd., Fukui, Japan) and seven elements of thermo-luminescent dosimeters (TLDs; MSO-S, Kasei Optonix, Co. Ltd., Kanagawa, Japan). These Bio PADLES packages were kept in three samples of the space experiments (Fig. 4). As a control, three samples were kept in each Culture Bag Holder in a grand facility of NASA KSC at –80˚C. The TLD elements for these space experiments were chosen so that their response deviations were within ± 3%. Each Bio PADLES package included a reference CR-39 PNTD plate exposed to heavy ions (274 MeV/u ^{12}C, 410 MeV/u ^{56}Fe) from the HIMAC heavy ion accelerator of NIRS in Japan to check sensitivity stability of the CR-39 PNTDs during space experiments. The track formation sensitivities for the pre-irradiated Carbon-ions and Fe-ions were in good agreement with the calibration curve. This meant that the aging effect of CR-39 PNTDs during this space experiments was negligible. CR-39 PNTDs can detect nuclear tracks with LET of 4 keV/µm or more. We took pictures of etch pits corresponding to nuclear tracks which were produced during space flight. We calculated differential particle fluxes as a function of LET measured with CR-39 PNTDs in flight packages (Fig. 4).



c		
Total absorbed dose		43.5 ± 2.8 mGy
	≤ 10 keV/µm	40.9 ± 3.2 mGy
	> 10 keV/µm	2.7 ± 0.5 mGy
Total dose equivalent		71.2 ± 2.5 mSv
		0.5 mSv/day

Fig. 4. Physical dosimetry for space radiations. ***a***,PADLES package; ***b***, etching-treated CR-39. Number and direction of space radiations were detected. ***c***, summary of physical dosimeter; data represent doses in different energy range.

Histological studies of histone H2AX phosphorylation

The fixed cells were blocked with 3% skim milk in PBS for 10 min, and washed in TPBS (PBS containing 0.05% Tween 20) for suppression of background noise. The cells were incubated with anti-phospho-H2AX (ser139) mouse monoclonal antibody (Upstate Biotechnology, Lake Placid, NY, USA) at a 300-fold dilution. The cells were then incubated with an AlexaFluor 488-conjugated anti-mouse IgG second antibody (Molecular Probes, Eugene, OR, USA) at a 400-fold dilution for 60 min at room temperature, and washed in TPBS. The slides were stained and mounted with 1 µg/ml 4’, 6-diamidino-2-phenylindole dihydrochloride (DAPI) in SlowFade® (Molecular Probes). Photographs of the cells were taken with a fluorescence microscope (OLYMPUS BX51, Olympus Optical, Tokyo, Japan). Untreated background noise was suppressed by lowering fluorescent sensitivity. To allow direct comparisons, all of the images were captured using the same parameters.

Gene expression analysis using DNA array

For gene expression during space flight, the frozen cells and the ground control cells were thawed, washed with PBS at 4˚C and preserved in RNA*later*® solutions (Ambion, Austin, TX, USA) at 4˚C (Figs. 5***a*** and 5***b***). For gene expression after spaceflight in a frozen state, the frozen cells and the ground control cells were thawed, immediately suspended in medium, and cultured for 6 h (Figs. 5***c*** and 5***d***). Cells were washed with PBS at 4˚C and preserved in RNA*later*® solutions. Extraction of RNA, microarray hybridization and imaging were performed at DNA array Research Inc. (Yokohama, Japan). Briefly, total RNA was extracted using a RNeasy mini kits (Qiagen Inc, Germantown, MD, USA), and labeled using a Quick Amp Labeling Kit, One-Color (Agilent Technologies, Palo Alto, CA, USA), an Agilent One Color Spike Mix Kit (Agilent Technologies) and a Hi-RPM Gene

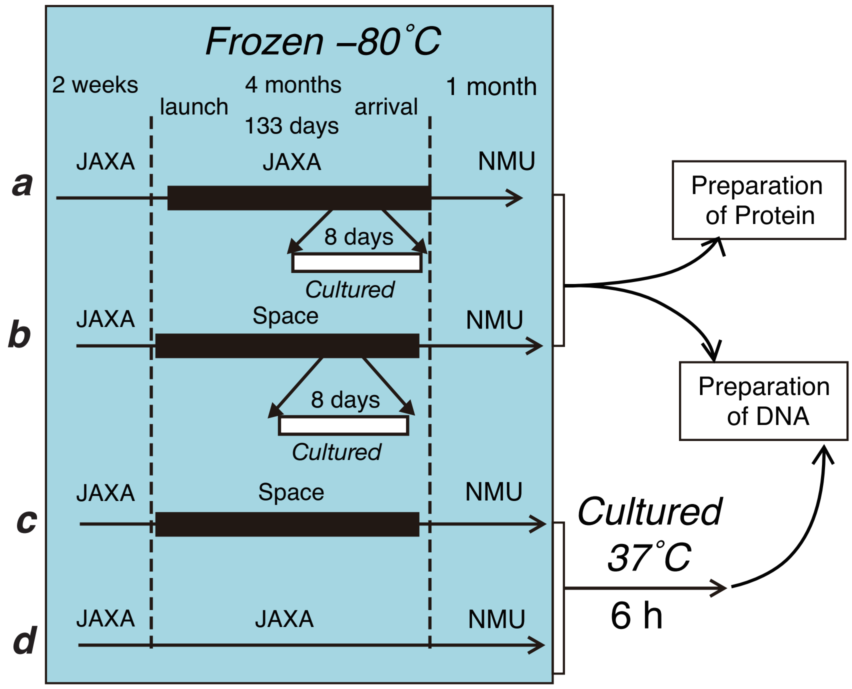


Fig. 5. Preparation of RNA and protein from space samples. ***a***, ground control of cultured samples; ***b***, cultured samples in space under microgravity and 1*G*; ***c***, frozen samples during spaceflight; ***d***, ground control of frozen samples.

Expression Hybridization Kit (Largevolume) (Agilent Technologies). Cy3-labeled cRNA was hybridized to a 44k whole human genome microarray which includes 41,000 genes (Agilent Technologies) according to the manufacturer's instructions. Slides were dried under nitrogen and scanned on a DNA Micro Array Scanner (Agilent Technologies). Microarray images were analyzed with Agilent Feature Extraction software. GeneSpring GX software (Agilent Technologies) was used to analyze the microarray data.

p53-Dependent up-regulated genes in cells during or after spaceflight

Up-regulated genes were considered to show increased expression when the ratio of gene expression increased more than two-fold (ratio ≧ 2.0) of that in wt*p53* cells, and when the ratio was less than two-fold (ratio ≦ 2.0) of that in m*p53* cells. In addition, the ratio had to be more than two-fold (ratio ≧ 2.0) of that in wt*p53* cells when compared with m*p53* cells. *p53*-Dependent up-regulated gene expression values were calculated by dividing the wt*p53* cell expression values by the m*p53* cell expression values.

p53-Dependent down-regulated genes in cells during or after spaceflight

Down-regulated genes were considered to have a depressed level of expression when the ratio of gene expression was less than half (ratio ≦ 0.5) of that in wt*p53* cells, and when the ratio was more than half (ratio ≧ 0.5) of that in m*p53* cells. In addition, the ratio was less than half (ratio ≦ 0.5) of that in wt*p53* cells when compared with m*p53* cells. *p53*-Dependent down-regulated gene expression values were calculated by dividing the wt*p53* cell expression values by the m*p53* cell expression values.

Protein expression analysis using protein array

The flight frozen cells and the control ground cells were thawed, washed with PBS at 4˚C, immediately frozen in liquid N_2 and stored at –80˚C (Figs. 5***a*** and 5***b***). Extraction of protein, Antibody microarray hybridization and imaging were performed at Filgen. Inc. (Nagoya, Japan). Briefly, proteins were extracted using Lysis Buffer (including dithiothreitol and protease inhibitors), and labeled with a Cy3 and Cy5 Mono-Reactive Dye Packs (GE Healthcare UK Ltd, Buckinghamshire, England). Cy3- or Cy5-labeled proteins were hybridized to a Panorama™ Ab MicroArray (XPRESS Profiler) (Sigma-Aldrich Co., St. Louis, MO, USA) according to the manufacturer's instructions. Slides were dried and scanned on a GenePix® 4000B scanner (Molecular Devices Co., Tokyo, Japan). Microarray images were analyzed with Array-Pro Analyzer® Ver.4.5 (Media Cybernetics Inc., Bethesda, MD, USA).

p53-Dependent up-regulated proteins in cells cultured in space

The up-regulated proteins were considered to be increased in the ratio of protein expression more than 1.5-fold (ratio ≧ 1.5) in wt*p53* cells, and in the ratio less than 1.5-fold (ratio ≦ 1.5) in m*p53* cells. In addition, the ratio was more than 1.5-fold (ratio ≧ 1.5) in wt*p53* cells when compared with m*p53* cells.

p53-Dependent down-regulated proteins in cells

cultured in space

The down-regulated proteins were considered to be depressed in the ratio of protein expression less than 0.66-fold (ratio ≦ 0.66) in wt*p53* cells, and in the ratio more than 0.66-fold (ratio ≧ 0.66) in m*p53* cells. In addition, the ratio was less than 0.66-fold (ratio ≦ 0.66) in wt*p53* cells when compared with m*p53* cells.

Classification of results

Results were interpreted to be responses to space radiation in comparisons between the 1*G* space samples and the ground samples. Results were interpreted to be responses to the space environment in comparisons between the space μ*G* samples and ground samples. Results were interpreted to be responses to microgravity after comparisons between the space μ*G* samples and the space 1*G* samples.

Analysis of the number of viable cells

The space flown cells at a frozen state (Fig. 6***a***) and the ground control cells (Fig. 6***b***) were thawed and immediately suspended in medium. The cells were cultured for 6 h, and then sham-irradiated or exposed to 2 Gy of X-rays as a challenging dose. The sham-irradiated and X-irradiated cells were cultured for 52 h after thawing. A 50 μl cell suspension sample was obtained at each time point from the cultures and mixed with an equal volume of 0.5% trypan blue, and then kept for 5 min at room temperature. The number of unstained viable cells in a 10 μl sample of stained cells was counted. The cell number ratio (%) was calculated using the following formula; cell number ratio (%) = 100 × $(N_X–N_0)/(N_U–N_0)$ where N_0, N_U and N_X represent the number of unirradiated viable cells at the initial and at terminal points of the experiment, and the number of X-irradiated viable cells at the terminal point of the experiment, respectively. In all cases, over 200 cells were counted in three random fields by three individuals who counted the samples in a blind manner.

Analysis of apoptosis

The frequency of apoptosis was analyzed by the detection of apoptotic bodies (Takahashi, 2001). Twenty-four hours after a 2 Gy X-ray-irradiation, cells were collected, fixed with 1% glutaraldehyde in PBS at 4˚C, washed with PBS, stained with 0.2 mM Hoechst33342 and then observed under a fluorescence microscope. In all cases, a total of 300 cells including normal and apoptotic cells were counted under a fluorescence microscope (Fig. 6). Apoptosis was characterized by nuclear and cytoplasmic condensation with blebbing leading to the formation and release of apoptotic bodies. Three independent experiments were performed for each point.

Analysis of chromosome aberrations

Induction of chromosome aberrations was detected by the observation of dicentrics (Takahashi *et al.*, 2008b). Twenty-four hours after a 2 Gy X-ray-irradiation, to enrich cultures for mitotic cells, cells were incubated in medium containing 0.2 μg/ml of colcemid for 2 h, and were then harvested (Fig. 6). Cells were allowed to swell for 20 min in 75 mmol/l of KCl, were fixed with three changes of methanol/acetic acid (3:1), and were dropped onto a clean microscope slide glass with the pipette. Slides were stained in 2% Giemsa for 30 min, and metaphase spreads were scored for dicentrics per cell. In all cases, the number of dicentrics was counted in a minimum of 1,000 chromosomes.

Analysis of hprt mutations

Frozen cells were thawed in culture medium and

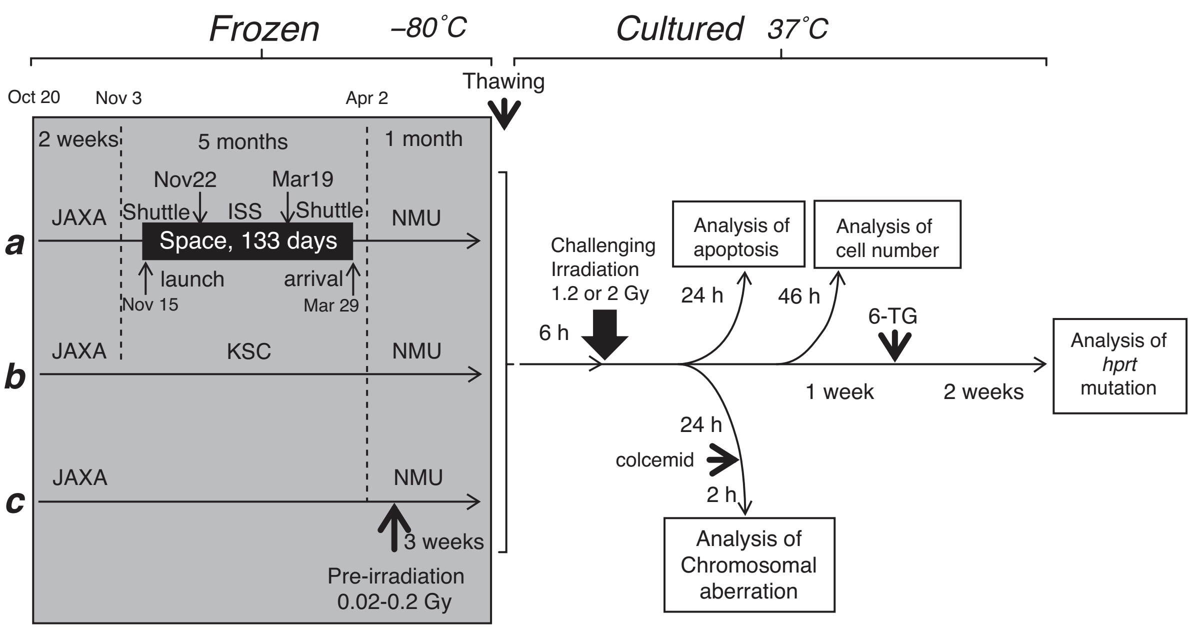


Fig. 6. Experimental procedure for radio-adaptive response after spaceflight. ***a***, flight samples; ***b*** and ***c***, the ground control samples.

cultured for 6 h, then irradiated with 1.2 Gy of X-rays as a challenging irradiation because *hprt* mutants were not obtained after a 2 Gy X-ray-irradiation (data not shown). The cells were then cultured for 1 week to fix mutations. Mutation induction at the *hprt* locus was assayed as previously described (Suzuki *et al.*, 1996) with a slight modification using a soft agar method. Cells were suspended in 0.33% agar medium containing 10% fetal bovine serum and 40 μM 6-thioguanine (6-TG). The suspension was seeded onto the top of 20 dishes with 0.50% base layer at a concentration of 1×10^6 cells/dish. After a two-week culture period in a CO_2 incubator, colonies larger than 0.2 mm in diameter were counted as 6-TG resistant mutant clones (Fig. 6).

Statistical analysis

Significance levels were calculated using the Student's t-test. Values of $p<0.05$ were considered statistically significant.

Results and discussion

Detection of space radiation-induced double strand breaks as a track in cell nucleus

The γH2AX-positive foci following exposure to IR have been reported to be mediated by ATM and DNA-PK (Stiff *et al.*, 2004). The phosphorylation of H2AX by ATM occurs at sites of DSBs in the cell nucleus whereas ATM auto-phosphorylation is thought to take place throughout the nucleoplasm. This assay is quite sensitive and is a specific indicator for the existence of a DSB (Rogakou *et al.*, 1999; Rothkamm and Lobrich, 2003). Although the particle track structure (*i.e.* radial extension) of the ions should be taken account into the interpretation of expected numbers of tracks and foci, the number of tracks corresponded well with calculated values. It was reported that the spatial distribution of DNA lesions within the cell nucleus produced by charged particles depended on the ion track structure as well as the random nature of ion impact parameters relative to the cell nucleus, and on the Poisson distribution of the number of hits per cell (Goodhead, 1994; Cucinotta *et al.*, 2000). We already reported that flow cytometry histograms of radiation-induced phosphorylation of H2AX; a graph plotting the mean values of γH2AX expression of cells treated with X-rays or Fe-ions *vs* different doses in G_1-, S- and G_2/M-phases (Takahashi *et al.*, 2008a). The dose-response for γH2AX was also reported to be similar with exposure to Fe-ions or X-rays in among phases. In addition, the time course of the γH2AX signal was observed to increase rapidly and reach a maximum at 30 min after either type of irradiation. Therefore, we applied the time of 30 min after the incubation from space flight.

γH2AX foci were observed with anti-γH2AX antibodies (*green*) and the nuclei were stained with DAPI (*blue*). The formation of γH2AX foci was not detected in the unirradiated ground control TSCE5 cells of a control (Fig.

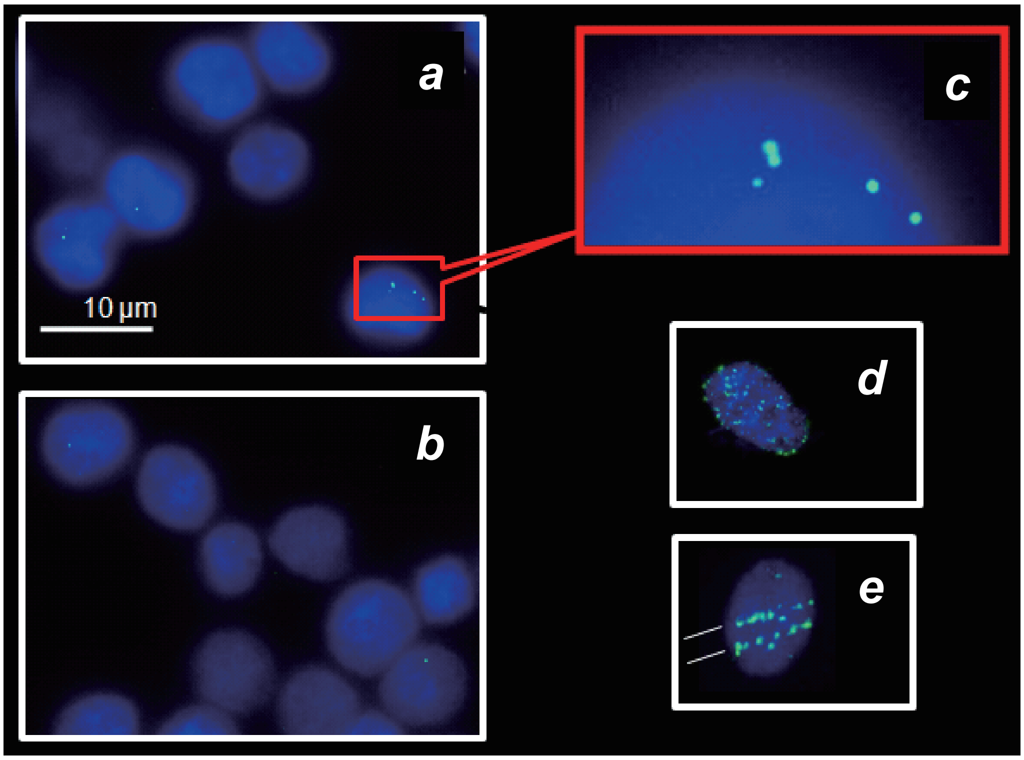


Fig. 7. Visualization of ionizing radiation tracks in nucleus by γH2AX-staining. ***a***, space samples; ***b***, ground control samples; ***c***, large scale (x 5) of ***a***; ***d***, X-rays (3 Gy); ***e***, 200 keV/μm Fe-ion beam (0.6 Gy); ***a-c***, TSCE5 cells; ***d*** and ***e***, AG1522 cells. The cells after irradiation or return back from space were cultured for 30 min, and then stained with immune-cytochemical methods for γH2AX. The white lines in E are direction of Fe-ion beam.

7***b***). Typical images of Fe-ion induced in γH2AX foci are shown in Fig. 7***e***. All the detected trajectories were almost parallel to each other inside of individual nuclei. At least 100 nuclei were counted from the microscopic images. From a result of the measurement by Scion Image (Scion Corp, Frederick, MD, USA), the area size of the nuclei was 152.9 ± 42.2 μm^2. The parameters used for calculation were nuclear area, LET and irradiation dose. The number of tracks induced by Fe-ions corresponded well with calculated values. Fe-ion (500 MeV/u; 200 keV/μm) doses of 0.3, 0.6, 0.9 and 1.2 Gy produced an average number of 1, 2, 3 or 4 tracks in each cell nucleus, respectively (data nor shown) (Takahashi *et al*., 2008a). The clear visualization of parallel tracks inside individual nuclei, and the alignment of all the strips or tracks within individual samples provides indirect evidence that these patterns were generated by individual particles from the irradiating Fe-ions (Fig. 7***e***). In Fig. 7, we found the tracks of γH2AX only in space samples (Figs. 7***a*** and 7***c***), not in ground samples (Fig. 7***b***). Therefore, we can speculate these tracks might produced by space radiations. The similar tracks were found by Fe-ions (500 MeV/u, 200 keV/μm at the target entrance) produced by the HIMAC at the NIRS in Chiba, Japan (Fig. 7***e***). In the case of low LET radiations such as X-ray, we could not find such tracks (Fig. 7***d***). It is assumed scattering action of X-rays character and indirect DSBs through radicals induced by X-rays. However, it has been reported that high LET radiations can penetrate in nuclei (Goodhead 1994). In fact, we found the tracks of γH2AX induced by Fe-ion beams. Here, it is the first report that space radiation-induced the γH2AX track in nuclei of space samples frown in space. We investigated the frequency of the track of γH2AX, that is, about one track per 100 cells in the both in wt*p53* and m*p53* cells flown in space. Almost the same number of γH2AX positive foci was found in the both cell lines. In addition, most positive cells have a track of γH2AX positive foci. From these results, we can speculate that total dose equivalent of high LET radiations was calculated to be about 94.5 mSv when 100 μm^2 cell nuclei were exposed space radiations such as 200 keV/μm Fe-ions as a relatively high energy component among space radiation species. The exposure dose rate was calculated to be 0.7 mSv per day.

Space radiation dosimetry was analyzed with Bio PADLES comprised four plates of CR-39 plastic nuclear track detectors (Fig. 4) (Ohnishi *et al*., 2009b). We separately measured two kinds of energy ranges of ≤ 10 keV/μm and > 10 keV/μm which were 40.9 ± 3.2 mGy and 2.7 ± 0.5 mGy, respectively. Total absorbed dose was 43.5 ± 2.8 mGy for 133 days flight (Fig. 4). They were calculated as 71.2 ± 2.5 mSv as total dose equivalent, that is, dose rate was 0.5 mSv per day. Although a real time physical monitoring facility of space radiations was detected to be about 1 mSv per day at about 15 years ago (Doke *et al*., 1995; Hayashi *et al*., 1996), recently, the daily exposure dose for a human body in the ISS was estimated to be about 0.5 mSv by NASA group (Cucinotta *et al*., 2008) as the same as our data (Ohnishi *et al*., 2009b).

These results confirmed that the space flown cells could be supplied for biological dosimetry. The exposure doses between biological and physical dosimetries may be dependent on the speculation for relatively high energy of Fe-ion beams by a biological dosimetry with γH2AX positive foci analysis. In fact, a characteristic of galactic cosmic rays and solar particles are containing high energy particles with several percent of heavy particles (Ohnishi and Ohnishi, 2004). Though biological dosimetry with γH2AX positive foci analysis might be overestimation, the dose rate was quite similar to the dose rate of physical dosimetry. In addition, these results confirmed that the flight cells have DSBs like a truck in nuclei as a memory of space radiation exposure even at such low dose.

p53-Dependent gene expression in cultured mammalian cells after spaceflight in a frozen state

The aim of this study was to compare gene expression profiles in wt*p53* and m*p53* cells after spaceflight in a frozen state. In this space experiment, gene expression was induced by space radiations alone because of frozen state. In this flight for 133 days, total equivalent doses of space radiations were 71.2 mSv by a Bio PADLES in space by JAXA (Ohnishi *et al*., 2009b). Gene expression

Table 1 *p53*-Dependent up-regulated gene expression after spaceflight.

No	Gene symbol	Ratio	No	Gene symbol	Ratio	No	Gene symbol	Ratio	No	Gene symbol	Ratio
1	*HSPA6*	10.56	14	*THC2766373*	2.59	27	*HSP90AB1*	2.29	40	*CXCL9*	2.10
2	*IL7R*	4.60	15	*A_24_P585660*	2.57	28	*ENST00000390258*	2.26	41	*ENST00000360548*	2.09
3	*HSPA1A*	4.02	16	*ASPH*	2.55	29	*TNFSF10*	2.24	42	*AK023645*	2.08
4	*SYT4*	3.46	17	*A_24_P560332*	2.54	30	*HSP90AB3P*	2.21	43	*CEBPA*	2.08
5	*LOC730211*	3.20	18	*SLC24A3*	2.53	31	*FAM90A1*	2.21	44	*IL18BP*	2.08
6	*LOC85391*	3.17	19	*A_24_P631625*	2.44	32	*UCN2*	2.21	45	*POP1*	2.06
7	*FAM90A9*	3.04	20	*SCEL*	2.41	33	*ST8SIA2*	2.19	46	*AI015919*	2.05
8	*HSP90AA1*	2.96	21	*AK090827*	2.38	34	*KIAA0319L*	2.16	47	*CLGN*	2.05
9	*KCNQ1*	2.95	22	*ZNF205*	2.36	35	*THC2563387*	2.16	48	*THC2543840*	2.03
10	*LATS2*	2.92	23	*ENST00000378770*	2.34	36	*MUM1*	2.16	49	*KCNG4*	2.02
11	*LOC727891*	2.81	24	*XAF1*	2.34	37	*BC036435*	2.15	50	*GPR171*	2.00
12	*EDN1*	2.80	25	*PTPRE*	2.29	38	*LAMP3*	2.14			
13	*CSF2*	2.72	26	*CXCL11*	2.29	39	*AF007192*	2.14			

profiles were measured using Agilent Technologies gene array technology. Analysis demonstrated *p53*-dependent up-regulated gene expression for 50 genes (Table 1) and down-regulated gene expression for 94 genes (Table 2). The profiling number of *p53*-dependent up- and down-regulated genes reaches 0.35% of 41,000 kinds of whole genomes examined here.

Genes which have been reported to be regulated by *p53* are apoptosis-related genes [*e.g.* AIF, apoptosis inducing-factor (Stambolsky *et al*., 2006); *Bax*, Bcl-2 associated X protein (Miyashita and Reed, 1995); *DR4*, death receptor 4 (Guan *et al*., 2001); *DR5*, death receptor 5 (Sheikh *et al*., 1998); *Noxa*, noxious stress inducible pro-apoptotic gene (Oda *et al*., 2000a); *PERP*, p53 apoptosis effector related to PMP-22 (Attardi *et al*., 2000); *PIDD*, *p53*-induced death-domain-containing protein (Lin *et al*., 2000); *PUMA*, *p53*-upregulated modulator of apoptosis (Nakano and Vousden, 2001); *p53AIP1*, *p53*-regulated apoptosis induced-protein 1 (Oda *et al*., 2000b); *p53DINP1*, *p53*-dependent damage-inducible nuclear protein 1 (Okamura *et al*., 2001)]; cell cycle-regulated genes [*e.g. Cdkn1a*, cyclin-dependent kinase inhibitor 1A, formerly known as *Waf1* (El-Deiry *et al*., 1993); *cyclin D* (Bito *et al*., 1995); *cyclin G* (Okamoto and Beach 1994); *PCNA*, proliferating cell nuclear antigen (Mercer *et al*., 1991); *PTEN*, phosphatase and tensin homolog deleted from chromosome 10 (Stambolic *et al*., 2001); *RB*, retinoblastoma gene product (Bito *et al*., 1995); and *14-3-3 sigma* (Hermeking *et al*., 1997)]; DNA repair-regulated genes [*e.g. Gadd45*, or growth arrest and DNA-damage-inducible gene 45 (Kastan *et al*., 1992); *Msh2*, mismatch repair protein MutS homolog 2 (Scherer *et al*., 1996); and *p53R2*, p53-inducible ribonucleotide reductase small subunit (Tanaka *et al*., 2000)]. Other genes are known to be regulated by *p53,* too [*e.g. Hdm2*, the human homolog of Mdm2 (Böttger *et al*., 1997)]. In this experiment, alterations of expression of *p53* or of these prominent *p53*-regulated genes were not detected (Tables 1 and 2). The direct accumulation of p53 protein was reported in rats muscle and skin after spaceflight (Ohnishi *et al*., 1996a, 1999a). The main difference might be brought from animal and cell culture systems. In addition, the results might be caused by not only space radiations and microgravity but also hypergravity during the launching and landing, and psychological problems in animals. However, at least the genes profiled in this report may possibly include newly observed *p53*-regulated genes.

On the other hand, heat shock protein (HSP)-related genes such as *HSPA6*, *HSPA1A*, *HSP90AA1*, *HSP90AB1* and *HSP90AB3P* were detected among the *p53*-dependent up-regulated genes in cells exposed to space in a frozen state (Table 1). These HSPs are called stress proteins which respond to altered genotoxic and non-genotoxic environments. During the heat-shock response, a group of chaperones, exemplified by Hsp90 and Hsp70, control various cellular processes, including protein folding, the assembly of multi-component protein complexes, translocation across cellular compartments, and targeting protein degradation through the proteasome (Nollen and Morimoto 2002). An accumulation of Hsp72 is induced not only by heat, but also by inducers of DNA damage in cultured human cells (Muramatsu *et al*.,

Table 2 *p53*-Dependent down-regulated gene expression after spaceflight.

No	Gene symbol	Ratio	No	Gene symbol	Ratio	No	Gene symbol	Ratio	No	Gene symbol	Ratio
1	*TMPRSS6*	0.12	25	*GRIN2C*	0.34	49	*MTTP*	0.40	73	*ACP2*	0.47
2	*AF234262*	0.13	26	*OR2B6*	0.34	50	*MAOA*	0.40	74	*RREB1*	0.47
3	*RP4-621O15.2*	0.13	27	*LOC126536*	0.34	51	*BX116163*	0.40	75	*A_32_P38806*	0.48
4	*FGFR2*	0.18	28	*TLX2*	0.34	52	*ASGR1*	0.41	76	*IFT80*	0.48
5	*DNASE1*	0.19	29	*CD44*	0.34	53	*AK123107*	0.41	77	*XRN1*	0.48
6	*THC2669878*	0.20	30	*H2AFB2*	0.34	54	*A_24_P932220*	0.41	78	*THC2742226*	0.48
7	*IRX6*	0.20	31	*A_32_P71171*	0.35	55	*PRKCZ*	0.42	79	*STC2*	0.48
8	*LOC338328*	0.21	32	*GDF15*	0.35	56	*AK022339*	0.42	80	*ENST00000372493*	0.49
9	*GALNACT-2*	0.22	33	*HSFX1*	0.35	57	*THC2617584*	0.43	81	*THC2733296*	0.49
10	*AF217970*	0.22	34	*THC2649341*	0.36	58	*AOC3*	0.43	82	*LOC647500*	0.49
11	*BX100437*	0.22	35	*BI913527*	0.36	59	*MYO5B*	0.43	83	*BBC3*	0.49
12	*CES7*	0.24	36	*AVIL*	0.36	60	*SEC61A2*	0.43	84	*BC042026*	0.49
13	*KLHDC8B*	0.25	37	*LOC497190*	0.36	61	*CHAC1*	0.44	85	*LOC55565*	0.49
14	*FUT1*	0.27	38	*CBLN3*	0.37	62	*HAMP*	0.44	86	*ICAM3*	0.49
15	*ADMR*	0.28	39	*AF283771*	0.38	63	*BE835321*	0.45	87	*BC021677*	0.49
16	*THC2717023*	0.28	40	*THC2520867*	0.38	64	*C10orf38*	0.45	88	*C10orf10*	0.49
17	*PRKCQ*	0.28	41	*AF318328*	0.38	65	*MGC4655*	0.45	89	*ZNF66*	0.49
18	*SH2D3C*	0.29	42	*DDIT3*	0.38	66	*LOC402573*	0.45	90	*MBD2*	0.49
19	*TRIM7*	0.29	43	*ZDHHC11*	0.38	67	*IGHD*	0.46	91	*TAGLN*	0.50
20	*TNFAIP2*	0.29	44	*GRB10*	0.39	68	*TSC22D3*	0.46	92	*SESN2*	0.50
21	*C9orf167*	0.30	45	*CYP2E1*	0.39	69	*KIAA1324L*	0.47	93	*MTF1*	0.50
22	*SLFNL1*	0.32	46	*BE716310*	0.39	70	*TNFRSF17*	0.47	94	*TTYH2*	0.50
23	*INHBE*	0.32	47	*TXLNB*	0.39	71	*THC2550463*	0.47			
24	*CB250445*	0.32	48	*A_23_P158868*	0.40	72	*DYNLRB2*	0.47			

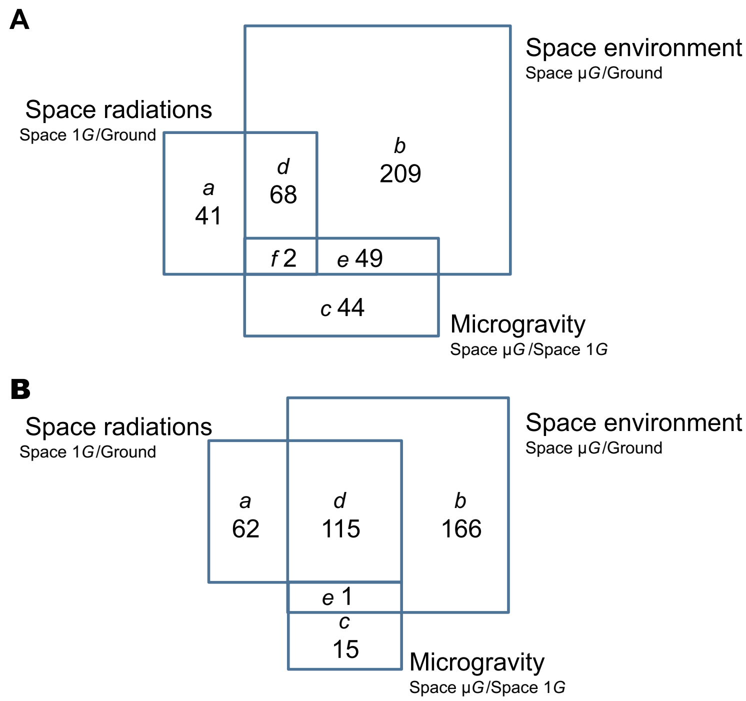


Fig. 8. *p53*-Dependent gene expression in cultured cells in space. **A**, up-regulated genes; **B**, down-regulated genes.

1992, 1993, 1996). Although it was reported that *HSP* gene expression was down-regulated by spaceflight in the rat muscle related to a reduction in the mechanical and neural activity levels (Ishihara *et al*., 2008), in this experiment, gene expression was *p53*-dependent up-regulated by space radiations alone because the cells exposed in space were frozen. Interestingly, Hsp72 accumulations induced in the muscle, skin and spleen of orbiting goldfish were confirmed by comparisons to control goldfish (Ohnishi *et al*. 1998b). In addition, it was reported that *Hsp27* gene expression was *p53*-dependent up-regulated by spaceflight in human lymphocytes (Cubano and Lewis, 2001). Our data are in agreement with these recent reports that the stress-responsive activator of p300 stimulates the transcription of *HSPs* genes through p53 (Barlev *et al*., 2001; Espinosa and Emerson, 2001; Xu *et al*., 2008).

There was a cell adhesion-related gene, *CD44*, among the *p53*-dependent down-regulated genes in the cells exposed to space in a frozen state (Table 2). Recently, it was reported that under conditions of basal physiologic and cell culture stresses, p53 inhibits expression of the CD44 cell-surface molecule *via* binding to a non-canonical p53-binding sequence in the *CD44* promoter (Godar *et al*., 2008). Moreover, a depression of CD44 in bone marrow cells was detected in mice after a 13 day flight on the space shuttle (Ortega *et al*., 2009). Tumor necrosis factor (TNF)-related genes such as *TNFAIP2* and *TNFRSF17* were down-regulated in a *p53*-dependent manner after spaceflight (Table 2), although the relationship between *p53* gene status and gene expression of *TNF* is unknown. A down-regulation of *TNF* in T lymphocytes from mice after a 13 day flight on the space shuttle has also been reported (Gridley *et al*., 2009).

Here, we would like to emphasize that we can detect the gene expression of *p53*-dependent regulated genes by exposure to complex space radiations accumulated during long term ISS stays in a frozen state. In the future, it is expected that data from this type of work will be helpful in designing physical protection from the deleterious effects of space radiations during long term stays in space.

The expression of p53-dependent regulated genes in the cultured mammalian cells during spaceflight

The aim of this study was to compare the gene expression profiles in wt*p53* and m*p53* cells during spaceflight. In this flight for 133 days, total equivalent doses of space radiations were 71.2 mSv by a Bio PADLES by JAXA (Ohnishi *et al*., 2009b). When calculating as the result, the space samples before cell culture may be exposed to space radiations with about 52 mSv in a frozen state for 97 days. The cells were cultured with the accumulated damage by these space radiations. During culture condition for 8 days, the space samples may be exposed to them with about 4 mSv. After re-frozen, the effect of space radiations on gene expression should be able to neglect. Gene expression profiles were defined using Agilent Technologies gene array technology. The expression of genes were increased (Fig. 8**A**) or depressed (Fig. 8**B**) in only wt*p53* cells, not but m*p53* cells, that is, the genes expressed both in wt*p53* and m*p53* cells are eliminated (Takahashi *et al*., 2010).

Therefore, we defined as *p53*-dependent regulated genes. DNA array analysis has indicated that a relatively large number of changes in gene expression can be detected. *p53*-Dependent up-regulated gene expression was found for 111 (Table S1), 95 (Table S2), and 328 genes (Table S3) in response to space radiations, microgravity and both space environment, respectively. In addition, *p53*-dependent down-regulated gene expression was found for 177 (Table S4), 16 (Table S5), and 282 genes (Table S6) in response to space radiations, microgravity and both space environment, respectively (Takahashi *et al.*, 2010). The number of profiled *p53*-dependent up- and down-regulated genes was about 2% of the 41,000 genes examined here.

Genes which have been reported to be regulated by *p53* are apoptosis-related genes, cell cycle-regulated genes, DNA repair-regulated genes and *p53*-regulated gene. In this experiment, alterations of expression of *p53* or of these prominent *p53*-regulated genes were not detected (Tables 1 and 2, Fig. 8).

On the other hand, changes in the expression of other *p53*-regulated genes expression was detected (Tables S1-S6). For example, *ALDH4*, aldehyde dehydrogenase 4 (Yoon *et al.*, 2004); *BTG3*, B-cell translocation gene 3 (Ou *et al.*, 2007); *FEN1*, flap endonuclease 1 (Christmann *et al.*, 2005); and *PRG3*, *p53*-responsive gene 3 (Ohiro *et al.*, 2002) are *p53*-dependent up-regulated genes whose expression were increased by space radiations and the space environment (Tables S1 and S3). In addition, the expression of *SOD2*, superoxide dismutase 2, also known as manganese superoxide dismutase (MnSOD) was increased by space radiations (Tables S1 and S3) although there are conflicting reports of *p53*-up-regulation (Hussain *et al.*, 2004) and down-regulation of *SOD2* (Drane *et al.*, 2001, Dhar *et al.*, 2006). The well documented *p53*-induced genes *TP53I3* and *TP53I11* (*PIG3* and *PIG11*)) (Polyak *et al.*, 1997) also show increased expression in the space environment (Table S3). In particular, *HSPA8*, well known as *Hsp70* was increased by space environment (Table S3). In addition, it was also reported that *Hsp27* gene expression was up-regulated by spaceflight in human lymphocytes (Cubano and Lewis, 2001). In addition, *CKB*, the brain creatine kinase gene (Zhao *et al.*, 1994) and *ID1*, an inhibitor of differentiation/DNA binding (Qian and Chen, 2008) are *p53*-dependent down-regulated genes, *EFEMP2*, EGF-containing fibulin-like extracellular matrix protein 2, and mutant p53-binding protein 1 (*MBP1*) (Gallagher *et al.*, 1999) expression levels are decreased after exposure to space radiations and in the space environment (Tables S4 and S6). Although *H19*, known as insulin-like growth factor II (*Igf2*) (Dugimont *et al.*, 1998) and CDKN2A, known as *p16/INK4a* (Leong *et al.*, 2009) were *p53*-down-regulated genes, the expression of these genes was increased in a *p53*-dependent manner in these space experiments (Tables S1-S3). In addition, although *Noxa*, noxious stress inducible pro-apoptotic gene (Oda *et al.*, 2000a); *CDH1*, cadherin 1 known as *E-cadherin* (Bukholm *et al.*, 1997); *HMOX1*, heme oxygenase 1 known as HO-1 (Meiller *et al.*, 2007); *CKM*, muscle creatine kinase gene (Zhao *et al.*, 1994); *Gadd45*, growth arrest and DNA-damage-inducible gene 45 (Kastan *et al.*, 1992); *SMAD7*, Sma- and Mad-related protein family member 7 (Zhang *et al.*, 2006) and *BNIP3L*, BCL2/adenovirus E1B 19kDa interacting protein 3-like (Fei *et al.*, 2004) were reported to be *p53*-dependent up-regulated genes, whereas these genes were depressed in a *p53* dependent manner in these space experiments (Tables S4 and S6). It is possible that the profiled genes reported here may represent newly observed *p53*-regulated genes or factors in *p53* signaling pathways which have not been documented until this report.

It was shown that there were a lot of genes increased (Fig. 8**A**) or decreased (Fig. 8**B**) by both compared with space radiations and microgravity alone. In gene expression level, it is considered that the synergistic effect of space radiations and microgravity on enhancement and depression of gene expression cannot be disregarded though the synergistic effect of them on gene instability is not clear.

For the following genes, there was a large quantitative change in gene expression of 5-fold or more. However, the relationship between the cellular *p53* gene status and the levels of their expression is still unknown. *SLC39A5*, solute carrier family 39 (metal ion transporter), member 5 (Taylor and Nicholson, 2003) and *UNG*, uracil-DNA glycosylase (Haug *et al.*, 1996) were *p53*-dependent up-regulated by space radiations alone (Table S1). *SLC39A5* belongs to a subfamily of proteins which has the structural characteristics of zinc transporters (Taylor and Nicholson, 2003). Zinc is involved in protein, nucleic acid, carbohydrate, and lipid metabolism, as well as in the control of gene transcription, growth, development, differentiation and DNA repair, too. *UNG* are DNA-repair genes that catalyse the removal of promutagenic uracil from single- and double-stranded DNA, thereby initiating the base-excision repair pathway. Interestingly, the radiation-resistant bacterium *Deinococcus radiodurans* has an elevated number of uracil-DNA glycosylases when compared with most other organisms (Sandigursky *et al.*, 2004). *GjB7*, gap junction protein beta 7 (Bondarev *et al.*, 2001); *A_32_P73413*; *AYTL2*, lysophosphatidylcholine acyltransferase 1 (Nakanishi *et al.*, 2006); and *AVIL*, the encoded gene of advillin which is a member of the gelsolin/villin family of actin regulatory proteins (Hasegawa *et al.*, 2007) were *p53*-dependent up-regulated by space radiations and the space environment (Tables S1 and S3). *CX869207* was induced by microgravity alone (Table S2). *A_24_P654649*; *WDR52*, WD repeat domain 52; and *APC2*, adenomatosis polyposis coli 2 (Jarrett *et al.*, 2001) were *p53*-dependent up-regulated by microgravity and the space environment (Tables S2 and S3). *A_24_P358406* was up-regulated by the space environment (Table S3). In addition, having a ratio of gene expression of 0.2 or less was *ADAM11*, a disintegrin and metalloproteinase-11 (Xie *et al.*, 2004) which was affected by space radiations alone (Table S4); *GARNL4*, GTPase activating RANGAP domain-like 4 (Hoffmeister *et al.*, 2008) and *PPP1R1B*,

Table 3 *p53*-Dependent protein synthesis in cultured cells in space.

***p53*-Dependent**	**Gene symbol**	**Value**	**Sample comparison**	**Cause**
Up-regulated proteins	MeCP2	1.85	Space 1*G*/Ground	Space radiations
	Notch1	1.63	Space μ*G*/Ground	Space environment
Down-regulated proteins	DR4	0.66	Space 1*G*/Ground	Space radiations
	PRMT	0.63		
	ROCK-2	0.64		
	ROCK-2	0.59	Space μ*G*/Ground	Space environment
	TGF-β	0.63	Space μ*G*/Space 1*G*	Microgravity
	TWEAK R	0.55		
	Phospho-Pyk2	0.49		
	14-3-3θ/τ	0.42		

A classification of *p53*-dependency means there were changes in protein synthesis levels in wt*p53* cells when compared to levels in m*p53* cells after cells were grown in space.

protein phosphatase 1, regulatory (inhibitor) subunit 1B (Reuter *et al*., 2009) by space radiations and space environment (Tables S4 and S6), and *AK056365*; and *C14orf1* were affected by microgravity alone (Table S5). Notably, *SLC39A5* expression was increased by space radiations alone (Table S1), and was decreased by microgravity alone (Table S6). The functions of these genes are not yet well understood enough.

In the work described here, the emphasize was on examining the behavior of *p53*-regulated genes after exposure to space radiations, microgravity and the space environment during spaceflight. The initial goal of this space experiment was achieved. It is expected that data from this type of work will be helpful in designing physical protection from the deleterious effects of space radiations during long term stays in space.

Protein array analysis of p53-dependent up-regulated proteins in cultured mammalian cells during spaceflight

The aim of this study was to compare protein expression profiles in wt*p53* and m*p53* cells during spaceflight (Table 3). After re-frozen state, the effect of space radiations on protein expression should be able to neglect. Protein expression profiles were measured using Sigma-Aldrich protein array technology including 642 human protein-recognizing antibodies and about 80 *p53*-related proteins. The number of *p53*-dependent up-regulated protein is only 2, and that of down-regulated protein is also 7. Still, the profiling number reaches about 1.4% of this protein array as well as DNA array. It was different between the profiled proteins and genes (data not shown). Perhaps, the protein expression might be depend on translation or stabilization level not transcription level. *p53*-Dependent up-regulated proteins were MeCP2 (mutations in methyl DNA binding protein 2) in response to space radiations, and Notch1 in response to the space environment. On the other hand, *p53*-dependent down-regulated proteins were TGF-β (Transforming growth factor-β), TWEAKR (tumor necrosis factor-like weak inducer of apoptosis receptor), phospho-Pyk2 (Proline-rich tyrosine kinase 2) and 14-3-3θ/τ in response to microgravity environments, and DR4, PRMT1 (protein arginine methyltransferase 1) and ROCK-2 (Rho-kinase) in response to space radiations. ROCK-2 was also down-regulated independently of *p53* status in the space environment (Table 3). In this experiment, alterations of expression of p53 or of these prominent *p53*-regulated proteins were not detected (Table 1). Although DR4 (Guan *et al*., 2001), TGF-β (Fujiwara *et al*., 1994) and 14-3-3 (Hermeking *et al*., 1997) were reported to be DNA damage-inducible *p53*-regulated genes, these proteins were down-regulated in a *p53*-dependent manner in these space experiments. The direct accumulation of p53 proteins was observed in rats muscle and skin after spaceflight (Ohnishi *et al*., 1996a, 1999a), but was not seen in space flown cells in this experiment. The main difference might be brought from animal and cell culture systems. In addition, the results might be caused by not only space radiations and microgravity but also hypergravity during lunch and landing, and psychosocial problems in animals.

It is possible that the profiled proteins reported here may represent newly observed *p53*-regulated proteins or the factor of *p53* signaling pathway which have not been documented until this report. In fact in support of this, it was recently demonstrated the up-regulation of *Notch1* gene expression by p53 (Yugawa *et al*., 2007) through negative regulation of ROCK-2 (Lefort *et al*., 2007) as well as our result (Table 1). After genotoxic stresses, Notch signaling determines cell fate and affects cell proliferation, differentiation, and apoptosis during cell development (Dotto, 2008). Therefore, it is interesting to note that Notch1 appeared to accumulate in a *p53*-dependent manner by space environment. Compared to *p53*-dependent space radiation-up-regulated protein, the mechanisms that microgravity induces gene activity are unknown. Progress in these fields should advance in the near future.

Here, we would like to emphasize that we can profile the *p53*-dependent regulated proteins by exposure to space radiations, microgravity and a space environment during spaceflight. As well as the *p53*-dependent regulated gene expression, the initial goal of this space experiment as for protein-array analysis was achieved.

Radio-adaptive responses in human cells exposed to space radiations

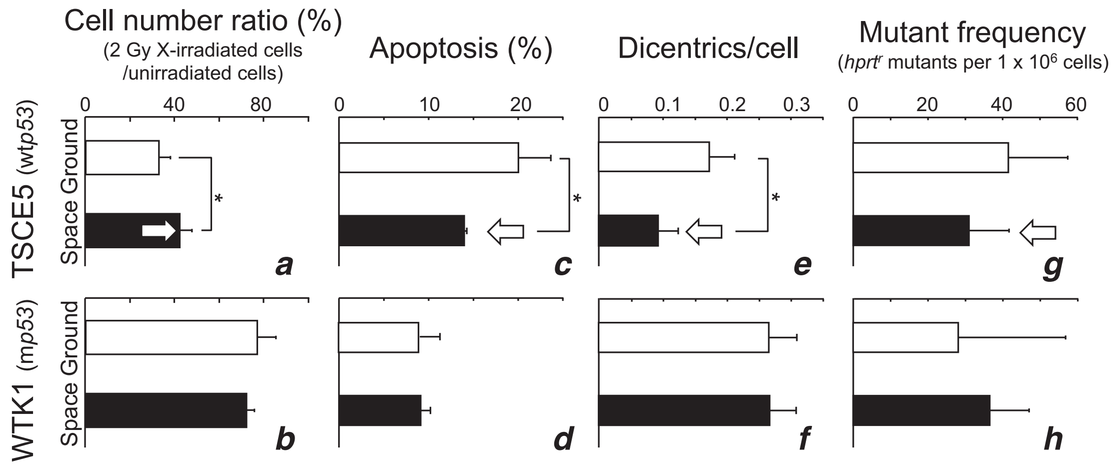


Fig. 9. Radio-adaptive response in flown cells in space at frozen state. ***a*** and b, surviving cell number; ***c*** and ***d***, induction frequency of apoptosis; ***e*** and ***f***, induction frequency of chromosomal aberrations; ***g*** and ***h***, induction frequency of mutations; ***a***, ***c***, ***e*** and ***g***, wt*p53* cells; ***b***, ***d***, ***f*** and ***h***, m*p53* cells. Arrows indicate positive in radio-adaptive response.

The induced radio-resistance reached a maximum at 50 mGy in pre-irradiated wt*p53* cells. In contrast, there was no change seen in responses to a challenging irradiation following a priming irradiation in m*p53* cells. Space experiments (Fig. 6***a***) with wt*p53* and m*p53* cells were performed with the ground control experiments (Figs. 6***b*** and 6***c***). In the samples exposed in space, induction of radio-resistance in wt*p53* cells was found (p<0.05) (Fig. 9***a***). In contrast, other than additive treatment effects in m*p53* cells, a radio-adaptive response was not found (Fig. 9***b***). The effect of a priming irradiation on radiation-induced apoptosis in the frozen cells was analyzed (Figs. 9***c*** and 9***d***). Radiation-induced apoptosis was observed more frequently in wt*p53* cells (Fig. 9***c***), but not in m*p53* cells (Fig. 9***d***). The effect of a priming irradiation delivered to frozen cells on radiation-induced chromosomal aberrations was analyzed (Figs. 9***e*** and 9***f***). In the space samples, radiation-induced dicentrics were depressed in wt*p53* cells (p<0.05) (Fig. 9***e***). In contrast, a radio-adaptive response was not observed in m*p53* cells (Fig. 9***f***). In the space samples, it was found that the induced mutation frequency resulting from a challenging irradiation was depressed in wt*p53* cells (Fig. 9***g***), but increased in m*p53* cells (Fig. 9***h***).

The radio-adaptive response has been induced at 6 h after a priming irradiation with 20-50 mGy in wt*p53* cells. However, in m*p53* cells, induction of the radio-adaptive response was not observed. p53, which plays a key role in protecting the genome (Lane, 1992), is the most important factor in the signaling pathway of the radio-adaptive response because various endpoints used to study this response such as changes in apoptosis levels (Wang *et al*., 2000; Hendrikse *et al*., 2000; Takahashi, 2001; Okazaki *et al*., 2007), micronuclei induction (Sasaki *et al*., 2002) and chromosome aberrations (Takahashi *et al*., 2008b) are not observed in *p53*-null and m*p53* cells. It should be noted that an accumulation of p53 was induced after a high-dose irradiation alone, but was not induced after a priming irradiation followed by a subsequent challenging irradiation in wt*p53* cells (Takahashi *et al*., 2008b). Under the same conditions, there was also no induction of *p53*-target gene products such as p21/WAF1 and Bax, and *p53*-dependent apoptosis (Takahashi, 2001). Taking these observations into consideration, the radio-adaptive response seems to be a universal phenomenon which can be down-regulated by cellular *p53* responses to small doses of ionizing radiation. A conditioning radiation exposure has also been reported to suppress p53 function (Ohnishi *et al*., 1999b). These findings led to a proposal suggesting that this repressed *p53*-dependent response is one of the mechanisms likely to be involved in the radio-adaptive response (Takahashi, 2002). The p53 which accumulates after irradiation can attenuate the induction of inducible nitric oxide (NO) synthase (iNOS, or alternatively, NOS2), which catalyzes the conversion of *L*-arginine into *L*-citrulline, resulting in the secretion of NO radicals as a byproduct of the reaction. This occurs through an interaction between p53 and the TATA binding protein (TBP) and/or the nuclear factor *κB* (NF-*κB*) which are essential for *iNOS* expression (Forrester *et al*., 1996; Matsumoto *et al*., 2000, 2001). Moreover, NO radicals secreted from irradiated cells with m*p53* were able to induce radio-resistance in unirradiated wt*p53* cells through intercellular signaling (Matsumoto *et al*., 2000, 2001). Recent reports have shown that NO radicals are an initiator of radio-resistance and of the depression of chromosome aberrations, and act through the activation of HDM2, the depression of p53 accumulation and iNOS which is observed following a priming irradiation (Matsumoto *et al*., 2007; Takahashi *et al*., 2008b; Takahashi and Ohnishi, 2009b).

In space, space radiations penetrated the ISS and possessed a low dose-rate (Fig. 1). In the interior of the ISS, there will be a high level of exposure to space radiations which consist of various types of particles: electrons, γ-rays, and high LET particles such as protons, neutrons, and *α*-particles. In addition, exposure doses will be higher than those experienced on the Earth's surface.

These types of radiations will originate primarily from the sun’s solar winds, from supernova, and from other galaxies. The dose rate may depend largely on occasional intensely energetic solar particle events. On the surface of the earth, most space radiations are diminished by the atmosphere and the geomagnetic field. Radiation damage in the frozen cells can be accumulated during a long stay in the ISS. Cells do not repair at frozen state, just accumulate DNA damage. In fact, space radiation-induced DNA double strand breaks were detected as a track in cell nucleus in this spaceflight (Ohnishi *et al.*, 2009b). Fortunately, the radiation doses about 71 mSv in this space experiment (Ohnishi *et al.*, 2009b) were in the possible range of priming doses about 20-100 mGy. In fact, it was found that the radio-adaptive response was present in wt*p53* cells. It was also possible to confirm an exposure from space radiations with a specific range of low doses, and to observe that the cells remember or retain the effects of their radiation exposure, even at low doses. In this spaceflight, this radio-adaptive response might not be induced by other stress such as gravity change and freezing/thawing stress, but it was induced by the space radiations alone because all samples were exposed to the same freezing/thawing stress. Therefore, it was confirmed that the radio-adaptive response may be induced by space radiations with a specific range of low doses.

Conclusion

To clarify the biological effects of space environment, especially space radiations, a proposal of “Rad Gene” were performed as the first life science experiment with two human lymphoblastoid cell lines bearing wt*p53* and m*p53* in an ISS for 133 days. We scheduled four projects; (1) DNA damage and biological and physical dosimetries, (2) gene and protein expression under microgravity and 1*G* during space flight, (3) gene expression after frozen stage, (4) a radio-adaptive response. (1) DNA damage induced by space radiations including the high LET particles was detected as a track of γH2AX foci in the nuclei of these frozen cells. High LET particles are suggested to induce DSBs as a track. From the frequency track formation, the exposure dose rate as biological dosimetry was calculated to be 0.7 mSv per day. From the physical dosimetry with CR39 and TLDs plastics, dose rate was 0.5 mSv per day. These values the exposed dose rate were similar between biological and physical dosimetries. (2) To examine the biological effects of microgravity and space radiations on gene and protein expression of *p53*-dependent regulated genes, these cells were grown under microgravity and 1*G* in ISS for 8 days and analyzed by DNA and protein arrays. *p53*-Dependent up-regulated gene expression was found for 111, 95, and 328 genes and *p53*-dependent down-regulated gene expression was found for 177, 16, and 282 genes by space radiations, microgravity and space environment including both conditions, respectively. In the analysis for protein expression, it was found that *p53*-dependent up-regulated proteins were MeCP2 in response to space radiations, and Notch1 in response to space environment. On the other hand, *p53*-dependent down-regulated proteins were TGF-β, TWEAKR, phospho-Pyk2, and 14-3-3*θ/τ* which were affected by microgravity and DR4, PRMT1 and ROCK-2 in response to space radiations. ROCK-2 was also down-regulated in a *p53*-dependent manner in response to the space environment. (3) For the gene expression of *p53*-dependent regulated genes, the gene expression profiles were analyzed in these cells from space samples in a frozen state. *p53*-Dependent up-regulated gene expression was found for 50 genes and *p53*-dependent down-regulated gene expression was found for 94 genes. (4) A pre-irradiation with low-dose in the range of 20-100 mGy has been reported to induce a *p53*-dependent radio-adaptive response in mammalian cells. To clarify the effects of space radiations on the radio-adaptive response, these two cell lines were analyzed for the induction of radio-resistance and the depression of radiation-induced apoptosis, chromosome aberrations and mutations. After the flight in a frozen state, the cells were cultured for 6 h, and then exposed to challenging X-ray-irradiation. In the cells exposed to a space environment, all of the radio-adaptive responses investigated here were found only in wt*p53* cells, but not in the m*p53* cells. These results confirmed that the cells exposed to a space environment were likely to the exposed cells to radiation in the specific low dose range which can lead to an adaptive response on ground-base experiments, and that the cells were confirmed to obtain space-radiations with low dose in space for radio-adaptive response.

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Appendix

Table S1 Space radiation-induced *p53*-dependent genes. 1-41, by space radiations alone; 42-109, by space radiations and space environment; 110 and 111, by microgravity alone. Red letters (54, 78, 107, 109), previously reported genes as up-regulated genes.

	Gene symbol	Ratio		Gene symbol	Ratio		Gene symbol	Ratio		Gene symbol	Ratio
1	SLC39A5	14.68	29	ENST00000380946	2.22	57	BX110908	3.44	85	U09197	2.54
2	UNG	5.12	30	MEGF9	2.14	58	SUSD1	3.43	86	BU507302	2.53
3	C14orf1	4.72	31	LOC644422	2.14	59	FCRL5	3.33	87	NT5E	2.53
4	FN3K	4.44	32	GRRP1	2.13	60	A_24_P782102	3.25	88	RAB27A	2.49
5	KCNK3	3.71	33	ENST00000334827	2.12	61	FOSL2	3.15	89	DA438590	2.48
6	THC2706493	3.26	34	ATPAF1	2.11	62	AK026881	3.14	90	ITPKA	2.48
7	DMBT1	3.20	35	SLC26A2	2.10	63	IL20RB	3.12	91	ENST00000369158	2.44
8	ATP2A1	3.12	36	ANXA1	2.04	64	HIST1H2AL	3.09	92	THC2659519	2.40
9	NR4A3	3.06	37	AK057116	2.03	65	SLA	3.08	93	RDH10	2.38
10	RGNEF	3.05	38	SMPD3	2.03	66	SGOL1	3.04	94	THC2724046	2.36
11	NBPF1	3.01	39	KCTD11	2.03	67	CB049993	2.97	95	CCDC96	2.34
12	USP41	2.86	40	NPCDR1	2.01	68	PIK3AP1	2.92	96	FLJ40172	2.32
13	SCRT1	2.79	41	ESPNL	2.00	69	AK023526	2.92	97	TRAα	2.31
14	PITX3	2.79	42	GJB7	8.79	70	AK092508	2.89	98	MYLK2	2.29
15	PTGFRN	2.66	43	A_32_P73413	7.35	71	THC2512545	2.88	99	TAF9B	2.26
16	KIAA1913	2.63	44	AYTL2	6.11	72	CG012	2.87	100	C9orf3	2.24
17	H19	2.63	45	AVIL	5.35	73	LRRC31	2.86	101	AB046850	2.23
18	TRIM23	2.60	46	FOSL1	4.65	74	RSU1	2.86	102	FAM111B	2.23
19	MGC35440	2.58	47	THC2617584	4.14	75	AK074097	2.84	103	DQ680071	2.18
20	PPHLN1	2.57	48	TCTE3	4.11	76	CN408247	2.72	104	SLC27A4	2.15
21	CLCN4	2.52	49	NUPR1	4.06	77	AF086429	2.66	105	THC2654231	2.14
22	AK056365	2.46	50	LMO2	3.99	78	ALDH4A1	2.65	106	AK123439	2.12
23	BM690036	2.44	51	A_32_P164637	3.86	79	USP18	2.65	107	BTG3	2.11
24	JRK	2.43	52	CCL4	3.69	80	PPM1F	2.60	108	DIDO1	2.10
25	BX092067	2.41	53	AY831680	3.68	81	STAU2	2.59	109	FEN1	2.05
26	SRGN	2.40	54	SOD2	3.68	82	SSH3	2.58	110	AK000038	3.76
27	MGC29891	2.35	55	PLEKHA7	3.55	83	LRRC16	2.57	111	AK090480	2.59
28	ENST00000370857	2.26	56	ACTL8	3.54	84	CXCR3	2.57			

Table S2 Microgravity-induced *p53*-dependent genes. 1-44, by microgravity alone; 45-93, by microgravity and space environment; 94 and 95, by microgravity and space radiations. Blue letter (88), previously reported gene as down-regulated gene.

	Gene symbol	Ratio		Gene symbol	Ratio		Gene symbol	Ratio		Gene symbol	Ratio
1	CX869207	5.98	25	THC2650029	2.24	49	MGC45491	4.66	73	A_32_P78395	2.58
2	A_32_P13442	4.16	26	BC000228	2.23	50	PRG3	4.54	74	LOC133874	2.58
3	ZNF179	3.71	27	C20orf12	2.23	51	tcag7.1017	4.51	75	LOC647502	2.50
4	SLC46A1	3.46	28	A_32_P56726	2.21	52	A_32_P124887	4.31	76	NTRK1	2.48
5	A_24_P315885	2.88	29	LOC652309	2.20	53	THC2689950	3.72	77	C6orf134	2.48
6	ZNRF4	2.81	30	ENST00000355232	2.18	54	A_32_P71456	3.56	78	AI167420	2.48
7	LOC730957	2.73	31	NM_001018022	2.18	55	LOC441245	3.52	79	BX449754	2.46
8	ZIC4	2.67	32	S75896	2.18	56	FAM131B	3.48	80	STRN3	2.43
9	ADAM20	2.62	33	CHI3L2	2.17	57	C10orf4	3.26	81	THC2588113	2.39
10	GPR56	2.52	34	A_24_P410256	2.15	58	BX089851	3.25	82	A_23_P108534	2.32
11	AF007192	2.51	35	ND2	2.13	59	FGF9	3.17	83	IFI27	2.30
12	A_24_P7494	2.50	36	A_24_P384239	2.11	60	A_24_P333077	3.03	84	GRIN2C	2.29
13	THC2692434	2.45	37	LOC389634	2.10	61	ENST00000343519	3.03	85	A_24_P930327	2.28
14	DEPDC4	2.44	38	ATF7IP	2.08	62	AI435484	3.01	86	ITGAM	2.26
15	PSD	2.42	39	GOLGA8E	2.07	63	AK095886	2.94	87	BQ185350	2.24
16	A_32_P125219	2.39	40	THC2676657	2.06	64	THC2721275	2.75	88	CDKN2A	2.23
17	FRMD4A	2.31	41	HERC2	2.06	65	LRRC41	2.73	89	AK057071	2.23
18	A_24_P350196	2.30	42	THC2541992	2.05	66	THC2577459	2.68	90	A_24_P204334	2.14
19	VCX	2.29	43	LOC389607	2.05	67	SLC22A18AS	2.65	91	AK131288	2.14
20	CLEC2D	2.29	44	RAPH1	2.02	68	A_32_P225768	2.64	92	FSCN2	2.13
21	A_24_P916853	2.26	45	A_24_P654649	7.89	69	KIAA1324L	2.63	93	NPDC1	2.13
22	BC035751	2.25	46	WDR52	5.05	70	WWTR1	2.63	94	AK000038	3.76
23	KLF2	2.25	47	APC2	5.02	71	LARP7	2.59	95	AK090480	2.59
24	FLJ11235	2.24	48	CCR3	4.68	72	OR5L2	2.58			

Table S3 Space environment-induced *p53*-dependent genes. 1-209, by space environment alone; 210-277, by space environment and space radiations; 278-326, by space environment and microgravity; 327 and 328, by space environment, radiations and microgravity. Red letters (41, 42, 168, 222, 246, 275, 277), previously reported genes as up-regulated genes; blue letter (321), previously reported gene as down- regulated gene.

	Gene symbol	Ratio		Gene symbol	Ratio		Gene symbol	Ratio		Gene symbol	Ratio
1	*A_24_P358406*	9.82	42	*TP53I11 (PIG11)*	2.74	83	*BC031957*	2.38	124	*LOC200420*	2.21
2	*SLC30A3*	4.59	43	*A_24_P238819*	2.71	84	*SOBP*	2.38	125	*THC2664742*	2.21
3	*SOLH*	4.57	44	*THC2729109*	2.71	85	*ENST00000329156*	2.37	126	*TIA1*	2.21
4	*BX103037*	4.44	45	*RELL1*	2.67	86	*PYGM*	2.36	127	*AK023816*	2.21
5	*AK027069*	4.39	46	*AK091308*	2.66	87	*UNQ1940*	2.36	128	*A_32_P141938*	2.20
6	*THC2708422*	4.22	47	*THC2725153*	2.63	88	*CENPP*	2.35	129	*PTGER4*	2.20
7	*THC2652466*	4.20	48	*TSGA14*	2.62	89	*THC2507805*	2.35	130	*CR611122*	2.19
8	*THC2672475*	3.76	49	*KCNRG*	2.62	90	*FAIM*	2.34	131	*FOXD4*	2.19
9	*PRRT2*	3.70	50	*THC2631150*	2.60	91	*A_24_P912404*	2.34	132	*THC2618074*	2.17
10	*THC2673554*	3.39	51	*BX099788*	2.60	92	*CCL3L3*	2.34	133	*BX100717*	2.16
11	*SPTLC3*	3.30	52	*ENST00000316131*	2.59	93	*CTNND1*	2.33	134	*ENST00000371081*	2.16
12	*AI433842*	3.27	53	*THC2627008*	2.57	94	*A_32_P101420*	2.33	135	*A_24_P170309*	2.16
13	*THC2685727*	3.27	54	*SRGAP2*	2.55	95	*LOC85391*	2.32	136	*PHF6*	2.15
14	*AY172962*	3.26	55	*KIF24*	2.55	96	*A_24_P492919*	2.32	137	*THC2503151*	2.14
15	*BE008305*	3.26	56	*UGCGL2*	2.54	97	*C9orf39*	2.32	138	*C17orf76*	2.14
16	*LOC51336*	3.20	57	*CHAC2*	2.54	98	*DQ786194*	2.32	139	*RNF213*	2.14
17	*KBTBD8*	3.20	58	*TMEM2*	2.53	99	*THC2659953*	2.31	140	*A_24_P674118*	2.14
18	*UCP3*	3.18	59	*C14orf145*	2.53	100	*THEM4*	2.31	141	*SFXN2*	2.14
19	*POLQ*	3.16	60	*BE710245*	2.48	101	*LOC440839*	2.30	142	*THC2740317*	2.13
20	*AV721971*	3.04	61	*BY796363*	2.48	102	*SLC23A3*	2.30	143	*A_24_P631625*	2.13
21	*THC2633136*	3.04	62	*AMAC1L2*	2.48	103	*THC2725553*	2.30	144	*BX641009*	2.13
22	*BI026064*	3.04	63	*KIAA1641*	2.47	104	*LOC150166*	2.30	145	*FCRL2*	2.13
23	*MYBPC2*	3.00	64	*RASL10A*	2.47	105	*LOC647500*	2.30	146	*POLE2*	2.13
24	*CHRNA10*	2.96	65	*C8orf15*	2.47	106	*AL832142*	2.28	147	*POP1*	2.13
25	*THC2647005*	2.95	66	*A_23_P46070*	2.47	107	*TIPIN*	2.28	148	*CD104030*	2.12
26	*THC2733296*	2.94	67	*BC032409*	2.45	108	*AL110257*	2.27	149	*CCL3*	2.12
27	*M87790*	2.93	68	*DNAJB6*	2.44	109	*OSBPL3*	2.26	150	*THC2660562*	2.12
28	*BC035392*	2.92	69	*NPFF*	2.44	110	*LOC730211*	2.26	151	*THC2748223*	2.11
29	*THC2564393*	2.92	70	*A_32_P167212*	2.43	111	*LOC728688*	2.26	152	*E2F7*	2.11
30	*PPP3CA*	2.91	71	*DKFZP586P0123*	2.43	112	*LOC402360*	2.26	153	*NARG2*	2.10
31	*FAM83E*	2.90	72	*AK022109*	2.43	113	*AK092134*	2.25	154	*THC2586764*	2.10
32	*GLDC*	2.87	73	*AI820751*	2.43	114	*AK058065*	2.25	155	*MCM10*	2.10
33	*CCL23*	2.85	74	*KIF1B*	2.42	115	*TTC26*	2.25	156	*THC2726026*	2.10
34	*CYP4V2*	2.82	75	*AP3B1*	2.41	116	*MGC24975*	2.24	157	*A_24_P752999*	2.10
35	*CBS*	2.80	76	*AK123333*	2.41	117	*LOC134145*	2.24	158	*THC2659414*	2.10
36	*FAM65A*	2.77	77	*EGR3*	2.41	118	*ALMS1*	2.23	159	*BM932034*	2.10
37	*CLSPN*	2.77	78	*DKFZp667E0512*	2.40	119	*PTPRE*	2.23	160	*RAB3IP*	2.10
38	*A_24_P357986*	2.76	79	*LOC283868*	2.40	120	*TNF*	2.23	161	*C2orf34*	2.10
39	*TNFSF4*	2.76	80	*ELAC1*	2.39	121	*FAM43B*	2.23	162	*BU930679*	2.09
40	*PARP11*	2.75	81	*MGC26718*	2.39	122	*SH2D2A*	2.23	163	*AJ293393*	2.09
41	*TP53I3 (PIG3)*	2.75	82	*AK090897*	2.38	123	*AK125170*	2.22	164	*LRRC14*	2.09

Table S3 (continued)

	Gene symbol	Ratio
165	THC2719547	2.09
166	C5orf34	2.09
167	THC2655811	2.09
168	HSPA8	2.08
169	A_32_P101799	2.08
170	FAM81A	2.08
171	COQ2	2.08
172	FUT7	2.08
173	THC2664391	2.08
174	ZBTB1	2.07
175	WWOX	2.07
176	LOC392545	2.07
177	FAM13A1	2.07
178	LOC442075	2.07
179	POLR2L	2.06
180	C15orf42	2.06
181	A_24_P161655	2.06
182	A_24_P281175	2.06
183	A_24_P332911	2.06
184	LOC150759	2.05
185	POLA1	2.05
186	AA465699	2.05
187	MFSD2	2.04
188	RBL1	2.04
189	PRKAR1B	2.04
190	APOB48R	2.04
191	AK021745	2.04
192	AA642112	2.03
193	AK022443	2.03
194	AK000420	2.03
195	FLJ11292	2.03
196	THC2701140	2.02
197	CENTB5	2.02
198	RILP	2.02
199	THC2760643	2.02
200	BC007917	2.02
201	A_32_P174385	2.02
202	AL832146	2.02
203	BE835321	2.02
204	SLC37A2	2.01
205	THC2526402	2.01
206	PRKDC	2.01
207	THC2639689	2.01
208	A_24_P914102	2.00
209	TNFRSF12A	2.00
210	GJB7	8.79
211	A_32_P73413	7.35
212	AYTL2	6.11
213	AVIL	5.35
214	FOSL1	4.65
215	THC2617584	4.14
216	TCTE3	4.11
217	NUPR1	4.06
218	LMO2	3.99
219	A_32_P164637	3.86
220	CCL4	3.69
221	AY831680	3.68
222	SOD2	3.68
223	PLEKHA7	3.55
224	ACTL8	3.54
225	BX110908	3.44
226	SUSD1	3.43
227	FCRL5	3.33
228	A_24_P782102	3.25
229	FOSL2	3.15
230	AK026881	3.14
231	IL20RB	3.12
232	HIST1H2AL	3.09
233	SLA	3.08
234	SGOL1	3.04
235	CB049993	2.97
236	PIK3AP1	2.92
237	AK023526	2.92
238	AK092508	2.89
239	THC2512545	2.88
240	CG012	2.87
241	LRRC31	2.86
242	RSU1	2.86
243	AK074097	2.84
244	CN408247	2.72
245	AF086429	2.66
246	ALDH4A1	2.65
247	USP18	2.65
248	PPM1F	2.60
249	STAU2	2.59
250	SSH3	2.58
251	LRRC16	2.57
252	CXCR3	2.57
253	U09197	2.54
254	BU507302	2.53
255	NT5E	2.53
256	RAB27A	2.49
257	DA438590	2.48
258	ITPKA	2.48
259	ENST00000369158	2.44
260	THC2659519	2.40
261	RDH10	2.38
262	THC2724046	2.36
263	CCDC96	2.34
264	FLJ40172	2.32
265	TRA@	2.31
266	MYLK2	2.29
267	TAF9B	2.26
268	C9orf3	2.24
269	AB046850	2.23
270	FAM111B	2.23
271	DQ680071	2.18
272	SLC27A4	2.15
273	THC2654231	2.14
274	AK123439	2.12
275	BTG3	2.11
276	DIDO1	2.10
277	FEN1	2.05
278	A_24_P654649	7.89
279	WDR52	5.05
280	APC2	5.02
281	CCR3	4.68
282	MGC45491	4.66
283	PRG3	4.54
284	tcag7.1017	4.51
285	A_32_P124887	4.31
286	THC2689950	3.72
287	A_32_P71456	3.56
288	LOC441245	3.52
289	FAM131B	3.48
290	C10orf4	3.26
291	BX089851	3.25
292	FGF9	3.17
293	A_24_P333077	3.03
294	ENST00000343519	3.03
295	AI435484	3.01
296	AK095886	2.94
297	THC2721275	2.75
298	LRRC41	2.73
299	THC2577459	2.68
300	SLC22A18zAS	2.65
301	A_32_P225768	2.64
302	KIAA1324L	2.63
303	WWTR1	2.63
304	LARP7	2.59
305	OR5L2	2.58
306	A_32_P78395	2.58
307	LOC133874	2.58
308	LOC647502	2.50
309	NTRK1	2.48
310	C6orf134	2.48
311	AI167420	2.48
312	BX449754	2.46
313	STRN3	2.43
314	THC2588113	2.39
315	A_23_P108534	2.32
316	IFI27	2.30
317	GRIN2C	2.29
318	A_24_P930327	2.28
319	ITGAM	2.26
320	BQ185350	2.24
321	CDKN2A	2.23
322	AK057071	2.23
323	A_24_P204334	2.14
324	AK131288	2.14
325	FSCN2	2.13
326	NPDC1	2.13
327	AK000038	3.76
328	AK090480	2.59

Table S4 Space radiation-depressed *p53*-dependent genes. 1-62, by space radiations alone; 63-177, by radiations and space environment. Red letter (41), previously reported genes as up-regulated gene blue letters (96, 114, 138), previously reported genes as down- regulated genes.

	Gene symbol	Ratio
1	*ADAM11*	0.20
2	*VCX*	0.21
3	*A_24_P7494*	0.23
4	*MUC1*	0.24
5	*REST*	0.24
6	*STRC*	0.25
7	*LOC391271*	0.29
8	*MGC51025*	0.29
9	*VCX2*	0.30
10	*VCX3A*	0.31
11	*CDH15*	0.32
12	*PTPRF*	0.33
13	*OVGP1*	0.35
14	*ALDH2*	0.35
15	*PNCK*	0.35
16	*PAGE2*	0.37
17	*C1QTNF6*	0.37
18	*AGBL2*	0.37
19	*EPHB6*	0.37
20	*ROBO1*	0.37
21	*COL16A1*	0.38
22	*ENST00000390556*	0.38
23	*SPOCD1*	0.38
24	*UNC5CL*	0.38
25	*PLAC2*	0.38
26	*ZNF179*	0.38
27	*ANKRD24*	0.39
28	*A_23_P21393*	0.39
29	*ENST00000390622*	0.40
30	*PLA2G4C*	0.40
31	*C13orf16*	0.40
32	*AK094786*	0.40
33	*ENST00000360329*	0.41
34	*TRIM74*	0.41
35	*SGCA*	0.42
36	*AL832786*	0.42
37	*EVC*	0.42
38	*ZNF765*	0.43
39	*A_24_P290109*	0.43
40	*DDO*	0.43
41	*NOXA1*	0.43
42	*SCT*	0.44
43	*FAM101A*	0.44
44	*NOXO1*	0.45
45	*CLDN9*	0.45
46	*SUGT1L1*	0.45
47	*CD7*	0.46
48	*LOC643100*	0.46
49	*ENST00000357802*	0.46
50	*A_24_P6850*	0.47
51	*SSX5*	0.47
52	*NRN1L*	0.47
53	*FCGBP*	0.48
54	*A_32_P158543*	0.48
55	*DMXL1*	0.49
56	*MUC20*	0.49
57	*AF146694*	0.49
58	*PRPH*	0.49
59	*VAT1*	0.49
60	*DKFZP564J102*	0.49
61	*JAG2*	0.50
62	*ENST00000379855*	0.50
63	*GARNL4*	0.12
64	*PPP1R1B*	0.17
65	*THC2708687*	0.20
66	*ATP11B*	0.21
67	*GAL3ST1*	0.24
68	*GLIS1*	0.24
69	*IGF1*	0.24
70	*A_32_P67355*	0.24
71	*GH1*	0.26
72	*AK096685*	0.27
73	*A_32_P224040*	0.27
74	*MUC19*	0.28
75	*MT1X*	0.28
76	*MT1E*	0.28
77	*MT1L*	0.28
78	*MT1H*	0.28
79	*ECAT8*	0.28
80	*MT1B*	0.28
81	*MT1G*	0.28
82	*AL832534*	0.28
83	*CLRN1*	0.28
84	*MT2A*	0.28
85	*BC015836*	0.29
86	*BLK*	0.29
87	*ZDHHC11*	0.29
88	*JPH2*	0.29
89	*CSH2*	0.29
90	*SLC6A13*	0.29
91	*ATCAY*	0.29
92	*THC2582296*	0.29
93	*ALDH7A1*	0.29
94	*CSH1*	0.30
95	*KIAA1462*	0.30
96	*CKB*	0.30
97	*C21orf63*	0.31
98	*AF217970*	0.31
99	*KRTAP17-1*	0.31
100	*CABP1*	0.31
101	*CSHL1*	0.32
102	*PPEF1*	0.32
103	*ARHGAP8*	0.32
104	*SEZ6L2*	0.33
105	*MT1A*	0.33
106	*PAGE5*	0.33
107	*AK022892*	0.33
108	*TMEM112*	0.33
109	*AY358804*	0.33
110	*GIMAP1*	0.33
111	*THC2550353*	0.33
112	*APOE*	0.33
113	*BC045163*	0.34
114	*ID1*	0.34
115	*IGSF21*	0.34
116	*CD274*	0.35
117	*FLJ42342*	0.35
118	*AK090499*	0.35
119	*ENST00000361259*	0.35
120	*TNFAIP2*	0.35
121	*GPR174*	0.36
122	*C1orf170*	0.36
123	*TF*	0.37
124	*DSCAML1*	0.37
125	*SPINT2*	0.37
126	*PIGZ*	0.37
127	*BAIAP2*	0.37
128	*THC2529684*	0.37
129	*RPL10*	0.37
130	*THC2525505*	0.37
131	*OR10J5*	0.37
132	*GRM8*	0.38
133	*SLAMF1*	0.38
134	*GPR30*	0.38
135	*CBLN3*	0.38
136	*ADARB2*	0.38
137	*KLK1*	0.38
138	*EFEMP2*	0.39
139	*MTL5*	0.39
140	*SLC22A18*	0.39
141	*ATP8B3*	0.39
142	*A_24_P686263*	0.40
143	*KIR2DL4*	0.40
144	*TMPIT*	0.40
145	*DRD1IP*	0.40
146	*BM008292*	0.41
147	*IL3RA*	0.41
148	*THC2694227*	0.41
149	*FAM132A*	0.41
150	*TRIM50*	0.42
151	*THC2650352*	0.42
152	*SELS*	0.42
153	*LSS*	0.42
154	*SEC24A*	0.42
155	*RNF43*	0.42
156	*ST7L*	0.42
157	*IFT140*	0.43
158	*PLD1*	0.43
159	*ENST00000381800*	0.43
160	*C19orf41*	0.43
161	*HSD17B14*	0.44
162	*CXCR7*	0.44
163	*MGC31957*	0.44
164	*SLC12A7*	0.44
165	*A_23_P106814*	0.44
166	*ERN1*	0.44
167	*CDA*	0.45
168	*SH3YL1*	0.45
169	*ENST00000354349*	0.45
170	*CRIP1*	0.46
171	*TCP10L*	0.46
172	*NMU*	0.46
173	*THC2616715*	0.46
174	*LHPP*	0.46
175	*TTYH1*	0.46
176	*PHF21A*	0.47
177	*RALGPS1*	0.48

Table S5 Microgravity-depressed *p53*-dependent genes. 1-15, by microgravity alone; 16, by microgravity and space environment.

	Gene symbol	Ratio		Gene symbol	Ratio		Gene symbol	Ratio		Gene symbol	Ratio
1	*SLC39A5*	0.12	5	*NBPF1*	0.25	9	*THC2706493*	0.33	13	*A_23_P111766*	0.37
2	*AK056365*	0.13	6	*ATP2A1*	0.25	10	*UNG*	0.33	14	*ELA2B*	0.42
3	*C14orf1*	0.19	7	*PITX3*	0.30	11	*RGNEF*	0.34	15	*THC2482457*	0.49
4	*ENST00000380946*	0.24	8	*FN3K*	0.31	12	*ATPAF1*	0.36	16	*ENG*	0.33

Table S6 Space environment-depressed *p53*-dependent genes. 1-166, by space environment alone; 167-281, by space environment and space radiations; 282, by space environment and microgravity. Red letters (2, 3, 5, 74, 99, 147), previously reported genes as up-regulated genes; blue letters (200, 218, 242), previously reported genes as down-regulated genes.

	Gene symbol	Ratio		Gene symbol	Ratio		Gene symbol	Ratio		Gene symbol	Ratio
1	*AK096020*	0.27	36	*A_32_P113462*	0.37	71	*CD244*	0.42	106	*LAMA5*	0.45
2	*CDH1*	0.29	37	*CLYBL*	0.38	72	*THC2567672*	0.42	107	*EBI2*	0.46
3	*HMOX1*	0.29	38	*THC2530832*	0.38	73	*CXCR4*	0.42	108	*BF217859*	0.46
4	*EML1*	0.29	39	*ENST00000334994*	0.38	74	*GADD45B*	0.42	109	*INHBE*	0.46
5	*CKM*	0.29	40	*DQ655984*	0.38	75	*TMOD1*	0.42	110	*LMO7*	0.46
6	*AI028577*	0.30	41	*GNB5*	0.38	76	*CN430296*	0.42	111	*AK093691*	0.46
7	*AA585242*	0.31	42	*BI828537*	0.39	77	*LOC441161*	0.43	112	*ZMYND12*	0.46
8	*AHNAK*	0.32	43	*MAGEA12*	0.39	78	*OPLAH*	0.43	113	*THC2505349*	0.46
9	*AK022971*	0.32	44	*SYT12*	0.39	79	*FLJ35220*	0.43	114	*AK056182*	0.46
10	*LINCR*	0.32	45	*GJE1*	0.39	80	*DIO3*	0.43	115	*THC2528572*	0.46
11	*LRRC62*	0.32	46	*P2RX5*	0.39	81	*HSD11B1L*	0.43	116	*AF131798*	0.46
12	*AKR1C1*	0.30	47	*CA431756*	0.39	82	*ITGB3*	0.43	117	*SENP6*	0.46
13	*TSC22D3*	0.33	48	*TMC4*	0.39	83	*GPSM1*	0.43	118	*PIM2*	0.46
14	*TTYH2*	0.33	49	*AK092715*	0.39	84	*ZNF358*	0.43	119	*STAMBP*	0.46
15	*THC2661917*	0.33	50	*ZNF235*	0.39	85	*NDST1*	0.44	120	*VN1R1*	0.46
16	*GPR146*	0.34	51	*CACNG3*	0.40	86	*ANKRD29*	0.44	121	*FCGRT*	0.46
17	*RAB15*	0.34	52	*CX3CL1*	0.40	87	*C15orf27*	0.44	122	*THC2704037*	0.46
18	*TJP2*	0.34	53	*HIG2*	0.40	88	*RIN3*	0.44	123	*A_24_P933538*	0.46
19	*TPRXL*	0.34	54	*MMACHC*	0.40	89	*CHST6*	0.44	124	*BTG1*	0.47
20	*SLCO5A1*	0.34	55	*LOC644186*	0.40	90	*THC2713795*	0.44	125	*C10orf54*	0.47
21	*ATP4A*	0.34	56	*THC2582897*	0.40	91	*PRDM2*	0.44	126	*CF139200*	0.47
22	*KCNQ1*	0.34	57	*RHCE*	0.40	92	*RHOV*	0.44	127	*ALDOC*	0.47
23	*AI650285*	0.35	58	*HRASLS2*	0.40	93	*TNXB*	0.44	128	*TRAPPC6A*	0.47
24	*EDG5*	0.35	59	*ODF3L1*	0.40	94	*STARD13*	0.44	129	*RARRES3*	0.47
25	*EPB41L1*	0.35	60	*GCET2*	0.40	95	*S81916*	0.45	130	*FGFRL1*	0.47
26	*TBC1D2B*	0.35	61	*THC2668815*	0.40	96	*ANXA8*	0.45	131	*THC2684625*	0.47
27	*LOC439938*	0.35	62	*LOC339240*	0.40	97	*DFNB31*	0.45	132	*NEURL2*	0.47
28	*FILIP1*	0.36	63	*VLDLR*	0.40	98	*WDR33*	0.45	133	*RUFY3*	0.47
29	*AF088026*	0.36	64	*SDC3*	0.40	99	*SMAD7*	0.45	134	*NF1*	0.47
30	*LOC440356*	0.36	65	*RRAGB*	0.41	100	*THC2511028*	0.45	135	*THC2526432*	0.47
31	*ENST00000366784*	0.36	66	*TEP1*	0.41	101	*C14orf49*	0.45	136	*KAL1*	0.48
32	*S100A3*	0.36	67	*NHEDC1*	0.41	102	*SAT1*	0.45	137	*NFKBIL1*	0.48
33	*MCTP1*	0.36	68	*MAGEA2B*	0.41	103	*PIK3IP1*	0.45	138	*A_24_P470782*	0.48
34	*BICD1*	0.36	69	*CCDC19*	0.41	104	*OTOA*	0.45	139	*STARD10*	0.48
35	*CBX7*	0.37	70	*DHDH*	0.42	105	*TIMD4*	0.45	140	*HBD*	0.48

Table S6 (continued)

	Gene symbol	Ratio
141	*NRN1*	0.48
142	*SLC37A3*	0.48
143	*PLTP*	0.48
144	*LPGAT1*	0.48
145	*PHTF2*	0.48
146	*PFN2*	0.48
147	*BNIP3L*	0.48
148	*FHIT*	0.48
149	*CALN1*	0.49
150	*AK125540*	0.49
151	*AK021629*	0.49
152	*AK057720*	0.49
153	*LRRC23*	0.49
154	*CA11*	0.49
155	*MPPE1*	0.49
156	*REPS2*	0.49
157	*CCDC115*	0.49
158	*A_24_P938006*	0.49
159	*MAL*	0.50
160	*PPAPDC3*	0.50
161	*BX647619*	0.50
162	*P4HA1*	0.50
163	*RRAS*	0.50
164	*BHLHB2*	0.50
165	*MYLIP*	0.50
166	*AI801879*	0.50
167	*GARNL4*	0.12
168	*PPP1R1B*	0.17
169	*THC2708687*	0.20
170	*ATP11B*	0.21
171	*GAL3ST1*	0.24
172	*GLIS1*	0.24
173	*IGF1*	0.24
174	*A_32_P67355*	0.24
175	*GH1*	0.26
176	*AK096685*	0.27
177	*A_32_P224040*	0.27
178	*MUC19*	0.28
179	*MT1X*	0.28
180	*MT1E*	0.28
181	*MT1L*	0.28
182	*MT1H*	0.28
183	*ECAT8*	0.28
184	*MT1B*	0.28
185	*MT1G*	0.28
186	*AL832534*	0.28
187	*CLRN1*	0.28
188	*MT2A*	0.28
189	*BC015836*	0.29
190	*BLK*	0.29
191	*ZDHHC11*	0.29
192	*JPH2*	0.29
193	*CSH2*	0.29
194	*SLC6A13*	0.29
195	*ATCAY*	0.29
196	*THC2582296*	0.29
197	*ALDH7A1*	0.29
198	*CSH1*	0.30
199	*KIAA1462*	0.30
200	*CKB*	0.30
201	*C21orf63*	0.31
202	*AF217970*	0.31
203	*KRTAP17-1*	0.31
204	*CABP1*	0.31
205	*CSHL1*	0.32
206	*PPEF1*	0.32
207	*ARHGAP8*	0.32
208	*SEZ6L2*	0.33
209	*MT1A*	0.33
210	*PAGE5*	0.33
211	*AK022892*	0.33
212	*TMEM112*	0.33
213	*AY358804*	0.33
214	*GIMAP1*	0.33
215	*THC2550353*	0.33
216	*APOE*	0.33
217	*BC045163*	0.34
218	*ID1*	0.34
219	*IGSF21*	0.34
220	*CD274*	0.35
221	*FLJ42342*	0.35
222	*AK090499*	0.35
223	*ENST00000361259*	0.35
224	*TNFAIP2*	0.35
225	*GPR174*	0.36
226	*C1orf170*	0.36
227	*TF*	0.37
228	*DSCAML1*	0.37
229	*SPINT2*	0.37
230	*PIGZ*	0.37
231	*BAIAP2*	0.37
232	*THC2529684*	0.37
233	*RPL10*	0.37
234	*THC2525505*	0.37
235	*OR10J5*	0.37
236	*GRM8*	0.38
237	*SLAMF1*	0.38
238	*GPR30*	0.38
239	*CBLN3*	0.38
240	*ADARB2*	0.38
241	*KLK1*	0.38
242	*EFEMP2*	0.39
243	*MTL5*	0.39
244	*SLC22A18*	0.39
245	*ATP8B3*	0.39
246	*A_24_P686263*	0.40
247	*KIR2DL4*	0.40
248	*TMPIT*	0.40
249	*DRD1IP*	0.40
250	*BM008292*	0.41
251	*IL3RA*	0.41
252	*THC2694227*	0.41
253	*FAM132A*	0.41
254	*TRIM50*	0.42
255	*THC2650352*	0.42
256	*SELS*	0.42
257	*LSS*	0.42
258	*SEC24A*	0.42
259	*RNF43*	0.42
260	*ST7L*	0.42
261	*IFT140*	0.43
262	*PLD1*	0.43
263	*ENST00000381800*	0.43
264	*C19orf41*	0.43
265	*HSD17B14*	0.44
266	*CXCR7*	0.44
267	*MGC31957*	0.44
268	*SLC12A7*	0.44
269	*A_23_P106814*	0.44
270	*ERN1*	0.44
271	*CDA*	0.45
272	*SH3YL1*	0.45
273	*ENST00000354349*	0.45
274	*CRIP1*	0.46
275	*TCP10L*	0.46
276	*NMU*	0.46
277	*THC2616715*	0.46
278	*LHPP*	0.46
279	*TTYH1*	0.46
280	*PHF21A*	0.47
281	*RALGPS1*	0.48
282	*ENG*	0.33